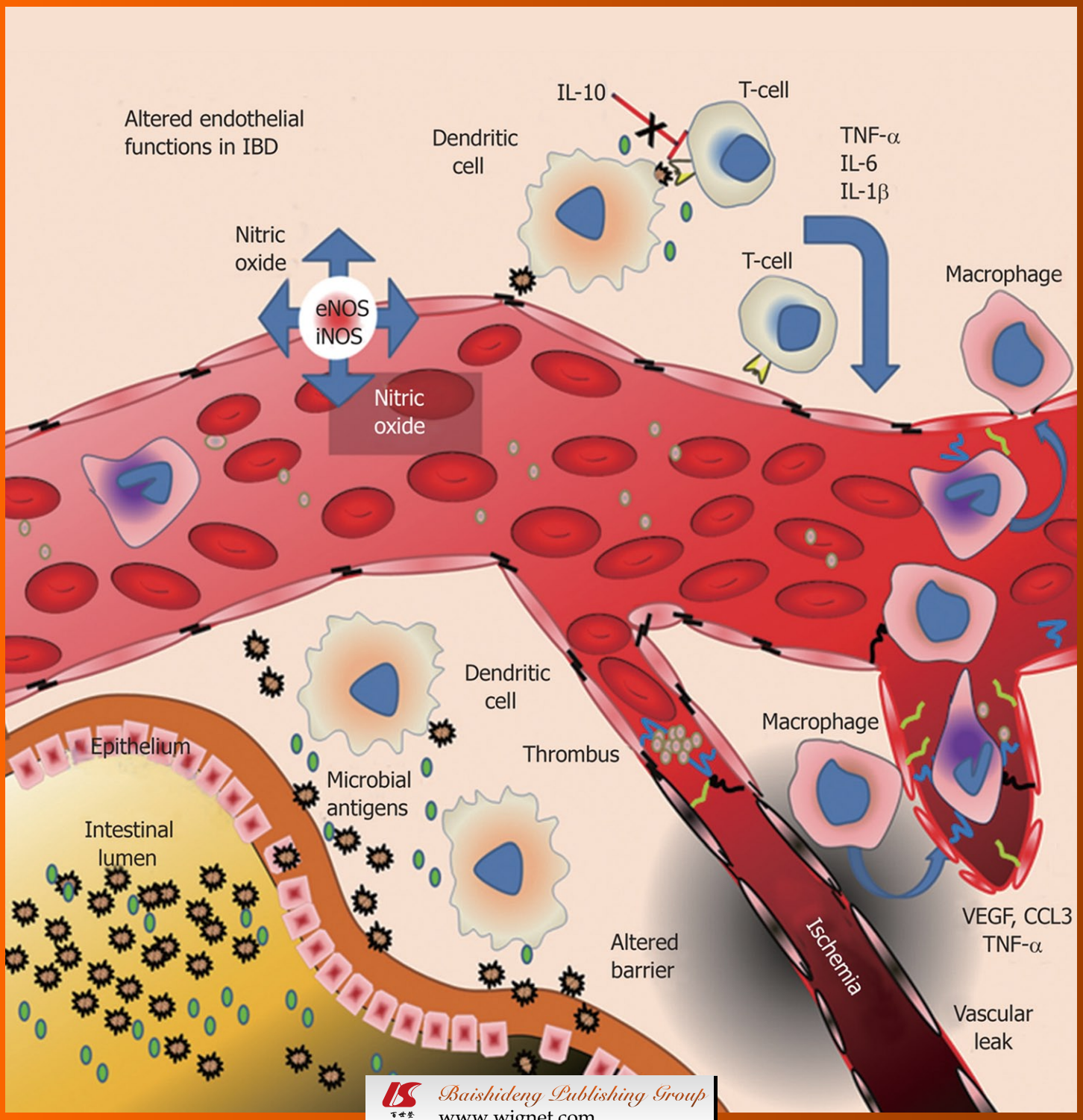
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2011 February 7; 17(5): 545-680



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

A peer-reviewed, online, open-access journal of gastroenterology and hepatology

Editorial Board

2010-2013

The *World Journal of Gastroenterology* Editorial Board consists of 1144 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 60 countries, including Albania (1), Argentina (8), Australia (29), Austria (14), Belgium (12), Brazil (10), Brunei Darussalam (1), Bulgaria (2), Canada (20), Chile (3), China (69), Colombia (1), Croatia (2), Cuba (1), Czech (4), Denmark (8), Ecuador (1), Egypt (2), Estonia (2), Finland (8), France (24), Germany (75), Greece (14), Hungary (10), India (26), Iran (6), Ireland (7), Israel (12), Italy (101), Japan (112), Jordan (1), Kuwait (1), Lebanon (3), Lithuania (2), Malaysia (1), Mexico (10), Moldova (1), Netherlands (29), New Zealand (2), Norway (11), Pakistan (2), Poland (11), Portugal (4), Romania (3), Russia (1), Saudi Arabia (3), Serbia (3), Singapore (10), South Africa (2), South Korea (32), Spain (38), Sweden (18), Switzerland (11), Thailand (1), Trinidad and Tobago (1), Turkey (24), United Arab Emirates (2), United Kingdom (82), United States (249), and Uruguay (1).

Robert JL Fraser, *Daw Park*
Jacob George, *Westmead*
Mark D Gorrell, *Sydney*
Alexander G Heriot, *Melbourne*
Michael Horowitz, *Adelaide*
John E Kellow, *Sydney*
William Kemp, *Melbourne*
Finlay A Macrae, *Victoria*
Daniel Markovich, *Brisbane*
Vance Matthews, *Melbourne*
Phillip S Oates, *Perth*
Shan Rajendra, *Tasmania*
Rajvinder Singh, *Elizabeth Vale*
Ross C Smith, *Sydney*
Kevin J Spring, *Brisbane*
Nathan Subramaniam, *Brisbane*
Phil Sutton, *Melbourne*
Cuong D Tran, *North Adelaide*
Debbie Trinder, *Fremantle*
David Ian Watson, *Bedford Park*

Austria

Herwig R Cerwenka, *Graz*
Ashraf Dahaba, *Graz*
Peter Ferenci, *Vienna*
Valentin Fuhrmann, *Vienna*
Alfred Gangl, *Vienna*
Alexander M Hirschl, *Wien*
Kurt Lenz, *Linz*
Dietmar Öfner, *Salzburg*
Markus Peck-Radosavljevic, *Vienna*
Markus Raderer, *Vienna*
Stefan Riss, *Vienna*
Georg Roth, *Vienna*
Michael Trauner, *Graz*
Thomas Wild, *Kapellerfeld*

Belgium

Rudi Beyaert, *Gent*
Benedicte Y De Winter, *Antwerp*
Inge I Depoortere, *Leuven*
Olivier Detry, *Liège*
Philip Meuleman, *Ghent*
Marc Peeters, *De Pintelaan*
Freddy Penninckx, *Leuven*
Jean-Yves L Reginster, *Liège*
Mark De Ridder, *Brussels*
Etienne M Sokal, *Brussels*
Kristin Verbeke, *Leuven*
Eddie Wisse, *Keerbergen*

Brazil

José LF Caboclo, *São José do Rio Preto*
Roberto J Carvalho-Filho, *São Paulo*
Jaime Natan Eisig, *São Paulo*
Andre Castro Lyra, *Salvador*
Marcelo Lima Ribeiro, *Braganca Paulista*
Joao Batista Teixeira Rocha, *Santa Maria*
Heitor Rosa, *Goiania*
Damiao C Moraes Santos, *Rio de Janeiro*
Ana Cristina Simões e Silva, *Belo Horizonte*
Eduardo Garcia Vilela, *Belo Horizonte*

Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*

Bulgaria

Zahariy Krastev, *Sofia*
Mihaela Petrova, *Sofia*

Canada

Alain Bitton, *Montreal*
Michael F Byrne, *Vancouver*
Kris Chadee, *Calgary*
Wangxue Chen, *Ottawa*
Ram Prakash Galwa, *Ottawa*
Philip H Gordon, *Montreal*
Waliul Khan, *Ontario*
Qiang Liu, *Saskatoon*
John K Marshall, *Ontario*
Andrew L Mason, *Alberta*
Kostas Pantopoulos, *Quebec*
Nathalie Perreault, *Sherbrooke*
Baljinder Singh Salh, *Vancouver*
Eldon Shaffer, *Calgary*
Martin Storr, *Calgary*
Pingchang Yang, *Hamilton*
Eric M Yoshida, *Vancouver*
Claudia Zwingmann, *Montreal*

Chile

Marcelo A Beltran, *La Serena*
Xabier De Aretxabala, *Santiago*
Silvana Zanlungo, *Santiago*

China

Hui-Jie Bian, *Xi'an*
San-Jun Cai, *Shanghai*
Guang-Wen Cao, *Shanghai*
Xiao-Ping Chen, *Wuhan*
Chi-Hin Cho, *Hong Kong*
Zong-Jie Cui, *Beijing*
Jing-Yuan Fang, *Shanghai*
De-Liang Fu, *Shanghai*
Ze-Guang Han, *Shanghai*
Chun-Yi Hao, *Beijing*
Ming-Liang He, *Hong Kong*
Ching-Lung Lai, *Hong Kong*
Simon Law, *Hong Kong*
Yuk-Tong Lee, *Hong Kong*
En-Min Li, *Shantou*
Fei Li, *Beijing*
Yu-Yuan Li, *Guangzhou*
Zhao-Shen Li, *Shanghai*
Xing-Hua Lu, *Beijing*
Yi-Min Mao, *Shanghai*
Qin Su, *Beijing*
Paul Kwong-Hang Tam, *Hong Kong*
Yuk Him Tam, *Hong Kong*
Ren-Xiang Tan, *Nanjing*
Wei-Dong Tong, *Chongqing*
Eric WC Tse, *Hong Kong*
Fu-Sheng Wang, *Beijing*
Xiang-Dong Wang, *Shanghai*
Nathalie Wong, *Hong Kong*
Justin CY Wu, *Hong Kong*
Wen-Rong Xu, *Zhenjiang*
An-Gang Yang, *Xi'an*
Wei-Cheng You, *Beijing*
Chun-Qing Zhang, *Jinan*
Jian-Zhong Zhang, *Beijing*
Xiao-Peng Zhang, *Beijing*
Xuan Zhang, *Beijing*

Colombia

Germán Campuzano-Maya, *Medellín*

Croatia

Tamara Cacev, *Zagreb*
Marko Duvnjak, *Zagreb*

Cuba

Damian C Rodriguez, *Havana*

Czech

Jan Bures, *Hradec Kralove*
Milan Jirsa, *Praha*
Marcela Kopacova, *Hradec Kralove*
Pavel Trunečka, *Prague*

Denmark

Leif Percival Andersen, *Copenhagen*
Asbjørn M Drewes, *Aalborg*
Morten Frisch, *Copenhagen*
Jan Mollenhauer, *Odense*
Morten Hylander Møller, *Holte*
Søren Rafaelsen, *Vejle*
Jorgen Rask-Madsen, *Skodsborg*
Peer Wille-Jørgensen, *Copenhagen*

Ecuador

Fernando E Sempértegui, *Quito*

Egypt

Zeinab Nabil Ahmed, *Cairo*
Hussein M Atta, *El-Minia*

Estonia

Riina Salupere, *Tartu*
Tamara Vorobjova, *Tartu*

Finland

Saila Kauhanen, *Turku*

Thomas Kietzmann, *Oulu*
Kaija-Leena Kolho, *Helsinki*
Jukka-Pekka Mecklin, *Jyvaskyla*
Minna Nyström, *Helsinki*
Pauli Antero Puolakkainen, *Turku*
Juhani Sand, *Tampere*
Lea Veijola, *Helsinki*

France

Claire Bonithon-Kopp, *Dijon*
Lionel Bueno, *Toulouse*
Sabine Colnot, *Paris*
Catherine Daniel, *Lille Cedex*
Alexis Desmoulière, *Limoges*
Thabut Dominique, *Paris*
Francoise L Fabiani, *Angers*
Jean-Luc Faucheron, *Grenoble*
Jean Paul Galmiche, *Nantes cedex*
Boris Guiu, *Dijon*
Paul Hofman, *Nice*
Laurent Huwart, *Paris*
Juan Iovanna, *Marseille*
Abdel-Majid Khatib, *Paris*
Philippe Lehours, *Bordeaux*
Flavio Maina, *Marseille*
Patrick Marcellin, *Paris*
Rene Gerolami Santandera, *Marseille*
Annie Schmid-Alliana, *Nice cedex*
Alain L Servin, *Châtenay-Malabry*
Stephane Supiot, *Nantes*
Baumert F Thomas, *Strasbourg*
Jean-Jacques Tuech, *Rouen*
Frank Zerbib, *Bordeaux Cedex*

Germany

Erwin Biecker, *Siegburg*
Hubert Blum, *Freiburg*
Thomas Bock, *Tuebingen*
Dean Bogoevski, *Hamburg*
Elfriede Bollschweiler, *Köln*
Jürgen Borlak, *Hannover*
Christa Buechler, *Regensburg*
Jürgen Büning, *Lübeck*
Elke Cario, *Essen*
Bruno Christ, *Halle/Saale*
Christoph F Dietrich, *Bad Mergentheim*
Ulrich R Fölsch, *Kiel*
Nikolaus Gassler, *Aachen*
Markus Gerhard, *Munich*
Dieter Glebe, *Giessen*
Ralph Graeser, *Freiburg*
Axel M Gressner, *Aachen*
Nils Habbe, *Marburg*
Thilo Hackert, *Heidelberg*
Wolfgang Hagmann, *Heidelberg*
Dirk Haller, *Freising*
Philip D Hard, *Giessen*
Claus Hellerbrand, *Regensburg*
Klaus R Herrlinger, *Stuttgart*
Eberhard Hildt, *Berlin*
Andrea Hille, *Goettingen*
Joerg C Hoffmann, *Berlin*
Philipe N Khalil, *Munich*
Andrej Khandoga, *Munich*
Jorg Kleeff, *Munich*
Ingmar Königsrainer, *Tübingen*
Peter Konturek, *Erlangen*
Stefan Kubicka, *Hannover*
Joachim Labenz, *Siegen*
Michael Linnebacher, *Rostock*
Jutta Elisabeth Lüttges, *Riegelsberg*
Peter Malfertheiner, *Magdeburg*
Oliver Mann, *Hamburg*
Peter N Meier, *Hannover*
Sabine Mihm, *Göttingen*
Klaus Mönkemüller, *Bottrop*
Jonas Mudter, *Erlangen*
Sebastian Mueller, *Heidelberg*
Robert Obermaier, *Freiburg*
Matthias Ocker, *Erlangen*
Stephan Johannes Ott, *Kiel*
Gustav Paumgartner, *Munich*
Christoph Reichel, *Bad Brückenau*
Markus Reiser, *Bochum*
Steffen Rickes, *Magdeburg*
Elke Roeb, *Giessen*
Christian Rust, *Munich*
Hans Scherubl, *Berlin*
Martin K Schilling, *Homburg*
Joerg F Schlaak, *Essen*
Rene Schmidt, *Freiburg*
Andreas G Schreyer, *Regensburg*
Karsten Schulmann, *Bochum*
Henning Schulze-Bergkamen, *Mainz*
Manfred V Singer, *Mannheim*
Jens Standop, *Bonn*
Jurgen M Stein, *Frankfurt*
Ulrike S Stein, *Berlin*
Wolfgang R Stremmel, *Heidelberg*
Harald F Teutsch, *Ulm*
Hans L Tillmann, *Leipzig*
Christian Trautwein, *Aachen*
Joerg Trojan, *Frankfurt*
Arndt Vogel, *Hannover*
Siegfried Wagner, *Deggendorf*
Frank Ulrich Weiss, *Greifswald*
Fritz von Weizsäcker, *Berlin*
Thomas Wex, *Magdeburg*
Stefan Wirth, *Wuppertal*
Marty Zdichavsky, *Tübingen*

Greece

Helen Christopoulou-Aletra, *Thessaloniki*
T Choli-Papadopoulou, *Thessaloniki*
Tsianos Epameinondas, *Ioannina*
Ioannis Kanellos, *Thessaloniki*
Elias A Kouroumalis, *Heraklion*
Ioannis E Koutroubakis, *Heraklion*
Michael Koutsilieris, *Athens*
Andreas Larentzakis, *Athens*
Emanuel K Manesis, *Athens*
Spilios Manolakopoulos, *Athens*
Konstantinos Mimidis, *Alexandroupolis*
George Papatheodoridis, *Athens*
Spiros Sgouros, *Athens*
Evangelos Tsiambas, *Ag Paraskevi Attiki*

Hungary

György M Buzás, *Budapest*
László Czakó, *Szeged*
Gyula Farkas, *Szeged*
Peter Hegyi, *Szeged*
Peter L Lakatos, *Budapest*
Yvette Mándi, *Szeged*
Zoltan Rakonczay, *Szeged*
Ferenc Sipos, *Budapest*
Zsuzsa Szondy, *Debrecen*
Gabor Veres, *Budapest*

India

Philip Abraham, *Mumbai*
Vineet Ahuja, *New Delhi*
Giriraj Ratan Chandak, *Hyderabad*
Devinder Kumar Dhawan, *Chandigarh*
Radha K Dhiman, *Chandigarh*
Pankaj Garg, *Panchkula*
Pramod Kumar Garg, *New Delhi*
Debidas Ghosh, *Midnpore*
Uday C Ghoshal, *Lucknow*
Bhupendra Kumar Jain, *Delhi*
Ashok Kumar, *Lucknow*
Bikash Medhi, *Chandigarh*
Sri P Misra, *Allahabad*
Gopal Nath, *Varanasi*
Samiran Nundy, *New Delhi*
Jagannath Palepu, *Mumbai*
Vandana Panda, *Mumbai*
Benjamin Perakath, *Tamil Nadu*
Ramesh Roop Rai, *Jaipur*
Nageshwar D Reddy, *Hyderabad*
Barjesh Chander Sharma, *New Delhi*
Virendra Singh, *Chandigarh*
Rupjyoti Talukdar, *Guwahati*
Rakesh Kumar Tandon, *New Delhi*
Jai Dev Wig, *Chandigarh*

Iran

Mohammad Abdollahi, *Tehran*
Peyman Adibi, *Isfahan*
Seyed-Moayed Alavian, *Tehran*
Seyed Mohsen Dehghani, *Shiraz*
Reza Malekzadeh, *Tehran*
Alireza Mani, *Tehran*

Ireland

Billy Bourke, *Dublin*
Ted Dinan, *Cork*
Catherine Greene, *Dublin*
Ross McManus, *Dublin*
Anthony P Moran, *Galway*
Marion Rowland, *Dublin*

Israel

Simon Bar-Meir, *Hashomer*
Alexander Becker, *Afula*
Abraham R Eliakim, *Haifa*
Sigal Fishman, *Tel Aviv*
Boris Kirshtein, *Beer Sheva*
Eli Magen, *Ashdod*
Menachem Moshkowitz, *Tel-Aviv*
Assy Nimer, *Safed*
Shmuel Odes, *Beer Sheva*
Mark Pines, *Bet Dagan*
Ron Shaoul, *Haifa*
Ami D Sperber, *Beer-Sheva*

Italy

Donato F Altomare, *Bari*
Piero Amodio, *Padova*
Angelo Andriulli, *San Giovanni Rotondo*
Paolo Angeli, *Padova*
Bruno Annibale, *Rome*
Paolo Aurello, *Rome*
Salvatore Auricchio, *Naples*
Antonio Basoli, *Rome*
Claudio Bassi, *Verona*
Gabrio Bassotti, *Perugia*
Mauro Bernardi, *Bologna*
Alberto Biondi, *Rome*
Luigi Bonavina, *Milano*
Guglielmo Borgia, *Naples*
Roberto Berni Canani, *Naples*
Maria Gabriella Caruso, *Bari*
Fausto Catena, *Bologna*
Giuseppe Chiarioni, *Valeggio*
Michele Cicala, *Rome*
Dario Conte, *Milano*
Francesco Costa, *Pisa*
Antonio Craxì, *Palermo*
Salvatore Cucchiara, *Rome*
Giuseppe Currò, *Messina*
Mario M D'Elios, *Florence*
Mirko D'Onofrio, *Verona*
Silvio Danese, *Milano*
Roberto de Franchis, *Milano*
Paola De Nardi, *Milan*
Giovanni D De Palma, *Naples*
Giuliana Decorti, *Trieste*
Gianlorenzo Dionigi, *Varese*
Massimo Falconi, *Verona*
Silvia Fargion, *Milan*
Giammarco Fava, *Ancona*
Francesco Feo, *Sassari*
Alessandra Ferlini, *Ferrara*
Alessandro Ferrero, *Torino*
Mirella Fraquelli, *Milan*
Luca Frulloni, *Verona*
Giovanni B Gaeta, *Napoli*
Antonio Gasbarrini, *Rome*
Edoardo G Giannini, *Genoa*
Alessandro Granito, *Bologna*
Fabio Grizzi, *Milan*
Salvatore Gruttadauria, *Palermo*
Pietro Invernizzi, *Milan*
Achille Iolascon, *Naples*
Angelo A Izzo, *Naples*
Ezio Laconi, *Cagliari*
Giovanni Latella, *L'Aquila*
Massimo Levrero, *Rome*
Francesco Luzza, *Catanzaro*
Lucia Malaguarnera, *Catania*
Francesco Manguso, *Napoli*
Pier Mannuccio Mannucci, *Milan*
Giancarlo Mansueto, *Verona*
Giulio Marchesini, *Bologna*
Mara Massimi, *Coppito*
Giovanni Milito, *Rome*
Giuseppe Montalto, *Palermo*
Giovanni Monteleone, *Rome*
Luca Morelli, *Trento*
Giovanni Musso, *Torino*
Mario Nano, *Torino*
Gerardo Nardone, *Napoli*
Riccardo Nascimbeni, *Brescia*
Valerio Nobili, *Rome*
Fabio Pace, *Milan*
Nadia Peparini, *Rome*
Marcello Persico, *Naples*
Mario Pescatori, *Rome*
Raffaele Pezzilli, *Bologna*
Alberto Piperno, *Monza*
Anna C Piscaglia, *Rome*
Piero Portincasa, *Bari*
Michele Reni, *Milan*
Vittorio Ricci, *Pavia*
Oliviero Riggio, *Rome*
Mario Rizzetto, *Torino*
Ballarin Roberto, *Modena*
Gerardo Rosati, *Potenza*
Franco Roviello, *Siena*
Cesare Ruffolo, *Treviso*
Massimo Rugge, *Padova*
Marco Scarpa, *Padova*
C armelo Scarpignato, *Parma*
Giuseppe Sica, *Rome*
Marco Silano, *Rome*
Pierpaolo Sileri, *Rome*
Vincenzo Stanghellini, *Bologna*
Fiorucci Stefano, *Perugia*
Giovanni Tarantino, *Naples*
Alberto Tommasini, *Trieste*
Guido Torzilli, *Rozzano Milan*
Cesare Tosetti, *Porretta Terme*
Antonello Trecca, *Rome*
Vincenzo Villanacci, *Brescia*
Lucia Ricci Vitiani, *Rome*
Marco Vivarelli, *Bologna*

Japan

Kyoichi Adachi, *Izumo*
Yasushi Adachi, *Sapporo*
Takafumi Ando, *Nagoya*
Akira Andoh, *Otsu*
Masahiro Arai, *Tokyo*
Hitoshi Asakura, *Tokyo*
Kazuo Chijiiwa, *Miyazaki*
Yuichiro Eguchi, *Saga*
Itaru Endo, *Yokohama*
Munechika Enjoji, *Fukuoka*
Yasuhiro Fujino, *Akashi*
Mitsuhiro Fujishiro, *Tokyo*
Kouhei Fukushima, *Sendai*
Masanori Hatakeyama, *Tokyo*
Keiji Hirata, *Kitakyushu*
Toru Hiyama, *Higashihiroshima*
Masahiro Iizuka, *Akita*
Susumu Ikehara, *Osaka*
Kenichi Ikejima, *Bunkyo-ku*
Yutaka Inagaki, *Kanagawa*
Hiromi Ishibashi, *Nagasaki*
Shunji Ishihara, *Izumo*
Toru Ishikawa, *Niigata*
Toshiyuki Ishiwata, *Tokyo*
Hajime Isomoto, *Nagasaki*
Yoshiaki Iwasaki, *Okayama*
Satoru Kakizaki, *Gunma*
Terumi Kamisawa, *Tokyo*
Mototsugu Kato, *Sapporo*
Naoya Kato, *Tokyo*
Takumi Kawaguchi, *Kurume*
Yohei Kida, *Kainan*
Shogo Kikuchi, *Aichi*
Tsuneo Kitamura, *Chiba*
Takashi Kobayashi, *Tokyo*
Yasuhiro Koga, *Isehara*
Takashi Kojima, *Sapporo*
Norihiro Kokudo, *Tokyo*
Masatoshi Kudo, *Osaka*
Shin Maeda, *Tokyo*
Satoshi Mamori, *Hyogo*
Atsushi Masamune, *Sendai*
Yasushi Matsuzaki, *Tsukuba*
Kenji Miki, *Tokyo*
Toshihiro Mitaka, *Sapporo*
Hiroto Miwa, *Hyogo*
Kotaro Miyake, *Tokushima*
Manabu Morimoto, *Yokohama*
Yoshiharu Motoo, *Kanazawa*
Yoshiaki Murakami, *Hiroshima*
Yoshiki Murakami, *Kyoto*
Kunihiko Murase, *Tusima*
Akihito Nagahara, *Tokyo*
Yuji Naito, *Kyoto*
Atsushi Nakajima, *Yokohama*
Hisato Nakajima, *Tokyo*
Hiroki Nakamura, *Yamaguchi*
Shotaro Nakamura, *Fukuoka*
Akimasa Nakao, *Nagogya*
Shuhei Nishiguchi, *Hyogo*
Mikio Nishioka, *Niihama*
Keiji Ogura, *Tokyo*
Susumu Ohmada, *Maebashi*
Hirohide Ohnishi, *Akita*
Kenji Okajima, *Nagoya*
Kazuichi Okazaki, *Osaka*
Morikazu Onji, *Ehime*
Satoshi Osawa, *Hamamatsu*
Hidetsugu Saito, *Tokyo*
Yutaka Saito, *Tokyo*
Naoaki Sakata, *Sendai*
Yasushi Sano, *Chiba*
Tokihiko Sawada, *Tochigi*
Tomohiko Shimatan, *Hiroshima*
Yukihiro Shimizu, *Kyoto*
Shinji Shimoda, *Fukuoka*
Yoshio Shirai, *Niigata*
Masayuki Sho, *Nara*
Shoichiro Sumi, *Kyoto*
Hidekazu Suzuki, *Tokyo*
Masahiro Tajika, *Nagoya*
Yoshihisa Takahashi, *Tokyo*
Toshinari Takamura, *Kanazawa*
Hiroaki Takeuchi, *Kochi*
Yoshitaka Takuma, *Okayama*
Akihiro Tamori, *Osaka*
Atsushi Tanaka, *Tokyo*
Shinji Tanaka, *Hiroshima*
Satoshi Tanno, *Hokkaido*
Shinji Togo, *Yokohama*
Hitoshi Tsuda, *Tokyo*
Hiroyuki Uehara, *Osaka*
Masahito Uemura, *Kashihara*
Yoshiyuki Ueno, *Sendai*
Mitsuyoshi Urashima, *Tokyo*
Takuya Watanabe, *Niigata*
Satoshi Yamagiwa, *Niigata*
Taketo Yamaguchi, *Chiba*
Mitsunori Yamakawa, *Yamagata*
Takayuki Yamamoto, *Yokkaichi*
Yutaka Yata, *Maebashi*
Hiroshi Yoshida, *Tokyo*
Norimasa Yoshida, *Kyoto*
Yuichi Yoshida, *Osaka*
Kentaro Yoshika, *Toyoake*
Hitoshi Yoshiji, *Nara*
Katsutoshi Yoshizato, *Higashihiroshima*
Tomoharu Yoshizumi, *Fukuoka*

Jordan

Ismail Matalka, *Irbid*

Kuwait

Islam Khan, *Safat*

Lebanon

Bassam N Abboud, *Beirut*
Ala I Sharara, *Beirut*
Rita Slim, *Beirut*

Lithuania

Giedrius Barauskas, *Kaunas*
Limas Kupcinskas, *Kaunas*

Malaysia

Andrew Seng Boon Chua, *Ipoh*

Mexico

Richard A Awad, *Mexico*
Aldo Torre Delgadillo, *Mexico*
Diego Garcia-Compean, *Monterrey*
Paulino M Hernández Magro, *Celaya*
Miguel Angel Mercado, *Distrito Federal*
Arturo Panduro, *Jalisco*
Omar Vergara-Fernandez, *Tlalpan*
Saúl Villa-Trevio, *Mexico*

Moldova

Igor Mishin, *Kishinev*

Netherlands

Ulrich Beuers, *Amsterdam*
Lee Bouwman, *Leiden*
Albert J Bredenoord, *Nieuwegein*
Lodewijk AA Brosens, *Utrecht*
J Bart A Crusius, *Amsterdam*
Wouter de Herder, *Rotterdam*
Pieter JF de Jonge, *Rotterdam*
Robert J de Knegt, *Rotterdam*
Wendy W Johanna de Leng, *Utrecht*
Annemarie de Vries, *Rotterdam*
James CH Hardwick, *Leiden*
Frank Hoentjen, *Haarlem*
Misha Luyer, *Sittard*
Jeroen Maljaars, *Maastricht*
Gerrit A Meijer, *Amsterdam*
Servaas Morré, *Amsterdam*
Chris JJ Mulder, *Amsterdam*
John Plukker, *Groningen*
Albert Frederik Pull ter Gunne, *Tilburg*
Paul E Sijens, *Groningen*
BW Marcel Spanier, *Arnhem*
Shiri Sverdlov, *Maastricht*
Maarten Tushuizen, *Amsterdam*
Jantine van Baal, *Heidelberglaan*
Astrid van der Velde, *The Hague*
Karel van Erpecum, *Utrecht*
Loes van Keimpema, *Nijmegen*
Robert Christiaan Verdonk, *Groningen*
Erwin G Zoetendal, *Wageningen*

New Zealand

Andrew S Day, *Christchurch*

Norway

Olav Dalgard, *Oslo*
Trond Peder Flaten, *Trondheim*
Reidar Fossmark, *Trondheim*
Rasmus Goll, *Tromso*
Ole Høie, *Arendal*
Asle W Medhus, *Oslo*
Espen Melum, *Oslo*
Trine Olsen, *Tromso*
Eyvind J Paulssen, *Tromso*
Jon Arne Søreide, *Stavanger*
Kjetil Soreide, *Stavanger*

Pakistan

Shahab Abid, *Karachi*
Syed MW Jafri, *Karachi*

Poland

Marek Bebenek, *Wroclaw*
Tomasz Brzozowski, *Cracow*
Halina Cichoż-Lach, *Lublin*
Andrzej Dabrowski, *Bialystok*
Hanna Gregorek, *Warsaw*
Marek Hartleb, *Katowice*
Beata Jolanta Jabłońska, *Katowice*
Stanislaw J Konturek, *Krakow*
Jan Kulig, *Krakow*
Dariusz M Lebensztejn, *Bialystok*
Julian Swierczynski, *Gdansk*

Portugal

Raquel Almeida, *Porto*
Ana Isabel Lopes, *Lisboa Codex*
Ricardo Marcos, *Porto*
Guida Portela-Gomes, *Estoril*

Romania

Dan L Dumitrascu, *Cluj*
Adrian Saftoiu, *Craiova*
Andrada Seicean, *Cluj-Napoca*

Russia

Vasiliy I Reshetnyak, *Moscow*

Saudi Arabia

Ibrahim A Al Mofleh, *Riyadh*
Abdul-Wahed Meshikhes, *Qatif*
Faisal Sanai, *Riyadh*

Serbia

Tamara M Alempijevic, *Belgrade*
Dusan M Jovanovic, *Sremska Kamenica*
Zoran Krivokapic, *Belgrade*

Singapore

Madhav Bhatia, *Singapore*
Kong Weng Eu, *Singapore*
Brian Kim Poh Goh, *Singapore*
Khek-Yu Ho, *Singapore*
Kok Sun Ho, *Singapore*
Fock Kwong Ming, *Singapore*
London Lucien Ooi, *Singapore*
Nagarajan Perumal, *Singapore*
Francis Seow-Choen, *Singapore*

South Africa

Rosemary Joyce Burnett, *Pretoria*
Michael Kew, *Cape Town*

South Korea

Sang Hoon Ahn, *Seoul*
Sung-Gil Chi, *Seoul*
Myung-Gyu Choi, *Seoul*
Hoon Jai Chun, *Seoul*
Yeun-Jun Chung, *Seoul*
Young-Hwa Chung, *Seoul*
Kim Donghee, *Seoul*
Ki-Baik Hahm, *Incheon*
Sun Pyo Hong, *Geonggi-do*
Seong Gyu Hwang, *Seongnam*
Hong Joo Kim, *Seoul*
Jae J Kim, *Seoul*
Jin-Hong Kim, *Suwon*
Nayoung Kim, *Seongnam-si*
Sang Geon Kim, *Seoul*
Seon Hahn Kim, *Seoul*
Sung Kim, *Seoul*
Won Ho Kim, *Seoul*
Jeong Min Lee, *Seoul*
Kyu Taek Lee, *Seoul*
Sang Kil Lee, *Seoul*
Sang Yeoup Lee, *Gyeongsangnam-do*
Yong Chan Lee, *Seoul*
Eun-Yi Moon, *Seoul*
Hyoung-Chul Oh, *Seoul*
Seung Woon Paik, *Seoul*
Joong-Won Park, *Goyang*
Ji Kon Ryu, *Seoul*
Si Young Song, *Seoul*
Marie Yeo, *Suwon*
Byung Chul Yoo, *Seoul*
Dae-Yeul Yu, *Daejeon*

Spain

Maria-Angeles Aller, *Madrid*
Raul J Andrade, *Málaga*
Luis Aparisi, *Valencia*
Gloria González Aseguinolaza, *Navarra*
Matias A Avila, *Pamplona*

Fernando Azpiroz, *Barcelona*
Ramon Bataller, *Barcelona*
Belén Beltrán, *Valencia*
Adolfo Benages, *Valencia*
Josep M Bordas, *Barcelona*
Lisardo Boscá, *Madrid*
Luis Bujanda, *San Sebastián*
Juli Busquets, *Barcelona*
Matilde Bustos, *Pamplona*
José Julián calvo Andrés, *Salamanca*
Andres Cardenas, *Barcelona*
Antoni Castells, *Barcelona*
Fernando J Corrales, *Pamplona*
J E Domínguez-Muñoz, *Santiago de Compostela*
Juan Carlos Laguna Egea, *Barcelona*
Isabel Fabregat, *Barcelona*
Antoni Farré, *Barcelona*
Vicente Felipo, *Valencia*
Laureano Fernández-Cruz, *Barcelona*
Luis Grande, *Barcelona*
Angel Lanas, *Zaragoza*
Juan-Ramón Larrubia, *Guadalajara*
María IT López, *Jaén*
Juan Macías, *Seville*
Javier Martin, *Granada*
José Manuel Martin-Villa, *Madrid*
Julio Mayol, *Madrid*
Mireia Miquel, *Sabadell*
Albert Parés, *Barcelona*
Jesús M Prieto, *Pamplona*
Pedro L Majano Rodriguez, *Madrid*
Joan Roselló-Catafau, *Barcelona*
Eva Vaquero, *Barcelona*

Sweden

Lars Erik Agréus, *Stockholm*
Mats Andersson, *Stockholm*
Roland Andersson, *Lund*
Mauro D'Amato, *Huddinge*
Evangelos Kalaitzakis, *Gothenburg*
Greger Lindberg, *Stockholm*
Annika Lindblom, *Stockholm*
Sara Lindén, *Göteborg*
Hanns-Ulrich Marschall, *Stockholm*
Pär Erik Myrelid, *Linköping*
Åke Nilsson, *Lund*
Helena Nordenstedt, *Stockholm*
Kjell Öberg, *Uppsala*
Lars A Pahlman, *Uppsala*
Stefan G Pierzynowski, *Lund*
Sara Regnér, *Malmö*
Bobby Tingstedt, *Lund*
Zongli Zheng, *Stockholm*

Switzerland

Pascal Bucher, *Geneva*
Michelangelo Foti, *Geneva*
Jean L Frossard, *Geneva*
Andreas Geier, *Zürich*
Pascal Gervaz, *Geneva*
Gerd A Kullak-Ublick, *Zürich*
Fabrizio Montecucco, *Geneva*
Paul M Schneider, *Zürich*
Felix Stickel, *Berne*
Bruno Stieger, *Zürich*
Inti Zlobec, *Basel*

Trinidad and Tobago

Shivananda Nayak, *Mount Hope*

Turkey

Sinan Akay, *Tekirdag*
Metin Basaranoglu, *Istanbul*
Yusuf Bayraktar, *Ankara*
A Mithat Bozdayi, *Ankara*
Hayrullah Derici, *Balıkesir*
Eren Ersoy, *Ankara*
Mukaddes Esrefoglu, *Malatya*
Can Goen, *Kutahya*
Selin Kapan, *Istanbul*
Aydin Karabacakoglu, *Konya*
Cuneyt Kayaalp, *Malatya*
Kemal Kismet, *Ankara*
Seyfettin Köklü, *Ankara*
Mehmet Refik Mas, *Etlik-Ankara*
Osman C Ozdogan, *Istanbul*
Bülent Salman, *Ankara*
Orhan Sezgin, *Mersin*
Ilker Tasci, *Ankara*
Müge Tecder-Ünal, *Ankara*
Ahmet Tekin, *Mersin*
Mesut Tez, *Ankara*
Ekmel Tezel, *Ankara*
Özlem Yilmaz, *Izmir*

United Arab Emirates

Fikri M Abu-Zidan, *Al-Ain*
Sherif M Karam, *Al-Ain*

United Kingdom

Simon Afford, *Birmingham*
Navneet K Ahluwalia, *Stockport*
Mohamed H Ahmed, *Southampton*
Basil Ammori, *Salford*
Lesley A Anderson, *Belfast*
Chin Wee Ang, *Liverpool*
Yeng S Ang, *Wigan*
Anthony TR Axon, *Leeds*
Kathleen B Bamford, *London*
Jim D Bell, *London*
John Beynon, *Swansea*
Chris Briggs, *Sheffield*
Geoffrey Burnstock, *London*
Alastair D Burt, *Newcastle*
Jeff Butterworth, *Shrewsbury*
Jeremy FL Cobbold, *London*
Jean E Crabtree, *Leeds*
Tatjana Crnogorac-Jurcevic, *London*
William Dickey, *Londonderry*
Sunil Dolwani, *Cardiff*
Emad M El-Omar, *Aberdeen*
A M El-Tawil, *Birmingham*
Charles B Ferguson, *Belfast*
Andrew Fowell, *Southampton*
Piers Gatenby, *London*
Daniel R Gaya, *Edinburgh*
Anil George, *London*
Rob Glynne-Jones, *Northwood*
Jason CB Goh, *Birmingham*
Gianpiero Gravante, *Leicester*
Brian Green, *Belfast*
William Greenhalf, *Liverpool*
Indra N Guha, *Nottingham*
Stefan G Hübscher, *Birmingham*
Robin Hughes, *London*
Pali Hungin, *Stockton*
Nawfal Hussein, *Nottingham*
Clement W Imrie, *Glasgow*
Janusz AZ Jankowski, *Oxford*
Sharad Karandikar, *Birmingham*
Peter Karayiannis, *London*
Shahid A Khan, *London*
Patricia F Lalor, *Birmingham*
John S Leeds, *Sheffield*
Ian Lindsey, *Oxford*
Hong-Xiang Liu, *Cambridge*
Dileep N Lobo, *Nottingham*
Graham MacKay, *Glasgow*
Mark Edward McAlindon, *Sheffield*
Anne McCune, *Bristol*
Donald Campbell McMillan, *Glasgow*
Giorgina Mieli-Vergani, *London*
Jamie Murphy, *London*
Guy Fairbairn Nash, *Poole*
James Neuberger, *Birmingham*
Patrick O'Dwyer, *Glasgow*
Christos Paraskeva, *Bristol*
Richard Parker, *North Staffordshire*
Thamara Perera, *Birmingham*
Kondragunta Rajendra Prasad, *Leeds*
D Mark Pritchard, *Liverpool*
Alberto Quaglia, *London*
Akhilesh B Reddy, *Cambridge*
Kevin Robertson, *Glasgow*
Sanchoy Sarkar, *Liverpool*
John B Schofield, *Kent*
Marco Senzolo, *Padova*
Venkatesh Shanmugam, *Derby*
Paul Sharp, *London*
Chew Thean Soon, *Manchester*
Aravind Suppiah, *East Yorkshire*
Noriko Suzuki, *Middlesex*
Simon D Taylor-Robinson, *London*
Frank I Tovey, *London*
A McCulloch Veitch, *Wolverhampton*
Vamsi R Velchuru, *Lowestoft*
Sumita Verma, *Brighton*
Catherine Walter, *Cheltenham*
Julian RF Walters, *London*
Roger Williams, *London*

United States

Kareem M Abu-Elmagd, *Pittsburgh*
Sami R Achem, *Florida*
Golo Ahlenstiel, *Bethesda*
Bhupinder S Anand, *Houston*
M Ananthanarayanan, *New York*
Balamurugan N Appakalal, *Minneapolis*
Dimitrios V Avgerinos, *New York*
Shashi Bala, *Worcester*
Anthony J Bauer, *Pittsburgh*
Kevin E Behrns, *Gainesville*
Roberto Bergamaschi, *New York*
Henry J Binder, *New Haven*
Edmund J Bini, *New York*
Wojciech Blonski, *Philadelphia*
Mark Bloomston, *Columbus*
Edward L Bradley III, *Sarasota*
Carla W Brady, *Durham*

World Journal of Gastroenterology

Contents

Weekly Volume 17 Number 5 February 7, 2011

World Journal of Gastroenterology
Volume 17 Number 5 February 7, 2011

Contents

NAME OF JOURNAL
World Journal of Gastroenterology

LAUNCH DATE
October 1, 1995

RESPONSIBLE INSTITUTION
Department of Science and Technology of Shanxi Province

SPONSOR
Taiyuan Research and Treatment Center for Digestive Diseases, 77 Shuangta Xijie, Taiyuan 030001, Shanxi Province, China

EDITING
Editorial Board of *World Journal of Gastroenterology*,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0039
Fax: +86-10-8538-1893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHING
Baishideng Publishing Group Co., Limited,
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-3115-8812
Telephone: +852-5804-2046
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

SUBSCRIPTION
Beijing Baishideng BioMed Scientific Co., Ltd.,
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-8538-1892
Fax: +86-10-8538-1893
E-mail: baishideng@wjgnet.com
http://www.wjgnet.com

PRINT SUBSCRIPTION
RMB 245 Yuan for each issue, RMB 11760 Yuan for one year.

ONLINE SUBSCRIPTION
One-Year Price 864.00 USD

PUBLICATION DATE
February 7, 2011

CSSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

EDITORIAL OFFICE
Jian-Xia Cheng, Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-5908-0039
Fax: +86-10-8538-1893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm. If you do not have web access please contact the editorial office.

ONLINE SUBMISSION
http://www.wjgnet.com/1007-9327office

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.545

World J Gastroenterol 2011 February 7; 17(5): 545-546
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

What's hot in inflammatory bowel disease in 2011?

Silvio Danese

Silvio Danese, IBD Center, Division of Gastroenterology, IRCCS Istituto Clinico Humanitas, Via Manzoni 56, 20089, Rozzano, Milan, Italy
Author contributions: Danese S contributed all to the paper.
Correspondence to: Dr. Silvio Danese, MD, PhD, IBD Center, Division of Gastroenterology, IRCCS Istituto Clinico Humanitas, Via Manzoni 56, 20089, Rozzano, Milan, Italy. sdanese@hotmail.com
Telephone: +39-2-82244771 Fax: +39-2-82244590
Received: January 4, 2011 Revised: January 18, 2011
Accepted: January 25, 2011
Published online: February 7, 2011

Abstract

Ulcerative colitis and Crohn's disease (CD) are the two major forms of inflammatory bowel disease (IBD). In this highlight topic series of articles, the most recent advances in the IBD field are reviewed, especially the newly described cytokines, including the therapeutic implications for their manipulation. In addition, the interplay between the intestinal microbiota and the host is reviewed, including the role of defensins and dysbiosis in CD pathogenesis. Finally, the importance of the non immune systems such as endothelial cells and the hemostatic system are highlighted as new players in IBD pathogenesis.

Key words: Crohn's disease; Ulcerative colitis; Inflammatory bowel disease; Immunology; Pathogenesis

Danese S. What's hot in inflammatory bowel disease in 2011? *World J Gastroenterol* 2011; 17(5): 545-546 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/545.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.545

In this special issue of *World Journal of Gastroenterology* (*WJG*), we have selected an expert group that is actively involved in the investigation of inflammatory bowel disease (IBD) pathogenesis.

Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of IBD.

These diseases still pose major clinical and therapeutic challenges to the gastroenterological comunity.

It is now clear that CD and UC represent two distinct forms of chronic inflammation of the gastrointestinal tract and have different causes and pathogenic mechanisms. Still, the factors underlying the appearance of both CD and UC are roughly the same, and include a temporal association with progressive changes in the environment, an intrinsic genetic predisposition, the existence of a rich enteric flora, and an abnormal immune reactivity which is ultimately responsible for damaging the gut and causing clinical manifestations. Even though the categories of underlying factors are roughly the same, there are variations in each category as well as differences in how the underlying factors interact. The end result is two related but distinct disorders named CD and UC. In this special issue of *WJG*, differences and similarities of the etiopathogenic factors in each form of IBD will be illustrated and discussed in each review assessing the newly described cytokines[1], the interplay between the intestinal microbiota and the host[2], the role of defensins and dysbiosis[3] and the importance of extraluminal factors[4] and non immune systems such as endothelial cells[5] and the hemostatic system[6] as new players in IBD pathogenesis.

Since the recognition of IBD as a perplexing and challenging clinical entity, the investigation of its pathogenic mechanisms has gone through repeated cycles of new hopes, new knowledge, and new realities. Infectious, allergic, dietary, psychosocial, environmental, microbial, vascular, metabolic, immune and other basic theories have been put forward, most of them to be rebuked, if not ridiculed. At the moment, we appear to have settled down on a unifying but still wide-ranging hypothesis that IBD results from complex interactions between evolv-

ing environmental changes induced by society progress, a still undefined number of predisposing genetic mutations, an incredibly complex gut microbiota that may be constantly varying, and the intricacies of individual immune systems. The ability to integrate all these various components into a single cohesive and logical pathway of disease that explains all aspects of IBD appears still a bit distant at the moment. On the other hand, if we look back at where we stood only two or three decades ago, the progress achieved in our understanding of IBD pathogenesis and the way it has changed our approach to therapy is just short of spectacular.

Although we have made tremendous advances in disease pathogenesis, among the many diseases that exist, IBD is the one for which the exact etiology remains obscure and the mechanisms underlying tissue injury appear to be exceedingly complex. This certainly seems to be the case for the two main forms of IBD, namely CD and UC.

REFERENCES

1 **Bouguen G**, Chevaux JB, Peyrin-Biroulet L. Recent advances in cytokines: Therapeutic implications for inflammatory bowel diseases. *World J Gastroenterol* 2011; **17**: 547-556

2 **Fava F**, Danese S. Intestinal microbiota in inflammatory bowel disease: Friends or foes? *World J Gastroenterol* 2011; **17**: 557-566

3 **Chamaillard M**, Dessein R. Defensins couple dysbiosis to primary immunodeficiency in Crohn's disease. *World J Gastroenterol* 2011; **17**: 567-571

4 **Batra A**, Stroh T, Siegmund B. Extraluminal factors contributing to inflammatory bowel disease. *World J Gastroenterol* 2011; **17**: 572-577

5 **Cromer WE**, Mathis JM, Granger DN, Chaitanya GV, Alexander JS. Role of the endothelium in inflammatory bowel diseases. *World J Gastroenterol* 2011; **17**: 578-593

6 **Scaldaferri F**, Lancellotti S, Pizzoferrato M, De Cristofaro R. Haemostatic system in inflammatory bowel diseases: New players in gut inflammation. *World J Gastroenterol* 2011; **17**: 594-608

S- Editor Cheng JX **L- Editor** Wang XL **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.547

World J Gastroenterol 2011 February 7; 17(5): 547-556
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

Silvio Danese, MD, PhD, Head, *Series Editor*

Recent advances in cytokines: Therapeutic implications for inflammatory bowel diseases

Guillaume Bouguen, Jean-Baptiste Chevaux, Laurent Peyrin-Biroulet

Guillaume Bouguen, Inserm UMR991, Department of Hepato-Gastroenterology, University Hospital of Rennes, Pontchaillou, 35000 Rennes, France
Jean-Baptiste Chevaux, Laurent Peyrin-Biroulet, Inserm U954, Department of Hepato-Gastroenterology, University Hospital of Nancy, 54511 Vandoeuvre-les-Nancy, France
Author contributions: Bouguen G wrote the first draft of the paper; Chevaux JB and Peyrin-Biroulet L revised the manuscript.
Correspondence to: Laurent Peyrin-Biroulet, MD, PhD, Professor, Inserm U954, Department of Hepato-Gastroenterology, University Hospital of Nancy, Allée du Morvan, 54511 Vandoeuvre-les-Nancy, France. peyrin-biroulet@netcourrier.com
Telephone: +33-3-83153631 Fax: +33-3-83153633
Received: March 1, 2010 Revised: March 22, 2010
Accepted: March 29, 2010
Published online: February 7, 2011

Abstract

Inflammatory bowel diseases (IBDs) are complex and chronic disabling conditions resulting from a dysregulated dialogue between intestinal microbiota and components of both the innate and adaptive immune systems. Cytokines are essential mediators between activated immune and non-immune cells, including epithelial and mesenchymal cells. They are immunomodulatory peptides released by numerous cells and these have significant effects on immune function leading to the differentiation and survival of T cells. The physiology of IBD is becoming a very attractive field of research for development of new therapeutic agents. These include cytokines involved in intestinal immune inflammation. This review will focus on mechanisms of action of cytokines involved in IBD and new therapeutic opportunities for these diseases.

Key words: Inflammatory bowel disease; Ulcerative colitis; Crohn's disease; Cytokine; Pathophysiology; Biological therapy

Peer reviewer: Takayuki Yamamoto, MD, Inflammatory Bowel Disease Center, Yokkaichi Social Insurance Hospital, 10-8 Hazuyamacho, Yokkaichi 510-0016, Japan

Bouguen G, Chevaux JB, Peyrin-Biroulet L. Recent advances in cytokines: Therapeutic implications for inflammatory bowel diseases. *World J Gastroenterol* 2011; 17(5): 547-556 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/547.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.547

INTRODUCTION

Ulcerative colitis (UC) and Crohn's disease (CD) are immune-mediated disorders of the intestine[1]. Accumulating data suggests that inflammatory bowel disease (IBD) results from an inappropriate inflammatory response to intestinal microbes in a genetically susceptible host[2]. Emerging evidence suggests that disease development implicates a dysregulated dialogue between the intestinal flora and components of both the innate and adaptive immune systems[3,4].

Active IBD is defined as an infiltration of the lamina propria by innate immune cells [neutrophils, macrophages, dendritic and natural killer (NK) T cells] and adaptive immune cells (B and T cells). Increased numbers and activation of these cells in the intestinal mucosa enhance local levels of tumor necrosis factor-α (TNF-α) and several proinflammatory interleukins (IL)[2,5]. Cytokines are essential mediators of the interaction between activated immune cells and non-immune cells, including epithelial and mesenchymal cells[6,7].

Recent advances in the study of the regulation of key cytokines during major forms of IBD promise the development of more effective mechanism-based therapies[8]. Given that many of these involve regulation of dynamic biological processes, it is likely that the most effective agents will fall within the broad rubric of biologic therapy.

The prototypic example of the ability of a biologic agent to effectively change the therapeutic landscape is provided by anti-TNF-α, first demonstrated through clinical validation of the prototypic agent infliximab[8]. The advent of anti-TNF-α agents has changed the way of treating IBD refractory to standard medications[3,9].

Advances in the understanding of IBD pathophysiology have become a very active area for the development of novel therapeutic agents. New targets for biologics include cytokines involved in intestinal immune inflammation that have led to new therapeutic opportunities[10,11]. Although IBD etiology is unknown, some molecules which are involved in the physiopathology have been identified and can be targeted by biological therapies[12]. This review will focus on cytokines involved in the dysregulated inflammatory response in IBD and targeted by biological therapies.

CYTOKINE NETWORK AND IMMUNITY

Cytokines (from greek cyto: cell; kinos: movement) are substances that are secreted by specific cells of the immune system and carry signals locally between cells, with extensive use in cellular communication. The term "cytokine" encompasses a large and diverse family of polypeptide regulators that are produced widely throughout the body by cells of diverse embryological origin. Basically, the term "cytokine" has been used to refer to the immunomodulating agent. Interferon was the first cytokine to be described in 1957[13]. The clinical efficacy of targeting TNF-α indicates that cytokines are potential therapeutic targets in IBD[6].

Cytokines have profound effects on immune functions[14]. Beyond the classical T helper Th1/Th2 paradigm indicating predominant Th1-mediated responses dominated by the production of interferon-γ (IFN-γ) in CD and an exaggerated Th2-like inflammation in UC characterized by an increased production of IL-13[2,15], there has been a surge of information with regard to the role of innate immunity in IBD pathogenesis. Thus new data on adaptive immunity are emerging, indicating that: (1) the mucosal Th1 and Th2 responses of CD and UC may be actually secondary to defects of the innate immune response; (2) the dysfunction of regulatory T cells may be contributing to mucosal immune abnormalities; and (3) the newly described Th17 cells are also prominently involved in the gut inflammatory response in both forms of IBD[5,15].

The differentiation and survival of T cells depend on the relative amount of key regulatory cytokines produced mainly by macrophages and dendritic cells[12]. In the presence of IL-12 and IFN-γ, naive CD4+ T cells adopt a Th1 phenotype which then activate macrophages that release IL-1, IL-6 and TNF-α. Thus this creates a positive feedback loop[3,6,12]. In the presence of IL-4, naive CD4-T cells adopt a Th2 phenotype[12,16]. The Th17 development is triggered by both IL-6, IL-21, IL-23 and transforming growth factor-β (TGF-β), leading to secretion of the IL-17 cytokine family and IL-22[6]. Although the function of Th17 cells is not clearly known, there is probably an important part of this T cell population which expresses IL-23 receptors. This has been recently demonstrated as an IBD susceptibility gene in genome-wide association studies.

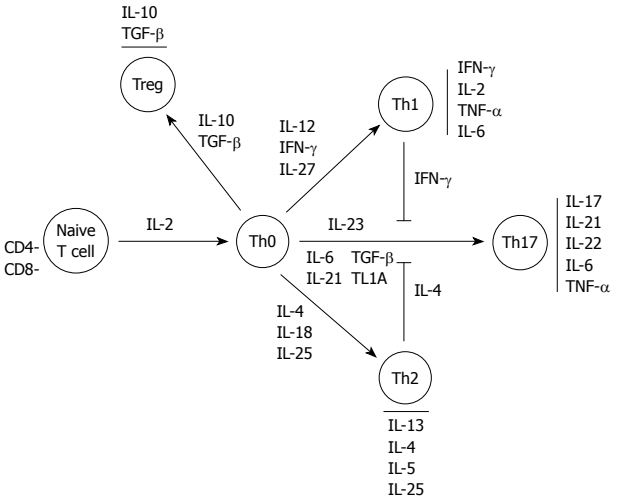


Figure 1 Overview of T cell differentiation and interleukin pathways. IL: Interleukin; IFN: Interferon; TGF: Transforming growth factor; TNF: Tumor necrosis factor; TL1A: TNF-like factor 1A.

In contrast, TGF-β and IL-10 modulate differentiation of naïve T cells to T regulatory cell subgroups leading to high amounts of IL-10 and TGF-β, and are able to suppress bystander T cell activation. This could be defective in IBD[17-20]. There is a complex network between these different cell populations in the case of inflammation as, for example, in the negative crossregulation of the differentiation of Th17 cells by Th2 cells (IL-4, IL-27) and Th1 cells (IFN-γ) (Figure 1)[21].

PROINFLAMMATORY CYTOKINES

TNF family: TNF-α and TNF-like factor 1A

Mechanisms of action: TNF-α is a major mediator of inflammation in the gut[22-25]. It is synthesized by several cells including intestinal epithelial cells but predominantly by cells of the monocyte line and T lymphocytes[14]. TNF-α is a homotrimeric protein that mediates its diverse biologic effects through 2 distinct receptors known as TNF-α receptor type I expressed on all nucleated cells and TNF-α receptor type Ⅱ restricted to cells of hematopoietic lineage[26]. Through the activation of nuclear factor-κB (NF-κB), TNF-α induces the expression of various genes such as urokinase plasminogen activator, cyclooxygenase Ⅱ (COX Ⅱ) and vascular endothelial growth factor (VEGF)[26]. By this method, TNF-α has multiple biological effects such as increasing leukocyte recruitment (induction of leukocyte adhesion molecules)[27,28], modulation of nitric oxide (NO) production (increasing the vascular permeability)[29,30], induction of secretion of proinflammatory cytokines[31], and the proliferation and differentiation of immune cells[26]. *TNFSF15* encodes TNF-like factor 1A (TL1A), which is a TNF-like molecule that mediates co-stimulation of Th1 and Th17 cells. It is required for optimal differentiation of

Table 1 Clinical efficacy and marketing approval for anti-tumor necrosis factor-α agents

Drug name	Efficacy (% of induction of remission/% sustained remission)			Approved (FDA/Europe)		
	Luminal CD	Fistulizing CD	UC	Luminal CD	Fistulizing CD	UC
Infliximab (Remicade®)	33/45	55/36	38.8/23.1	Yes/Yes	Yes/Yes	Yes/Yes
Adalimumab (Humira®)	36/36	No RCT	No RCT	Yes/Yes	No/No	No/No
Certolizumab (Cimzia®)	35/48	No RCT	No RCT	Yes/No	No/No	No/No

CD: Crohn's disease; UC: Ulcerative colitis; FDA: US food and drug administration; RCT: Randomized controlled trial.

Th17 cells[21,32]. Variants in the *TNFSF15* gene contribute to overall CD susceptibility[33,34] and an increased production of TL1A has been observed in CD[35]. Interestingly, in mice, colitis was prevented and attenuated by an anti-TL1A antibody[36].

Results of clinical trials (Table 1): Three anti-TNF agents, namely infliximab, adalimumab and certolizumab pegol have been approved by the US Food and Drug Administration for the treatment of luminal CD. In Europe, certolizumab has not yet received approval for IBD. Infliximab has also been approved for fistulizing CD and UC. In luminal CD, infliximab was effective in inducing clinical remission in 33% of patients compared with only 4% of a placebo group at week 4 ($P = 0.005$)[37], and in maintaining clinical remission (45% in the infliximab group *vs* 21% in the placebo group, $P < 0.005$). Adalimumab was also significantly more effective than placebo in inducing clinical remission (36% *vs* 12%, $P < 0.001$)[38], and more effective than placebo in maintaining clinical remission at week 56 (36% *vs* 16%). Infliximab and adalimumab have also been shown to be more effective than placebo in maintaining steroid-free remission at 1 year[39,40]. Regarding certolizumab pegol, results from large randomized, placebo-controlled trials are more controversial, with no improvement at week 6 and different long-term response rates between trials[41,42]. In fistulizing CD, 55% of the patients who received 5 mg/kg infliximab had complete fistula closure, as compared with only 13% of the patients assigned to placebo ($P = 0.001$)[43]. In UC, 2 large randomized, placebo-controlled studies, namely the ACT 1 and ACT 2 trials, evaluated the efficacy of infliximab for induction and maintenance therapy in UC[44]. In both trials, at week 8, nearly two-thirds of patients in the group receiving 5 mg of infliximab had had a clinical response, as compared with one-third of patients in the placebo group ($P < 0.001$).

Regarding the safety of anti-TNF agents, the Crohn's Therapy, Resource, Evaluation, and Assessment Tool registry, including 3179 CD patients who received infliximab, demonstrated that this agent was not an independent predictive factor of serious infections[45]. In a meta-analysis of 21 placebo-controlled trials enrolling 5356 individuals, anti-TNF therapy did not increase the risk of death, malignancy or serious infection when compared to control arms[9]. However, a longer duration of follow-up and a larger number of patients are required to better assess the safety profile of anti-TNF agents in CD.

Mechanisms of action of anti-TNF-α agents remain poorly known. Neutralization of TNF-α in the inflamed mucosa is unlikely to be a sufficient explanation. Antibody-dependent cytotoxicity also induces apoptosis or lysis of TNF-α-producing cells. This mechanism involves the Fc portion of antibodies that increases the pro-apoptotic factor caspase-3[46].

IL-12, p40/IL-23, p40

Mechanisms of action (Figure 2): IL-12 is a key cytokine that drives the inflammatory response mediated by Th1 cells[47,48]. As such, it underlies both normal host responses to a variety of intracellular bacterial, fungal and protozoan pathogens, and abnormal inflammatory responses linked to many autoimmune diseases, such as CD[49]. Indeed CD is characterized by increased production of IL-12 by antigen-presenting cells in intestinal tissue[50,51]. IL-23, secreted by antigen-presenting cells, is also a central cytokine involved in the differentiation and function of Th17 cells[2]. The IL-23-Th17 interaction mediates microbial defenses and intestinal inflammation[52,53]. Individual properties of IL-23 are also underscored by identification of the gene encoding the receptor for this cytokine as modifying host susceptibility[8,54,55]. These 2 most potent Th1- and Th17-activating cytokines, IL-12 and IL-23 are both composed of a p40 subunit and therefore, a p40 antibody may have therapeutic potential in inhibiting both Th1-activating IL-12 and Th17-activating IL-23[21].

Results of clinical trials (Figure 2 and Table 2): IL-12 and IL-23 are targeted by one humanized IL-12/23 antibody, ABT-874. It has shown promising results in a phase Ⅱ dose-ranging study comprising 79 patients with CD[49]. Seven weeks of uninterrupted treatment with 3 mg/kg ABT-874 resulted in higher response rates than placebo (75% *vs* 25%, $P = 0.03$). Another dose-ranging study comparing efficacy, safety and pharmacokinetic of intravenous infusions of ABT-874 *vs* placebo in subjects with active CD is ongoing. A double-blind, placebo-controlled, parallel-group, crossover study, assessing ustekinumab in 104 patients with CD has been completed[56]. The clinical response to ustekinumab was significantly greater than the group given placebo at weeks 4 and 6 (52%-54% *vs* 22%-39%, $P < 0.05$) but not at week 8 (49% *vs* 40%, $P = 0.34$). Interestingly, the effect was most prominent in patients treated previously with infliximab at weeks 4, 6 and 8 (59% in the ustekinumab group *vs* 25%-26% in the placebo group, $P < 0.05$). A phase 2, randomized,

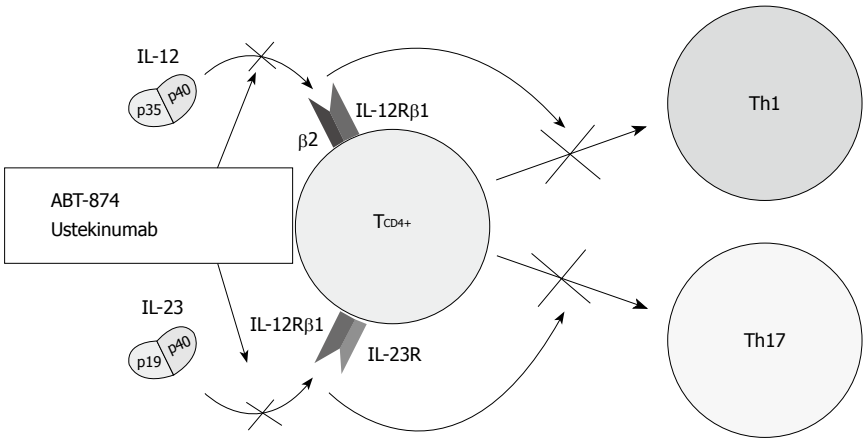


Figure 2 Therapeutic blockade of the interleukin-12/interleukin-23 pathway at the common p40 subunit of both cytokines. IL: Interleukin.

Table 2 Summary of safety and efficacy of anti-cytokine therapies in randomized, controlled trials

Study	Drug name	Targeted cytokine	Indication	No. of patients	Follow-up	Clinical response (%)	Clinical remission (%)	SAE (%)
Mannon *et al*[49]	ABT-874	IL-12/23	CD	79	8 wk	69	38	10
Sandborn *et al*[56]	Ustekinumab	IL-12/23	CD	131	8 wk	49	19	4
Sands *et al*[57]	Apilimod mesylate	IL-12/23	CD	220	168 d	25.7	NA	NS[b]
Ito *et al*[64]	Tocilizumab	IL-6	CD	36	12 wk	80	20	13
Hommes *et al*[68]	Fontolizumab	IFN-γ	CD	133	28 d	38-44	19-31	4.5
Reinisch *et al*[69]	Fontolizumab	IFN-γ	CD	201	29 d	31-38	21	13.6
Van assche *et al*[79]	Daclizumab	IL-2	UC	159	8 wk	25-33	2-7	4.3-12.5
Schreiber *et al*[85]	rhuIL-10	IL-10	CD	320	29 d	NA	23.5	7
Schreiber *et al*[86]	rhuIL-10	IL-10	UC	94	28 d	NA[1]	NA[1]	7.5
Colombel *et al*[87]	Tenovil	IL-10	Postoperative CD	65	12 wk	46% of patients with endoscopic recurrence		9
Sands *et al*[95]	rhIL-11	IL11	CD	148	8 wk	31.5[4]	36.7[4]	NS[2]
Herrlinger *et al*[90]	rhIL-11	IL-11	CD	51	12 wk	19[3]	4[3]	8
Pena-Rossi *et al*[96]	IFN-β1a	IFN-β1a	UC	194	12 wk	NA	20-29.2	12.3
Tilg *et al*[97]	PEG-IFN-α	IFN-α	UC	60	12 wk	NA	40	15

[1]No difference between placebo and rhuIL-10 treatment; [2]More headache, edema and increased platelet count; [3]Significantly inferior than prednisolone; [4]Significantly superior than placebo at a dose of 15 microg/kg weekly. SAE: Severe adverse event; IL: Interleukin; IFN: Interferon; CD: Crohn's disease; UC: Ulcerative colitis; NA: Not available; NS: Not significant.

double-blinded, placebo-controlled study has evaluated the efficacy of apilimod mesylate, an oral IL-12 and IL-23 inhibitor in treating 220 patients with moderate-to-severe CD. The enrollment was closed early because it did not demonstrate efficacy over placebo[57].

IL-6

Mechanisms of action: IL-6 is produced by various cells such as T cells, B cells, monocytes, fibroblasts, osteoblasts, keratinocytes, endothelial cells, mesangial cells and some tumor cells[58]. This cytokine specifically binds to the IL-6 receptor (IL-6R) or a soluble IL-6R, forming the IL-6/IL-6R complex that binds to gp130 and activates intracellular pathways including JAK/STAT signaling, tyrosine phosphatase SHP2 and NF-κB[59]. Many cells express gp130, hence IL-6 is a pleiotropic multi-functional cytokine acting as both a proinflammatory and an antiinflammatory cytokine[12,59]. It is involved in terminal differentiation of B cells, differentiation and activation of T cells, induction of a hepatic acute-phase response, hematopoiesis and fever[60,61]. Thus activated IL-6 plays a major role in its own amplification and then in the chronic phase of inflammation helped by mononuclear cell accumulation at the site of injury, through continuous monocyte chemoattractant protein-1 secretion, angioproliferation and antiapoptotic functions of T cells[59,62]. Plasma soluble IL-6R is increased in patient with CD and IL-6 plasma concentrations increase in active CD[63].

Results of clinical trials (Table 2): Tocilizumab binds to both the membrane-bound and the soluble forms of human IL-6R with high affinity and specificity[3,64]. Tocilizumab has shown promising results in a small phase I / II study (n = 36) that met its primary endpoint. At 12 wk, the response rate was higher in patients given an 8 mg/kg infusion of tocilizumab every 2 wk than in those given placebo (80% *vs* 31%, P = 0.019) and is accompanied by a decrease in C-reactive protein concentration[3,64]. However, only 2 of 10 patients went into remission, compared with none of 13 in the placebo group (P = 0.092), without significant improvement in mucosal healing[3,64]. Improvement in disease activity in a patient with UC associated with Takayasu arteritis has been reported after treatment with tocilizumab[65]. A placebo-controlled phase I study on the safety and biological effects of c326, an inhibitor of IL-6, in CD is ongoing.

IFN-γ

Mechanisms of action: Type Ⅱ INF, also called IFN-γ, is a proinflammatory cytokine secreted by Th1-cells[66]. IFN-γ drives expression of major histocompatibility complex class Ⅱ on antigen-presenting cells, modulates lipopolysaccharide responsiveness in intestinal epithelial cells, and increases chemokine secretion. It also activates macrophages, Th1 lymphocytes in a positive feedback loop, NK cells and endothelial cells[12,66,67]. Concentrations of IFN-γ are increased both in UC and CD.

Results of clinical trials (Table 2): Fontolizumab has been assessed in 3 phase Ⅰ / Ⅱ dose-ranging studies enrolling a total of 374 patients with moderate to severe CD[68-70]. Fontolizumab at doses of up to 4 mg/kg improved endoscopic lesions and decreased concentrations of C-reactive protein[68-70], but no study met its primary endpoint, which was defined as induction of clinical response at 1 mo[68-70]; thus the development of fontolizumab for CD has been stopped[3].

IL-2 family

Mechanisms of action: IL-2 is produced mainly by activated T cells[71]. In addition to promoting T cell proliferation and activation, IL-2 increases cytokine production and modifies the functional properties of B cells, NK cells, and macrophages. Thus, it improves the activated macrophage microbicidal and cytotoxic activities and promotes secretion of hydrogen peroxide, TNF-α and IL-6[72]. IL-2 signals through a heterodimeric (αγ) or trimeric αβγ high-affinity receptor complex[72]. Studies have proved a role for IL-2 in IBD pathogenesis, for example the calcineurin inhibitor cyclosporin, which inhibits IL-2 production, is effective in the treatment of severely active UC[73]. IL-21, an IL-2 cytokine family member expressed by activated CD4+ T cells and NK T cells, is a key regulator in production of Th17 cells. It also increases the proliferation of Th1 cells, CD4+ and CD8+ lymphocytes and regulates the profile of cytokines secreted by these cells[19,74]. Indeed, IL-21-deficient mice are protected from experimental colitis, possibly through the failure to generate the Th17 response[75]. Furthermore, blockade of endogenous IL-21, with an antagonisitic IL-21R/Fc, ameliorated dextran sulphate sodium colitis in mice[75]. No studies have been performed in humans as yet.

Results of clinical trials (Table 2): Two antibodies against the α-chain of the IL-2 receptor (CD25), namely daclizumab and basiliximab, have been studied to mimic the activity of cyclosporine[76-78]. Despite promising response rates observed in an uncontrolled trial, a randomized, double-blind, placebo-controlled, dose-ranging trial failed to demonstrate an increased remission or clinical response both at high (2 mg/kg intravenously at weeks 0, 2, 4, and 6) and low doses (1 mg/kg intravenously at weeks 0 and 4) in 159 treated patients with daclizumab for active UC[79].

ANTIINFLAMMATORY CYTOKINES

IL-10

Mechanisms of action: IL-10 is secreted by a wide variety of cells and has pleiotropic effects on T cells, B cells, myeloid cells, and other cell types[80]. IL-10 has suppressive antiinflammatory activity on T cells, macrophages, and dendritic cells (among other cells) in humans, as well as in animal models of inflammatory diseases[80]. In particular, mice deficient in IL-10 or the IL-10 receptor undergo spontaneous development of intestinal inflammation, similar to human disease[81,82]. Even though IL-10 effectively treats colitis in mouse models and suppresses inflammatory cytokine production *in vitro* in intestinal cells from IBD patients[83], unfortunately clinical trials using recombinant IL-10 to treat IBD in humans have been largely disappointing[84].

Results of clinical trials: A placebo-controlled study was conducted in 329 patients with moderate-to-severe CD and in 94 patients with UC and did not demonstrate any significant improvement in response and remission rates compared to placebo[85,86]. Also, no evidence of prevention of endoscopic recurrence in CD by subcutaneous IL-10 injections was observed in a placebo-controlled trial of 65 CD patients[87]. Animal studies showed that local administration of IL-10 to the colon *via* genetically engineered *Lactococcus lactis* bacteria administered orally allowed for the achievement of high colonic mucosal concentrations of IL-10, potentially resulting in increased efficacy[12,88].

IL-11

Mechanisms of action: IL-11 is a pleiotropic cytokine from mesenchymal cell origin[89]. It exhibits potent antiinflammatory activity on macrophages and T cells by inhibiting the secretion of pro-inflammatory cytokines[90-92] and has shown beneficial effects on intestinal mucosa in several animal IBD models[89,90]. However one study suggested that the expression of the IL-11 receptor α-chain in the mucosa was restricted to epithelial cells, and although reducing apoptosis, it had no antiinflammatory effects on these cells[93].

Results of clinical trials: In a placebo-controlled study in 76 active CD patients, subcutaneously administered recombinant human IL-11 was shown to be safe and well tolerated[94]. In a second placebo-controlled study in 148 patients comparing 2 doses of subcutaneously administered recombinant human IL-11, it was significantly superior in inducing remission after 6 wk when compared to placebo[95]. In contrast, a recent trial showed significant inferiority of recombinant human IL-11 when compared to prednisolone in inducing remission in active CD and in obtaining a clinical response[90].

Type I IFNs

Mechanisms of action: Type I IFNs consist of 14 α

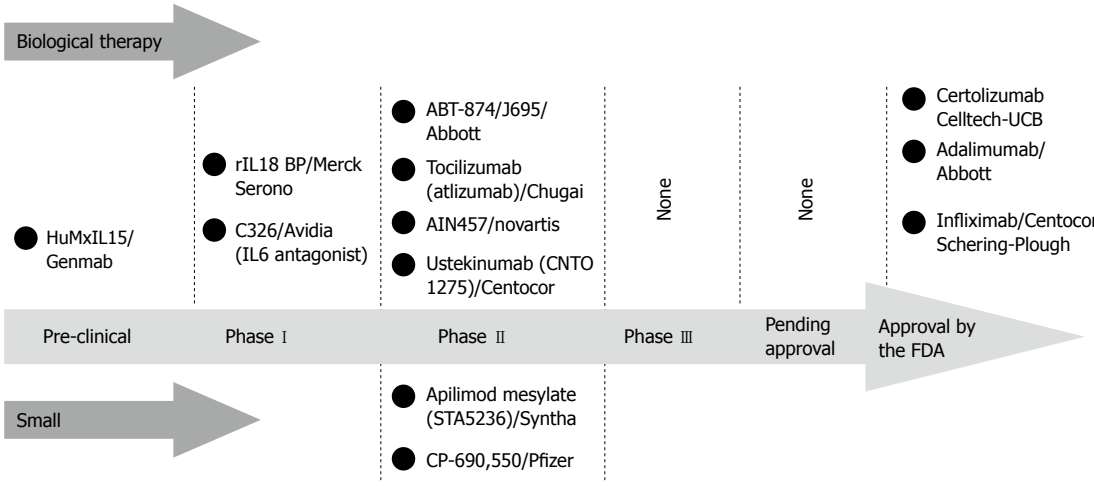


Figure 3 Cytokine therapies and inflammatory bowel disease: pipeline compounds.

isoforms and β, ε, ω, and κ isoforms[66]. Immunoregulatory therapy with type Ⅰ IFNs such as IFN-α or IFN-β can inhibit production of TNF-α and IFN-γ, antagonize the IFN-γ signaling pathway and increase production of the antiinflammatory cytokine IL-10. It has also been shown to be immunoregulated by enhanced regulatory T lymphocyte and NK cell activity[66].

Results of clinical trials: Several type Ⅰ IFNs have been studied in UC. A phase 2 placebo-controlled, dose-ranging trial, studied IFN-β1a in 194 patients with moderately active UC. Clinical outcomes, including the proportion of patients achieving endoscopically confirmed remission, were not statistically significantly superior in the IFN-β1a treatment groups over placebo[96]. A randomized, placebo-controlled trial of pegylated IFN-α in 60 patients with active UC did not show any efficacy in clinical response and response rate despite a significant decrease in levels of C reactive protein[97].

WHERE DO WE GO FROM HERE?

In 2010, infliximab represents the pinnacle of the therapeutic pyramid of IBD treatment. However, this anti-TNF agent has several limitations. First, despite its widespread use in IBD, 20% of patients still require surgery[98]. Second, about 10% of patients are primary non-responders to infliximab and only one-third of IBD patients are in clinical remission at 1 year[9,98]. Third, the annual risk of loss of response is 13% per patient-year[99]. Finally, infliximab treatment optimization with combination therapy can be considered, but this must be weighed against the increased risk of serious infections and perhaps lymphoma. These data underscore the urgent need to develop new drug classes.

Humanized IL-12/23 antibodies seem the most promising therapy for the future: (1) IL-23 is an essential mediator for the differentiation and amplification of the proinflammatory Th17 pathway; (2) its role is underscored by the increased host susceptibility for IBD in cases of polymorphism of the gene encoding the receptor for this cytokine; and (3) the effective results observed in a recent randomized, controlled trial, particularly in cases of infliximab withdrawal. Phase Ⅲ trials are ongoing in IBD patients.

Recent advances in the pathophysiology of IBD have led to the identification of additional cytokine pathways representing potential therapeutic targets. Numerous other cytokines are currently under investigation: IL-27, produced mainly by dendritic cells, acting in the differentiation of both Th1 and Th2 cells; IL-32, produced by NK cell-activated lymphocytes and epithelial cells, providing a proinflammatory amplification pathway in the innate immune responses to bacteria[7]; IL-31, preferentially produced by T cells skewed towards a Th2 phenotype, playing a role in the acute phase of inflammation by maintaining proliferation of B and T cells[6]. Further studies are needed to fully explore their different roles in human IBD, and their biological significance, to eventually determine the therapeutic implications (Figure 3).

To overcome anti-TNF therapy failure in IBD, one way would be to develop more targeted therapy[100]. A humanized TNF receptor-1 specific antagonistic antibody for selective inhibition of TNF action has shown interesting results in animal experiments[100]. Avimer proteins or nanobodies look promising, offering multiple advantages with a low immunogenicity, a high ligand affinity, a high specificity, oral bioavailability and a low cost[101]. Another way would be to use cytokine therapy in association with other anti-cytokine agents. The efficacy of TNF-α antagonist agents alone reflects probably the pleiotropic effects of TNF-α[2]. An effective treatment strategy for patients might therefore involve the blockade of multiple cytokines in order to intervene in several pathways[102]. Animal studies in rheumatoid arthritis showed that anti-CD4 therapy acts synergistically with anti-TNF-α in improving established collagen-induced arthritis[103]. In IBD, a safety study suggested several positive trends in improving efficacy when natalizumab was added to infliximab treatment[104]. Further investigations are necessary to better evaluate the cost-effectiveness and long-term safety profile of these associations.

CONCLUSION

Despite recent advances in the pathophysiology of IBD, leading to the identification and understanding of several cytokine pathways, anti-TNF-α agents still represent the pinnacle of the therapeutic pyramid of IBD treatment. The humanized IL-12/23 antibodies appear to be the most promising therapy. Future directions could include the development of more targeted therapy or therapeutic blockade of multiple cytokines in order to intervene in several pathways.

REFERENCES

1 **Monteleone G**, Caprioli F. Why are molecular mechanisms of immune activation important in IBD? *Inflamm Bowel Dis* 2008; **14** Suppl 2: S106-S107
2 **Abraham C**, Cho JH. Inflammatory bowel disease. *N Engl J Med* 2009; **361**: 2066-2078
3 **Peyrin-Biroulet L**, Desreumaux P, Sandborn WJ, Colombel JF. Crohn's disease: beyond antagonists of tumour necrosis factor. *Lancet* 2008; **372**: 67-81
4 **Xavier RJ**, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. *Nature* 2007; **448**: 427-434
5 **Bamias G**, Nyce MR, De La Rue SA, Cominelli F. New concepts in the pathophysiology of inflammatory bowel disease. *Ann Intern Med* 2005; **143**: 895-904
6 **Andoh A**, Yagi Y, Shioya M, Nishida A, Tsujikawa T, Fujiyama Y. Mucosal cytokine network in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 5154-5161
7 **Fantini MC**, Monteleone G, Macdonald TT. New players in the cytokine orchestra of inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 1419-1423
8 **Podolsky DK**. Beyond tumor necrosis factor: next-generation biologic therapy for inflammatory bowel disease. *Dig Dis* 2009; **27**: 366-369
9 **Peyrin-Biroulet L**, Deltenre P, de Suray N, Branche J, Sandborn WJ, Colombel JF. Efficacy and safety of tumor necrosis factor antagonists in Crohn's disease: meta-analysis of placebo-controlled trials. *Clin Gastroenterol Hepatol* 2008; **6**: 644-653
10 **Baumgart DC**, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007; **369**: 1627-1640
11 **Korzenik JR**, Podolsky DK. Evolving knowledge and therapy of inflammatory bowel disease. *Nat Rev Drug Discov* 2006; **5**: 197-209
12 **Rutgeerts P**, Vermeire S, Van Assche G. Biological therapies for inflammatory bowel diseases. *Gastroenterology* 2009; **136**: 1182-1197
13 **Isaacs A**, Lindenmann J. Virus interference. I. The interferon. *Proc R Soc Lond B Biol Sci* 1957; **147**: 258-267
14 **Armstrong AM**, Gardiner KR, Kirk SJ, Halliday MI, Rowlands BJ. Tumour necrosis factor and inflammatory bowel disease. *Br J Surg* 1997; **84**: 1051-1058
15 **Scaldaferri F**, Fiocchi C. Inflammatory bowel disease: progress and current concepts of etiopathogenesis. *J Dig Dis* 2007; **8**: 171-178
16 **Targan SR**, Karp LC. Defects in mucosal immunity leading to ulcerative colitis. *Immunol Rev* 2005; **206**: 296-305
17 **Hvas CL**, Kelsen J, Agnholt J, Höllsberg P, Tvede M, Møller JK, Dahlerup JF. Crohn's disease intestinal CD4+ T cells have impaired interleukin-10 production which is not restored by probiotic bacteria. *Scand J Gastroenterol* 2007; **42**: 592-601
18 **Saruta M**, Yu QT, Fleshner PR, Mantel PY, Schmidt-Weber CB, Banham AH, Papadakis KA. Characterization of FOXP3+CD4+ regulatory T cells in Crohn's disease. *Clin Immunol* 2007; **125**: 281-290
19 **Shih DQ**, Targan SR. Insights into IBD Pathogenesis. *Curr Gastroenterol Rep* 2009; **11**: 473-480
20 **Zhou L**, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 2007; **8**: 967-974
21 **Brand S**. Crohn's disease: Th1, Th17 or both? The change of a paradigm: new immunological and genetic insights implicate Th17 cells in the pathogenesis of Crohn's disease. *Gut* 2009; **58**: 1152-1167
22 **Braegger CP**, Nicholls S, Murch SH, Stephens S, MacDonald TT. Tumour necrosis factor alpha in stool as a marker of intestinal inflammation. *Lancet* 1992; **339**: 89-91
23 **Breese EJ**, Michie CA, Nicholls SW, Murch SH, Williams CB, Domizio P, Walker-Smith JA, MacDonald TT. Tumor necrosis factor alpha-producing cells in the intestinal mucosa of children with inflammatory bowel disease. *Gastroenterology* 1994; **106**: 1455-1466
24 **Esposito E**, Cuzzocrea S. TNF-alpha as a therapeutic target in inflammatory diseases, ischemia-reperfusion injury and trauma. *Curr Med Chem* 2009; **16**: 3152-3167
25 **Murch SH**, Braegger CP, Walker-Smith JA, MacDonald TT. Location of tumour necrosis factor alpha by immunohistochemistry in chronic inflammatory bowel disease. *Gut* 1993; **34**: 1705-1709
26 **Paul AT**, Gohil VM, Bhutani KK. Modulating TNF-alpha signaling with natural products. *Drug Discov Today* 2006; **11**: 725-732
27 **Pober JS**, Lapierre LA, Stolpen AH, Brock TA, Springer TA, Fiers W, Bevilacqua MP, Mendrick DL, Gimbrone MA Jr. Activation of cultured human endothelial cells by recombinant lymphotoxin: comparison with tumor necrosis factor and interleukin 1 species. *J Immunol* 1987; **138**: 3319-3324
28 **Slowik MR**, De Luca LG, Fiers W, Pober JS. Tumor necrosis factor activates human endothelial cells through the p55 tumor necrosis factor receptor but the p75 receptor contributes to activation at low tumor necrosis factor concentration. *Am J Pathol* 1993; **143**: 1724-1730
29 **Estrada C**, Gómez C, Martín C, Moncada S, González C. Nitric oxide mediates tumor necrosis factor-alpha cytotoxicity in endothelial cells. *Biochem Biophys Res Commun* 1992; **186**: 475-482
30 **Yoshizumi M**, Perrella MA, Burnett JC Jr, Lee ME. Tumor necrosis factor downregulates an endothelial nitric oxide synthase mRNA by shortening its half-life. *Circ Res* 1993; **73**: 205-209
31 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429
32 **Pappu BP**, Borodovsky A, Zheng TS, Yang X, Wu P, Dong X, Weng S, Browning B, Scott ML, Ma L, Su L, Tian Q, Schneider P, Flavell RA, Dong C, Burkly LC. TL1A-DR3 interaction regulates Th17 cell function and Th17-mediated autoimmune disease. *J Exp Med* 2008; **205**: 1049-1062
33 **Tremelling M**, Berzuini C, Massey D, Bredin F, Price C, Dawson C, Bingham SA, Parkes M. Contribution of TNFSF15 gene variants to Crohn's disease susceptibility confirmed in UK population. *Inflamm Bowel Dis* 2008; **14**: 733-737
34 **Yamazaki K**, McGovern D, Ragoussis J, Paolucci M, Butler H, Jewell D, Cardon L, Takazoe M, Tanaka T, Ichimori T, Saito S, Sekine A, Iida A, Takahashi A, Tsunoda T, Lathrop M, Nakamura Y. Single nucleotide polymorphisms in TNFSF15 confer susceptibility to Crohn's disease. *Hum Mol Genet* 2005; **14**: 3499-3506
35 **Bamias G**, Martin C 3rd, Marini M, Hoang S, Mishina M, Ross WG, Sachedina MA, Friel CM, Mize J, Bickston SJ, Pizarro TT, Wei P, Cominelli F. Expression, localization, and functional activity of TL1A, a novel Th1-polarizing cytokine in inflammatory bowel disease. *J Immunol* 2003; **171**: 4868-4874
36 **Takedatsu H**, Michelsen KS, Wei B, Landers CJ, Thomas LS, Dhall D, Braun J, Targan SR. TL1A (TNFSF15) regulates the development of chronic colitis by modulating both T-helper 1 and T-helper 17 activation. *Gastroenterology* 2008; **135**: 552-567

37 **Targan SR**, Hanauer SB, van Deventer SJ, Mayer L, Present DH, Braakman T, DeWoody KL, Schaible TF, Rutgeerts PJ. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997; **337**: 1029-1035

38 **Hanauer SB**, Sandborn WJ, Rutgeerts P, Fedorak RN, Lukas M, MacIntosh D, Panaccione R, Wolf D, Pollack P. Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology* 2006; **130**: 323-333; quiz 591

39 **Colombel JF**, Sandborn WJ, Rutgeerts P, Enns R, Hanauer SB, Panaccione R, Schreiber S, Byczkowski D, Li J, Kent JD, Pollack PF. Adalimumab for maintenance of clinical response and remission in patients with Crohn's disease: the CHARM trial. *Gastroenterology* 2007; **132**: 52-65

40 **Hanauer SB**, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, Rachmilewitz D, Wolf DC, Olson A, Bao W, Rutgeerts P. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002; **359**: 1541-1549

41 **Sandborn WJ**, Feagan BG, Stoinov S, Honiball PJ, Rutgeerts P, Mason D, Bloomfield R, Schreiber S. Certolizumab pegol for the treatment of Crohn's disease. *N Engl J Med* 2007; **357**: 228-238

42 **Schreiber S**, Khaliq-Kareemi M, Lawrance IC, Thomsen OØ, Hanauer SB, McColm J, Bloomfield R, Sandborn WJ. Maintenance therapy with certolizumab pegol for Crohn's disease. *N Engl J Med* 2007; **357**: 239-250

43 **Present DH**, Rutgeerts P, Targan S, Hanauer SB, Mayer L, van Hogezand RA, Podolsky DK, Sands BE, Braakman T, DeWoody KL, Schaible TF, van Deventer SJ. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999; **340**: 1398-1405

44 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476

45 **Lichtenstein GR**, Feagan BG, Cohen RD, Salzberg BA, Diamond RH, Chen DM, Pritchard ML, Sandborn WJ. Serious infections and mortality in association with therapies for Crohn's disease: TREAT registry. *Clin Gastroenterol Hepatol* 2006; **4**: 621-630

46 **Stokkers PC**, Hommes DW. New cytokine therapeutics for inflammatory bowel disease. *Cytokine* 2004; **28**: 167-173

47 **Gately MK**, Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, Presky DH. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* 1998; **16**: 495-521

48 **Trinchieri G**. Proinflammatory and immunoregulatory functions of interleukin-12. *Int Rev Immunol* 1998; **16**: 365-396

49 **Mannon PJ**, Fuss IJ, Mayer L, Elson CO, Sandborn WJ, Present D, Dolin B, Goodman N, Groden C, Hornung RL, Quezado M, Yang Z, Neurath MF, Salfeld J, Veldman GM, Schwertschlag U, Strober W. Anti-interleukin-12 antibody for active Crohn's disease. *N Engl J Med* 2004; **351**: 2069-2079

50 **Monteleone G**, Biancone L, Marasco R, Morrone G, Marasco O, Luzza F, Pallone F. Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology* 1997; **112**: 1169-1178

51 **Parronchi P**, Romagnani P, Annunziato F, Sampognaro S, Becchio A, Giannarini L, Maggi E, Pupilli C, Tonelli F, Romagnani S. Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn's disease. *Am J Pathol* 1997; **150**: 823-832

52 **Mangan PR**, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* 2006; **441**: 231-234

53 **McGeachy MJ**, Cua DJ. The link between IL-23 and Th17 cell-mediated immune pathologies. *Semin Immunol* 2007; **19**: 372-376

54 **Barrett JC**, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008; **40**: 955-962

55 **Duerr RH**, Taylor KD, Brant SR, Rioux JD, Silverberg MS, Daly MJ, Steinhart AH, Abraham C, Regueiro M, Griffiths A, Dassopoulos T, Bitton A, Yang H, Targan S, Datta LW, Kistner EO, Schumm LP, Lee AT, Gregersen PK, Barmada MM, Rotter JI, Nicolae DL, Cho JH. A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. *Science* 2006; **314**: 1461-1463

56 **Sandborn WJ**, Feagan BG, Fedorak RN, Scherl E, Fleisher MR, Katz S, Johanns J, Blank M, Rutgeerts P. A randomized trial of Ustekinumab, a human interleukin-12/23 monoclonal antibody, in patients with moderate-to-severe Crohn's disease. *Gastroenterology* 2008; **135**: 1130-1141

57 **Sands BE**, Jacobson EW, Sylwestrowicz T, Younes Z, Dryden G, Fedorak R, Greenbloom S. Randomized, double-blind, placebo-controlled trial of the oral interleukin-12/23 inhibitor apilimod mesylate for treatment of active Crohn's disease. *Inflamm Bowel Dis* 2010; **16**: 1209-1218

58 **Kishimoto T**. The biology of interleukin-6. *Blood* 1989; **74**: 1-10

59 **Fonseca JE**, Santos MJ, Canhão H, Choy E. Interleukin-6 as a key player in systemic inflammation and joint destruction. *Autoimmun Rev* 2009; **8**: 538-542

60 **Akira S**, Taga T, Kishimoto T. Interleukin-6 in biology and medicine. *Adv Immunol* 1993; **54**: 1-78

61 **Veldhuis GJ**, Willemse PH, Sleijfer DT, van der Graaf WT, Groen HJ, Limburg PC, Mulder NH, de Vries EG. Toxicity and efficacy of escalating dosages of recombinant human interleukin-6 after chemotherapy in patients with breast cancer or non-small-cell lung cancer. *J Clin Oncol* 1995; **13**: 2585-2593

62 **Atreya R**, Mudter J, Finotto S, Müllberg J, Jostock T, Wirtz S, Schütz M, Bartsch B, Holtmann M, Becker C, Strand D, Czaja J, Schlaak JF, Lehr HA, Autschbach F, Schürmann G, Nishimoto N, Yoshizaki K, Ito H, Kishimoto T, Galle PR, Rose-John S, Neurath MF. Blockade of interleukin 6 trans signaling suppresses T-cell resistance against apoptosis in chronic intestinal inflammation: evidence in crohn disease and experimental colitis in vivo. *Nat Med* 2000; **6**: 583-588

63 **Nancey S**, Hamzaoui N, Moussata D, Graber I, Bienvenu J, Flourie B. Serum interleukin-6, soluble interleukin-6 receptor and Crohn's disease activity. *Dig Dis Sci* 2008; **53**: 242-247

64 **Ito H**, Takazoe M, Fukuda Y, Hibi T, Kusugami K, Andoh A, Matsumoto T, Yamamura T, Azuma J, Nishimoto N, Yoshizaki K, Shimoyama T, Kishimoto T. A pilot randomized trial of a human anti-interleukin-6 receptor monoclonal antibody in active Crohn's disease. *Gastroenterology* 2004; **126**: 989-996; discussion 947

65 **Nishimoto N**, Nakahara H, Yoshio-Hoshino N, Mima T. Successful treatment of a patient with Takayasu arteritis using a humanized anti-interleukin-6 receptor antibody. *Arthritis Rheum* 2008; **58**: 1197-1200

66 **Ghosh S**, Chaudhary R, Carpani M, Playford R. Interfering

with interferons in inflammatory bowel disease. *Gut* 2006; **55**: 1071-1073

67 **Suzuki M**, Hisamatsu T, Podolsky DK. Gamma interferon augments the intracellular pathway for lipopolysaccharide (LPS) recognition in human intestinal epithelial cells through coordinated up-regulation of LPS uptake and expression of the intracellular Toll-like receptor 4-MD-2 complex. *Infect Immun* 2003; **71**: 3503-3511

68 **Hommes DW**, Mikhajlova TL, Stoinov S, Stimac D, Vucelic B, Lonovics J, Zákuciová M, D'Haens G, Van Assche G, Ba S, Lee S, Pearce T. Fontolizumab, a humanised anti-interferon gamma antibody, demonstrates safety and clinical activity in patients with moderate to severe Crohn's disease. *Gut* 2006; **55**: 1131-1137

69 **Reinisch W**, de Villiers W, Bene L, Simon L, Rácz I, Katz S, Altorjay I, Feagan B, Riff D, Bernstein CN, Hommes D, Rutgeerts P, Cortot A, Gaspari M, Cheng M, Pearce T, Sands BE. Fontolizumab in moderate to severe Crohn's disease: a phase 2, randomized, double-blind, placebo-controlled, multiple-dose study. *Inflamm Bowel Dis* 2010; **16**: 233-242

70 **Reinisch W**, Hommes DW, Van Assche G, Colombel JF, Gendre JP, Oldenburg B, Teml A, Geboes K, Ding H, Zhang L, Tang M, Cheng M, van Deventer SJ, Rutgeerts P, Pearce T. A dose escalating, placebo controlled, double blind, single dose and multidose, safety and tolerability study of fontolizumab, a humanised anti-interferon gamma antibody, in patients with moderate to severe Crohn's disease. *Gut* 2006; **55**: 1138-1144

71 **Gordon J**, MacLean LD. A lymphocyte-stimulating factor produced in vitro. *Nature* 1965; **208**: 795-796

72 **Chavez AR**, Buchser W, Basse PH, Liang X, Appleman LJ, Maranchie JK, Zeh H, de Vera ME, Lotze MT. Pharmacologic administration of interleukin-2. *Ann N Y Acad Sci* 2009; **1182**: 14-27

73 **Lichtiger S**, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994; **330**: 1841-1845

74 **Strengell M**, Sareneva T, Foster D, Julkunen I, Matikainen S. IL-21 up-regulates the expression of genes associated with innate immunity and Th1 response. *J Immunol* 2002; **169**: 3600-3605

75 **Fina D**, Sarra M, Fantini MC, Rizzo A, Caruso R, Caprioli F, Stolfi C, Cardolini I, Dottori M, Boirivant M, Pallone F, Macdonald TT, Monteleone G. Regulation of gut inflammation and th17 cell response by interleukin-21. *Gastroenterology* 2008; **134**: 1038-1048

76 **Creed TJ**, Norman MR, Probert CS, Harvey RF, Shaw IS, Smithson J, Anderson J, Moorghen M, Gupta J, Shepherd NA, Dayan CM, Hearing SD. Basiliximab (anti-CD25) in combination with steroids may be an effective new treatment for steroid-resistant ulcerative colitis. *Aliment Pharmacol Ther* 2003; **18**: 65-75

77 **Creed TJ**, Probert CS, Norman MN, Moorghen M, Shepherd NA, Hearing SD, Dayan CM. Basiliximab for the treatment of steroid-resistant ulcerative colitis: further experience in moderate and severe disease. *Aliment Pharmacol Ther* 2006; **23**: 1435-1442

78 **Van Assche G**, Dalle I, Noman M, Aerden I, Swijsen C, Asnong K, Maes B, Ceuppens J, Geboes K, Rutgeerts P. A pilot study on the use of the humanized anti-interleukin-2 receptor antibody daclizumab in active ulcerative colitis. *Am J Gastroenterol* 2003; **98**: 369-376

79 **Van Assche G**, Sandborn WJ, Feagan BG, Salzberg BA, Silvers D, Monroe PS, Pandak WM, Anderson FH, Valentine JF, Wild GE, Geenen DJ, Sprague R, Targan SR, Rutgeerts P, Vexler V, Young D, Shames RS. Daclizumab, a humanised monoclonal antibody to the interleukin 2 receptor (CD25), for the treatment of moderately to severely active ulcerative colitis: a randomised, double blind, placebo controlled, dose ranging trial. *Gut* 2006; **55**: 1568-1574

80 **Moore KW**, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; **19**: 683-765

81 **Kühn R**, Löhler J, Rennick D, Rajewsky K, Müller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993; **75**: 263-274

82 **Spencer SD**, Di Marco F, Hooley J, Pitts-Meek S, Bauer M, Ryan AM, Sordat B, Gibbs VC, Aguet M. The orphan receptor CRF2-4 is an essential subunit of the interleukin 10 receptor. *J Exp Med* 1998; **187**: 571-578

83 **Schreiber S**, Heinig T, Thiele HG, Raedler A. Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. *Gastroenterology* 1995; **108**: 1434-1444

84 **Kelsall B**. Interleukin-10 in inflammatory bowel disease. *N Engl J Med* 2009; **361**: 2091-2093

85 **Schreiber S**, Fedorak RN, Wild G, Gangl A, Targan S, Jacyna M, Wright JP, Kilian A, Cohard M, Lebeaut A, Tremaine WJ, the Ulcerative Colitis IL-10 Cooperative Study Group. Safety and tolerance of rHuIL-10 treatment in patients with mild/moderate active ulcerative colitis. *Gastroenterology* 1998; **114**: A1080-A1081

86 **Schreiber S**, Fedorak RN, Nielsen OH, Wild G, Williams CN, Nikolaus S, Jacyna M, Lashner BA, Gangl A, Rutgeerts P, Isaacs K, van Deventer SJ, Koningsberger JC, Cohard M, LeBeaut A, Hanauer SB. Safety and efficacy of recombinant human interleukin 10 in chronic active Crohn's disease. Crohn's Disease IL-10 Cooperative Study Group. *Gastroenterology* 2000; **119**: 1461-1472

87 **Colombel JF**, Rutgeerts P, Malchow H, Jacyna M, Nielsen OH, Rask-Madsen J, Van Deventer S, Ferguson A, Desreumaux P, Forbes A, Geboes K, Melani L, Cohard M. Interleukin 10 (Tenovil) in the prevention of postoperative recurrence of Crohn's disease. *Gut* 2001; **49**: 42-46

88 **Steidler L**, Hans W, Schotte L, Neirynck S, Obermeier F, Falk W, Fiers W, Remaut E. Treatment of murine colitis by Lactococcus lactis secreting interleukin-10. *Science* 2000; **289**: 1352-1355

89 **Du X**, Williams DA. Interleukin-11: review of molecular, cell biology, and clinical use. *Blood* 1997; **89**: 3897-3908

90 **Herrlinger KR**, Witthoeft T, Raedler A, Bokemeyer B, Krummenerl T, Schulzke JD, Boerner N, Kueppers B, Emmrich J, Mescheder A, Schwertschlag U, Shapiro M, Stange EF. Randomized, double blind controlled trial of subcutaneous recombinant human interleukin-11 vs prednisolone in active Crohn's disease. *Am J Gastroenterol* 2006; **101**: 793-797

91 **Leng SX**, Elias JA. Interleukin-11 inhibits macrophage interleukin-12 production. *J Immunol* 1997; **159**: 2161-2168

92 **Trepicchio WL**, Ozawa M, Walters IB, Kikuchi T, Gilleaudeau P, Bliss JL, Schwertschlag U, Dorner AJ, Krueger JG. Interleukin-11 therapy selectively downregulates type I cytokine proinflammatory pathways in psoriasis lesions. *J Clin Invest* 1999; **104**: 1527-1537

93 **Kiessling S**, Muller-Newen G, Leeb SN, Hausmann M, Rath HC, Strater J, Spottl T, Schlottmann K, Grossmann J, Montero-Julian FA, Scholmerich J, Andus T, Buschauer A, Heinrich PC, Rogler G. Functional expression of the interleukin-11 receptor alpha-chain and evidence of antiapoptotic effects in human colonic epithelial cells. *J Biol Chem* 2004; **279**: 10304-10315

94 **Sands BE**, Bank S, Sninsky CA, Robinson M, Katz S, Singleton JW, Miner PB, Safdi MA, Galandiuk S, Hanauer SB, Varilek GW, Buchman AL, Rodgers VD, Salzberg B, Cai B, Loewy J, DeBruin MF, Rogge H, Shapiro M, Schwertschlag US. Preliminary evaluation of safety and activity of recombinant human interleukin 11 in patients with active Crohn's disease. *Gastroenterology* 1999; **117**: 58-64

95 **Sands BE**, Winston BD, Salzberg B, Safdi M, Barish C, Wruble L, Wilkins R, Shapiro M, Schwertschlag US. Randomized, controlled trial of recombinant human interleukin-11 in patients with active Crohn's disease. *Aliment Pharmacol Ther* 2002; **16**: 399-406

96 **Pena-Rossi C**, Schreiber S, Golubovic G, Mertz-Nielsen A, Panes J, Rachmilewitz D, Shieh MJ, Simanenkov VI, Stanton D, Graffner H. Clinical trial: a multicentre, randomized, double-blind, placebo-controlled, dose-finding, phase II study of subcutaneous interferon-beta-1a in moderately active ulcerative colitis. *Aliment Pharmacol Ther* 2008; **28**: 758-767

97 **Tilg H**, Vogelsang H, Ludwiczek O, Lochs H, Kaser A, Colombel JF, Ulmer H, Rutgeerts P, Krüger S, Cortot A, D'Haens G, Harrer M, Gasche C, Wrba F, Kuhn I, Reinisch W. A randomised placebo controlled trial of pegylated interferon alpha in active ulcerative colitis. *Gut* 2003; **52**: 1728-1733

98 **Schnitzler F**, Fidder H, Ferrante M, Noman M, Arijs I, Van Assche G, Hoffman I, Van Steen K, Vermeire S, Rutgeerts P. Long-term outcome of treatment with infliximab in 614 patients with Crohn's disease: results from a single-centre cohort. *Gut* 2009; **58**: 492-500

99 **Gisbert JP**, Panés J. Loss of response and requirement of infliximab dose intensification in Crohn's disease: a review. *Am J Gastroenterol* 2009; **104**: 760-767

100 **Kontermann RE**, Münkel S, Neumeyer J, Müller D, Branschädel M, Scheurich P, Pfizenmaier K. A humanized tumor necrosis factor receptor 1 (TNFR1)-specific antagonistic antibody for selective inhibition of tumor necrosis factor (TNF) action. *J Immunother* 2008; **31**: 225-234

101 **He MM**, Smith AS, Oslob JD, Flanagan WM, Braisted AC, Whitty A, Cancilla MT, Wang J, Lugovskoy AA, Yoburn JC, Fung AD, Farrington G, Eldredge JK, Day ES, Cruz LA, Cachero TG, Miller SK, Friedman JE, Choong IC, Cunningham BC. Small-molecule inhibition of TNF-alpha. *Science* 2005; **310**: 1022-1025

102 **Cominelli F**. Cytokine-based therapies for Crohn's disease--new paradigms. *N Engl J Med* 2004; **351**: 2045-2048

103 **Williams RO**, Mason LJ, Feldmann M, Maini RN. Synergy between anti-CD4 and anti-tumor necrosis factor in the amelioration of established collagen-induced arthritis. *Proc Natl Acad Sci USA* 1994; **91**: 2762-2766

104 **Sands BE**, Kozarek R, Spainhour J, Barish CF, Becker S, Goldberg L, Katz S, Goldblum R, Harrigan R, Hilton D, Hanauer SB. Safety and tolerability of concurrent natalizumab treatment for patients with Crohn's disease not in remission while receiving infliximab. *Inflamm Bowel Dis* 2007; **13**: 2-11

S- Editor Tian L **L- Editor** Cant MR **E- Editor** Lin YP

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.557

World J Gastroenterol 2011 February 7; 17(5): 557-566
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

Silvio Danese, MD, PhD, Head, *Series Editor*

Intestinal microbiota in inflammatory bowel disease: Friend of foe?

Francesca Fava, Silvio Danese

Francesca Fava, Silvio Danese, Inflammatory Bowel Disease Unit, Istituto Clinico Humanitas, Via Manzoni 113, 20089 Rozzano, Milano, Italy
Author contributions: Fava F and Danese S both generated the ideas and contributed to the writing of this manuscript.
Correspondence to: Silvio Danese, MD, PhD, Inflammatory Bowel Disease Unit, Istituto Clinico Humanitas, Via Manzoni 113, 20089 Rozzano, Milano, Italy. silvio.danese@humanitas.it
Telephone: +39-2-82244771 Fax: +39-2-82245101
Received: June 28, 2010 Revised: August 18, 2010
Accepted: August 25, 2010
Published online: February 7, 2011

Abstract

Inflammatory bowel disease (IBD) arises from disruption of immune tolerance to the gut commensal microbiota, leading to chronic intestinal inflammation and mucosal damage in genetically predisposed hosts. In healthy individuals the intestinal microbiota have a symbiotic relationship with the host organism and possess important and unique functions, including a metabolic function (i.e. digestion of dietary compounds and xenobiotics, fermentation of undigestible carbohydrates with production of short chain fatty acids), a mucosal barrier function (i.e. by inhibiting pathogen invasion and strengthening epithelial barrier integrity), and an immune modulatory function (i.e. mucosal immune system priming and maintenance of intestinal epithelium homeostasis). A fine balance regulates the mechanism that allows coexistence of mammals with their commensal bacteria. In IBD this mechanism of immune tolerance is impaired because of several potential causative factors. The gut microbiota composition and activity of IBD patients are abnormal, with a decreased prevalence of dominant members of the human commensal microbiota (i.e. *Clostridium* IXa and IV groups, *Bacteroides*, bifidobacteria) and a concomitant increase in detrimental bacteria (i.e. sulphate-reducing bacteria, *Escherichia coli*). The observed dysbiosis is concomitant with defective innate immunity and bacterial killing (i.e. reduced mucosal defensins and IgA, malfunctioning phagocytosis) and overaggressive adaptive immune response (due to ineffective regulatory T cells and antigen presenting cells), which are considered the basis of IBD pathogenesis. However, we still do not know how the interplay between these parameters causes the disease. Studies looking at gut microbial composition, epithelial integrity and mucosal immune markers in genotyped IBD populations are therefore warranted to shed light on this obscure pathogenesis.

Key words: Microbiota; Inflammatory bowel disease; Microbial dysbiosis; Immune tolerance; Innate immunity; Mucosal barrier

Peer reviewers: Tauseef Ali, MD, Assistant Professor, Section of Digestive Diseases and Nutrition, University of Oklahoma Health Sciences Center, 920 SL Young Blvd, Oklahoma City, OK 73104, United States; Jürgen Büning, MD, Internal Medicine I, Department of Gastroenterology, University Hospital of Schleswig-Holstein, Ratzeburger Allee 160, D-23538 Lübeck, Germany

Fava F, Danese S. Intestinal microbiota in inflammatory bowel disease: Friend of foe? *World J Gastroenterol* 2011; 17(5): 557-566 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/557.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.557

INTRODUCTION

Inflammatory bowel disease (IBD) is a chronic, relapsing inflammatory disorder affecting the gastrointestinal tract which involves an imbalanced host-commensal microbiota interaction. Crohn's disease (CD) and ulcerative colitis (UC) are commonly included in the collec-

tive term IBD, although the two diseases present with distinct pathogenesis, symptomatology, inflammatory profiles and gut microbiota composition. Inflammation associated with CD is discontinuous, may extend deeply into the submucosal regions and occurs anywhere along the alimentary canal. In UC, inflammation involves only the superficial layers of the intestinal mucosa and is localised to regions of the gut most highly colonized by bacteria, starting at the distal colon and moving proximally along the large bowel[1]. CD is predominantly associated with a type 1 helper-T-cell (Th1) and type 17 helper-T-cell (Th17) immune responses, characterized by increased production of interleukin (IL)-12, IL-23, IL-27, interferon-γ (IFN-γ) and tumor necrosis factor (TNF)-α. Diversely, UC seems to be associated with a type 2 helper-T cell (Th2) immune response, mainly leading to raised levels of IL-5 and transforming growth factor-β (TGF-β)[2]. The etiology of IBD is complex and multifactorial, where environmental, genetic and immunological components appear to play a role[3].

A consistent body of evidence implicates the gut microbiota in the pathogenesis of IBD, including the consideration that inflammation mainly occurs in the intestinal sites with the highest bacterial concentration (in UC), that antibiotic treatment often results in amelioration of disease symptoms[4], and that germ-free mice do not spontaneously initiate colitis[5]. The most extensively investigated hypothesis is that IBD development might be due to an altered immune response and a disrupted mechanism of host tolerance to the non-pathogenic resident microbiota, leading to an elevated inflammatory response.

THE HUMAN INTESTINAL MICROBIOTA

The adult human gut contains around 10^{14} bacterial cells and up to an estimated 1000 different bacterial species, thus constituting the largest microbial community associated with the human body[6]. Recent studies using culture-independent molecular microbiological techniques have shown that the most abundant bacterial phyla found in the healthy human large intestine are the Gram-negative *Bacteroidetes* and the Gram-positive, low GC% *Firmicutes*[6,7]. *Proteobacteria*, *Actinobacteria*, *Fusobacteria* and *Verrucomicrobia* phyla are relatively less abundant, but nonetheless are known to play important roles in human health[6]. The same studies have described the vast diversity of bacterial species and identified the dominant bacterial groups to be *Clostridium coccoides* (*C. coccoides*)-*Eubacterium rectale*, *Clostridium leptum* (*C. leptum*), *Bacteroides-Prevotella*, *Bifidobacterium* species and *Atopobium* species[8]. The gut microbial species composition varies greatly between individuals, with each individual harboring a unique collection of bacterial species, which is highly stable over time[9]. Zoetendal *et al*[10] also showed that the gut microbiota composition of spouses, who were living in the same environment and had similar eating habits, showed the least degree of species similarity, while siblings showed increased similarity in species make-up. Interestingly, the gut microbiota profiles of identical twins showed a high degree of similarity, but were yet distinct. These findings highlight that genetic factors play an important role in gut microbiota development, although environment also drives species acquisition. Studies have shown that the vast majority of intestinal bacteria are novel, new to science and so far resist cultivation using traditional culture techniques, necessitating the use of culture-independent molecular microbiology techniques, such as 16S rRNA gene probing and polymerase chain reaction (PCR)-based strategies.

Recently, the human body together with its gut microbiota has been referred to as a "superorganism" comprised of human and bacterial genes[11]. It has been estimated that the human gut microbiome consists of 100 times more genes than the human genome. Therefore, the presence of the intestinal microbiota enriches the human organism with important functions, especially functions involved in deriving energy from nutrients which escape digestion in the upper gut and the metabolism of xenobiotics. The gut microbiota acts as a "metabolic organ", through breakdown of complex indigestible dietary carbohydrates and proteins, with consequent generation of fermentation end-products (short chain fatty acids, ethanol and gas) and also through production of vitamins, ion absorption and conversion of dietary polyphenolic compounds into their active form[12,13]. The commensal microbiota contribute to the "barrier effect", which constitutes a real obstacle to pathogen invasion of the intestinal mucosa. Recent studies have shown that a modulation of the gut microbiota through dietary supplementation with a prebiotic (i.e. oligofructose) increases epithelial barrier integrity by increasing the expression of tight junctions proteins (i.e. ZO-1 and occludin), with a mechanism that is dependent on the augmented secretion of the GLP-2 gut hormone[14]. The immune regulatory function of the intestinal microbiota consists of priming the mucosal immune system and maintenance of intestinal epithelium homeostasis. Studies in germ-free animals have demonstrated that the normal functioning of intestinal epithelial cells (IEC) and of the underlying immune cells are impaired in the absence of the gut microbiota. IEC expression of microbial recognition receptors, defensins and antimicrobial peptides are reduced in germ-free animals[15,16]. Defective development of gut-associated lymphoid tissues, antibody production (i.e. sIgA) and maturation of isolated lymphoid follicles have also been shown in germ-free animals, together with reduced Peyer's patches and mesenteric lymph node number and dimension[17,18].

IMMUNE TOLERANCE TO THE COMMENSAL MICROBIOTA

In health, finely balanced mechanisms regulate the host's immunological tolerance to the continuous stimulus of the resident gut microbiota and their metabolic end-products. Microbial recognition by antigen presenting cells (i.e. dendritic cells, DC) and epithelial cells is mainly carried out through sensing of conserved microbial-associated molecular patterns (MAMPs) by toll-like receptors (TLR), capable of detecting a variety of bacterial components, such

as lipopolysaccharide (LPS), lipoproteins, CpG DNA[19], and by nucleotide-binding oligomerisation domain (NOD)-like receptors (NLR), which recognise peptidoglycan molecules on the bacterial cell wall[20]. In healthy hosts the pro-inflammatory pathways associated with TLR and NLR are suppressed by inhibitory molecules of both human and bacterial origin [i.e. cyclooxygenase-2 (COX-2) inhibitors; LPS; A20; peroxisome proliferator-activated receptor-γ (PPAR-γ); nuclear factor-κB (NF-κB) inhibitor IκB-α; interferon-α/β (IFN-α/β); interleukin-10 (IL-10); TGF-β; eicosanoids][21,22]. Activated innate immune cells, such as mucosal DC, constantly sample luminal microbial antigens and present them to adaptive immune cells. Recent studies have shown that the intestine is home to specialised DC, whose function it is to induce a highly tolerogenic response from T and B cells, through induction of regulatory T cells (Treg) and secretion of IgA, respectively[23,24]. Commensal bacteria actively coordinate the host tolerogenic response, either through DC-mediated conversion of naïve T cells into Treg, or through direct ligation of TLRs on the surface of Treg. Certain resident bacterial populations, often referred to as "beneficial bacteria" (i.e. lactobacilli and bifidobacteria) can influence DC differentiation towards a more undifferentiated and monocyte-like phenotype, which may account for DC immune tolerance[25]. Moreover, incubation of monocyte-derived DC with probiotic bacteria was shown to induce DC maturation and cytokine secretion, with strain-specific cytokine secretive profiles[26]. Repetitive TLR stimulation due to commensal bacterial exposure induces down-regulation of the NF-κB pathway and stimulates production of antimicrobial peptides (i.e. defensins)[27]. Also, chronic NOD-2 stimulation has been demonstrated to lead to down-regulation of pro-inflammatory cytokines (TNF-α, IL-8, IL-1β) in primary human monocyte-derived macrophages after pre-treatment with muramyl dipeptide (MDP) and re-stimulation with NOD-2, TLR-2 and TLR-4 ligands[28]. Therefore, the host's mechanism of tolerance to the resident microbiota offers, at the same time, protection from unwanted inflammatory responses and from pathogen invasion. Microbial ligands have also been shown to modulate the expression levels of miR-155, a miRNA that is involved in immune homeostasis and whose absence causes a reduction in Treg numbers in miR-155-deficient mice[29]. However, since commensal and pathogenic bacteria possess many common motifs that are immunologically recognised by the host, how the host can tolerate resident bacteria whilst being able to mount an effective inflammatory response to invading pathogens is still not fully understood. Nonetheless, pathogenic bacteria do differentiate themselves from commensals by their behaviour; breaching the intestinal epithelial barrier and, in healthy individuals, eliciting strong inflammatory reactions when they trigger MAMPs basolaterally on epithelial cells[30].

In IBD, the homeostatic mechanisms that allow co-existence of the host organism and the commensal microbiota are disrupted. Polymorphisms in TLR (*TLR4 D299G* associated with CD and UC; *TLR1 L80P* and *TLR2 R753G*, associated with pancolitis) and NLR (i.e. three mutations in *NOD 2/CARD15* gene, *Arg702Trp*, *Gly908Arg*, and a frameshift deletion mutation at *Leu1007*, accounting for about 80% of all CD-associated mutations) have been implicated in increased susceptibility to IBD[19,31-33]. However, not everyone who carries these mutations develops IBD, indicating that other etiologic mechanisms might underlie IBD pathogenesis.

INTESTINAL MICROBIOTA IN IBD

Evidence from several recent studies has highlighted that gut microbiota composition and activity in IBD patients are abnormal. In particular, several studies have demonstrated that IBD patients are characterized by a reduced abundance of dominant members of the gut microbiota. Through a combination of PCR of total bacterial genomic DNA with universal bacterial primers and clone sequencing of 16S rRNA genes, Frank *et al*[34] showed that in mucosal biopsies taken from CD and UC patients there was reduced abundance of rRNA sequences associated with *Firmicutes* and *Bacteroidetes*, and a concomitant increase in 16S rRNA sequences of *Proteobacteria* and *Actinobacteria,* compared to non-IBD controls. In particular, the decreased relative abundance of the *Firmicutes* phylum was due to decreases in populations of *Clostridium* IXa and IV groups. As a consequence of this dysbiosis, the relative abundance of *Enterobacteriaceae* was increased in IBD patients compared to healthy controls, although their absolute numbers remained unaltered. No differences were observed in fecal and mucosal bacterial population numbers between CD and UC patients. These findings are common to several other studies, which also observed decreased clostridia concentrations in IBD[35,36], although not always accompanied by a decrease in *Bacteroides*[34,37].

Aberrancies in *Bifidobacterium* populations in IBD have also been previously observed in another study, where significantly lower counts of bifidobacteria were found in rectal biopsies of patients with UC compared to patients without UC[38]. By employing fluorescent *in situ* hybridization (FISH), Macfarlane *et al*[38] showed that bacteria belonging to the *C. leptum* phylogenetic group were significantly less abundant in fecal samples of CD patients compared to healthy individuals. Moreover, through a metagenomic approach, the same authors reported a conspicuous loss of microbial diversity in CD, mainly due to a reduction of operational taxonomic units (OTU) within the *C. coccoides* group and the *C. leptum* group. A reduction in bacterial diversity was also previously observed by Ott *et al*[39] after analysis of mucosa-associated microbiota of CD and UC patients through a combination of single strand conformation polymorphism (SSCP) fingerprint, cloning and real time PCR. Additionally, Zhang *et al*[40] more recently showed that bacterial diversity of lactobacilli and *C. leptum* group as determined by denaturing gradient gel electrophoresis (DGGE) analysis was also lower in ulcerated tissues compared to the non-ulcerated tissues within the same UC individual. These results suggest that microbial alteration in IBD patients might be caused by the physiological state of the intestinal mucosa. How-

ever, little is known about how inflammatory mediators (e.g. pro-inflammatory cytokines and chemokines) on the gut wall affect bacterial populations *in vivo*. We do know, however, that altered microbial composition may impact on important physiological processes in the intestinal environment. *Clostridium* and *Bacteroides* species are the main producers of short chain fatty acids (SCFA) in the human colon. Decreased clostridia of groups Ⅳ and XⅣa, the main butyrate-producing bacteria in the gut, could therefore explain the decreased SCFA concentrations found in fecal samples of IBD patients. Among the SCFA produced upon carbohydrate fermentation, butyrate serves as a major source of energy for colonic epithelial cells[41] and as an inhibitor of pro-inflammatory cytokine expression in the intestinal mucosa, through a mechanism that involves hyperacetylation of histones and suppression of NF-κB signaling[42]. Moreover, butyrate reinforces the mucosal barrier by inducing production of mucin and antimicrobial peptides, and by strengthening epithelial barrier integrity through directly increasing the expression of tight junction proteins[43]. A decrease of butyrate levels could therefore be involved in the increased inflammatory state characteristic of IBD, and butyrate is already considered to be of possible therapeutic value in treating IBD[44-46]. Stimulation of butyric acid production could be achieved through repopulation of clostridial clusters Ⅳ and XⅣa, or even through probiotic therapy with lactic acid bacteria, by increasing butyrate production through enhancement of carbohydrate fermentation (i.e. by supplementation with butyrogenic prebiotics such as inulin or oligofructose). Lactic acid can be employed as substrate for the production of high concentrations of butyrate by clostridial cluster XⅣa, in a process also known as cross-feeding[47]. *Faecalibacterium prausnitzii* (*F. prausnitzii*), a prevalent member of the human gut microbiota belonging to clostridial cluster Ⅳ and an important butyrate producer, has been recently shown to be less abundant in the intestinal microbiota of IBD patients[48,49]. *In vitro* and *in vivo* animal studies have also demonstrated the anti-inflammatory and anti-colitic properties of supernatants from *F. prausnitzii* cultures in peripheral blood mononuclear cells or in mouse models of colitis, respectively[48]. This effect appeared to be due to an as yet unidentified metabolite produced by the microorganism, but was shown to be independent of butyrate production.

Overgrowth of a class of microorganisms referred to as sulphate-reducing bacteria (SRB) was also previously observed in IBD gut microbiota in concomitance with a decrease in clostridia of groups Ⅳ and XⅣa, especially in UC and pouchitis patients[50]. SRB metabolize sulphate into hydrogen sulphide, which is toxic to colonocytes, blocks butyrate utilization, induces cell hyperproliferation, and inhibits phagocytosis and bacterial killing[51]. It was previously demonstrated that the presence of intestinal microorganisms is necessary for induction of dextran sodium sulphate (DSS) colitis in animal models, thus emphasizing the possible role of SRB in IBD, through their reduction of sulphate in DSS into the cytotoxic and inflammatory trigger molecule H_2S[52]. SRB numbers or their metabolic activity were found to be significantly higher in studies comparing UC patients to controls or to UC patients in remission[53-55].

In the search for a putative microbial cause of IBD, the theory of bacterial pathogen-induced intestinal inflammation has also been put forward. A wide range of microorganisms have been suggested as etiologic agents of IBD, including mycobacteria, *Listeria monocytogenes* (*L. monocytogenes*), *Chlamydia*, *Enterobacteriaceae* [including strains of *Escherichia coli* (*E. coli*) and *Helicobacter*] and also reoviruses and paramyxovirus[56-58]. However, when considering the diversity of IBD lesions and disease course, and the fact that no single pathogenic agent can routinely be isolated from diseased tissue, there is no conclusive evidence that a single pathogen is the cause of the disease. Among the *Enterobacteriaceae* genus, *E. coli* is the bacterium most commonly related to IBD. It was observed that IBD patients harbor increased *Enterobacteriaceae*, in particular *E. coli* belonging to the B2+D group (i.e. with increased virulent potential), compared to controls[59]. Adherent invasive *E. coli* was commonly found in ileal CD patients, particularly associated with ileal mucosal lesions[60,61]. On the other hand, *E. coli* isolated from UC patients was less invasive compared to CD[62]. *Mycobacterium avium* subspecies *paratuberculosis* (MAP) is an obligate intracellular pathogen that causes spontaneous granulomatous enterocolitis in cattle by evading phagocytosis. Therefore, MAP infection would be favored in those individuals with defective innate immunological defenses, such as CD patients. MAP presence was found with significantly higher frequency in CD patients compared to non-IBD controls, but not in all individuals[63]. No significant correspondence was found between CD-associated *NOD-2* polymorphisms, especially in ileal CD, and MAP infection[64,65]. Moreover, clinical studies failed to demonstrate the efficacy of antimycobacterium triple antibiotic therapy in inducing persistent response in CD patients[66]. Detection of MAP by molecular techniques (i.e. detection of insertion element-900 (IS-900) by PCR) has the limitation of picking up environmental mycobacteria and presents high variability among laboratories[67-69]. Hence, the etiologic role of MAP in IBD pathogenesis remains to be demonstrated.

Therefore, microbial dysbiosis consisting of a decrease in beneficial bacteria and their metabolic end-products, together with an increase of detrimental bacterial populations and their toxic metabolites, might alter gut luminal environment; thus contributing to the pathogenesis of IBD.

COMPROMISED EPITHELIAL BARRIER FUNCTION, DEFECTIVE INNATE IMMUNE RESPONSE TO BACTERIA AND LOSS OF IMMUNOTOLERANCE

Efficient functioning of the gut mucosa is achieved by means of a combination of intact epithelial barrier and effective bacterial killing through secretion of antimicrobial peptides (e.g. defensins), secretory IgA and phagocytosis.

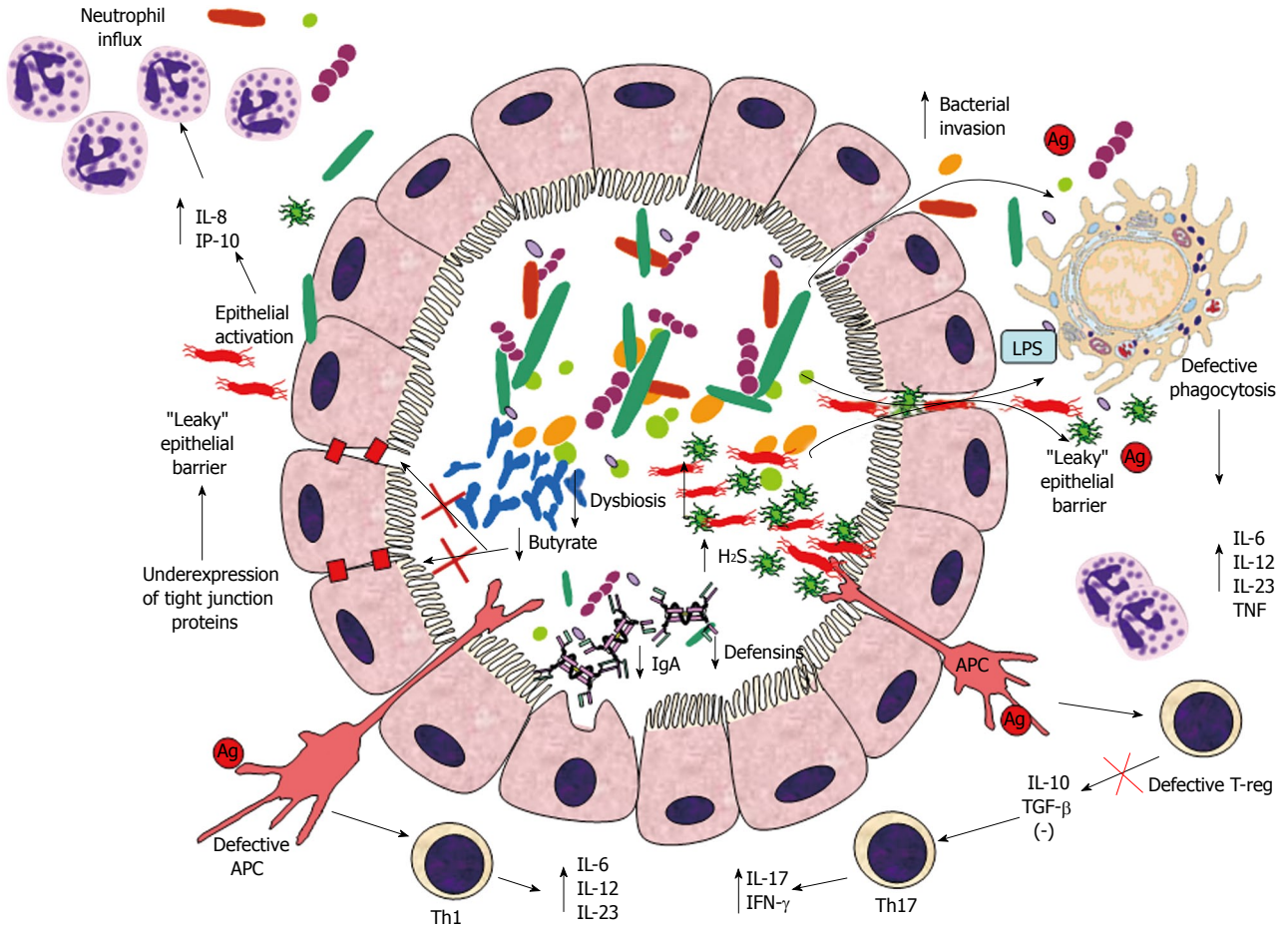


Figure 1 Suggested mechanism of inflammatory bowel disease pathogenesis. Intestinal dysbiosis in inflammatory bowel disease (IBD) consists of decreased prevalence of putative beneficial bacteria (e.g. bifidobacteria) and concomitant increase in detrimental bacterial (e.g. sulphate-reducing bacteria). This microbial imbalance causes reduced intraluminal levels of butyrate (because of decreased production through fermentation and decreased utilization due to increased H_2S levels), thus contributing to down-regulation of epithelial tight junction protein expression and increased epithelial permeability. Epithelial barrier dysfunction brings about increased bacterial translocation through the lamina propria, which is worsened by decreased luminal IgA and defensin concentrations. Killing of bacteria reaching the lamina propria through the "leaky" epithelium is also impaired by a genetically predisposed defective phagocytosis by macrophages. Ineffective bacterial clearance leads to excessive toll-like receptor (TLR) stimulation, secretion of pro-inflammatory cytokines and activation of innate and T-cell mediated immune responses. The disrupted mechanism of tolerance in epithelial cells and antigen presenting cells (APC) amplifies innate immune cell recruitment (i.e. neutrophils). Additionally, defective T-reg and APC cause excessive T-cell response (Th1 and Th17), with consequential intensification of the inflammatory response and granulomatous reaction. IL: Interleukin; IFN-γ: Interferon-γ; TNF: Tumor necrosis factor; TGF-β: Transforming growth factor-β; LPS: Lipopolysaccharide.

In IBD these mechanisms of mucosal defence are compromised at all levels and they all contribute to disease progression. A potential mechanism of pathogenesis of IBD is summarized in Figure 1. Disease arises from the initial epithelial barrier dysfunction that brings about increased bacterial translocation through the lamina propria, where microbial antigens elicit a strong inflammatory response, due to ectopic (i.e. basolateral) TLR stimulation, activation of the NF-κB pathway and consequent induction of pro-inflammatory chemokine and cytokine secretion. This inflammatory process is aggravated by the decreased innate immune defense (i.e. reduced luminal defensin and IgA, defective phagocytosis in IBD), which amplifies the magnitude of bacterial translocation through the "leaky" epithelial layer. Disease progression mainly results from a more global defective immunoregulation and immunotolerance in response to the initial inflammatory insult, due to overaggressive T cell reaction, dysfunctional regulatory T cells and antigen presenting cells (APC) (Figure 1).

IBD, and especially CD, presents with a characteristic increased epithelial permeability, due to underexpression of certain tight junction proteins [e.g. claudins, junction adhesion molecule-A (JAM-A)] concomitant with up-regulation of other pore-forming proteins (i.e. claudin-2)[70,71]. Defective bacterial clearance due to impaired defensin and IgA production contributes to increased bacterial translocation from the gut lumen across the lamina propria. α-Defensins (i.e. human defensin 5 and 6 (HD5 and HD6)) are antibactericidal compounds produced by Paneth cells efficacious against *Enterobacteriaceae* (e.g. *E. coli*, *Salmonella typhimurium*, *L. monocytogenes*) and *Bacteroides vulgatus*, and were found significantly reduced in association with ileal CD, in particular in patients with *NOD-2* mutations[72,73]. On the other hand, colonic CD, but not UC, was observed to be associated with lower copy number of β-defensins 2 and 3, which are the main antimicrobial peptides found in the colon. This reduction in β-defensins was shown to be due to a chromosomic polymorphism,

since chromosome 8 presented with a lower copy number of β-defensin 2 in colonic CD[74,75].

Microbial clearance can also be impaired because of reduced levels of protective secretory IgA (SIgA) in IBD. IgA constitutes the most abundant immunoglobulin phenotype present in the human body[76]. In the gut, IgA is produced by lamina propria B cells, then translocates to the lumen by attaching to a basolateral receptor on epithelial cells, and finally is transported to the luminal surface of epithelial cells, where it forms SIgA clusters that elicit multiple roles in the intestinal lumen. Firstly, IgA in the mucus layer entraps bacteria and dietary antigens, down-regulates epitope expression on the bacterial cell surface and, therefore, regulates microbial intestinal colonization[77-79]. Moreover, SIgA prevents pathogen attachment and invasion of epithelial cells and removes bacteria breaching the epithelial barrier by translocating them back to the lumen and by promoting their clearance by dendritic cells, neutrophils and phagocytes[80-82]. In IBD, intestinal IgA is usually reduced and this is compensated for by increased secretion of IgG, which induces pro-inflammatory cytokine production and mounting of adaptive immune responses to the resident microbiota[83]. Mucosal secretory IgG was found to be significantly higher in UC and CD patients compared to control patients with irritable bowel syndrome[84]. In addition, the same study showed that both CD and UC patients presented with increased mucosal IgG bound to fecal bacterial cytoplasmic antigens compared to control patients with irritable bowel syndrome and to non-IBD controls with intestinal inflammation[84].

Malfunctioning bacterial killing in IBD has also recently been linked to dysfunctional autophagy. Autophagy is a constitutive pathway of cellular homeostasis and organelle turnover. However, it has recently been demonstrated that autophagy plays a key role in innate and adaptive immunity. Macrophages use autophagy to capture and effectively kill intracellular and extracellular invading bacterial pathogens, including *Legionella*, *E. coli, Streptococcus* and *Mycobacterium* species, by fusion of the phagocytic compartment with the lysosome[85,86]. Epithelial cells also employ autophagy to kill invading bacteria and the gene *ATG 16L1* has been shown to be necessary for starting the autophagic process against the cytoplasmic invasion of *Salmonella typhimurium*[87]. Mutations in *ATG 16L1* have recently been associated with CD, thus implicating defective bacterial killing by autophagy in IBD[87]. Autophagy impairment might also influence the adaptive immune response to bacteria, since autophagy is involved in major histocompatibility complex (MHC) class Ⅱ loading in the lysosome, where the autophagic cytoplasmic content is also delivered[88]. Therefore, a defect in the autophagy pathway could influence antigen presentation by APC, epithelial cells and immune surveillance. Finally, autophagy has been implicated in the regulation of T cell death and proliferation, and *ATG 16L1* is central to these autophagy-regulated processes[89]. Alteration of *ATG 16L1* in CD might therefore, at least in part, explain the pathologic behaviour of T cells in IBD. In IBD the coexistence of compromised epithelial barrier and defective innate immunity aggravates the impaired mechanism of tolerance to the resident microbiota and causes inflammatory granulomatous reaction (Figure 1). Defective interaction between regulatory T lymphocytes in the lamina propria and epithelial cells is central to the process of loss of tolerance, through a mechanism that involves NF-κB signaling. Epithelial NF-κB activation in healthy hosts is normally suppressed by anti-inflammatory cytokines produced by the underlying T lymphocytes, such as TGF-β and IL-10, while in IBD Th1- and Th17-type immune responses are predominant and lead to chronic inflammation and worsening of the epithelial layer damage[90]. Perpetuation of the epithelial damage causes increased basolateral as opposed to physiological apical stimulation of TLR-9 receptors, thus causing activation, rather than blockade, of NF-κB signaling[30]. This leads to a vicious cycle of aberrant immune response, mucosal inflammation, altered microbiota composition and/or activity and increased mucosal permeability, which would explain the persistent and recurrent nature of IBD.

THERAPEUTIC IMPLICATIONS OF GUT MICROBIOTA-HUMAN HOST INTERACTION

The increasing understanding of the gut microbiota-host immune system interaction has recently drawn interest towards a modulation of intestinal bacterial communities as a novel potential adjuvant in IBD therapy. Although antibiotic therapy constitutes an established therapeutic tool for the treatment of specific IBD-associated symptoms (e.g. abscesses and fistulae), as well as a possible preventive measure, research studies that demonstrate antibiotic efficacy in IBD are still limited[91]. Promising outcomes have been observed after gut microbiota modulation through probiotic, prebiotic and synbiotic supplementation in CD and UC to change IBD-associated dysbiosis. Treating CD patients with the probiotic strain *E. coli* Nissle 1917 has been shown to induce remission more rapidly than untreated control patients, although it did not influence the number of patients achieving remission[92]. In UC, *E. coli* Nissle 1917 was proven as effective as mesalazine in maintaining remission[93,94]. Maintenance of remission after probiotic supplementation was observed in a study with the yeast probiotic *Saccharomyces boulardii* (reduced percentage of relapses in probiotic + mesalamine-treated CD patients, compared to control mesalamine-treated CD patients), although the significance of the study is somewhat restricted because of the low number of subjects involved (n = 32)[95,96]. Positive results were also observed in a double-blind, randomised controlled trial with *Bifidobacterium breve* and *Bifidobacterium bifidum* fermented milk supplementation in 20 UC subjects for 12 wk, where a significant decrease of clinical indices was observed compared to unsupplemented controls[97]. The probiotic mixture VSL#3 showed convincing effects in the maintenance of remission in UC patients[98-100], and it was later shown to prevent the onset of pouchitis[101]. On the other

hand, the data with regard to VSL#3 supplementation in CD are still preliminary. VSL#3 supplementation did not result in a reduction in post-surgical relapse when administered to pediatric CD patients, compared to control mesalamine-treated patients[102]. In general, it appears that this probiotic supplementation is more effective in reducing disease onset or recurrence, rather than diminishing active inflammatory symptoms.

Prebiotic supplementation with inulin was shown to improve clinical condition in pouchitis patients, and to increase tolerance (i.e. through decreased TLR-2 and TLR-4 expression on DC) and fecal bifidobacteria levels in CD patients[103-105]. Synbiotics (i.e. a synergy of pro- and pre-biotics in a single preparation) also showed potential therapeutic effect, although the number of studies in IBD is still limited. Supplementation of the inulin-derived prebiotic, Synergy-1, together with *Bifidobacterium longum,* in 18 UC patients for 4 wk significantly decreased rectal pro-inflammatory cytokine levels and down-regulated the expression of inflammation-associated β-defensins[106].

In summary, some evidence has already indicated a promising therapeutic effect of pro-, pre- and synbiotics in IBD. However, the studies are still very few, underpowered and their design and selection of active agent are sometimes less than optimal. Indeed, this topic deserves further investigation in studies using an adequate number of subjects and employing functional food products targeting the gut microbiota, that have been specifically selected for their anti-inflammatory properties from preliminary *in vitro* and animal studies.

CONCLUSION

Despite the observation that IBD is associated with an abnormal gut microbiota composition, the question as to whether the altered gut microbial dysbiosis is a cause of disease or a consequence of the inflammatory state of the intestinal environment still remains unanswered. Although several studies implicate the gut microbiota in IBD pathogenesis, so far no pathogenic/infectious microorganism has been identified as sole disease causing agent. It is more likely that microbial dysbiosis and lack of beneficial bacteria, together with genetically predisposed increased epithelial permeability, bacterial translocation into the lamina propria, defective innate immunity and loss of tolerance to the resident microbiota, may lead to the abnormal inflammatory response and granulomatous reaction characteristic of IBD. A modulation of the gut microbiota through pro-, pre- and synbiotics, specifically designed to reduce IBD-associated dysbiosis and inflammation, constitutes an interesting approach in the field of novel therapeutic approaches for IBD.

REFERENCES

1 **Cho JH**. The genetics and immunopathogenesis of inflammatory bowel disease. *Nat Rev Immunol* 2008; **8**: 458-466
2 **Sartor RB**. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 390-407
3 **Danese S**, Fiocchi C. Etiopathogenesis of inflammatory bowel diseases. *World J Gastroenterol* 2006; **12**: 4807-4812
4 **Greenberg GR**. Antibiotics should be used as first-line therapy for Crohn's disease. *Inflamm Bowel Dis* 2004; **10**: 318-320
5 **Sellon RK**, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998; **66**: 5224-5231
6 **Eckburg PB**, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, Gill SR, Nelson KE, Relman DA. Diversity of the human intestinal microbial flora. *Science* 2005; **308**: 1635-1638
7 **Louis P**, Scott KP, Duncan SH, Flint HJ. Understanding the effects of diet on bacterial metabolism in the large intestine. *J Appl Microbiol* 2007; **102**: 1197-1208
8 **Zoetendal EG**, Vaughan EE, de Vos WM. A microbial world within us. *Mol Microbiol* 2006; **59**: 1639-1650
9 **Zoetendal EG**, Akkermans AD, De Vos WM. Temperature gradient gel electrophoresis analysis of 16S rRNA from human fecal samples reveals stable and host-specific communities of active bacteria. *Appl Environ Microbiol* 1998; **64**: 3854-3859
10 Zoetendal EG, Akkermans ADL, Akkermans-van Vliet WM, de Visser JAGM, de Vos WM. The host genotype affects the bacterial community in the human gastronintestinal tract. *Microb Ecol Health Dis* 2001; **13**: 129-134
11 **Ley RE**, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 2006; **124**: 837-848
12 **Macfarlane S**, Macfarlane GT. Regulation of short-chain fatty acid production. *Proc Nutr Soc* 2003; **62**: 67-72
13 **Falony G**, Vlachou A, Verbrugghe K, De Vuyst L. Cross-feeding between Bifidobacterium longum BB536 and acetate-converting, butyrate-producing colon bacteria during growth on oligofructose. *Appl Environ Microbiol* 2006; **72**: 7835-7841
14 **Cani PD**, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM, Burcelin R. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes* 2008; **57**: 1470-1481
15 **Cash HL**, Whitham CV, Behrendt CL, Hooper LV. Symbiotic bacteria direct expression of an intestinal bactericidal lectin. *Science* 2006; **313**: 1126-1130
16 **Bry L**, Falk PG, Midtvedt T, Gordon JI. A model of host-microbial interactions in an open mammalian ecosystem. *Science* 1996; **273**: 1380-1383
17 **Macpherson AJ**, Harris NL. Interactions between commensal intestinal bacteria and the immune system. *Nat Rev Immunol* 2004; **4**: 478-485
18 **Bouskra D**, Brézillon C, Bérard M, Werts C, Varona R, Boneca IG, Eberl G. Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* 2008; **456**: 507-510
19 **Cario E**. Bacterial interactions with cells of the intestinal mucosa: Toll-like receptors and NOD2. *Gut* 2005; **54**: 1182-1193
20 **Strober W**, Murray PJ, Kitani A, Watanabe T. Signalling pathways and molecular interactions of NOD1 and NOD2. *Nat Rev Immunol* 2006; **6**: 9-20
21 **Neish AS**, Gewirtz AT, Zeng H, Young AN, Hobert ME, Karmali V, Rao AS, Madara JL. Prokaryotic regulation of epithelial responses by inhibition of IkappaB-alpha ubiquitination. *Science* 2000; **289**: 1560-1563
22 **Fukata M**, Chen A, Klepper A, Krishnareddy S, Vamadevan AS, Thomas LS, Xu R, Inoue H, Arditi M, Dannenberg AJ, Abreu MT. Cox-2 is regulated by Toll-like receptor-4 (TLR4) signaling: Role in proliferation and apoptosis in the intestine. *Gastroenterology* 2006; **131**: 862-877
23 **Coombes JL**, Siddiqui KR, Arancibia-Cárcamo CV, Hall J,

Sun CM, Belkaid Y, Powrie F. A functionally specialized population of mucosal CD103+ DCs induces Foxp3+ regulatory T cells via a TGF-beta and retinoic acid-dependent mechanism. *J Exp Med* 2007; **204**: 1757-1764
24 **Tezuka H**, Abe Y, Iwata M, Takeuchi H, Ishikawa H, Matsushita M, Shiohara T, Akira S, Ohteki T. Regulation of IgA production by naturally occurring TNF/iNOS-producing dendritic cells. *Nature* 2007; **448**: 929-933
25 **Davies JM**, Sheil B, Shanahan F. Bacterial signalling overrides cytokine signalling and modifies dendritic cell differentiation. *Immunology* 2009; **128**: e805-e815
26 **Latvala S**, Pietila TE, Veckman V, Kekkonen RA, Tynkkynen S, Korpela R, Julkunen I. Potentially probiotic bacteria induce efficient maturation but differential cytokine production in human monocyte-derived dendritic cells. *World J Gastroenterol* 2008; **14**: 5570-5583; discussion 5581-5582
27 **Zeng H**, Wu H, Sloane V, Jones R, Yu Y, Lin P, Gewirtz AT, Neish AS. Flagellin/TLR5 responses in epithelia reveal intertwined activation of inflammatory and apoptotic pathways. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G96-G108
28 **Hedl M**, Li J, Cho JH, Abraham C. Chronic stimulation of Nod2 mediates tolerance to bacterial products. *Proc Natl Acad Sci USA* 2007; **104**: 19440-19445
29 **Lu LF**, Thai TH, Calado DP, Chaudhry A, Kubo M, Tanaka K, Loeb GB, Lee H, Yoshimura A, Rajewsky K, Rudensky AY. Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. *Immunity* 2009; **30**: 80-91
30 **Lee J**, Mo JH, Katakura K, Alkalay I, Rucker AN, Liu YT, Lee HK, Shen C, Cojocaru G, Shenouda S, Kagnoff M, Eckmann L, Ben-Neriah Y, Raz E. Maintenance of colonic homeostasis by distinctive apical TLR9 signalling in intestinal epithelial cells. *Nat Cell Biol* 2006; **8**: 1327-1336
31 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603
32 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606
33 **Pierik M**, Joossens S, Van Steen K, Van Schuerbeek N, Vlietinck R, Rutgeerts P, Vermeire S. Toll-like receptor-1, -2, and -6 polymorphisms influence disease extension in inflammatory bowel diseases. *Inflamm Bowel Dis* 2006; **12**: 1-8
34 **Frank DN**, St Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc Natl Acad Sci USA* 2007; **104**: 13780-13785
35 **Gophna U**, Sommerfeld K, Gophna S, Doolittle WF, Veldhuyzen van Zanten SJ. Differences between tissue-associated intestinal microfloras of patients with Crohn's disease and ulcerative colitis. *J Clin Microbiol* 2006; **44**: 4136-4141
36 **Manichanh C**, Rigottier-Gois L, Bonnaud E, Gloux K, Pelletier E, Frangeul L, Nalin R, Jarrin C, Chardon P, Marteau P, Roca J, Dore J. Reduced diversity of faecal microbiota in Crohn's disease revealed by a metagenomic approach. *Gut* 2006; **55**: 205-211
37 **Baumgart M**, Dogan B, Rishniw M, Weitzman G, Bosworth B, Yantiss R, Orsi RH, Wiedmann M, McDonough P, Kim SG, Berg D, Schukken Y, Scherl E, Simpson KW. Culture independent analysis of ileal mucosa reveals a selective increase in invasive Escherichia coli of novel phylogeny relative to depletion of Clostridiales in Crohn's disease involving the ileum. *ISME J* 2007; **1**: 403-418
38 **Macfarlane S**, Furrie E, Cummings JH, Macfarlane GT. Chemotaxonomic analysis of bacterial populations colonizing the rectal mucosa in patients with ulcerative colitis. *Clin Infect Dis* 2004; **38**: 1690-1699
39 **Ott SJ**, Musfeldt M, Wenderoth DF, Hampe J, Brant O, Fölsch UR, Timmis KN, Schreiber S. Reduction in diversity of the colonic mucosa associated bacterial microflora in patients with active inflammatory bowel disease. *Gut* 2004; **53**: 685-693
40 **Zhang M**, Liu B, Zhang Y, Wei H, Lei Y, Zhao L. Structural shifts of mucosa-associated lactobacilli and Clostridium leptum subgroup in patients with ulcerative colitis. *J Clin Microbiol* 2007; **45**: 496-500
41 **Fleming LL**, Floch MH. Digestion and absorption of fiber carbohydrate in the colon. *Am J Gastroenterol* 1986; **81**: 507-511
42 **Segain JP**, Raingeard de la Blétière D, Bourreille A, Leray V, Gervois N, Rosales C, Ferrier L, Bonnet C, Blottière HM, Galmiche JP. Butyrate inhibits inflammatory responses through NFkappaB inhibition: implications for Crohn's disease. *Gut* 2000; **47**: 397-403
43 **Vanhoutvin SA**, Troost FJ, Hamer HM, Lindsey PJ, Koek GH, Jonkers DM, Kodde A, Venema K, Brummer RJ. Butyrate-induced transcriptional changes in human colonic mucosa. *PLoS One* 2009; **4**: e6759
44 **Breuer RI**, Soergel KH, Lashner BA, Christ ML, Hanauer SB, Vanagunas A, Harig JM, Keshavarzian A, Robinson M, Sellin JH, Weinberg D, Vidican DE, Flemal KL, Rademaker AW. Short chain fatty acid rectal irrigation for left-sided ulcerative colitis: a randomised, placebo controlled trial. *Gut* 1997; **40**: 485-491
45 **Kanauchi O**, Suga T, Tochihara M, Hibi T, Naganuma M, Homma T, Asakura H, Nakano H, Takahama K, Fujiyama Y, Andoh A, Shimoyama T, Hida N, Haruma K, Koga H, Mitsuyama K, Sata M, Fukuda M, Kojima A, Bamba T. Treatment of ulcerative colitis by feeding with germinated barley foodstuff: first report of a multicenter open control trial. *J Gastroenterol* 2002; **37** Suppl 14: 67-72
46 **Galvez J**, Rodríguez-Cabezas ME, Zarzuelo A. Effects of dietary fiber on inflammatory bowel disease. *Mol Nutr Food Res* 2005; **49**: 601-608
47 **Duncan SH**, Louis P, Flint HJ. Lactate-utilizing bacteria, isolated from human feces, that produce butyrate as a major fermentation product. *Appl Environ Microbiol* 2004; **70**: 5810-5817
48 **Sokol H**, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottière HM, Doré J, Marteau P, Seksik P, Langella P. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 2008; **105**: 16731-16736
49 **Sokol H**, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm Bowel Dis* 2009; **15**: 1183-1189
50 **Roediger WE**, Duncan A, Kapaniris O, Millard S. Reducing sulfur compounds of the colon impair colonocyte nutrition: implications for ulcerative colitis. *Gastroenterology* 1993; **104**: 802-809
51 **Gardiner KR**, Halliday MI, Barclay GR, Milne L, Brown D, Stephens S, Maxwell RJ, Rowlands BJ. Significance of systemic endotoxaemia in inflammatory bowel disease. *Gut* 1995; **36**: 897-901
52 **Ohkusa T**, Yamada M, Takenaga T, Kitazume C, Yamamoto N, Sasabe M, Takashimizu I, Tamura Y, Sakamoto E, Kurosawa H. [Protective effect of metronidazole in experimental ulcerative colitis induced by dextran sulfate sodium] *Nippon Shokakibyo Gakkai Zasshi* 1987; **84**: 2337-2346
53 **Gibson GR**, Macfarlane GT, Cummings JH. Sulphate reducing bacteria and hydrogen metabolism in the human large intestine. *Gut* 1993; **34**: 437-439
54 **Loubinoux J**, Bronowicki JP, Pereira IA, Mougenel JL, Faou AE. Sulfate-reducing bacteria in human feces and their asso-

ciation with inflammatory bowel diseases. *FEMS Microbiol Ecol* 2002; **40**: 107-112
55 **Pitcher MC**, Beatty ER, Cummings JH. The contribution of sulphate reducing bacteria and 5-aminosalicylic acid to faecal sulphide in patients with ulcerative colitis. *Gut* 2000; **46**: 64-72
56 **Munro J**, Mayberry JF, Matthews N, Rhodes J. Chlamydia and Crohn's disease. *Lancet* 1979; **2**: 45-46
57 **Burnham WR**, Lennard-Jones JE, Stanford JL, Bird RG. Mycobacteria as a possible cause of inflammatory bowel disease. *Lancet* 1978; **2**: 693-696
58 **Liu Y**, van Kruiningen HJ, West AB, Cartun RW, Cortot A, Colombel JF. Immunocytochemical evidence of Listeria, Escherichia coli, and Streptococcus antigens in Crohn's disease. *Gastroenterology* 1995; **108**: 1396-1404
59 **Kotlowski R**, Bernstein CN, Sepehri S, Krause DO. High prevalence of Escherichia coli belonging to the B2+D phylogenetic group in inflammatory bowel disease. *Gut* 2007; **56**: 669-675
60 **Darfeuille-Michaud A**, Neut C, Barnich N, Lederman E, Di Martino P, Desreumaux P, Gambiez L, Joly B, Cortot A, Colombel JF. Presence of adherent Escherichia coli strains in ileal mucosa of patients with Crohn's disease. *Gastroenterology* 1998; **115**: 1405-1413
61 **Rolhion N**, Darfeuille-Michaud A. Adherent-invasive Escherichia coli in inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 1277-1283
62 **Sasaki M**, Sitaraman SV, Babbin BA, Gerner-Smidt P, Ribot EM, Garrett N, Alpern JA, Akyildiz A, Theiss AL, Nusrat A, Klapproth JM. Invasive Escherichia coli are a feature of Crohn's disease. *Lab Invest* 2007; **87**: 1042-1054
63 **Abubakar I**, Myhill D, Aliyu SH, Hunter PR. Detection of Mycobacterium avium subspecies paratuberculosis from patients with Crohn's disease using nucleic acid-based techniques: a systematic review and meta-analysis. *Inflamm Bowel Dis* 2008; **14**: 401-410
64 **Bernstein CN**, Wang MH, Sargent M, Brant SR, Collins MT. Testing the interaction between NOD-2 status and serological response to Mycobacterium paratuberculosis in cases of inflammatory bowel disease. *J Clin Microbiol* 2007; **45**: 968-971
65 **Sechi LA**, Gazouli M, Ikonomopoulos J, Lukas JC, Scanu AM, Ahmed N, Fadda G, Zanetti S. Mycobacterium avium subsp. paratuberculosis, genetic susceptibility to Crohn's disease, and Sardinians: the way ahead. *J Clin Microbiol* 2005; **43**: 5275-5277
66 **Selby W**, Pavli P, Crotty B, Florin T, Radford-Smith G, Gibson P, Mitchell B, Connell W, Read R, Merrett M, Ee H, Hetzel D. Two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Crohn's disease. *Gastroenterology* 2007; **132**: 2313-2319
67 **Englund S**, Bölske G, Johansson KE. An IS900-like sequence found in a Mycobacterium sp. other than Mycobacterium avium subsp. paratuberculosis. *FEMS Microbiol Lett* 2002; **209**: 267-271
68 **Bull TJ**, McMinn EJ, Sidi-Boumedine K, Skull A, Durkin D, Neild P, Rhodes G, Pickup R, Hermon-Taylor J. Detection and verification of Mycobacterium avium subsp. paratuberculosis in fresh ileocolonic mucosal biopsy specimens from individuals with and without Crohn's disease. *J Clin Microbiol* 2003; **41**: 2915-2923
69 **Collins MT**, Lisby G, Moser C, Chicks D, Christensen S, Reichelderfer M, Høiby N, Harms BA, Thomsen OO, Skibsted U, Binder V. Results of multiple diagnostic tests for Mycobacterium avium subsp. paratuberculosis in patients with inflammatory bowel disease and in controls. *J Clin Microbiol* 2000; **38**: 4373-4381
70 **Vetrano S**, Rescigno M, Cera MR, Correale C, Rumio C, Doni A, Fantini M, Sturm A, Borroni E, Repici A, Locati M, Malesci A, Dejana E, Danese S. Unique role of junctional adhesion molecule-a in maintaining mucosal homeostasis in inflammatory bowel disease. *Gastroenterology* 2008; **135**: 173-184
71 **Zeissig S**, Bürgel N, Günzel D, Richter J, Mankertz J, Wahnschaffe U, Kroesen AJ, Zeitz M, Fromm M, Schulzke JD. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut* 2007; **56**: 61-72
72 **Nuding S**, Fellermann K, Wehkamp J, Stange EF. Reduced mucosal antimicrobial activity in Crohn's disease of the colon. *Gut* 2007; **56**: 1240-1247
73 **Wehkamp J**, Harder J, Weichenthal M, Schwab M, Schäffeler E, Schlee M, Herrlinger KR, Stallmach A, Noack F, Fritz P, Schröder JM, Bevins CL, Fellermann K, Stange EF. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut* 2004; **53**: 1658-1664
74 **Wehkamp J**, Harder J, Weichenthal M, Mueller O, Herrlinger KR, Fellermann K, Schroeder JM, Stange EF. Inducible and constitutive beta-defensins are differentially expressed in Crohn's disease and ulcerative colitis. *Inflamm Bowel Dis* 2003; **9**: 215-223
75 **Fellermann K**, Stange DE, Schaeffeler E, Schmalzl H, Wehkamp J, Bevins CL, Reinisch W, Teml A, Schwab M, Lichter P, Radlwimmer B, Stange EF. A chromosome 8 gene-cluster polymorphism with low human beta-defensin 2 gene copy number predisposes to Crohn disease of the colon. *Am J Hum Genet* 2006; **79**: 439-448
76 **Macpherson AJ**, McCoy KD, Johansen FE, Brandtzaeg P. The immune geography of IgA induction and function. *Mucosal Immunol* 2008; **1**: 11-22
77 **Fagarasan S**, Muramatsu M, Suzuki K, Nagaoka H, Hiai H, Honjo T. Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. *Science* 2002; **298**: 1424-1427
78 **Peterson DA**, McNulty NP, Guruge JL, Gordon JI. IgA response to symbiotic bacteria as a mediator of gut homeostasis. *Cell Host Microbe* 2007; **2**: 328-339
79 **Phalipon A**, Cardona A, Kraehenbuhl JP, Edelman L, Sansonetti PJ, Corthésy B. Secretory component: a new role in secretory IgA-mediated immune exclusion in vivo. *Immunity* 2002; **17**: 107-115
80 **Cerutti A**, Rescigno M. The biology of intestinal immunoglobulin A responses. *Immunity* 2008; **28**: 740-750
81 **Pasquier B**, Launay P, Kanamaru Y, Moura IC, Pfirsch S, Ruffié C, Hénin D, Benhamou M, Pretolani M, Blank U, Monteiro RC. Identification of FcalphaRI as an inhibitory receptor that controls inflammation: dual role of FcRgamma ITAM. *Immunity* 2005; **22**: 31-42
82 **Phalipon A**, Corthésy B. Novel functions of the polymeric Ig receptor: well beyond transport of immunoglobulins. *Trends Immunol* 2003; **24**: 55-58
83 **Sartor RB**. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008; **134**: 577-594
84 **Macpherson A**, Khoo UY, Forgacs I, Philpott-Howard J, Bjarnason I. Mucosal antibodies in inflammatory bowel disease are directed against intestinal bacteria. *Gut* 1996; **38**: 365-375
85 **Nakagawa I**, Amano A, Mizushima N, Yamamoto A, Yamaguchi H, Kamimoto T, Nara A, Funao J, Nakata M, Tsuda K, Hamada S, Yoshimori T. Autophagy defends cells against invading group A Streptococcus. *Science* 2004; **306**: 1037-1040
86 **Amer AO**, Byrne BG, Swanson MS. Macrophages rapidly transfer pathogens from lipid raft vacuoles to autophagosomes. *Autophagy* 2005; **1**: 53-58
87 **Kuballa P**, Huett A, Rioux JD, Daly MJ, Xavier RJ. Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant. *PLoS One* 2008; **3**: e3391
88 **Schmid D**, Pypaert M, Münz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* 2007; **26**: 79-92

89 **Dengjel J**, Schoor O, Fischer R, Reich M, Kraus M, Müller M, Kreymborg K, Altenberend F, Brandenburg J, Kalbacher H, Brock R, Driessen C, Rammensee HG, Stevanovic S. Autophagy promotes MHC class II presentation of peptides from intracellular source proteins. *Proc Natl Acad Sci USA* 2005; **102**: 7922-7927

90 **Ruiz PA**, Shkoda A, Kim SC, Sartor RB, Haller D. IL-10 gene-deficient mice lack TGF-beta/Smad-mediated TLR2 degradation and fail to inhibit proinflammatory gene expression in intestinal epithelial cells under conditions of chronic inflammation. *Ann N Y Acad Sci* 2006; **1072**: 389-394

91 **Kruis W**. Review article: antibiotics and probiotics in inflammatory bowel disease. *Aliment Pharmacol Ther* 2004; **20** Suppl 4: 75-78

92 **Malchow HA**. Crohn's disease and Escherichia coli. A new approach in therapy to maintain remission of colonic Crohn's disease? *J Clin Gastroenterol* 1997; **25**: 653-658

93 **Kruis W**, Schütz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 1997; **11**: 853-858

94 **Kruis W**, Fric P, Pokrotnieks J, Lukás M, Fixa B, Kascák M, Kamm MA, Weismueller J, Beglinger C, Stolte M, Wolff C, Schulze J. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004; **53**: 1617-1623

95 **Guslandi M**, Mezzi G, Sorghi M, Testoni PA. Saccharomyces boulardii in maintenance treatment of Crohn's disease. *Dig Dis Sci* 2000; **45**: 1462-1464

96 **Rolfe VE**, Fortun PJ, Hawkey CJ, Bath-Hextall F. Probiotics for maintenance of remission in Crohn's disease. *Cochrane Database Syst Rev* 2006; CD004826

97 **Kato K**, Mizuno S, Umesaki Y, Ishii Y, Sugitani M, Imaoka A, Otsuka M, Hasunuma O, Kurihara R, Iwasaki A, Arakawa Y. Randomized placebo-controlled trial assessing the effect of bifidobacteria-fermented milk on active ulcerative colitis. *Aliment Pharmacol Ther* 2004; **20**: 1133-1141

98 **Gionchetti P**, Rizzello F, Venturi A, Brigidi P, Matteuzzi D, Bazzocchi G, Poggioli G, Miglioli M, Campieri M. Oral bacteriotherapy as maintenance treatment in patients with chronic pouchitis: a double-blind, placebo-controlled trial. *Gastroenterology* 2000; **119**: 305-309

99 **Mimura T**, Rizzello F, Helwig U, Poggioli G, Schreiber S, Talbot IC, Nicholls RJ, Gionchetti P, Campieri M, Kamm MA. Once daily high dose probiotic therapy (VSL#3) for maintaining remission in recurrent or refractory pouchitis. *Gut* 2004; **53**: 108-114

100 **Venturi A**, Gionchetti P, Rizzello F, Johansson R, Zucconi E, Brigidi P, Matteuzzi D, Campieri M. Impact on the composition of the faecal flora by a new probiotic preparation: preliminary data on maintenance treatment of patients with ulcerative colitis. *Aliment Pharmacol Ther* 1999; **13**: 1103-1108

101 **Gionchetti P**, Rizzello F, Helwig U, Venturi A, Lammers KM, Brigidi P, Vitali B, Poggioli G, Miglioli M, Campieri M. Prophylaxis of pouchitis onset with probiotic therapy: a double-blind, placebo-controlled trial. *Gastroenterology* 2003; **124**: 1202-1209

102 **Campieri M**, Rizzello F, Venturi A, Poggioli G, Ugolini F, Helwig U. Combination of antibiotic and probiotic treatment is efficacious in prophylaxis of post-operative recurrence of Crohn's disease: a randomized controlled study versus mezalamine. *Gastroenterology* 2000; **118**: A781

103 **Welters CF**, Heineman E, Thunnissen FB, van den Bogaard AE, Soeters PB, Baeten CG. Effect of dietary inulin supplementation on inflammation of pouch mucosa in patients with an ileal pouch-anal anastomosis. *Dis Colon Rectum* 2002; **45**: 621-627

104 **Hussey TA**, Issenman RM, Persad R, Olley AR, Christensen BA. Nutrition therapy in pediatric Crohn's disease patients improves nutrition status and decreases inflammation. *J Pediatr Gastroenterol* 2003; **37**: A341

105 **Lindsay JO**, Whelan K, Stagg AJ, Gobin P, Al-Hassi HO, Rayment N, Kamm MA, Knight SC, Forbes A. Clinical, microbiological, and immunological effects of fructo-oligosaccharide in patients with Crohn's disease. *Gut* 2006; **55**: 348-355

106 **Furrie E**, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'neil DA, Macfarlane GT. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005; **54**: 242-249

S- Editor Shi ZF **L- Editor** Logan S **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.567

World J Gastroenterol 2011 February 7; 17(5): 567-571
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

Silvio Danese, MD, PhD, Head, *Series Editor*

Defensins couple dysbiosis to primary immunodeficiency in Crohn's disease

Mathias Chamaillard, Rodrigue Dessein

Mathias Chamaillard, Rodrigue Dessein, Institut Pasteur de Lille, Center for Infection and Immunity of Lille, F-59019 Lille, France
Mathias Chamaillard, Rodrigue Dessein, CNRS, UMR 8204, F-59021 Lille, France
Mathias Chamaillard, Rodrigue Dessein, Institut National de la Santé et de la Recherche Médicale, U1019, Team 7, Equipe FRM, F-59019 Lille, France
Mathias Chamaillard, Rodrigue Dessein, Univ Lille Nord de France, F-59000 Lille, France
Rodrigue Dessein, C.H.R.U. de Lille, F-59000 Lille, France
Author contributions: Chamaillard M and Dessein R wrote the paper.
Correspondence to: Mathias Chamaillard, PhD, Institut Pasteur de Lille, Center for Infection and Immunity of Lille, 1, rue du Professeur Calmette, F-59019 Lille, France. mathias.chamaillard@inserm.fr
Telephone: +33-3-59317427 Fax: +33-3-59317480
Received: May 8, 2010 Revised: June 4, 2010
Accepted: June 11, 2010
Published online: February 7, 2011

Abstract

Antimicrobial peptides, including defensins, are essential effectors in host defence and in the maintenance of immune homeostasis. Clinical studies have linked the defective expression of both α- and β-defensin to the reduced killing of certain microorganisms by the intestinal mucosa of patients suffering from ileal and colonic Crohn's disease (CD), respectively. Only recently have the events leading to defective expression of defensins in CD been further elucidated, and are discussed herein. These events may account for CD-associated alterations in the microbiome and may subsequently precipitate the development of granulomatous inflammatory lesions in genetically-predisposed patients. We also address how these discoveries may pave the way for the development of a molecular medicine aimed at restoring gut barrier function in CD.

Key words: Crohn's disease; Defensins; Primary immunodeficiencies

Peer reviewer: Yuji Naito, Professor, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566, Japan

Chamaillard M, Dessein R. Defensins couple dysbiosis to primary immunodeficiency in Crohn's disease. *World J Gastroenterol* 2011; 17(5): 567-571 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/567.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.567

INTRODUCTION

So far, *in silico* mining of human genomic databases have identified or predicted more than one hundred defensin-related sequences[1,2]. Based on their amino-acid sequences, these human peptides are divided into two categories, the α- and β-defensins (DEFA and DEFB, respectively). Although some defensins are not fully characterized, these molecules are cationic polypeptides smaller than 4 kDa. DEFA and DEFB possess six conserved cysteine residues that are linked in a 1-6, 2-4 and 3-5 pattern for DEFA and in a 1-5, 2-4 and 3-5 pattern for DEFB. Structural studies showed that the tertiary configuration of both DEFA and DEFB consist of triple-stranded β-sheet structures that are stabilized by three intramolecular disulfide bonds. DEFA and DEFB are stored within proteinaceous granules and are secreted through molecular mechanisms that still remain to be elucidated.

Of at least six human DEFA that are expressed within the gut mucosa, DEFA1-4, also referred to as Human Neutrophil Peptide 1 to 4, are predominantly secreted from azurophilic granules of polymorphonuclear leukocytes, while DEFA5 and DEFA6 are primarily contained in the apically-oriented secretory granules of Paneth cells.

The latter is one of the four major epithelial cell lineages that reside at the base of the crypt of Lieberkühn in the small intestine. In addition to DEFA, only four human β-defensins (DEFB1 to DEFB4) have been studied in the past few decades. DEFB1 to DEFB4 are primarily expressed by epithelia of diverse gastrointestinal tissues, including stomach, small intestine and colon[3].

Biologically-active defensins are released upon the proteolytic processing of their proforms by certain enzymes[4], including trypsin for DEFA5 and DEFA6 in humans[5] and matrilysin for cryptdins in mice[6]. This suggests that appropriate control of homeostatic quantities of both defensins and defensin-activating proteases may ultimately dictate the outcome of the gut immunological response to intruding pathogens and to commensal microorganisms that are permanently present. Our understanding of the regulatory mechanisms that maintain appropriate expression of these antimicrobial factors, which are discharged within the intestinal lumen, is at an early stage. However, recent experimental and clinical findings, which are discussed hereafter, provide us with preliminary answers on how failure to maintain optimal defensin function may lead to dysbiosis and to the development of chronic inflammatory lesions.

THE ANTIMICROBIAL ACTIVITY OF DEFENSINS

The membrane integrity of a broad spectrum of microorganisms, including enveloped viruses, protozoa, bacteria and some fungi, is sensitive to the amphiphilic nature of defensins (Figure 1). Over the past decade, several models have been proposed to explain how defensin may induce non-oxidative killing of microorganisms. Notably, the Shai-Matsuzaki-Huang model provides a reasonable structure-function explanation for the antimicrobial character of defensins[7], whereby the cationic property of these antimicrobial effectors may disrupt the phospholipid bilayer as a detergent. Defensins may promote the formation of micelles by electrostatic forces to negatively-charged components of the microbial membrane, including lipopolysaccharide from Gram negative bacteria and lipoteichoic acids from Gram positive bacteria. Conversely, the nosocomial Gram positive bacteria *Staphylococcus epidermidis* and *Staphylococcus aureus* are thought to repulse the killing activity of defensins by expressing a membrane-bound molecule with a high density of negative charge[8] and by modifying its lipid membrane through Mprf[9], respectively. Similarly, both the two-component system PhoP/PhoQ and lipopolysaccharide are involved in resistance to defensins in the facultative intracellular bacterium *Salmonella typhimurium* (*S. typhimurium*)[10], providing a mechanism whereby certain pathogens may circumvent innate immune mechanisms. Additional investigations are now eagerly awaited to determine whether luminal secretion of certain defensins may influence immune homeostasis in any part of the gastrointestinal tract.

Genetically engineered mice that express the human DEFA5 showed oral resistance to salmonellosis[11]. Conversely, matrilysin deficiency is linked to enhanced susceptibility to oral infection by *S. typhimurium*[6]. Similarly, genetic ablation of the Crohn's disease (CD) predisposing *NOD2* gene leads to reduced expression of certain cryptdins and to enhanced susceptibility to orogastric *Listeria monocytogenes* infection[12]. More importantly, molecular analyses point to profound changes in the composition of the gut microbiome from *DEFA5*-transgenic mice[13]. As a consequence, an abnormal T cell homeostasis, including fewer interleukin 17 (IL-17)-producing *lamina propria* T cells, and an overt resistance to colonization of the ileum by segmented filamentous bacteria were observed in *DEFA5*-transgenic mice[13]. The gastrointestinal tract, in a large proportion of the human population, is colonized by Gram positive anaerobic segmented filamentous bacteria that are thought to regulate both Th17 differentiation[14,15] and secretory immunoglobulin A production[16,17]. Besides DEFA5, a physiological role for mouse β-defensin 1 (mDefB1) in host defence has been primarily unveiled in the pulmonary and urinary tract. Genetic ablation of mDefB1 expression failed to impede the outcome of lung infection by *Haemophilus influenzae*[18], but also resulted in enhanced bacterial burden of *Staphylococcus* in the bladder when compared to controls[19]. However, even if certain pathogenic microorganisms inhibit the secretion and/or killing activity of defensins[20], the latter still provide partial protection against infection, suggesting another prophylactic role for these key elicitors of mammalian immunity.

THE IMMUNOREGULATORY FACET OF DEFENSINS

In essence, defensins are regarded as "natural antibiotics", but accumulating immunological investigations revealed that these long-held antimicrobial peptides may also educate the gut immune system through multiple mechanisms (Figure 1). Indeed, it has been known for some time that DEFA1 is not only involved in the attraction of monocytes to inflammatory sites[21], but also of T cells and immature dendritic cells[22]. Recent immunological investigations have now revealed that this phenomenon is not restricted to α-defensins. Notably, β-defensins were chemotactic for T cells[23], dendritic cells[23], monocytes[24,25] and mast cells[26]. Interestingly, the presence of a disulfide bridge was required for DEFB2 to recruit immunocytes through the C-C chemokine receptor 6[27]. Besides their direct chemoattracting function, certain defensins may also contribute to the recruitment of immune cells by eliciting the expression of co-stimulatory molecules and secretion of a subset of cytokines and chemokines by a myriad of cell types through receptor-dependent mechanisms[28]. Notably, DEFB2 and DEFB3 have been characterized as signalling ligands *per se* for some membrane-bound pathogen-recognition receptors, including the toll-like receptors 4 and 2, respectively[29,30]. The expression of the co-stimulatory molecules CD80, CD86 and CD40 is consistently enhanced after exposure of monocytes and dendritic cells to recombinant DEFB3 in a MyD88-dependent manner[29]. Likewise, treatment of immature

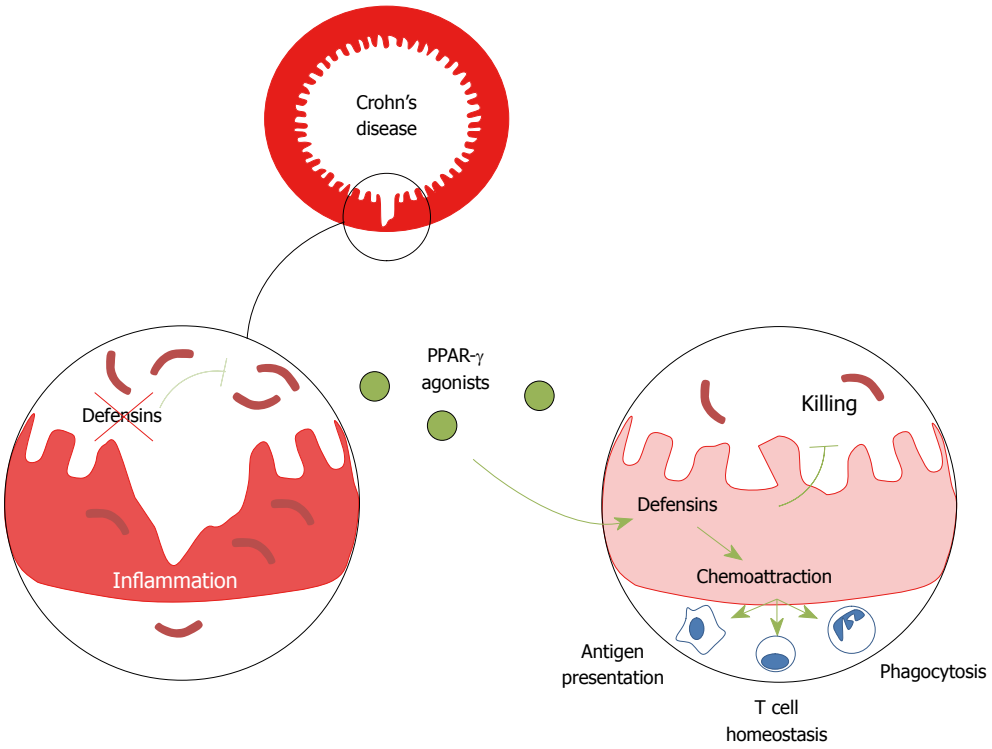


Figure 1 Model of defensin-mediated control of luminal microbiota and maintenance of immune homeostasis in the gut. PPAR: Peroxisome proliferator-activated receptor.

dendritic cells with exogenous recombinant DEFA1 and DEFB1 enhanced the expression of CD80, CD86, CD40, the maturation marker CD83, and HLA-DR[31]. Moreover, both neutrophil-derived defensins and DEFB2 have been involved as positive regulators of neovasculogenesis and wound healing[32-34], but our understanding of the underlying mechanisms still remains incomplete.

TOWARDS A CAUSAL LINK BETWEEN DEFENSINS AND CROHN'S DISEASE DEVELOPMENT

In recent years, defensins have been observed to be negative regulators of both infectious diseases and of chronic inflammatory diseases, including CD. As many as 4 million adults are affected by CD in Europe and North America, and there is no cure for this relapsing-remitting granulomatous illness. CD is traditionally characterized by the development of transmural inflammatory lesions that may affect any part of the bowel. The ileal and colonic mucosa of patients with CD show impaired antimicrobial activity against major components of the microbiota, which is not found in biopsies of healthy subjects or patients with ulcerative colitis, another inflammatory bowel disease[35,36]. In a clinical study by Wehkamp and collaborators, decreased expression of both DEFA5 and DEFA6 was linked to the development of CD-associated lesions in the small intestine[35,37]. More recently, decreased expression of DEFB1 was observed in CD patients with colonic involvement[38]. While the transcript level of the neutrophil chemoattractant IL-8 is a surrogate marker of inflammation, it failed to correlate with *DEFA5* and *DEFB1* transcript levels in Crohn's ileitis and colitis, respectively[35,38]. Similarly, levels of DEFA5 and DEFB1 were not modulated by most current therapies for CD[39]. Collectively, these findings reinforce the notion that decreased expression of defensins may result in excessive inflammation which is the basis of CD. Furthermore, consistent with the chemoattracting function of defensins, trauma to intestinal or cutaneous epithelia in CD patients is also associated with impaired neutrophil attraction when compared to controls[40]. Decreased expression of defensins in CD may account for defective local microbial killing and for reduced attraction of immunocytes to mucosal breaches, but what causes the impaired expression of defensins?

To determine the mechanisms underlying the defect in defensins in ileal and colonic CD, the promoter of both DEFA5 and DEFB1 were screened for potential disease-associated variants. Genetic errors in a regulatory region downstream of the human *DEFA5* gene were found to predispose to ileal CD development[41]. In the ileum, both NOD2 and the Wnt-signalling pathway transcription factor Tcf7l2 were characterized as positive regulatory molecules of DEFA5 expression in human and of certain cryptdins in mice[12,35,42]. Notably, patients carrying the 1007fs NOD2 mutation and those with lowered expression of Tcf7l2 showed a significantly decreased transcript level of DEFA5[43]. We recently showed in the colon, that engagement of the peroxisome proliferator-activated receptor peroxisome proliferator-activated receptor (PPAR)-γ with rosiglitazone triggered epithelial DEFB1 expression *in vitro* and *in vivo*[38]. Dysregulated PPAR-γ production consistently results in reduced antimicrobial activity of the

mucosa against major components of the microbiota in mice[38]. Furthermore, the single nucleotide polymorphism rs1800972, which is located within the promoter region of the human *DEFB1* gene, was also found to be protective towards colonic CD development[38,44] and to positively regulate the expression of DEFB1[45].

CONCLUSION

At present, the therapeutic management of CD is far from optimal. Approximately 25% of patients fail to respond to current biologics and/or to immunosuppressive drugs. Given the essential role of defensins in maintaining gut homeostasis, defensin deficiency requires to be corrected in CD. Recent experimental investigations provided preliminary answers to this problem by identifying the PPAR-γ agonist rosiglitazone as a potent inducer of a subset of β-defensin in mouse colon[38], opening the way for the potential correction of gut barrier function in CD. The development of a mucosal-delivery system for PPAR-γ agonists is now eagerly awaited to improve their therapeutic efficacy, while avoiding potential systemic side effects. Finally, it is worth noting the regulatory role of additional nuclear receptors, including vitamin D and PPAR-β, in regulating the expression of other antimicrobial peptides, including certain defensins[46-49]. The potential synergistic effects of these regulatory factors on the expression of intestinal defensins remains to be addressed. Drugs and diets which can modulate the expression of defensins may thereby protect against colitis and colitis-associated cancer by maintaining sufficient levels of these versatile antimicrobial peptides.

REFERENCES

1 **Dorin JR**, Jackson IJ. Genetics. Beta-defensin repertoire expands. *Science* 2007; **318**: 1395
2 **Nusbaum C**, Mikkelsen TS, Zody MC, Asakawa S, Taudien S, Garber M, Kodira CD, Schueler MG, Shimizu A, Whittaker CA, Chang JL, Cuomo CA, Dewar K, FitzGerald MG, Yang X, Allen NR, Anderson S, Asakawa T, Blechschmidt K, Bloom T, Borowsky ML, Butler J, Cook A, Corum B, DeArellano K, DeCaprio D, Dooley KT, Dorris L 3rd, Engels R, Glöckner G, Hafez N, Hagopian DS, Hall JL, Ishikawa SK, Jaffe DB, Kamat A, Kudoh J, Lehmann R, Lokitsang T, Macdonald P, Major JE, Matthews CD, Mauceli E, Menzel U, Mihalev AH, Minoshima S, Murayama Y, Naylor JW, Nicol R, Nguyen C, O'Leary SB, O'Neill K, Parker SC, Polley A, Raymond CK, Reichwald K, Rodriguez J, Sasaki T, Schilhabel M, Siddiqui R, Smith CL, Sneddon TP, Talamas JA, Tenzin P, Topham K, Venkataraman V, Wen G, Yamazaki S, Young SK, Zeng Q, Zimmer AR, Rosenthal A, Birren BW, Platzer M, Shimizu N, Lander ES. DNA sequence and analysis of human chromosome 8. *Nature* 2006; **439**: 331-335
3 **Cunliffe RN**, Mahida YR. Expression and regulation of antimicrobial peptides in the gastrointestinal tract. *J Leukoc Biol* 2004; **75**: 49-58
4 **Valore EV**, Martin E, Harwig SS, Ganz T. Intramolecular inhibition of human defensin HNP-1 by its propiece. *J Clin Invest* 1996; **97**: 1624-1629
5 **Ghosh D**, Porter E, Shen B, Lee SK, Wilk D, Drazba J, Yadav SP, Crabb JW, Ganz T, Bevins CL. Paneth cell trypsin is the processing enzyme for human defensin-5. *Nat Immunol* 2002; **3**: 583-590
6 **Wilson CL**, Ouellette AJ, Satchell DP, Ayabe T, López-Boado YS, Stratman JL, Hultgren SJ, Matrisian LM, Parks WC. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. *Science* 1999; **286**: 113-117
7 **Shai Y**. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides. *Biochim Biophys Acta* 1999; **1462**: 55-70
8 **Li M**, Lai Y, Villaruz AE, Cha DJ, Sturdevant DE, Otto M. Gram-positive three-component antimicrobial peptide-sensing system. *Proc Natl Acad Sci USA* 2007; **104**: 9469-9474
9 **Peschel A**, Jack RW, Otto M, Collins LV, Staubitz P, Nicholson G, Kalbacher H, Nieuwenhuizen WF, Jung G, Tarkowski A, van Kessel KP, van Strijp JA. Staphylococcus aureus resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with l-lysine. *J Exp Med* 2001; **193**: 1067-1076
10 **Groisman EA**, Parra-Lopez C, Salcedo M, Lipps CJ, Heffron F. Resistance to host antimicrobial peptides is necessary for Salmonella virulence. *Proc Natl Acad Sci USA* 1992; **89**: 11939-11943
11 **Salzman NH**, Ghosh D, Huttner KM, Paterson Y, Bevins CL. Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. *Nature* 2003; **422**: 522-526
12 **Kobayashi KS**, Chamaillard M, Ogura Y, Henegariu O, Inohara N, Nuñez G, Flavell RA. Nod2-dependent regulation of innate and adaptive immunity in the intestinal tract. *Science* 2005; **307**: 731-734
13 **Salzman NH**, Hung K, Haribhai D, Chu H, Karlsson-Sjöberg J, Amir E, Teggatz P, Barman M, Hayward M, Eastwood D, Stoel M, Zhou Y, Sodergren E, Weinstock GM, Bevins CL, Williams CB, Bos NA. Enteric defensins are essential regulators of intestinal microbial ecology. *Nat Immunol* 2010; **11**: 76-83
14 **Ivanov II**, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009; **139**: 485-498
15 **Gaboriau-Routhiau V**, Rakotobe S, Lécuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, Eberl G, Snel J, Kelly D, Cerf-Bensussan N. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 2009; **31**: 677-689
16 **Talham GL**, Jiang HQ, Bos NA, Cebra JJ. Segmented filamentous bacteria are potent stimuli of a physiologically normal state of the murine gut mucosal immune system. *Infect Immun* 1999; **67**: 1992-2000
17 **Umesaki Y**, Setoyama H, Matsumoto S, Imaoka A, Itoh K. Differential roles of segmented filamentous bacteria and clostridia in development of the intestinal immune system. *Infect Immun* 1999; **67**: 3504-3511
18 **Moser C**, Weiner DJ, Lysenko E, Bals R, Weiser JN, Wilson JM. beta-Defensin 1 contributes to pulmonary innate immunity in mice. *Infect Immun* 2002; **70:** 3068-3072
19 **Morrison G**, Kilanowski F, Davidson D, Dorin J. Characterization of the mouse beta defensin 1, Defb1, mutant mouse model. *Infect Immun* 2002; **70**: 3053-3060
20 **Peyrin-Biroulet L**, Vignal C, Dessein R, Simonet M, Desreumaux P, Chamaillard M. NODs in defence: from vulnerable antimicrobial peptides to chronic inflammation. *Trends Microbiol* 2006; **14**: 432-438
21 **Territo MC**, Ganz T, Selsted ME, Lehrer R. Monocyte-chemotactic activity of defensins from human neutrophils. *J Clin Invest* 1989; **84**: 2017-2020
22 **Yang D**, Chen Q, Chertov O, Oppenheim JJ. Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells. *J Leukoc Biol* 2000; **68**: 9-14

23 **Yang D**, Chertov O, Bykovskaia SN, Chen Q, Buffo MJ, Shogan J, Anderson M, Schröder JM, Wang JM, Howard OM, Oppenheim JJ. Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science* 1999; **286**: 525-528

24 **García JR**, Krause A, Schulz S, Rodríguez-Jiménez FJ, Klüver E, Adermann K, Forssmann U, Frimpong-Boateng A, Bals R, Forssmann WG. Human beta-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity. *FASEB J* 2001; **15**: 1819-1821

25 **García JR**, Jaumann F, Schulz S, Krause A, Rodríguez-Jiménez J, Forssmann U, Adermann K, Klüver E, Vogelmeier C, Becker D, Hedrich R, Forssmann WG, Bals R. Identification of a novel, multifunctional beta-defensin (human beta-defensin 3) with specific antimicrobial activity. Its interaction with plasma membranes of Xenopus oocytes and the induction of macrophage chemoattraction. *Cell Tissue Res* 2001; **306**: 257-264

26 **Niyonsaba F**, Iwabuchi K, Matsuda H, Ogawa H, Nagaoka I. Epithelial cell-derived human beta-defensin-2 acts as a chemotaxin for mast cells through a pertussis toxin-sensitive and phospholipase C-dependent pathway. *Int Immunol* 2002; **14**: 421-426

27 **Wu Z**, Hoover DM, Yang D, Boulègue C, Santamaria F, Oppenheim JJ, Lubkowski J, Lu W. Engineering disulfide bridges to dissect antimicrobial and chemotactic activities of human beta-defensin 3. *Proc Natl Acad Sci USA* 2003; **100**: 8880-8885

28 **Lillard JW Jr**, Boyaka PN, Chertov O, Oppenheim JJ, McGhee JR. Mechanisms for induction of acquired host immunity by neutrophil peptide defensins. *Proc Natl Acad Sci USA* 1999; **96**: 651-656

29 **Funderburg N**, Lederman MM, Feng Z, Drage MG, Jadlowsky J, Harding CV, Weinberg A, Sieg SF. Human -defensin-3 activates professional antigen-presenting cells via Toll-like receptors 1 and 2. *Proc Natl Acad Sci USA* 2007; **104**: 18631-18635

30 **Biragyn A**, Ruffini PA, Leifer CA, Klyushnenkova E, Shakhov A, Chertov O, Shirakawa AK, Farber JM, Segal DM, Oppenheim JJ, Kwak LW. Toll-like receptor 4-dependent activation of dendritic cells by beta-defensin 2. *Science* 2002; **298**: 1025-1029

31 **Presicce P**, Giannelli S, Taddeo A, Villa ML, Della Bella S. Human defensins activate monocyte-derived dendritic cells, promote the production of proinflammatory cytokines, and up-regulate the surface expression of CD91. *J Leukoc Biol* 2009; **86**: 941-948

32 **Chavakis T**, Cines DB, Rhee JS, Liang OD, Schubert U, Hammes HP, Higazi AA, Nawroth PP, Preissner KT, Bdeir K. Regulation of neovascularization by human neutrophil peptides (alpha-defensins): a link between inflammation and angiogenesis. *FASEB J* 2004; **18**: 1306-1308

33 **Aarbiou J**, Verhoosel RM, Van Wetering S, De Boer WI, Van Krieken JH, Litvinov SV, Rabe KF, Hiemstra PS. Neutrophil defensins enhance lung epithelial wound closure and mucin gene expression in vitro. *Am J Respir Cell Mol Biol* 2004; **30**: 193-201

34 **Baroni A**, Donnarumma G, Paoletti I, Longanesi-Cattani I, Bifulco K, Tufano MA, Carriero MV. Antimicrobial human beta-defensin-2 stimulates migration, proliferation and tube formation of human umbilical vein endothelial cells. *Peptides* 2009; **30**: 267-272

35 **Wehkamp J**, Salzman NH, Porter E, Nuding S, Weichenthal M, Petras RE, Shen B, Schaeffeler E, Schwab M, Linzmeier R, Feathers RW, Chu H, Lima H Jr, Fellermann K, Ganz T, Stange EF, Bevins CL. Reduced Paneth cell alpha-defensins in ileal Crohn's disease. *Proc Natl Acad Sci USA* 2005; **102**: 18129-18134

36 **Nuding S**, Fellermann K, Wehkamp J, Stange EF. Reduced mucosal antimicrobial activity in Crohn's disease of the colon. *Gut* 2007; **56**: 1240-1247

37 **Elphick D**, Liddell S, Mahida YR. Impaired luminal processing of human defensin-5 in Crohn's disease: persistence in a complex with chymotrypsinogen and trypsin. *Am J Pathol* 2008; **172**: 702-713

38 **Peyrin-Biroulet L**, Beisner J, Wang G, Nuding S, Oommen ST, Kelly D, Parmentier-Decrucq E, Dessein R, Merour E, Chavatte P, Grandjean T, Bressenot A, Desreumaux P, Colombel JF, Desvergne B, Stange EF, Wehkamp J, Chamaillard M. Peroxisome proliferator-activated receptor gamma activation is required for maintenance of innate antimicrobial immunity in the colon. *Proc Natl Acad Sci USA* 2010; **107**: 8772-8777

39 **Kübler I**, Koslowski MJ, Gersemann M, Fellermann K, Beisner J, Becker S, Rothfuss K, Herrlinger KR, Stange EF, Wehkamp J. Influence of standard treatment on ileal and colonic antimicrobial defensin expression in active Crohn's disease. *Aliment Pharmacol Ther* 2009; **30**: 621-633

40 **Smith AM**, Rahman FZ, Hayee B, Graham SJ, Marks DJ, Sewell GW, Palmer CD, Wilde J, Foxwell BM, Gloger IS, Sweeting T, Marsh M, Walker AP, Bloom SL, Segal AW. Disordered macrophage cytokine secretion underlies impaired acute inflammation and bacterial clearance in Crohn's disease. *J Exp Med* 2009; **206**: 1883-1897

41 **Koslowski MJ**, Kübler I, Chamaillard M, Schaeffeler E, Reinisch W, Wang G, Beisner J, Teml A, Peyrin-Biroulet L, Winter S, Herrlinger KR, Rutgeerts P, Vermeire S, Cooney R, Fellermann K, Jewell D, Bevins CL, Schwab M, Stange EF, Wehkamp J. Genetic variants of Wnt transcription factor TCF-4 (TCF7L2) putative promoter region are associated with small intestinal Crohn's disease. *PLoS One* 2009; **4**: e4496

42 **Wehkamp J**, Wang G, Kübler I, Nuding S, Gregorieff A, Schnabel A, Kays RJ, Fellermann K, Burk O, Schwab M, Clevers H, Bevins CL, Stange EF. The Paneth cell alpha-defensin deficiency of ileal Crohn's disease is linked to Wnt/Tcf-4. *J Immunol* 2007; **179**: 3109-3118

43 **Bevins CL**, Stange EF, Wehkamp J. Decreased Paneth cell defensin expression in ileal Crohn's disease is independent of inflammation, but linked to the NOD2 1007fs genotype. *Gut* 2009; **58**: 882-883; discussion 883-884

44 **KKocsis AK**, Lakatos PL, Somogyvári F, Fuszek P, Papp J, Fischer S, Szamosi T, Lakatos L, Kovacs A, Hofner P, Mándi Y. Association of beta-defensin 1 single nucleotide polymorphisms with Crohn's disease. *Scand J Gastroenterol* 2008; **43**: 299-307

45 **Kalus AA**, Fredericks LP, Hacker BM, Dommisch H, Presland RB, Kimball JR, Dale BA. Association of a genetic polymorphism (-44 C/G SNP) in the human DEFB1 gene with expression and inducibility of multiple beta-defensins in gingival keratinocytes. *BMC Oral Health* 2009; **9**: 21

46 **Varnat F**, Heggeler BB, Grisel P, Boucard N, Corthésy-Theulaz I, Wahli W, Desvergne B. PPARbeta/delta regulates paneth cell differentiation via controlling the hedgehog signaling pathway. *Gastroenterology* 2006; **131**: 538-553

47 **Liu PT**, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M, Bals R, Steinmeyer A, Zügel U, Gallo RL, Eisenberg D, Hewison M, Hollis BW, Adams JS, Bloom BR, Modlin RL. Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006; **311**: 1770-1773

48 **Schauber J**, Dorschner RA, Coda AB, Büchau AS, Liu PT, Kiken D, Helfrich YR, Kang S, Elalieh HZ, Steinmeyer A, Zügel U, Bikle DD, Modlin RL, Gallo RL. Injury enhances TLR2 function and antimicrobial peptide expression through a vitamin D-dependent mechanism. *J Clin Invest* 2007; **117**: 803-811

49 **Wang TT**, Nestel FP, Bourdeau V, Nagai Y, Wang Q, Liao J, Tavera-Mendoza L, Lin R, Hanrahan JW, Mader S, White JH. Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. *J Immunol* 2004; **173**: 2909-2912

S- Editor Tian L **L- Editor** Webster JR **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.572

World J Gastroenterol 2011 February 7; 17(5): 572-577
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

Silvio Danese, MD, PhD, Head, *Series Editor*

Extraluminal factors contributing to inflammatory bowel disease

Arvind Batra, Thorsten Stroh, Britta Siegmund

Arvind Batra, Thorsten Stroh, Britta Siegmund, Charité Universitätsmedizin Berlin, Campus Benjamin Franklin, Medical Department I (Gastroenterology/Rheumatology/Infectious Diseases), Hindenburgdamm 30, 12200 Berlin, Germany
Author contributions: Batra A, Stroh T and Siegmund B contributed equally to this work.
Supported by SFB 633 of the Deutsche Forschungsgemeinschaft
Correspondence to: Britta Siegmund, MD, Charité Universitätsmedizin Berlin, Campus Benjamin Franklin, Medical Department I (Gastroenterology/Rheumatology/Infectious Diseases), Hindenburgdamm 30, 12200 Berlin, Germany. britta.siegmund@charite.de
Telephone: +49-30-84454039 Fax: +49-30-84454017
Received: February 3, 2010 Revised: April 7, 2010
Accepted: April 14, 2010
Published online: February 7, 2011

Abstract

Many identified and yet unknown factors contribute to the pathogenesis of inflammatory bowel disease (IBD). The genome-wide association studies clearly support the earlier developed concept that IBD occurs in genetically predisposed individuals who are exposed to distinct environmental factors, which together result in dysregulation of the mucosal immune system. Thus, the majority of previous studies have focused on the immune response within the intestinal wall. The present review aims to emphasize the contribution of three extraluminal structures to this inflammatory process, namely the mesenteric fat tissue, the lymphatics and the microvasculature. Broadening our view across the intestinal wall will not only facilitate our understanding of the disease, but will also us to identify future therapeutic targets.

Key words: Inflammatory bowel disease; Extraluminal structures; Mesenteric fat tissue; Lymphatics; Microvasculature

Peer reviewer: Mitsunori Yamakawa, Professor, Department of Pathological Diagnostics, Yamagata University, Faculty of Medicine, 2-2-2 Iida-Nishi, Yamagata 990-9585, Japan

Batra A, Stroh T, Siegmund B. Extraluminal factors contributing to inflammatory bowel disease. *World J Gastroenterol* 2011; 17(5): 572-577 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/572.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.572

INTRODUCTION

The genome-wide association studies in recent years have contributed significantly to the understanding of the pathogenesis of inflammatory bowel disease (IBD)[1]. The results obtained from these studies have not only confirmed the relevance of earlier characterized pathways, but equally have opened novel avenues. One possible hypothesis for the etiology of IBD is that the mucosal immune system is hyper-responsive to luminal antigens (e.g. dietary factors, commensal bacteria) in genetically predisposed individuals[2]. This hypothesis is limited to the intestinal lumen and wall. Focusing on Crohn's disease, the inflammation is not restricted to the luminal side of the intestinal wall. Rather, transmural inflammation presents as the dominant phenotype, which leads to the question of whether extraintestinal/extraluminal structures contribute to the inflammatory process. In the present overview, three extraluminal structures are discussed, which have been demonstrated to play a role in the regulation of intestinal inflammation, namely the mesenteric fat tissue, microvasculature and lymphatics (Figure 1).

MESENTERIC FAT TISSUE

Historic view

Crohn BB himself provided the first evidence that the mesenteric fat tissue might play a role in the pathogen-

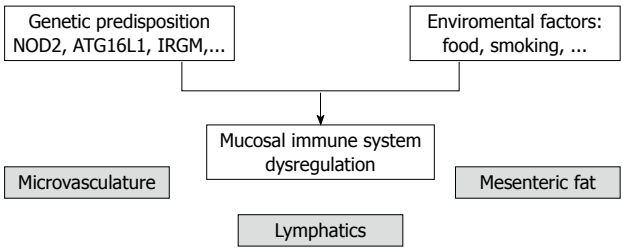


Figure 1 Extraluminal structures contributing to Crohn's disease. The figure illustrates the potential contribution of the extraluminal structures of mesenteric fat tissue, lymphatics and microvasculature to the dysregulation of the mucosal immune system.

esis of Crohn's disease, by describing local hypertrophy of the mesenteric fat adjacent to inflamed intestinal segments[3]. This phenomenon, which is also called "creeping fat" or "fat wrapping" is restricted to Crohn's disease, and is not observed in ulcerative colitis or other forms of chronic intestinal inflammation.

Anatomical view

The characteristic "fat wrapping" seen only in Crohn's disease represents fat hypertrophy that results in partial cover of the intestinal circumference, which is defined as > 50% coverage of the intestinal surface by adipose tissue and occurs in both the large and small bowel. The localization of this "creeping fat" correlates with transmural inflammation, ulceration, and stricture formation[3]. These observational results have been underlined by magnetic resonance imaging that quantified the amount of intra-abdominal fat in relation to total body fat, which indicates that the intra-abdominal fat but not total body fat increases[4]. Adipocytes within this hypertrophied fat are significantly smaller, but a fourfold increase in the total number of adipocytes is present in the mesentery of Crohn's disease patients as compared to controls[5]. How can we explain this observation and what might be the possible contribution to disease?

Adipocytes and chronic inflammation

Each lymph node in our body is in close proximity to adipose tissue. Once the lymph nodes are activated, the number of adipocytes increases, which allows for the supply of sufficient energy for a functional immune system[6-8]. However, is the role of the mesenteric fat tissue restricted to energy supply? In the first studies to analyze the expression of pro-inflammatory mediators in fat tissue, an increase of tumor necrosis factor (TNF)-α and the adipokine leptin was demonstrated in the fat tissue of Crohn's disease patients, in comparison to non-inflammatory controls[4]. In addition, adipocytes express C-reactive protein, and there is a significant correlation between serum C-reactive protein levels and increased mesenteric fat density in Crohn's disease[9]. What is the relevance of these mediators released by the adipose tissue?

Adipokines

Various adipokines are released by adipose tissue. The relevance of adipokines in IBD has been summarized recently in broad detail[10]. The adipokines characterized best with regard to intestinal inflammation are leptin and adiponectin, respectively.

Leptin is a 16-kDa peptide predominantly produced by adipocytes, which signals the status of satiety to the hypothalamus[11]. Leptin deficiency or non-function of the long isoform of the leptin receptor (OB-Rb) is associated with massive obesity in mice and humans. From a structural point of view, leptin can be classified as a helical cytokine[12]. Thus, the structure of leptin suggests a regulatory function within the immune system. In humans, leptin deficiency is rare, but results in impaired T-cell proliferation and is associated with increased mortality in childhood due to infection[13,14]. In mice, leptin deficiency has been associated with protection from dextran sodium sulfate (DSS)-, oxazolone- and trinitrobenzene sulfonic acid (TNBS)-induced colitis. In addition, results from the transfer model of colitis indicate that leptin serves as crucial T-cell stimulator in intestinal inflammation[15-17]. In addition, leptin stimulates the proliferation of naive $CD4^+$ T cells and affects T-cell polarization[15,18,19]. In Crohn's disease, increased expression of leptin mRNA as well as protein in the hypertrophic mesenteric fat has been reported[20,21]. Together with data from animal studies, a pro-inflammatory role for leptin in Crohn's disease has been suggested.

Adiponectin, a 30-kDa polypeptide, contributes 5-10 μg/mL to 0.01% of plasma proteins, and hence is the most abundant adipokine in the circulation[22]. Adiponectin has a high affinity to form trimers that can further multimerize to polymers, which results in various high and low molecular isoforms. The biological significance of the different high and low molecular forms is not finally understood. In Crohn's disease patients, adiponectin mRNA and protein release is upregulated in hypertrophied adipose tissue, as compared to normal adipose tissue from the same subjects, or mesenteric adipose tissue from ulcerative colitis patients and controls[23]. Data concerning the effects on disease severity in experimental models of colitis are conflicting. Whereas one group has observed increased susceptibility to the chemically induced model of DSS colitis[24], another has reported protection against DSS- as well as TNBS-induced colitis in adiponectin-deficient mice[25]. To confuse the issue even more, a third study has reported that adiponectin deficiency does not affect the outcome of disease in interleukin (IL)-10-deficient mice that develop colitis spontaneously[26]. In the model of chronic TNBS-induced colitis in rats, the size of mesenteric adipocytes is decreased, and production of adiponectin, besides other mediators, is increased in perinodal mesenteric fat[27]. As a result of the conflicting effects mediated by adiponectin on immune cells, both pro- and anti-inflammatory consequences of altered adiponectin production in IBD are possible. However, adiponectin does seem to modulate immune responses, and abnormal production could thus be involved in the altered responsiveness of immune cells that occurs in IBD.

Recent data from genetic studies have added independent support for such dysregulated production of adiponectin and leptin in Crohn's disease. In mice, deficiency

of the autophagy gene Atg16l1 results in upregulation of leptin as well as adiponectin mRNA expression[28]. In humans, the ATG16L1 risk allele is associated with an increased risk of developing Crohn's disease[28,29]. Further cross-population studies are needed to ascertain whether this mutation is the cause of the altered leptin and adiponectin production in the hypertrophic fat of Crohn's disease patients. However, so far, it is tempting to speculate that the ATG16L1 risk allele and the subsequent altered production of adipokines might contribute to the predisposition to Crohn's disease.

The data described above indicate that adipokines are able to regulate the acquired immune response and that the production of some is altered in mesenteric fat of IBD patients. What kind of stimulus is required to modify the production of these adipokines in patients with Crohn's disease? Translocation of luminal antigens (e.g. bacteria) from the intestinal lumen to the adipose tissue could offer this stimulus, presuming that adipocytes and pre-adipocytes express innate receptors.

Adipocytes as cells of the innate immune system

The release of free fatty acids by adipocytes following lipopolysaccharide (LPS) stimulation, and hence responsiveness of fat cells to bacterial components, was first detected over 30 years ago[30]. In line with these historic data, the expression of toll-like receptor (TLR)4 and TLR2 was described in adipocytes generated from the 3T3-L1 cell line[31]. Furthermore, our group and others have demonstrated that adipocytes and their precursors from mice and humans express TLR1-TLR11 and that specific stimulation induces secretion of immune regulatory mediators[32-34]. In addition, data from our group indicate expression of functional nucleotide oligomerization domain (NOD) proteins-1 and -2 on pre-adipocytes[35]. Expression of these NOD proteins in adipocytes and pre-adipocytes is further regulated by TNF-α or LPS (NOD2), respectively, IFNγ (NOD1)[35]. This observation is of particular interest, since mutations in the *NOD2* gene have been associated with an increased risk of developing Crohn's disease[36-38]. Thus, adipocytes and pre-adipocytes share functional properties of immune cells, which suggest an active role in defense against bacterial and viral antigens *in vivo*. Hence, adipocytes and pre-adipocytes could represent a yet ignored population of innate immune cells.

A working model could be that primary increased production of pro-inflammatory mediators in the mesenteric fat due to genetic predisposition might contribute to the development of Crohn's disease. Additionally, the massive cytokine production in the inflamed colon, in addition to translocalizing bacteria, could further induce the production of pro-inflammatory mediators in the adjacent adipose tissue, thus inducing a vicious cycle, in which inflammatory conditions in the intestine and the mesenteric fat support each other.

LYMPHATICS

The lymphatic system is closely connected to and within the intestine, and is a neglected structure. In 2008, Van Kruiningen *et al*[39] reminded us of their presence in a concise review. They reviewed the pathological descriptions of Crohn's disease in the era before antibiotic, corticosteroid, immunomodulatory and biological therapy. These pathologists described lesions in the basal portion of the lamina propria, in the superficial and deep submucosa, and in the subserosa, which suggested lymphatic disease. These lesions comprised lymphocytic thrombi within the lymphatics and multiple large aggregates of lymphocytes with or without multi-nucleated giant cells, a picture consistent with chronic lymphangitis[39]. The granulomas of Crohn's disease appear to be in and around the very thin-walled lymphatics that are found adjacent to small vessels[40]. This further supports the idea that lymphatics might directly contribute to the pathogenesis of this disease.

Almost more intriguing are the rat and pig models in which regional lymphatics of the small intestine were obstructed with sclerosing agents[41,42]. These animals subsequently developed segmental intestinal disease that was characterized by many of the alterations that occur in Crohn's disease, including lymphocytic and granulomatous changes. Remarkably, enteroenteric as well as enterocutaneous fistulas developed in these models, which are not seen in animal models routinely used today[41,42]. Additional observations have pointed out that the distribution and character of these lesions represent obstructive lymphocytic lymphangitis[43]. In these older studies, the connection between the shorter segments of Crohn's disease in the jejunum and the longer segments in the ileum, with the shorter vasa recta of the jejunum and the longer lymphatic collecting ducts of the ileum, was emphasized[39,43].

Very recent work by Vetrano *et al*[44] has provided further experimental data underlining the relevance of lymphatics in IBD[44]. They have concentrated on the expression of D6, a promiscuous decoy receptor and scavenger for CC chemokines that plays a non-redundant role in the control of the inflammatory response in various organs. Vetrano *et al*[44] have demonstrated upregulation of D6 in human colitis. The expression could be localized to lymphatic vessels and leukocytes in the mucosa. D6-deficient mice showed an increased susceptibility to experimental colitis when compared to wild-type mice. *Via* bone-marrow chimeras, the regulatory function of D6 in colitis has been tracked to the stromal/lymphatic compartment, and a contribution of hematopoietic cells could be excluded. Thus, these data further emphasize the regulatory role of the lymphatic system in intestinal inflammation.

In line with these observations, Van Kruiningen *et al*[39] have suggested recently to focus again on the lymphatic damage in Crohn's disease, and the identification of possible harmful agents that cause lymphangitis and lesions in the lymphatic endothelium. Although the lymphatics are not completely separated from the intestine, they represent the second structure that should be reevaluated in Crohn's disease.

MICROVASCULATURE

In similar close proximity to the intestinal wall is the

microvasculature that is embedded in the mesenteric fat tissue, which thus provides an additional link as outlined below.

Whether increased vascularization as assessed by mesenteric angiography or Doppler ultrasound reflects Crohn's disease activity is disputed[45]. Recent evidence for angiogenesis playing a role in IBD pathogenesis has prompted interest in anti-angiogenic therapies for IBD[46,47]. Remarkably, angiogenesis plays a crucial role in various chronic inflammatory disorders such as artherosclerosis, rheumatoid arthritis, peptic ulcer, IBD, psoriasis, and Alzheimer's disease[48]. Growth of new blood vessels is intrinsic to inflammation and is associated with structural changes, including activation and proliferation of endothelial cells and capillary and venule remodeling, all of which result in an expansion of the tissue microvascular bed[49-51]. As inflammation evolves, vessels expand to supply nutrients that sustain the accumulation of activated immune cells, and in the chronic phase, local immune cells overproduce endothelial cell growth factors[49].

This expansion of the vascular network facilitates several mechanisms. The influx of inflammatory cells increases the nutrient supply that allows the metabolically active immune response to take place, and the activated endothelium contributes to the local production of cytokines, chemokines, and matrix metalloproteinases[52,53]. In chronic inflammatory disorders, infiltration by macrophages and lymphocytes, tissue damage and repair occur concurrently, and the newly formed vessels become permanent[51,54]. The anatomical expansion and increased activation of the remodeled microvascular bed foster further influx of immune cells, and angiogenesis and inflammation become co-dependent processes[55]. Both innate as well as adaptive immune responses promote angiogenesis.

Thus, the endothelium, and more specifically, the endothelial cells of the microvasculature seem to assume a central function, because they are not only capable of generating a range of mediators, but also display distinct adhesive molecule patterns, to activate a unique sets of genes and form capillaries[56-58]. In addition, endothelial cells act depending on the body compartment heterogeneity[56,59-61]. An example is the expression of mucosal addressin cellular adhesion molecule-1 by Peyer's patches and high endothelial venules to recruit $\alpha 4\beta 7$ homing receptor-positive naïve lymphocytes[62]. Similarly, endothelial cells from brain, liver, and other organs express distinct surface markers, protein transporters, and intracellular enzymes[61-63]. This heterogeneity becomes of particular interest when considering the regulation of organ-specific inflammation; in our case, intestinal inflammation. The distribution and infiltration of leukocytes is tightly regulated by numerous homing and adhesion molecules on the surface of microvascular and immune cells[64]. At inflamed sites, endothelial cells still control the type and number of immune cells that extravasate into the interstitium in a dysregulated fashion[65,66].

An additional cell population has been identified to play a crucial role in the process of cell infiltration *via* the endothelium in areas of inflammation, namely platelets. They normally circulate without attaching to the endothelium, but do so when the endothelial cells become activated, and platelet adherence triggers inflammation[67]. Activated platelets produce massive amounts of pro-inflammatory mediators and interact with various other cell populations[68,69]. In inflamed areas, the microvasculature can recruit leukocytes through a platelet-dependent mechanism, but at the same time, platelet recruitment is leukocyte dependent[70]. The details of this crucial interaction have been summarized by other reviews[71].

A number of animal studies have proven that the process of angiogenesis can be taken advantage of as a therapeutic approach. Hence, in models of DSS-induced inflammation, as well as spontaneous colitis in IL-10-deficient mice, angiogenesis occurs. However, when this angiogenesis is inhibited, clinical severity and the signs of histological inflammation decrease significantly[47]. Furthermore, vascular endothelial growth factor A that induces angiogenesis has been recently shown to be up-regulated in samples from patients with IBD, and in mice with colitis. The overexpression of VEGF-A in mice exposed to DSS was followed by deterioration of disease and an additional increase in angiogenesis when compared to DSS-exposed wild-type mice[72].

PERSPECTIVE

Considering the factors that contribute to intestinal inflammation, particularly in Crohn's disease, we should avoid restricting ourselves to the luminal site and immune-cell infiltration, but rather include the extraluminal structures discussed in this review. This broadened view might help us in understanding the disease, and more importantly, in identifying novel therapeutic targets.

REFERENCES

1 **Mathew CG**. New links to the pathogenesis of Crohn disease provided by genome-wide association scans. *Nat Rev Genet* 2008; **9**: 9-14

2 **Baumgart DC**, Carding SR. Inflammatory bowel disease: cause and immunobiology. *Lancet* 2007; **369**: 1627-1640

3 **Sheehan AL**, Warren BF, Gear MW, Shepherd NA. Fat-wrapping in Crohn's disease: pathological basis and relevance to surgical practice. *Br J Surg* 1992; **79**: 955-958

4 **Desreumaux P**, Ernst O, Geboes K, Gambiez L, Berrebi D, Müller-Alouf H, Hafraoui S, Emilie D, Ectors N, Peuchmaur M, Cortot A, Capron M, Auwerx J, Colombel JF. Inflammatory alterations in mesenteric adipose tissue in Crohn's disease. *Gastroenterology* 1999; **117**: 73-81

5 **Peyrin-Biroulet L**, Chamaillard M, Gonzalez F, Beclin E, Decourcelle C, Antunes L, Gay J, Neut C, Colombel JF, Desreumaux P. Mesenteric fat in Crohn's disease: a pathogenetic hallmark or an innocent bystander? *Gut* 2007; **56**: 577-583

6 **Demas GE**. The energetics of immunity: a neuroendocrine link between energy balance and immune function. *Horm Behav* 2004; **45**: 173-180

7 **Long KZ**, Nanthakumar N. Energetic and nutritional regulation of the adaptive immune response and trade-offs in ecological immunology. *Am J Hum Biol* 2004; **16**: 499-507

8 **Pond CM**. Adipose tissue and the immune system. *Prostaglandins Leukot Essent Fatty Acids* 2005; **73**: 17-30

9 **Colombel JF**, Solem CA, Sandborn WJ, Booya F, Loftus EV Jr, Harmsen WS, Zinsmeister AR, Bodily KD, Fletcher JG.

Quantitative measurement and visual assessment of ileal Crohn's disease activity by computed tomography enterography: correlation with endoscopic severity and C reactive protein. *Gut* 2006; **55**: 1561-1567
10 **Batra A**, Zeitz M, Siegmund B. Adipokine signaling in inflammatory bowel disease. *Inflamm Bowel Dis* 2009; **15**: 1897-1905
11 **Vaisse C**, Halaas JL, Horvath CM, Darnell JE Jr, Stoffel M, Friedman JM. Leptin activation of Stat3 in the hypothalamus of wild-type and ob/ob mice but not db/db mice. *Nat Genet* 1996; **14**: 95-97
12 **Madej T**, Boguski MS, Bryant SH. Threading analysis suggests that the obese gene product may be a helical cytokine. *FEBS Lett* 1995; **373**: 13-18
13 **Farooqi IS**, Matarese G, Lord GM, Keogh JM, Lawrence E, Agwu C, Sanna V, Jebb SA, Perna F, Fontana S, Lechler RI, DePaoli AM, O'Rahilly S. Beneficial effects of leptin on obesity, T cell hyporesponsiveness, and neuroendocrine/metabolic dysfunction of human congenital leptin deficiency. *J Clin Invest* 2002; **110**: 1093-1103
14 **Ozata M**, Ozdemir IC, Licinio J. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. *J Clin Endocrinol Metab* 1999; **84**: 3686-3695
15 **Batra A**, Okur B, Glauben R, Erben U, Ihbe J, Stroh T, Fedke I, Chang HD, Zeitz M, Siegmund B. Leptin: a critical regulator of CD4+ T-cell polarization in vitro and in vivo. *Endocrinology* 2010; **151**: 56-62
16 **Siegmund B**, Lehr HA, Fantuzzi G. Leptin: a pivotal mediator of intestinal inflammation in mice. *Gastroenterology* 2002; **122**: 2011-2025
17 **Siegmund B**, Sennello JA, Jones-Carson J, Gamboni-Robertson F, Lehr HA, Batra A, Fedke I, Zeitz M, Fantuzzi G. Leptin receptor expression on T lymphocytes modulates chronic intestinal inflammation in mice. *Gut* 2004; **53**: 965-972
18 **Lord GM**, Matarese G, Howard JK, Baker RJ, Bloom SR, Lechler RI. Leptin modulates the T-cell immune response and reverses starvation-induced immunosuppression. *Nature* 1998; **394**: 897-901
19 **Lord GM**, Matarese G, Howard JK, Bloom SR, Lechler RI. Leptin inhibits the anti-CD3-driven proliferation of peripheral blood T cells but enhances the production of proinflammatory cytokines. *J Leukoc Biol* 2002; **72**: 330-338
20 **Barbier M**, Vidal H, Desreumaux P, Dubuquoy L, Bourreille A, Colombel JF, Cherbut C, Galmiche JP. Overexpression of leptin mRNA in mesenteric adipose tissue in inflammatory bowel diseases. *Gastroenterol Clin Biol* 2003; **27**: 987-991
21 **Paul G**, Schäffler A, Neumeier M, Fürst A, Bataillle F, Buechler C, Müller-Ladner U, Schölmerich J, Rogler G, Herfarth H. Profiling adipocytokine secretion from creeping fat in Crohn's disease. *Inflamm Bowel Dis* 2006; **12**: 471-477
22 **Arita Y**, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257**: 79-83
23 **Yamamoto K**, Kiyohara T, Murayama Y, Kihara S, Okamoto Y, Funahashi T, Ito T, Nezu R, Tsutsui S, Miyagawa JI, Tamura S, Matsuzawa Y, Shimomura I, Shinomura Y. Production of adiponectin, an anti-inflammatory protein, in mesenteric adipose tissue in Crohn's disease. *Gut* 2005; **54**: 789-796
24 **Nishihara T**, Matsuda M, Araki H, Oshima K, Kihara S, Funahashi T, Shimomura I. Effect of adiponectin on murine colitis induced by dextran sulfate sodium. *Gastroenterology* 2006; **131**: 853-861
25 **Fayad R**, Pini M, Sennello JA, Cabay RJ, Chan L, Xu A, Fantuzzi G. Adiponectin deficiency protects mice from chemically induced colonic inflammation. *Gastroenterology* 2007; **132**: 601-614
26 **Pini M**, Gove ME, Fayad R, Cabay RJ, Fantuzzi G. Adiponectin deficiency does not affect development and progression of spontaneous colitis in IL-10 knockout mice. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G382-G387
27 **Gambero A**, Maróstica M, Abdalla Saad MJ, Pedrazzoli J Jr. Mesenteric adipose tissue alterations resulting from experimental reactivated colitis. *Inflamm Bowel Dis* 2007; **13**: 1357-1364
28 **Cadwell K**, Liu JY, Brown SL, Miyoshi H, Loh J, Lennerz JK, Kishi C, Kc W, Carrero JA, Hunt S, Stone CD, Brunt EM, Xavier RJ, Sleckman BP, Li E, Mizushima N, Stappenbeck TS, Virgin HW 4th. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 2008; **456**: 259-263
29 **Cummings JR**, Cooney R, Pathan S, Anderson CA, Barrett JC, Beckly J, Geremia A, Hancock L, Guo C, Ahmad T, Cardon LR, Jewell DP. Confirmation of the role of ATG16L1 as a Crohn's disease susceptibility gene. *Inflamm Bowel Dis* 2007; **13**: 941-946
30 **Hikawyj-Yevich I**, Spitzer JA. Endotoxin influence on lipolysis in isolated human and primate adipocytes. *J Surg Res* 1977; **23**: 106-113
31 **Lin Y**, Lee H, Berg AH, Lisanti MP, Shapiro L, Scherer PE. The lipopolysaccharide-activated toll-like receptor (TLR)-4 induces synthesis of the closely related receptor TLR-2 in adipocytes. *J Biol Chem* 2000; **275**: 24255-24263
32 **Batra A**, Pietsch J, Fedke I, Glauben R, Okur B, Stroh T, Zeitz M, Siegmund B. Leptin-dependent toll-like receptor expression and responsiveness in preadipocytes and adipocytes. *Am J Pathol* 2007; **170**: 1931-1941
33 **Khazen W**, M'Bika JP, Collinet M, Tramoni M, Chany C, Achour A, Forest C. Differentiation-dependent expression of interferon gamma and toll-like receptor 9 in 3T3-F442A adipocytes. *Biochimie* 2007; **89**: 669-675
34 **Vitseva OI**, Tanriverdi K, Tchkonia TT, Kirkland JL, McDonnell ME, Apovian CM, Freedman J, Gokce N. Inducible Toll-like receptor and NF-kappaB regulatory pathway expression in human adipose tissue. *Obesity* (Silver Spring) 2008; **16**: 932-937
35 **Stroh T**, Batra A, Glauben R, Fedke I, Erben U, Kroesen A, Heimesaat MM, Bereswill S, Girardin S, Zeitz M, Siegmund B. Nucleotide oligomerization domains 1 and 2: regulation of expression and function in preadipocytes. *J Immunol* 2008; **181**: 3620-3627
36 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603
37 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606
38 **Hampe J**, Grebe J, Nikolaus S, Solberg C, Croucher PJ, Mascheretti S, Jahnsen J, Moum B, Klump B, Krawczak M, Mirza MM, Foelsch UR, Vatn M, Schreiber S. Association of NOD2 (CARD 15) genotype with clinical course of Crohn's disease: a cohort study. *Lancet* 2002; **359**: 1661-1665
39 **Van Kruiningen HJ**, Colombel JF. The forgotten role of lymphangitis in Crohn's disease. *Gut* 2008; **57**: 1-4
40 **Mooney EE**, Walker J, Hourihane DO. Relation of granulomas to lymphatic vessels in Crohn's disease. *J Clin Pathol* 1995; **48**: 335-338
41 **Kalima TV**. Experimental lymphatic obstruction in the ileum. *Ann Chir Gynaecol Fenn* 1970; **59**: 187-201

42 **Kalima TV**, Saloniemi H, Rahko T. Experimental regional enteritis in pigs. *Scand J Gastroenterol* 1976; **11**: 353-362
43 **Tonelli P**. Novita per la malattia di Crohn: la soluzione dei suoi misteri dottrinali ed il suo recupero come malattia di competenza chirurgica. Nota 1. Il processo crohniano non e quello di un enterite, ma di un linfedema inquinato del contenuto intestinale. *Chirurgia Italiana* 2000; **52**: 109-121
44 **Vetrano S**, Borroni EM, Sarukhan A, Savino B, Bonecchi R, Correale C, Arena V, Fantini M, Roncalli M, Malesci A, Mantovani A, Locati M, Danese S. The lymphatic system controls intestinal inflammation and inflammation-associated Colon Cancer through the chemokine decoy receptor D6. *Gut* 2010; **59**: 197-206
45 **Maconi G**, Parente F, Bollani S, Imbesi V, Ardizzone S, Russo A, Bianchi Porro G. Factors affecting splanchnic haemodynamics in Crohn's disease: a prospective controlled study using Doppler ultrasound. *Gut* 1998; **43**: 645-650
46 **Danese S**, Sans M, de la Motte C, Graziani C, West G, Phillips MH, Pola R, Rutella S, Willis J, Gasbarrini A, Fiocchi C. Angiogenesis as a novel component of inflammatory bowel disease pathogenesis. *Gastroenterology* 2006; **130**: 2060-2073
47 **Danese S**, Sans M, Spencer DM, Beck I, Doñate F, Plunkett ML, de la Motte C, Redline R, Shaw DE, Levine AD, Mazar AP, Fiocchi C. Angiogenesis blockade as a new therapeutic approach to experimental colitis. *Gut* 2007; **56**: 855-862
48 **Folkman J**. Angiogenesis in cancer, vascular, rheumatoid and other disease. *Nat Med* 1995; **1**: 27-31
49 **Carmeliet P**. Angiogenesis in health and disease. *Nat Med* 2003; **9**: 653-660
50 **Carmeliet P**. Angiogenesis in life, disease and medicine. *Nature* 2005; **438**: 932-936
51 **Majno G**. Chronic inflammation: links with angiogenesis and wound healing. *Am J Pathol* 1998; **153**: 1035-1039
52 **Firestein GS**. Starving the synovium: angiogenesis and inflammation in rheumatoid arthritis. *J Clin Invest* 1999; **103**: 3-4
53 **Szekanecz Z**, Koch AE. Vascular endothelium and immune responses: implications for inflammation and angiogenesis. *Rheum Dis Clin North Am* 2004; **30**: 97-114
54 **Frantz S**, Vincent KA, Feron O, Kelly RA. Innate immunity and angiogenesis. *Circ Res* 2005; **96**: 15-26
55 **Jackson JR**, Seed MP, Kircher CH, Willoughby DA, Winkler JD. The codependence of angiogenesis and chronic inflammation. *FASEB J* 1997; **11**: 457-465
56 **Binion DG**, West GA, Ina K, Ziats NP, Emancipator SN, Fiocchi C. Enhanced leukocyte binding by intestinal microvascular endothelial cells in inflammatory bowel disease. *Gastroenterology* 1997; **112**: 1895-1907
57 **Gerlach E**, Nees S, Becker BF. The vascular endothelium: a survey of some newly evolving biochemical and physiological features. *Basic Res Cardiol* 1985; **80**: 459-474
58 **Lang I**, Pabst MA, Hiden U, Blaschitz A, Dohr G, Hahn T, Desoye G. Heterogeneity of microvascular endothelial cells isolated from human term placenta and macrovascular umbilical vein endothelial cells. *Eur J Cell Biol* 2003; **82**: 163-173
59 **Belloni PN**, Carney DH, Nicolson GL. Organ-derived microvessel endothelial cells exhibit differential responsiveness to thrombin and other growth factors. *Microvasc Res* 1992; **43**: 20-45
60 **Garlanda C**, Dejana E. Heterogeneity of endothelial cells. Specific markers. *Arterioscler Thromb Vasc Biol* 1997; **17**: 1193-1202
61 **Ribatti D**, Nico B, Vacca A, Roncali L, Dammacco F. Endothelial cell heterogeneity and organ specificity. *J Hematother Stem Cell Res* 2002; **11**: 81-90
62 **Berlin C**, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, Weissman IL, Hamann A, Butcher EC. Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. *Cell* 1993; **74**: 185-195
63 **Pauli BU**, Lee CL. Organ preference of metastasis. The role of organ-specifically modulated endothelial cells. *Lab Invest* 1988; **58**: 379-387
64 **Butcher EC**, Picker LJ. Lymphocyte homing and homeostasis. *Science* 1996; **272**: 60-66
65 **Biedermann BC**. Vascular endothelium: checkpoint for inflammation and immunity. *News Physiol Sci* 2001; **16**: 84-88
66 **Granger DN**, Kubes P. The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion. *J Leukoc Biol* 1994; **55**: 662-675
67 **Weyrich AS**, Zimmerman GA. Platelets: signaling cells in the immune continuum. *Trends Immunol* 2004; **25**: 489-495
68 **Selak MA**. Neutrophil-platelet interactions in inflammation. *Receptor* 1994; **4**: 3-7
69 **Weyrich AS**, Prescott SM, Zimmerman GA. Platelets, endothelial cells, inflammatory chemokines, and restenosis: complex signaling in the vascular play book. *Circulation* 2002; **106**: 1433-1435
70 **Kirton CM**, Nash GB. Activated platelets adherent to an intact endothelial cell monolayer bind flowing neutrophils and enable them to transfer to the endothelial surface. *J Lab Clin Med* 2000; **136**: 303-313
71 **Danese S**, Dejana E, Fiocchi C. Immune regulation by microvascular endothelial cells: directing innate and adaptive immunity, coagulation, and inflammation. *J Immunol* 2007; **178**: 6017-6022
72 **Scaldaferri F**, Vetrano S, Sans M, Arena V, Straface G, Stigliano E, Repici A, Sturm A, Malesci A, Panes J, Yla-Herttuala S, Fiocchi C, Danese S. VEGF-A links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. *Gastroenterology* 2009; **136**: 585-595.e5

S- Editor Tian L **L- Editor** Kerr C **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.578

World J Gastroenterol 2011 February 7; 17(5): 578-593
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

Silvio Danese, MD, PhD, Head, *Series Editor*

Role of the endothelium in inflammatory bowel diseases

Walter E Cromer, J Michael Mathis, Daniel N Granger, Ganta V Chaitanya, J Steven Alexander

Walter E Cromer, J Michael Mathis, Department of Cellular Biology and Anatomy, LSU Health Sciences Center-Shreveport, 1501 Kings Highway, Shreveport, LA 71130-3932, United States
Daniel N Granger, Ganta V Chaitanya, J Steven Alexander, Department of Cellular and Molecular Physiology, LSU Health Sciences Center-Shreveport, 1501 Kings Highway, Shreveport, LA 71130-3932, United States
Author contributions: Cromer WE wrote the paper; Mathis JM, Granger DN and Chaitanya GV significantly contributed to the editing of this paper; Alexander JS significantly contributed to the outline, design and editing.
Supported by National Institute of Health, NIH DK 43785
Correspondence to: J Steven Alexander, PhD, Department of Cellular and Molecular Physiology, LSU Health Sciences Center-Shreveport, 1501 Kings Highway, Shreveport, LA 71130-3932, United States. jalexa@lsuhsc.edu
Telephone: +1-318-6754151 Fax: +1-318-6754156
Received: March 8, 2010 Revised: June 29, 2010
Accepted: July 6, 2010
Published online: February 7, 2011

Abstract

Inflammatory bowel diseases (IBD) are a complex group of diseases involving alterations in mucosal immunity and gastrointestinal physiology during both initiation and progressive phases of the disease. At the core of these alterations are endothelial cells, whose continual adjustments in structure and function coordinate vascular supply, immune cell emigration, and regulation of the tissue environment. Expansion of the endothelium in IBD (angiogenesis), mediated by inflammatory growth factors, cytokines and chemokines, is a hallmark of active gut disease and is closely related to disease severity. The endothelium in newly formed or inflamed vessels differs from that in normal vessels in the production of and response to inflammatory cytokines, growth factors, and adhesion molecules, altering coagulant capacity, barrier function and blood cell recruitment in injury. This review examines the roles of the endothelium in the initiation and propagation of IBD pathology and distinctive features of the intestinal endothelium contributing to these conditions.

Key words: Microvasculature; Endothelium; Inflammation; Nitric oxide; Adhesion molecules; Crohn's disease; Ulcerative colitis; Cytokines; Chemokines; Growth factors

Peer reviewers: Tauseef Ali, MD, Assistant Professor, Section of Digestive Diseases and Nutrition, University of Oklahoma Health Sciences Center, 920 SL Young Blvd, Oklahoma City, OK 73104, United States; John B Schofield, MB, BS, MRCP, FRCP, Department of Cellular Pathology, Preston Hall, Maidstone, Kent, ME20 7NH, United Kingdom

Cromer WE, Mathis JM, Granger DN, Chaitanya GV, Alexander JS. Role of the endothelium in inflammatory bowel diseases. *World J Gastroenterol* 2011; 17(5): 578-593 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/578.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.578

INTRODUCTION

Inflammatory bowel diseases (IBD) include Crohn's disease (CD), ulcerative colitis (UC) (and indeterminate colitis), which share several inflammatory characteristics with other chronic immune disturbances including immune activation, leukocyte infiltration into tissues and increased vascular density[1]. In UC, the colon shows a continuous, superficial inflammation, while CD occurs as patchy transmural inflammation which may affect any region of the gastrointestinal tract. Genetic susceptibilities may play an important role in the development of IBD[2-6] with polymorphisms in CARD15/NOD2 haplotypes (especially in Caucasians) and HLA-DR haplotypes (especially in Asian IBD) and possible defects in interleukin (IL)-23, IL-2, and IL-10 signaling[2,7-10]. IBD is more prevalent in developed nations[11], with several mechanisms being considered to explain disease pathology including environment, hygiene

and altered gut flora[11-13]. These different contributing causes may underlie divergent forms and patterns of IBD, which ultimately may lead to a redefinition of different sub forms of UC and CD.

While the mechanisms initiating and sustaining IBD may differ, both UC and CD may reflect dysfunction within antigen-presenting cells (e.g. dendritic cells) or excess activation of $CD4^+$ T-cells (resembling T-cell disturbances in psoriasis). Reduced activation of T-cells in some forms of CD appear to allow gut microbiota that have breached the gut epithelium to trigger microvascular inflammation[1,5,9,14-16]. The activation of immune responses in IBD release inflammatory cytokines [e.g. tumor necrosis factor (TNF)-α] and growth factors [e.g. vascular endothelial growth factor (VEGF)-A] into gut tissues provoking gut inflammation and injury[5,17]. Antibodies produced against "normal" gut antigens (e.g. anti-colon, anti-mucin, anti-tropomyosin) have been found in IBD and are suggested to activate cytotoxic T lymphocytes, further increasing inflammation[7]. As IBD progresses, cytokine-mediated inflammation and epithelial apoptosis disturb the intestinal barrier, to allow penetration of gut flora beyond the lamina propria causing intense inflammatory responses[18] while also provoking endothelial microvascular permeability[19].

Another key event in IBD progression is the expansion of the intestinal microvasculature. Angiogenesis in IBD sustains inflammation through alterations in the endothelial lining of these vessels. The endothelium regulates recruitment of inflammatory cells, tissue damage (e.g. vasogenic edema), and production of inflammatory mediators[19-22]. In this review we describe the key roles of the endothelium in mediating and aggravating inflammation in IBD (Figure 1).

ENDOTHELIAL CELLS IN IBD

Endothelial cells (ECs) are the major constituent of the microvasculature that line blood and lymphatic vessels. ECs during IBD undergo rapid and remarkable changes in response to elevated levels of cytokines and growth factors often producing injury to gut tissues. Normally ECs provide an anti-adhesive and selectively permeable exchange barrier[23]. Even though ECs have long been recognized as participants in inflammation their roles in intestinal inflammation during IBD are not yet clear. The unique physiological and molecular characteristics of gut microvessels may help explain several characteristics of IBD. The close relationships between gut metabolism, tissue perfusion, microvascular expansion and immune cell infiltration are unclear but suggest that microvascular alterations may be maladaptive in IBD. Intestinal vascular ECs basally exhibit unique properties which may contribute to IBD. Haraldsen *et al*[24] first described characteristics of human intestinal ECs (HIMECs) in long-term cultures and differences from ECs of different origin. For example lipopolysaccharide (LPS) only transiently increases HIMEC adhesion molecule expression, while causing long-lasting increases in human umbilical vein ECs (HUVECs)[25]. Nilson *et al*[26] found that HIMEC cultures produce different cytokines (IL-1β, IL-3 and IL-6) upon stimulation with inflammatory cytokines (e.g. TNF-α, IL-1) compared to HUVECs. Binion *et al*[27,28] have shown distinctive HIMEC properties such as constitutive inducible nitric oxide (NO) synthase (iNOS) as well as unique adhesive determinants, and that these properties were altered in IBD and may underlie endothelial dysfunction in IBD development.

ENDOTHELIAL NO IN IBD

Endothelial-derived NO reduces leukocyte and platelet adhesion to the endothelium[29,30], mediates flow-dependent and agonist-dependent vasodilatation, and couples VEGF-A signaling with NO-dependent permeability[31,32]. NO-mediated endothelial permeability involves 2 separate mechanisms: (1) increased guanylate cyclase and phospholipase C activity which increases intracellular Ca^{2+}; and (2) permeability mediated by Erk1/2 *via* Ras/Raf/PKC causing increased actin contractility[29,33,34]. Increased p38 mitogen-activated protein kinase (MAPK) signaling, Rho-GTPase activity and increased Ca^{2+} release mediated by upregulated cytokines and growth factors may also represent possible mechanisms for increased endothelial permeability[35-37].

Endothelial nitric oxide synthase (eNOS)-derived NO is a radical scavenger not only absorbing O_2^- but also generating the potent oxidant $ONOO^-$. eNOS expression is reduced in IBD; eNOS deficiency in IBD is exacerbated by arginase-mediated depletion of substrate as well as eNOS uncoupling[38-40]. Decreased eNOS activity in IBD reduces endothelium-dependent vasodilation, leading to ungoverned oxidant formation, prominent in IBD[41]. Deletion of eNOS ($eNOS^{-/-}$) increases severity of experimental IBD[42,43] consistent with protective roles of NO against inflammation. NO may prevent development of endothelial inflammatory and hyper-adhesive phenotype in IBD by suppressing cytokine-induced EC adhesion molecules (ECAMs) and matrix metalloproteinases (MMPs)[44]. Increased endothelial oxidant stress (e.g. in IBD) also disturbs tight junctional organization *via* p38, p42/44 MAPK[45-47].

Sera from patients with CD reduce, while UC sera increase eNOS in HUVECs; both UC and CD sera increase iNOS[48]. This may reflect differences in anatomic origins of the endothelium i.e. venous *vs* intestinal. HIMEC iNOS expression appears to be a unique feature of gut microvessels. In HIMECs, iNOS appears at least as important a source of NO as eNOS. Binion *et al*[30], have shown that HIMECs persistently express iNOS, and that iNOS-derived NO limits leukocyte adhesion in normal HIMECs. Paradoxically iNOS inhibition increases binding of leukocytes. Thus, while leukocyte-derived iNOS may drive inflammation, HIMEC expression of iNOS limits the inflammatory responses (leukocyte adhesion, permeability, vasodilatation) in the gut, and decreased endothelial iNOS abundance and activity in IBD may represent an

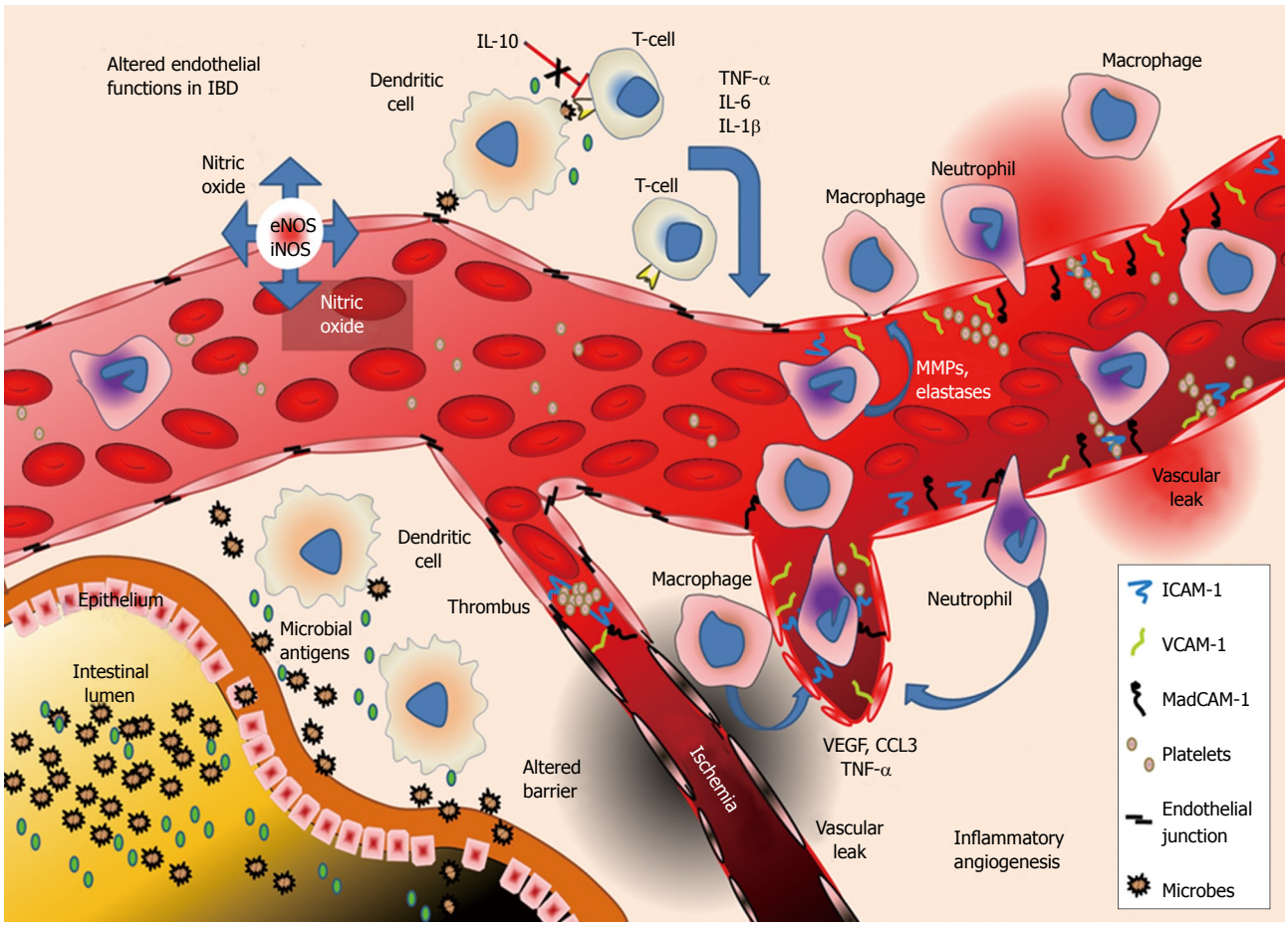


Figure 1 Inflammation triggers a change in the endothelium of the intestinal vasculature in response to the cytokines, chemokines and growth factors released by immune cells leading to increased angiogenesis, adhesion molecule expression, leukocyte extravasation, decreased endothelial barrier function and increased coagulation. TNF: Tumor necrosis factor; IL: Interleukin; iNOS: Inducible nitric oxide synthase; eNOS: Endothelial nitric oxide synthase; VEGF: Vascular endothelial growth factor; MMPs: Matrix metalloproteinases; VCAM: Vascular cell adhesive molecule; ICAM: Intracellular adhesive molecules.

underrated basis of IBD pathology[27,30]. HIMECs derived from CD patients also show a persistent loss of iNOS expression[27]. Interestingly, iNOS can be decreased by injury to normal HIMECs (opposite to most tissues which mobilize iNOS in response to injury[27]) suggesting that during injury, reduced iNOS might trigger inflammatory responses. Even with the loss of endothelial iNOS, there is often increased NO in tissues surrounding the area of inflammation. Despite decreased endothelial iNOS derived-NO, IBD frequently exhibits increased leukocyte recruitment and activation of gut epithelial cells to increase overall NO production[44]. Krieglstein *et al*[49] found that tissue-derived iNOS, and to some extent leukocyte iNOS, mediate colitis injury, but could not specifically distinguish between tissue and endothelial contributions of iNOS in colitis. Aoi *et al*[50] have suggested that iNOS-derived NO plays an important role in gut healing after injury through induction of VEGF, necessary for angiogenesis in wound healing. We have previously shown that excess NO may play an important role in IBD exacerbation. Using STAT-6$^{-/-}$ mice (which have high iNOS levels) in dextran sulfate sodium (DSS) colitis, we found more severe IBD in STAT-6$^{-/-}$ mice correlate with extraordinary NO flux suggesting that excess NO may also drive gut injury[51]. Despite elevated NO abundance, downstream guanylate cyclase signaling appears to be depressed in DSS colitis leading to decreased cGMP in the inflamed intestine[52]. Under these conditions, cGMP dependent protective NO effects may be masked by pro-oxidant effects of NO metabolites. Conner *et al*[53] and Grisham *et al*[54] revealed an important role of the 26S proteasome in the regulation of endothelial nuclear factor-κB (NF-κB) and cumulative iNOS NO production and adhesion molecule expression. Cumulatively, these studies suggest that intestinal homeostasis is controlled by distinctive and compartmentalized NO sources, and that excess NO formation may support the pathophysiology of IBD.

ENDOTHELIAL TOLL-LIKE RECEPTORS AND IBD

The gut is an organ supporting a high bacterial load; despite physical and chemical barriers, some bacterial antigens will ultimately penetrate the gut wall to activate gut microvascular ECs through Toll-like receptor (TLR) signaling[18,55]. The intestinal microvascular endothelium also differs from ECs of other origins in TLR responses. For example, repeated exposure and activation of TLR4 in HIMECs

leads to development of lipopolysaccharide tolerance; however HUVECs lack such a mechanism, indicating the importance in controlling endothelial-dependent inflammation and host commensal interactions[25,56,57]. Protease activated receptors activate transforming growth factor (TGF)-β to induce TLR4 and lead to increased disease severity in IBD[58,59]. TLR5 is constitutively expressed in all ECs, and is of particular interest in gut pathophysiology. TLR5, a receptor for flagellin[60], is constitutively expressed on the basolateral surface of the gut endothelial (an epithelial) layers[61]. TLR5 signaling induces endothelial intercellular adhesion molecule-1, TNF-α production and leukocyte binding and emigration[61]. Loss of TLR5 activity in murine models leads to the development of infectious as a result of deficient and improper responses to normal flora and pathological microorganisms[61,62]. Conversely, endothelial TLR3 has been shown to be protective in the DSS model of acute colitis. This process is mediated by interferon (IFN) type 1 induction of IL-10, a potent anti-inflammatory cytokine[63]. However, Heidemann *et al*[64] in 2007 found that IL-12 expression and its associated gene products were also induced by TLR3 signaling in addition to increased adhesion and transmigration of leukocytes and TLR functions in the gut remain complex, and requires further study.

IBD-ASSOCIATED CYTOKINES AND CHEMOKINES EFFECTS ON GUT ECs

During inflammation there is an increase in plasma levels of inflammatory cytokines, including IL-6, IL-23, IL-12 and TNF-α, in both human IBD and animal IBD models[1,2,15]. Kawachi *et al*[65,66] examined cytokine alterations in the adoptive T-cell transfer and the IL-10$^{-/-}$ IBD models and found IL-1, IL-6, IL-18 and TNF-α were upregulated in both models. Many of the inflammatory cytokines that are upregulated in IBD are pro-angiogenic, the best examples being IL-17 (produced by invasive Th17 cells) and TNF-α produced by several tissue types, including infiltrating immune cells (macrophages and monocytes)[67,68] and the endothelium[69]. EC produce inflammatory mediators in response to activation by immune cells and alterations in the tissue microenvironment[64,70].

TNF-α is one example of a cytokine with pleiotropic effects on the endothelium in IBD, ranging from adhesion molecule induction [vascular cellular adhesion molecule (VCAM)-1 and mucosal addressin cellular adhesion molecule (MAdCAM)-1], promoting interaction of platelets with ECs and inducing expression of pro-angiogenic growth factors such as VEGF-A[25,44,71-73]. Defects in the activity of the anti-inflammatory cytokines such as IL-10 may play a role in the establishment of some IBD, and IL-10 deficient mice (IL-10$^{-/-}$) develop IBD spontaneously, while other animal models of colitis show reduced injury when treated with exogenous IL-10[2,74-76]. Interestingly Oshima *et al*[19] observed that pretreatment of ECs with IL-10 prevented IFN-γ mediated endothelial barrier disruption, indicating that an important role of IL-10 may be to prevent cytokine mediated EC barrier disturbances which initiate and exacerbate disease. This is supported by the finding that several EC adhesion molecules such as intercellular adhesion molecule (ICAM)-1, VCAM-1 and MAdCAM-1 are increased in IL-10$^{-/-}$ mouse colitis and may mediate leukocyte recruitment in this model[66].

Over 40 chemokines in 4 separate families interact with as many as 19 receptors to regulate trafficking of leukocytes. Of these, several chemokines may mediate leukocyte trafficking to the gut and colon dysfunction in IBD. Papadakis *et al*[77] showed that CCL2 and CCL5$^{-/-}$ mice are protected from colitis. Interestingly, Barcelos *et al*[78] and Wu *et al*[79] showed that CCL5 and CCL3 can induce inflammatory angiogenesis in a murine sponge model and promote angiogenesis in murine tumors. Eyman *et al*[80] have also shown that CCL5 upregulates pro-angiogenic genes. CCL25 interacting with its receptor on CCR9^{+} leukocytes plays a major role in the early stages of experimental IBD pathogenesis[81]. CXCL8 (IL-8) another pro-angiogenic chemokine, is known to be stored in EC Weibel-Palade bodies, can be rapidly secreted, and induces HIMEC proliferation in culture *via* binding to CXCR2[82,83]. Although angiogenesis may support injury IBD, IL-8 may be dysregulated in some forms of IBD. IL-8 seems to be downregulated in leukocytes and in the endothelium of patients with CD. There appears to be no upregulation in the endothelium of UC patients, suggesting a possible link to TGF-β1 over expression in IBD[84-86]. In contrast, Scaldaferri *et al*[87,88] found that intestinal fibroblasts treated with TNF-α produce IL-8 and monocyte chemoattractant protein-1 *via* p38/p42/44 mitogen-activated protein kinase.

CX3CL1/fractalkine is a chemokine expressed by EC, can be upregulated by TNF-α, IL-1, LPS and IFN-γ, and is highly upregulated in IBD[89,90]. CX3CL1 can function as an endothelial adhesive determinant to recruit a subpopulation of dendritic cells and macrophages that have high CX3CR1 expression. CXCL1 can be shed from the surface of the ECs (in response to increased IL-1β in IBD). This form of CX3CL1 acts as a chemoattractant for CD4^{+} and CD8^{+} T-cells[90]. Sans *et al*[91] reported that in fact there is enhanced recruitment of CX3CR1 expressing T-cell to the gut *via* interactions with CX3CL1. CXCR4/SDF-1α and its ligand CXCL12 is an important chemokine/receptor pair in angiogenesis, but have received very little attention in IBD. Heidemann *et al*[92] reported that blocking this CXCR4/CXCL12 interaction is sufficient to inhibit migration and proliferation of HIMECs in response to VEGF-A. CXCR4/SDF-1α plays an important role in the recruitment of EC precursors to sites of angiogenesis, and may be impaired in IBD, leading to the conclusion that this pathway may be interrupted[93-95]. Midkine, another chemokine of great interest is increased in serum and is associated with tumor drug resistance and poor cancer prognosis[96-98]. Midkine is also upregulated in IBD serum, and has prognostic value like VEGF, TNF-α, sVCAM and VCAM[20,99-102]. Midkine has a pronounced

angiogenic effect, like some other inflammatory factors, and also increases the levels of surface glycosaminoglycans on ECs to favor recruitment of circulating leukocytes in IBD[103].

INCREASED ENDOTHELIAL ADHESION MOLECULE EXPRESSION IN IBD

Inflammation in IBD is characterized by increases in both blood and lymphatic vessels in the intestine. This increase in endothelial surface area provides a powerful means of increasing leukocyte recruitment with the mobilization of ECAMs including selectins[28]. Animal models of IBD (IL-10$^{-/-}$, IL-2$^{-/-}$, SAMP1/Yit and T-bet$^{-/-}$), like human IBD, all show ECAM upregulation is linked to disease severity[66,104-106], allowing use of adhesion antagonists in IBD therapy[55,102,107]. Endogenous endothelial-derived inhibitors of leukocyte binding (e.g. sVCAM-1) may also be downregulated in IBD[21,108-111] and could provide new diagnostic or anti-adhesive strategies.

P and E-selectins, glycoproteins expressed on the surface of platelets and other leukocytes, are also expressed on the surface of activated or inflamed endothelium in IBD. P-selectin can interact with ECAMs such as VCAM-1/ICAM-1, as well as with O-glycans collectively referred to as peripheral lymph node addressins (PNAds) containing sialyl Lewis X moieties[112,113]. P-selectin at least partially mediates rolling and recruitment of gut-infiltrating leukocytes in IBD, with approximately 50% increase in gut P-selectin in UC *vs* control groups; serum levels of soluble P-selectin, an inhibitor of selectin binding, are decreased in IBD patients[109,114,115]. Increased platelet P-selectin, with the enhanced prothrombotic surface of the gut EC in IBD increases thrombus formation and tissue damage by ischemic injury[115]. E-selectin, a relative of P-selectin is expressed solely on the surface of activated ECs during inflammation and is a major contributor to leukocyte rolling injury. E-selectin is not stored in Weibel-Palade bodies and must be produced in response to inflammatory stimuli such as IL-1, TNF-α and VEGF-A[116,117]. In contrast to sP-selectin, sE-selectin is not downregulated in IBD and in CD, and actually increases in comparison to controls[109].

High endothelial venules (HEV) are specialized post-capillary venules that allow trafficking of leukocytes between immune (e.g. Peyer's patches) and vascular compartments, and are increased in IBD[113]. L-selectin expressed on leukocytes (after activation) binds PNAd on HEV and recruits leukocytes expressing L-selectin in IBD. The gut and brain selective adhesion determinant, MAdCAM-1 is also expressed on HEV, and in UC MAdCAM-1 O-glycosylation increases, allowing greater L-selectin binding[118]. MAdCAM-1 interacts with α4β7 integrins on the surface of a subset of naive CD4$^+$ T-cells[119,120]. MAdCAM-1 induction is found only in chronically inflamed gut endothelium and suggests that in IBD there is a fundamental alteration in the phenotype and gene expression pattern in the inflamed intestinal EC[28]. Mizushima *et al*[121] demonstrated that inhibition of angiotensin-Ⅱ type 1 receptors reduced TNF-α dependent MAdCAM-1 expression and reduce the severity of DSS-induced colitis, possibly linking vasoregulation and inflammation. In HIMEC MAdCAM-1 is also expressed inversely with cell density, with proportionally greater levels of MAdCAM-1 found at low densities. This indicates that in newly formed vessels, larger amounts of MAdCAM-1 may be available to recruit leukocytes to these "leaky and permissive" vessels[122,123].

ICAM-1, another important ECAM in IBD binds LFA-1 (aLb2), Mac-1 (aMb2) and α4β2 integrins, and is expressed by inflamed ECs to mediate the firm adhesion of leukocytes to activated ECs[124,125]. ICAM-1 has a unique relationship with VEGF-A; Goebel *et al*[125] reported that HIMECs constitutively express ICAM-1, which is significantly upregulated following treatment with 50 ng/mL VEGF-A, linking inflammation and angiogenesis. In addition to direct activation and upregulation of ICAM-1 by VEGF, Zitterman *et al*[117] found that VEGF treatment also sensitizes cells to TNF-α induced ICAM-1 mobilization. Normally ICAM-1 concentrates at EC junctions, but is redistributed to apical surfaces of ECs under inflammatory conditions where it supports firm adhesion of leukocytes[25]. In the adoptive T-cell transfer model of murine IBD, Ostanin *et al*[126] found that T-cells that lack LFA-1, (a T-cell ICAM-1 ligand), fail to induce disease, revealing a critical role for EC modulated immune responses. ICAM-1 was the one of the first clinical targets in IBD, but an antisense IBD therapy showed limited success[21].

VCAM-1, an ECAM highly expressed on the luminal surface of activated ECs in IBD, mediates the adhesion of α4β1 expressing lymphocytes. In HIMECs, the expression of VCAM-1 is regulated by the PI3K/NF-κB signaling pathway and its stimulation by mediators can be inhibited by curcumin[127]. Like ICAM-1, VCAM-1 can also up regulated by VEGF-A *via* NF-κB[117,128]. Studies in the picrylsulfonic acid model of UC using radiolabeled anti-VCAM-1 antibodies show that leukocyte infiltration and histological damage are proportionate to VCAM-1 expression in the gut microvasculature[129]. In addition, in the DSS model of UC, there is an upregulation of VCAM-1 which if blocked (by specific antibodies) attenuates disease activity, while ICAM-1 and MAdCAM-1 blockade do not protect in this manner[129,130].

CD31/PECAM-1 expressed by ECs and leukocytes mediates homophilic binding between activated ECs and leukocytes especially during extravasation. CD31 is found on the endothelial surface and in endothelial junctions. Work by Romer *et al*[72] found that CD31 is not upregulated in response to inflammatory cytokines but is redistributed from cellular junctions. CD31 blockade inhibits leukocyte transmigration, and CD31 inhibition in IBD reduced leukocyte rolling and firm adhesion suggesting a unique role for CD31 in IBD or in the function of the gut microvasculature[131].

Originally considered a mesenchymal stem cell marker[132-134], CD146 is now described as a novel immunoglobulin super family adhesion molecule which is increased in

gut tissue of IBD patients[108]. The function of CD146 in IBD is not completely understood, but has potential roles in inflammation since it supports rolling and invasion of natural killer T-cells[135]. The upregulation of CD146 in IBD, like ICAM-1 and VCAM-1, may be driven by VEGF-A overexpression during IBD[100]. Additionally, the soluble form of CD146, regulates endothelial and leukocyte CD146 interactions with their ligands, and is reduced in IBD, enhancing leukocyte extravasation[100,108,135]. Interestingly Tsiolakidou *et al*[100] determined that new vessels formed in IBD are disproportionately $CD146^{+}$. Inflamed ECs from CD and UC patients show an increased ability to recruit naïve T-cells and macrophages to the intestinal immune compartment after stimulation with several inflammatory cytokines, but not with LPS[28,120]. These data are consistent with IBD not being initially driven by immune cells, but rather by the endothelial response to an increased inflammatory mediatory load.

PLATELETS AND COAGULATION IN IBD

Platelet and leukocyte aggregation as well as activation of the coagulation cascade increase during IBD, reflecting loss of the non-thrombogenic EC phenotype in IBD. Thrombi aggravate inflammation by binding of micro infarcts to the endothelial surface often leading to ischemic inflammation in the intestinal microvasculature[136]. Mesenteric venous thrombosis has been observed in a fraction of IBD cases, and thrombotic processes are being recognized in altered perfusion, inflammation and tissue injury in IBD[137]. Indeed, subclinical thrombosis is common in IBD, and is a major source of morbidity in approximately 25% of IBD deaths[136]. Increased markers of coagulation include thrombin anti-thrombin complex, tissue factor and fibrinopeptide B[55], and can be described early in IBD. Factor XIIIa, a fibrin-stabilizing coagulation factor (and agonist for VEGFR-2), is increased in IBD, while factor XIII TT has an increased number of mutations in IBD patients compared to controls suggesting links between thrombosis, angiogenesis and inflammation. However, Bernstein *et al*[138], Dardik *et al*[139] and Vrij *et al*[140] reported that factor XIII activity is reduced in IBD patients.

In addition to increased levels of coagulation cascade proteins in IBD, CD40, CD40L and soluble CD40L are increased in IBD. CD40, expressed on several cell types (including ECs) is involved in inflammatory and immune activation, and interacts with CD40L on T-cells. Danese *et al*[141] suggested that the primary source of sCD40L was from activated platelets. CD40 signaling increases production of pro-inflammatory cytokines and chemokines by ECs and surrounding tissue[142]. CD40L release also leads to binding of platelets and immune cells to ECs by increasing tissue factor, ECAM expression and pro-thrombotic phenotype in HIMECs[141-143]. Danese *et al*[71] suggested that a possible therapeutic benefit of TNF-α blockade was downregulation of CD40/CD40L signaling in IBD. A still unanswered question is whether coagulation is a secondary or initiating event in inflammation. It is worth mentioning that individuals with coagulation cascade disorders (e.g. hemophilia, factor V deficiency and von Willebrand disease) rarely develop IBD[55]. The opposite of the previous observation is also true; patients with IBD have an increased likelihood of having genetic pro-thrombotic disease Factor V Lieden[144]. This evidence strongly links thrombus formation as a possible trigger of IBD and suggests prognostic factors which may increase risk of IBD development.

ENDOTHELIAL BARRIER DYSFUNCTION IN IBD

The maintenance of normal vascular barrier supports nutrient and O_2 exchange, osmotic balance and leukocyte abundance in the extracellular compartment. In IBD, increased vascular permeability leads to tissue edema and damage in both human IBD and animal models of IBD[19]. This alteration in solute permeability of the vasculature is not restricted to the gut microcirculation but is widespread affecting the vasculature of other organs including the brain[145]. Several classes of mediators in IBD alter both solute permeability and angiogenic balance, including angiogenin (an angiogenic peptide with ribonuclease activity), chemokines (e.g. IL-8, IL-10), coagulation factors (thrombin), cytokines (IFN-γ, IL-13), and growth factors, most notably VEGF, the most potent and important blood vascular angiogenic growth factor and an important inflammatory mediator[19,36,37,47,146-148]. Tolstanova *et al*[149] found that VEGF-A inhibition by neutralizing antibodies reduced vessel permeability in the iodoacetamide model of colitis. Downregulation of anti-inflammatory cytokines e.g. IL-10 may play an equally important role in increasing endothelial permeability. Oshima *et al*[19] have shown increased vascular permeability in the $IL\text{-}10^{-/-}$ colitis model due to loss of IL-10 inhibition of IFN-γ induced junctional degradation; also IL-10 protects against IFN-γ mediated loss of human microvascular barrier.

Leukocytes, e.g. neutrophils and monocytes, can degrade endothelial junctions through protease secretion and upregulation. Cytokines and growth factors also induce MMP-9, MMMP-3 and MMP-1[150,151], resulting in degradation of junctional and matrix targets[152]. Neutrophil elastase is elevated in IBD and can degrade vascular endothelial cadherin, important in maintaining junctional apposition, adhesion and barrier function[153-156]. Endothelial junctional adhesion molecule-A is also dysregulated in IBD, and is closely linked to disease activity in DSS colitis[37,157].

ANGIOGENESIS IN IBD

Angiogenesis (increased blood vessel density) in IBD increases the area of endothelium available for exchange, but also for extravasation of blood constituents into surrounding tissue to increase disease severity in IBD[158]. Increased vessel formation in IBD may represent recruitment of endothelial progenitor cells, vascular intussuscep-

tion (splitting) and extension from existing vessels[159]. Increased angiogenesis is observed in animal (2,4,6-trinitrobenzene sulphonic acid (TNBS), DSS and iodoacetamide) colitis models and in human colitis. However, inflammatory angiogenesis in IBD does not simply match increased tissue mass. Vessels formed during inflammation are different from those formed during normal development. These vessels are immature, lacking investment with pericytes. They express ECAMs, leak, are hypoperfused, often stenose and are hyperthrombotic, with an elevated ability to respond to growth factors[160-163] actively supporting IBD progression[149,164-168]. Spalinger *et al*[158] and Maconi *et al*[169] concluded that there is an increased blood vessel density in the intestines of CD and UC patients and that increased vascular density in IBD was directly correlated with increased IBD disease severity. This is also true in animal models of IBD like TNBS- and DSS-induced colitis models[166,170].

Growth factors, especially VEGF-A, dramatically alter several aspects of the colon microvascular endothelial phenotype, resembling a de-differentiation (loss of maturity) of the vessels which can reflect changes in vascular support cells, e.g. pericytes/smooth muscle, that surrounds the capillaries. Inflamed tissues display increased vascular density resulting from the formation of new vessels during angiogenesis. These changes result in decreased perfusion, increased solute permeability (*via* cytokines and VEGF-A induced junction remodeling) and contractility, as well as increased leukocyte and platelet adhesiveness[161,171,172]. Ganta *et al*[163] have demonstrated that in angiotensin-2 knockout mice (using the DSS model of UC), loss of the pericytes around vessels resulted in diminished angiopoietin-1 signaling that destabilized the endothelial layer, increased leukocyte recruitment to the tissue, increased vessel permeability and induced vessel hyper-proliferation. Blood and lymphatic vessels are hyperstabilized by angiopoietin-2 deficiency, and show diminished inflammatory remodeling as well as decreased capacity to recruit leukocytes suggesting a link between maturity and inflammatory capacity[163].

ENDOTHELIAL CELL AND ANGIOGENIC GROWTH FACTOR INTERACTIONS IN IBD

VEGF-A is the first described and best known VEGF, which controls developmental angiogenesis, wound healing and pathology[173,174]. Bousvaros *et al*[175], Kapsoritakis *et al*[101] and Ozawa *et al*[176] all found elevated VEGF-A levels in plasma and tissue during active human and animal IBD, often twice normal[101,109,166,175,176]. However, Chidlow *et al*[166] have reported that DSS diminishes levels of VEGF-A as well as VEGF-C and VEGF-D, suggesting complex, concentration-dependent and inhibitor-regulated effects of VEGF in different animal models of IBD. Danese *et al*[177] and Scaldaferri *et al*[167] have shown that inhibition of VEGF signaling can attenuate disease activity in the DSS model of UC while overexpression of VEGF-A increases disease severity in the same model[167,177]. VEGF-A is released by several cell sources (e.g. neutrophils, platelets, macrophages, pericytes, fibroblasts, ECs, and colonic epithelial cells) and is transcriptionally activated by hypoxia through hypoxia inducible factor 1α, and message stabilization *via* eukaryotic translation initiation factor 4e[70,178-183]. Interestingly Birmingham *et al*[184] have shown that activated colonic epithelium represents an important source of VEGF-A, and injury or inflammation of the colon epithelium may provide a local stimulus for blood vessel growth. Invasive leukocytes, specifically neutrophils, granulocytes, macrophages and platelets, are increased in tissue during active IBD, and are also important sources of VEGF-A in inflamed tissues[178,179,185-187]. Salivary secretions also contain high levels of VEGF-A and VEGF-C, which have been suggested as important sources of these growth factors in IBD[188] released site-specifically during denudation. Apart from VEGFs, other angiogenic growth factors, e.g. basic fibroblast growth factor (bFGF), TGF-β and platelet-derived growth factor (PDGF) are upregulated in IBD and may be of clinical relevance[86,189].

TGF-β is an important regulator of the cell cycle and apoptosis, especially in mucosal immune cells. The expression of TGF-β and its 2 receptors (TGFR1 and TGFR2) are increased in IBD, specifically UC; however, it appears that the levels are decreased in CD[190]. In IBD either tachyphylaxis develops for TGF-β (UC), or the lack of TGF-β (CD) allows mucosal immune cells to proliferate when they would have undergone apoptosis[190,191]. Early studies on the role of TGF-β in IBD indicated a protective role; more recent studies may point to a pathological role of TGF-β signaling in IBD[191,192]. In fact, TGF-β is important in the formation of fibrosis in the colon of IBD patients by stimulating the transition of many cell types to fibroblasts[193]. Over one-third of the fibroblasts responsible for inflammatory fibrotic injury may actually originate from the transformation of ECs to fibroblasts (not counting contributions of pericytes to fibroblast formation). Therefore the vasculature may provide a significant proportion (if not the majority of fibroblasts) and associated fibrosis in IBD[194,195]. bFGF, a potent mitogen for the cells of mesodermal origin, stimulates EC proliferation, activates MMPs resulting in proteolysis of extracellular matrix, and increases cellular motility[191]. Even though levels of bFGF are elevated in IBD there is no correlation with the stage or severity of the disease. However, the contribution of bFGF in the initiation or maintenance of IBD should not be discounted[196]. PDGF is a close relative to VEGF and is upregulated in IBD. PDGF is predictive of both oxidative stress and angiogenesis in the intestine[189]. PDGF is released in response to inflammatory and thrombotic stimuli. PDGF increases P-selectin expression on ECs and induces histamine secretion which induces other effects such as increased vascular leakage[197,198]

ENDOTHELIAL PROGENITOR CELLS AND VESSEL SPROUTING IN IBD

Recruitment of endothelial progenitor cells (EPCs) may

contribute to angiogenesis in IBD, although reduced numbers of VEGFR2$^+$, CD34$^+$, CD133$^+$ cells (endothelial, bone marrow, and stem cell markers) have been reported in IBD[199], and EPCs from IBD have reduced antigenic activity[95]. These findings suggest that recruitment of EPCs is unlikely to be a source of increased vessels, however, these findings are from patients with established disease as initial angiogenesis in early stages of IBD may rely on EPCs. Apart from EPC recruitment, angiogenic sprouting is active in IBD; sprouting ECs referred to as "tip" cells, are highly motile with distinct gene expression compared to that in quiescent ECs[200]. VEGF-A induces the tip cell phenotype and also guides vessel sprouting, indicating that in IBD, VEGF might induce new vessel formation in this way[201]. Normally, not all sprouts survive, many undergoing apoptosis, (vessel "pruning") suggesting that high levels of VEGF prevent endothelial apoptosis resulting in increased numbers of surviving sprouts in IBD[201].

INHIBITORS OF VASCULAR ENDOTHELIAL EXPANSION IN IBD

While increased pro-angiogenic growth factors increase angiogenesis, reductions in anti-angiogenic factors (seen in the DSS model of colitis) may be as important for permitting expansion of the vascular endothelium[166,167]. Angiopoietin-1 a competitive inhibitor of Ang-2, binds to the Tie-2 receptor and inhibit vascular remodeling. Angiopoietin-2 is upregulated during inflammation and angiogenesis[163,202] and competes with angiopoietin-1, to allow ECs to maximally respond to cytokines and growth factors. Work by Ganta *et al*[163] found that angiopoietin-2 signaling also appears to be necessary for neutrophil infiltration, and blood and lymphatic vessel proliferation in DSS colitis. Interestingly angiopoietin-2 can be upregulated by both bFGF and VEGF, potent pro-angiogenic growth factors also upregulated in IBD[203-205].

Angiostatin, a fragment of plasminogen generated by MMPs has anti-angiogenic and anti-proliferative effects on ECs and blocks vessel maturation[206]. During IBD, levels of MMPs are elevated and generate angiostatin[153]. In fact 2 models of experimental colitis (iodoacetamide, TNBS) show increased angiostatin, and may represent a feedback control for angiogenesis[207]. Interestingly, the effect of angiostatin hinges less on inhibition of EC proliferation, but more on inhibiting final vessel maturity[208]. Much like angiostatin, endostatin results from the cleavage of collagen type XVIII yielding an anti-angiogenic fragment that is upregulated in experimental colitis[207,209]. Endostatin reduces EC migration and proliferation; however like angiostatin, endostatin fails to block angiogenesis in the TNBS model, but may play a role in disease progression and maintenance by impairing vessel maturity and tissue healing by antagonizing VEGF-A induced tissue repair[207]. Interestingly Deng *et al*[210] showed mesalamine treatment of iodoacetamide colitis restored levels of endogenous angiogenesis inhibitors, endostatin and angiostatin helping reduce disease severity.

Soluble VEGF receptors (sVEGFRs) are truncated forms of VEGFR1 or VEGFR2 genes[211] that under normal physiological conditions maintain tissue avascularity (e.g. in the cornea) and might be dysregulated in IBD. During inflammation, sVEGFR1 inhibition seems to be lost (e.g. in the case of an alkali burn)[211,212]. sVEGFR2 seems to plays an important role in the inhibition of lymphangiogenesis compared to sVEGFR1, but sVEGFR2 blocks transplant rejection which points out its greater immunomodulatory effect[213]. Additionally, Scaldaferri *et al*[167] found that over expression of sVEGFR1 reduced disease severity in the DSS model of colitis, suggesting that loss of this molecule in IBD would be detrimental. Interestingly the anti-angiogenic VEGFs, alternate splice variants of VEGFs, are downregulated in several inflammatory diseases, and are linked to the alteration of the cytokine milieu in the tissues[214-217]. These inhibitory VEGFs make up a majority of the VEGF load in the normal intestinal micro-environment with approximately 20 times greater levels in the healthy gut[217]. Currently, the levels of these inhibitory VEGFs are unknown in IBD, but may provide a new avenue for anti-angiogenic therapies, we are pursuing this possibility which is currently showing great promise (unpublished data).

IBD THERAPIES

It is increasingly clear that IBD therapies affect the microvasculature, and that the microvasculature is a central target in IBD, coordinating cell infiltration, solute permeability, cytokine/chemokine production and gut immunological responses. An increasing number of drugs that show efficacy in treating IBD have now been found to affect the endothelium. Accumulating evidence suggests inhibition of angiogenesis as a secondary mechanism of action for many IBD therapies including anti-TNF-α antibodies, and some immunosuppressive agents (cyclosporine A)[218-220]. Scaldaferri *et al*[87] found TNF-α mediated lymphocyte adhesion and chemotaxis across intestinal microvascular ECs depends on expression of ICAM-1, VCAM-1 and fractalkine in the affected ECs mediated by p38 MAPK, p42/44 MAPK and JNK. Danese *et al*[71] found that anti-TNF-α therapies can reduce thrombus formation and adhesion to the endothelium by interfering with CD40/CD40L signaling. Integrin-blocking antibodies have been used in the treatment of IBD, but not without a controversial side effect. Natalizumab (Tysabri), an α4-integrin blocking monoclonal antibody originally developed for use in the treatment of multiple sclerosis, but has recently been approved for the treatment CD[21,221]. AJM300, a peptide blocker for α4 integrins, successfully blocked α4 -VCAM-1 and MAdCAM-1 adhesion and prevented exacerbation in IBD models[20,21]. However, recent preclinical trials using AJM300 failed to inhibit disease progression[20,21]. Rafiee *et al*[222] found that 2 drugs used in IBD, thalidomide and cyclosporine-A, are anti-angiogenic; thalidomide targets TNF-α and VEGF-A, while cyclosporin-A targets VEGF-A alone[222,223]. Studies by Ogawa *et al*[224] determined that HIMEC expression of the inflammatory mediators IL-6 and cyclooxygenase (COX)-2

by LPS were inhibited by butyrate, and that butyrate also inhibited HIMEC angiogenesis[224-226]. Despite its anti-inflammatory properties[223,227,228], cyclosporin-A increases leukocyte binding, unlike thalidomide which reduces leukocyte binding to HIMECs[227,228].

IBD-INDUCED ANGIOGENESIS AND COLORECTAL CANCER

The risk of developing cancer is elevated by inflammation, and the link between IBD and colorectal cancer (CRC) is convincing[229-231]. Inhibition of angiogenesis in CRC by bevacizumab (anti-VEGF monoclonal antibody) improves clinical outcomes, revealing the importance of angiogenesis in the progression from IBD to CRC[232]. As stated before, IBD in human disease and animal models is associated with an increase in vascular density, and it is possible this vascular endothelial expansion may enable CRC[158,163,169]. CRC incidence may depend on COX expression (seen in adenomatous polyposis coli, pre-cancerous lesions enriched in COX[233-235]. COX-2 is increased in human IBD and IBD models, and may promote CRC through angiogenesis[236,237]. COX-2 promotes EC proliferation by prostaglandin induction of VEGF-A, important for tumor angiogenesis[237,238]. Chan *et al*[239] reported that the regular use of aspirin, a non-selective COX inhibitor, significantly reduced the risk of COX-2$^+$ CRC, which constitutes approximately 67% of human CRC[239,240]. Additionally COX-2 inhibition reduces tumor growth and increased tumor apoptosis, and is associated with reduced tumor angiogenesis[238,241,242]. Conversely, Ishikawa *et al*[243] found that COX-deficient animals were not protected from tumor formation in azoxymethane (tumor promoter)-induced colorectal cancer, and concluded that COX expression was not a major determinant of tumor formation in UC. While COX expression may not be not necessary for tumor formation in UC, COX-2 upregulation is only one mechanism for increased angiogenesis in IBD[86,166,189]. VEGF-A and other angiogenic factors are upregulated independent of COX-2 in IBD; therefore, while COX-2 may be important in CRC in the absence of IBD, expansion of the vasculature in IBD through other mechanisms may contribute to the development and growth of CRC[166,244].

CONCLUSION

A unique combination of genetic and environmental factors may contribute to development of IBD. ECs are now recognized as central and fundamental elements in IBD pathophysiology. ECs are indirectly affected by many IBD medications, which are increasingly targeting ECs directly. As treatments for IBD are developed and refined there will be an increased interest in inhibiting functions of ECs in IBD such as immune cell recruitment and inflammatory angiogenesis, and improving beneficial lymphatic function. Use of endogenous inhibitors of leukocyte binding (sVCAM) and peptides (AJM300) may become novel therapies which supplement or replace current anti-adhesion treatments. Additional studies on the interactions between the gut microvasculature, platelets and their regulation of inflammatory angiogenesis may provide new avenues for treatments that not only reduce thrombosis but also several clinical manifestations of IBD. Inhibition of inflammatory growth factors, cytokines and chemokines that promote angiogenesis by the use of "traps" or decoy receptors, alone or in combination, in addition to current treatments could provide greater anti-inflammatory effects by reducing endothelial expansion in IBD. More importantly, work in our laboratory suggests that endogenous angiogenic inhibitors (VEGF164b) have great potential in the treatment of IBD. Future studies promoting therapeutic intervention by combining anti-angiogenic, anti-immune and anti-inflammatory agents as treatment options focusing on the endothelium as core/vital for IBD pathogenesis will provide greater specificity and efficacy for treating CD and UC patients.

REFERENCES

1 **Sands BE**, Kaplan GG. The role of TNFalpha in ulcerative colitis. *J Clin Pharmacol* 2007; **47**: 930-941
2 **Parkes M**, Satsangi J, Jewell D. Contribution of the IL-2 and IL-10 genes to inflammatory bowel disease (IBD) susceptibility. *Clin Exp Immunol* 1998; **113**: 28-32
3 **Boyko EJ**, Perera DR, Koepsell TD, Keane EM, Inui TS. Coffee and alcohol use and the risk of ulcerative colitis. *Am J Gastroenterol* 1989; **84**: 530-534
4 **Langhorst J**, Cobelens PM, Kavelaars A, Heijnen CJ, Benson S, Rifaie N, Dobos GJ, Schedlowski M, Elsenbruch S. Stress-related peripheral neuroendocrine-immune interactions in women with ulcerative colitis. *Psychoneuroendocrinology* 2007; **32**: 1086-1096
5 **Langhorst J**, Wieder A, Michalsen A, Musial F, Dobos GJ, Rueffer A. Activated innate immune system in irritable bowel syndrome? *Gut* 2007; **56**: 1325-1326
6 **Pousa ID**, Gisbert JP. Gastric angiogenesis and Helicobacter pylori infection. *Rev Esp Enferm Dig* 2006; **98**: 527-541
7 **Hibi T**, Ogata H. Novel pathophysiological concepts of inflammatory bowel disease. *J Gastroenterol* 2006; **41**: 10-16
8 **Garrett WS**, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, Glickman JN, Glimcher LH. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. *Cell* 2007; **131**: 33-45
9 **Sands BE**. Inflammatory bowel disease: past, present, and future. *J Gastroenterol* 2007; **42**: 16-25
10 **Mayer L**. Evolving paradigms in the pathogenesis of IBD. *J Gastroenterol* 2010; **45**: 9-16
11 **Ruyssers NE**, De Winter BY, De Man JG, Loukas A, Herman AG, Pelckmans PA, Moreels TG. Worms and the treatment of inflammatory bowel disease: are molecules the answer? *Clin Dev Immunol* 2008; **2008**: 567314
12 **Fiasse R**, Latinne D. Intestinal helminths: a clue explaining the low incidence of inflammatory bowel diseases in Subsaharan Africa? Potential benefits and hazards of helminth therapy. *Acta Gastroenterol Belg* 2006; **69**: 418-422
13 **Moreels TG**, Pelckmans PA. Gastrointestinal parasites: potential therapy for refractory inflammatory bowel diseases. *Inflamm Bowel Dis* 2005; **11**: 178-184
14 **Sandborn WJ**, Targan SR. Biologic therapy of inflammatory bowel disease. *Gastroenterology* 2002; **122**: 1592-1608
15 **Sawa Y**, Oshitani N, Adachi K, Higuchi K, Matsumoto T, Arakawa T. Comprehensive analysis of intestinal cytokine messenger RNA profile by real-time quantitative polymerase chain reaction in patients with inflammatory bowel

disease. *Int J Mol Med* 2003; **11**: 175-179
16 **Latinne D**, Fiasse R. New insights into the cellular immunology of the intestine in relation to the pathophysiology of inflammatory bowel diseases. *Acta Gastroenterol Belg* 2006; **69**: 393-405
17 **Chidlow JH Jr**, Shukla D, Grisham MB, Kevil CG. Pathogenic angiogenesis in IBD and experimental colitis: new ideas and therapeutic avenues. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G5-G18
18 **Jiang HQ**, Thurnheer MC, Zuercher AW, Boiko NV, Bos NA, Cebra JJ. Interactions of commensal gut microbes with subsets of B- and T-cells in the murine host. *Vaccine* 2004; **22**: 805-811
19 **Oshima T**, Laroux FS, Coe LL, Morise Z, Kawachi S, Bauer P, Grisham MB, Specian RD, Carter P, Jennings S, Granger DN, Joh T, Alexander JS. Interferon-gamma and interleukin-10 reciprocally regulate endothelial junction integrity and barrier function. *Microvasc Res* 2001; **61**: 130-143
20 **Dharmani P**, Chadee K. Biologic therapies against inflammatory bowel disease: a dysregulated immune system and the cross talk with gastrointestinal mucosa hold the key. *Curr Mol Pharmacol* 2008; **1**: 195-212
21 **Yacyshyn BR**. Adhesion molecule therapeutics in IBD. *Inflamm Bowel Dis* 2008; **14** Suppl 2: S279-S280
22 **Danese S**, Dejana E, Fiocchi C. Immune regulation by microvascular endothelial cells: directing innate and adaptive immunity, coagulation, and inflammation. *J Immunol* 2007; **178**: 6017-6022
23 **Cines DB**, Pollak ES, Buck CA, Loscalzo J, Zimmerman GA, McEver RP, Pober JS, Wick TM, Konkle BA, Schwartz BS, Barnathan ES, McCrae KR, Hug BA, Schmidt AM, Stern DM. Endothelial cells in physiology and in the pathophysiology of vascular disorders. *Blood* 1998; **91**: 3527-3561
24 **Haraldsen G**, Rugtveit J, Kvale D, Scholz T, Muller WA, Hovig T, Brandtzaeg P. Isolation and longterm culture of human intestinal microvascular endothelial cells. *Gut* 1995; **37**: 225-234
25 **Haraldsen G**, Kvale D, Lien B, Farstad IN, Brandtzaeg P. Cytokine-regulated expression of E-selectin, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in human microvascular endothelial cells. *J Immunol* 1996; **156**: 2558-2565
26 **Nilsen EM**, Johansen FE, Jahnsen FL, Lundin KE, Scholz T, Brandtzaeg P, Haraldsen G. Cytokine profiles of cultured microvascular endothelial cells from the human intestine. *Gut* 1998; **42**: 635-642
27 **Binion DG**, Rafiee P, Ramanujam KS, Fu S, Fisher PJ, Rivera MT, Johnson CP, Otterson MF, Telford GL, Wilson KT. Deficient iNOS in inflammatory bowel disease intestinal microvascular endothelial cells results in increased leukocyte adhesion. *Free Radic Biol Med* 2000; **29**: 881-888
28 **Binion DG**, West GA, Volk EE, Drazba JA, Ziats NP, Petras RE, Fiocchi C. Acquired increase in leucocyte binding by intestinal microvascular endothelium in inflammatory bowel disease. *Lancet* 1998; **352**: 1742-1746
29 **Sessa WC**. Molecular control of blood flow and angiogenesis: role of nitric oxide. *J Thromb Haemost* 2009; **7** Suppl 1: 35-37
30 **Binion DG**, Fu S, Ramanujam KS, Chai YC, Dweik RA, Drazba JA, Wade JG, Ziats NP, Erzurum SC, Wilson KT. iNOS expression in human intestinal microvascular endothelial cells inhibits leukocyte adhesion. *Am J Physiol* 1998; **275**: G592-G603
31 **Petersson J**, Schreiber O, Steege A, Patzak A, Hellsten A, Phillipson M, Holm L. eNOS involved in colitis-induced mucosal blood flow increase. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G1281-G1287
32 **Spyridopoulos I**, Luedemann C, Chen D, Kearney M, Chen D, Murohara T, Principe N, Isner JM, Losordo DW. Divergence of angiogenic and vascular permeability signaling by VEGF: inhibition of protein kinase C suppresses VEGF-induced angiogenesis, but promotes VEGF-induced, NO-dependent vascular permeability. *Arterioscler Thromb Vasc Biol* 2002; **22**: 901-906
33 **Breslin JW**, Pappas PJ, Cerveira JJ, Hobson RW 2nd, Durán WN. VEGF increases endothelial permeability by separate signaling pathways involving ERK-1/2 and nitric oxide. *Am J Physiol Heart Circ Physiol* 2003; **284**: H92-H100
34 **Rousseau S**, Houle F, Kotanides H, Witte L, Waltenberger J, Landry J, Huot J. Vascular endothelial growth factor (VEGF)-driven actin-based motility is mediated by VEGFR2 and requires concerted activation of stress-activated protein kinase 2 (SAPK2/p38) and geldanamycin-sensitive phosphorylation of focal adhesion kinase. *J Biol Chem* 2000; **275**: 10661-10672
35 **Mihaescu A**, Santen S, Jeppsson B, Thorlacius H. p38 Mitogen-activated protein kinase signalling regulates vascular inflammation and epithelial barrier dysfunction in an experimental model of radiation-induced colitis. *Br J Surg* 2010; **97**: 226-234
36 **Kumar P**, Shen Q, Pivetti CD, Lee ES, Wu MH, Yuan SY. Molecular mechanisms of endothelial hyperpermeability: implications in inflammation. *Expert Rev Mol Med* 2009; **11**: e19
37 **Vetrano S**, Danese S. The role of JAM-A in inflammatory bowel disease: unrevealing the ties that bind. *Ann N Y Acad Sci* 2009; **1165**: 308-313
38 **Horowitz S**, Binion DG, Nelson VM, Kanaa Y, Javadi P, Lazarova Z, Andrekopoulos C, Kalyanaraman B, Otterson MF, Rafiee P. Increased arginase activity and endothelial dysfunction in human inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1323-G1336
39 **Förstermann U**. Janus-faced role of endothelial NO synthase in vascular disease: uncoupling of oxygen reduction from NO synthesis and its pharmacological reversal. *Biol Chem* 2006; **387**: 1521-1533
40 **Förstermann U**, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. *Circulation* 2006; **113**: 1708-1714
41 **Hatoum OA**, Binion DG, Otterson MF, Gutterman DD. Acquired microvascular dysfunction in inflammatory bowel disease: Loss of nitric oxide-mediated vasodilation. *Gastroenterology* 2003; **125**: 58-69
42 **Palatka K**, Serfozo Z, Veréb Z, Hargitay Z, Lontay B, Erdodi F, Bánfalvi G, Nemes Z, Udvardy M, Altorjay I. Changes in the expression and distribution of the inducible and endothelial nitric oxide synthase in mucosal biopsy specimens of inflammatory bowel disease. *Scand J Gastroenterol* 2005; **40**: 670-680
43 **Sasaki M**, Bharwani S, Jordan P, Elrod JW, Grisham MB, Jackson TH, Lefer DJ, Alexander JS. Increased disease activity in eNOS-deficient mice in experimental colitis. *Free Radic Biol Med* 2003; **35**: 1679-1687
44 **Oshima T**, Jordan P, Grisham MB, Alexander JS, Jennings M, Sasaki M, Manas K. TNF-alpha induced endothelial MAdCAM-1 expression is regulated by exogenous, not endogenous nitric oxide. *BMC Gastroenterol* 2001; **1**: 5
45 **Kevil CG**, Ohno N, Gute DC, Okayama N, Robinson SA, Chaney E, Alexander JS. Role of cadherin internalization in hydrogen peroxide-mediated endothelial permeability. *Free Radic Biol Med* 1998; **24**: 1015-1022
46 **Kevil CG**, Oshima T, Alexander B, Coe LL, Alexander JS. H(2)O(2)-mediated permeability: role of MAPK and occludin. *Am J Physiol Cell Physiol* 2000; **279**: C21-C30
47 **Kevil CG**, Okayama N, Alexander JS. H(2)O(2)-mediated permeability II: importance of tyrosine phosphatase and kinase activity. *Am J Physiol Cell Physiol* 2001; **281**: C1940-C1947
48 **Palatka K**, Serfozo Z, Veréb Z, Bátori R, Lontay B, Hargitay Z, Nemes Z, Udvardy M, Erdodi F, Altorjay I. Effect of IBD sera on expression of inducible and endothelial nitric oxide synthase in human umbilical vein endothelial cells. *World J Gastroenterol* 2006; **12**: 1730-1738

49 **Krieglstein CF**, Anthoni C, Cerwinka WH, Stokes KY, Russell J, Grisham MB, Granger DN. Role of blood- and tissue-associated inducible nitric-oxide synthase in colonic inflammation. *Am J Pathol* 2007; **170**: 490-496
50 **Aoi Y**, Terashima S, Ogura M, Nishio H, Kato S, Takeuchi K. Roles of nitric oxide (NO) and NO synthases in healing of dextran sulfate sodium-induced rat colitis. *J Physiol Pharmacol* 2008; **59**: 315-336
51 **Elrod JW**, Laroux FS, Houghton J, Carpenter A, Ando T, Jennings MH, Grisham M, Walker N, Alexander JS. DSS-induced colitis is exacerbated in STAT-6 knockout mice. *Inflamm Bowel Dis* 2005; **11**: 883-889
52 **Van Crombruggen K**, Van Nassauw L, Demetter P, Cuvelier C, Timmermans JP, Lefebvre RA. Influence of soluble guanylate cyclase inhibition on inflammation and motility disturbances in DSS-induced colitis. *Eur J Pharmacol* 2008; **579**: 337-349
53 **Conner EM**, Brand S, Davis JM, Laroux FS, Palombella VJ, Fuseler JW, Kang DY, Wolf RE, Grisham MB. Proteasome inhibition attenuates nitric oxide synthase expression, VCAM-1 transcription and the development of chronic colitis. *J Pharmacol Exp Ther* 1997; **282**: 1615-1622
54 **Grisham MB**, Palombella VJ, Elliott PJ, Conner EM, Brand S, Wong HL, Pien C, Mazzola LM, Destree A, Parent L, Adams J. Inhibition of NF-kappa B activation in vitro and in vivo: role of 26S proteasome. *Methods Enzymol* 1999; **300**: 345-363
55 **Deban L**, Correale C, Vetrano S, Malesci A, Danese S. Multiple pathogenic roles of microvasculature in inflammatory bowel disease: a Jack of all trades. *Am J Pathol* 2008; **172**: 1457-1466
56 **Ogawa H**, Rafiee P, Heidemann J, Fisher PJ, Johnson NA, Otterson MF, Kalyanaraman B, Pritchard KA Jr, Binion DG. Mechanisms of endotoxin tolerance in human intestinal microvascular endothelial cells. *J Immunol* 2003; **170**: 5956-5964
57 **Gewirtz AT**. Flag in the crossroads: flagellin modulates innate and adaptive immunity. *Curr Opin Gastroenterol* 2006; **22**: 8-12
58 **Hokari R**, Miura S. Neutrophil elastase in colitis: more than a marker of disease activity? *J Gastroenterol* 2006; **41**: 395-396
59 **Morohoshi Y**, Matsuoka K, Chinen H, Kamada N, Sato T, Hisamatsu T, Okamoto S, Inoue N, Takaishi H, Ogata H, Iwao Y, Hibi T. Inhibition of neutrophil elastase prevents the development of murine dextran sulfate sodium-induced colitis. *J Gastroenterol* 2006; **41**: 318-324
60 **Maaser C**, Heidemann J, von Eiff C, Lugering A, Spahn TW, Binion DG, Domschke W, Lugering N, Kucharzik T. Human intestinal microvascular endothelial cells express Toll-like receptor 5: a binding partner for bacterial flagellin. *J Immunol* 2004; **172**: 5056-5062
61 **Vijay-Kumar M**, Aitken JD, Gewirtz AT. Toll like receptor-5: protecting the gut from enteric microbes. *Semin Immunopathol* 2008; **30**: 11-21
62 **Vijay-Kumar M**, Sanders CJ, Taylor RT, Kumar A, Aitken JD, Sitaraman SV, Neish AS, Uematsu S, Akira S, Williams IR, Gewirtz AT. Deletion of TLR5 results in spontaneous colitis in mice. *J Clin Invest* 2007; **117**: 3909-3921
63 **Vijay-Kumar M**, Wu H, Aitken J, Kolachala VL, Neish AS, Sitaraman SV, Gewirtz AT. Activation of toll-like receptor 3 protects against DSS-induced acute colitis. *Inflamm Bowel Dis* 2007; **13**: 856-864
64 **Heidemann J**, Rüther C, Kebschull M, Domschke W, Brüwer M, Koch S, Kucharzik T, Maaser C. Expression of IL-12-related molecules in human intestinal microvascular endothelial cells is regulated by TLR3. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G1315-G1324
65 **Kawachi S**, Morise Z, Jennings SR, Conner E, Cockrell A, Laroux FS, Chervenak RP, Wolcott M, van der Heyde H, Gray L, Feng L, Granger DN, Specian RA, Grisham MB. Cytokine and adhesion molecule expression in SCID mice reconstituted with CD4+ T cells. *Inflamm Bowel Dis* 2000; **6**: 171-180
66 **Kawachi S**, Jennings S, Panes J, Cockrell A, Laroux FS, Gray L, Perry M, van der Heyde H, Balish E, Granger DN, Specian RA, Grisham MB. Cytokine and endothelial cell adhesion molecule expression in interleukin-10-deficient mice. *Am J Physiol Gastrointest Liver Physiol* 2000; **278**: G734-G743
67 **Polzer K**, Baeten D, Soleiman A, Distler J, Gerlag DM, Tak PP, Schett G, Zwerina J. Tumour necrosis factor blockade increases lymphangiogenesis in murine and human arthritic joints. *Ann Rheum Dis* 2008; **67**: 1610-1616
68 **Heidenreich R**, Röcken M, Ghoreschi K. Angiogenesis drives psoriasis pathogenesis. *Int J Exp Pathol* 2009; **90**: 232-248
69 **Hatoum OA**, Heidemann J, Binion DG. The intestinal microvasculature as a therapeutic target in inflammatory bowel disease. *Ann N Y Acad Sci* 2006; **1072**: 78-97
70 **Pufe T**, Lemke A, Kurz B, Petersen W, Tillmann B, Grodzinsky AJ, Mentlein R. Mechanical overload induces VEGF in cartilage discs via hypoxia-inducible factor. *Am J Pathol* 2004; **164**: 185-192
71 **Danese S**, Sans M, Scaldaferri F, Sgambato A, Rutella S, Cittadini A, Piqué JM, Panes J, Katz JA, Gasbarrini A, Fiocchi C. TNF-alpha blockade down-regulates the CD40/CD40L pathway in the mucosal microcirculation: a novel anti-inflammatory mechanism of infliximab in Crohn's disease. *J Immunol* 2006; **176**: 2617-2624
72 **Romer LH**, McLean NV, Yan HC, Daise M, Sun J, DeLisser HM. IFN-gamma and TNF-alpha induce redistribution of PECAM-1 (CD31) on human endothelial cells. *J Immunol* 1995; **154**: 6582-6592
73 **Kosmadaki MG**, Yaar M, Arble BL, Gilchrest BA. UV induces VEGF through a TNF-alpha independent pathway. *FASEB J* 2003; **17**: 446-448
74 **Scheinin T**, Butler DM, Salway F, Scallon B, Feldmann M. Validation of the interleukin-10 knockout mouse model of colitis: antitumour necrosis factor-antibodies suppress the progression of colitis. *Clin Exp Immunol* 2003; **133**: 38-43
75 **Lindsay JO**, Ciesielski CJ, Scheinin T, Brennan FM, Hodgson HJ. Local delivery of adenoviral vectors encoding murine interleukin 10 induces colonic interleukin 10 production and is therapeutic for murine colitis. *Gut* 2003; **52**: 981-987
76 **Lindsay J**, Van Montfrans C, Brennan F, Van Deventer S, Drillenburg P, Hodgson H, Te Velde A, Sol Rodriguez Pena M. IL-10 gene therapy prevents TNBS-induced colitis. *Gene Ther* 2002; **9**: 1715-1721
77 **Papadakis KA**. Chemokines in inflammatory bowel disease. *Curr Allergy Asthma Rep* 2004; **4**: 83-89
78 **Barcelos LS**, Coelho AM, Russo RC, Guabiraba R, Souza AL, Bruno-Lima G Jr, Proudfoot AE, Andrade SP, Teixeira MM. Role of the chemokines CCL3/MIP-1 alpha and CCL5/RANTES in sponge-induced inflammatory angiogenesis in mice. *Microvasc Res* 2009; **78**: 148-154
79 **Wu Y**, Li YY, Matsushima K, Baba T, Mukaida N. CCL3-CCR5 axis regulates intratumoral accumulation of leukocytes and fibroblasts and promotes angiogenesis in murine lung metastasis process. *J Immunol* 2008; **181**: 6384-6393
80 **Eyman D**, Damodarasamy M, Plymate SR, Reed MJ. CCL5 secreted by senescent aged fibroblasts induces proliferation of prostate epithelial cells and expression of genes that modulate angiogenesis. *J Cell Physiol* 2009; **220**: 376-381
81 **Rivera-Nieves J**, Ho J, Bamias G, Ivashkina N, Ley K, Oppermann M, Cominelli F. Antibody blockade of CCL25/CCR9 ameliorates early but not late chronic murine ileitis. *Gastroenterology* 2006; **131**: 1518-1529
82 **Heidemann J**, Ogawa H, Dwinell MB, Rafiee P, Maaser C, Gockel HR, Otterson MF, Ota DM, Lugering N, Domschke W, Binion DG. Angiogenic effects of interleukin 8 (CXCL8) in human intestinal microvascular endothelial cells are mediated by CXCR2. *J Biol Chem* 2003; **278**: 8508-8515
83 **Utgaard JO**, Jahnsen FL, Bakka A, Brandtzaeg P, Haraldsen G. Rapid secretion of prestored interleukin 8 from Weibel-

Palade bodies of microvascular endothelial cells. *J Exp Med* 1998; **188**: 1751-1756
84 **Gijsbers K**, Van Assche G, Joossens S, Struyf S, Proost P, Rutgeerts P, Geboes K, Van Damme J. CXCR1-binding chemokines in inflammatory bowel diseases: down-regulated IL-8/CXCL8 production by leukocytes in Crohn's disease and selective GCP-2/CXCL6 expression in inflamed intestinal tissue. *Eur J Immunol* 2004; **34**: 1992-2000
85 **Lügering N**, Kucharzik T, Gockel H, Sorg C, Stoll R, Domschke W. Human intestinal epithelial cells down-regulate IL-8 expression in human intestinal microvascular endothelial cells; role of transforming growth factor-beta 1 (TGF-beta1). *Clin Exp Immunol* 1998; **114**: 377-384
86 **Kanazawa S**, Tsunoda T, Onuma E, Majima T, Kagiyama M, Kikuchi K. VEGF, basic-FGF, and TGF-beta in Crohn's disease and ulcerative colitis: a novel mechanism of chronic intestinal inflammation. *Am J Gastroenterol* 2001; **96**: 822-828
87 **Scaldaferri F**, Sans M, Vetrano S, Correale C, Arena V, Pagano N, Rando G, Romeo F, Potenza AE, Repici A, Malesci A, Danese S. The role of MAPK in governing lymphocyte adhesion to and migration across the microvasculature in inflammatory bowel disease. *Eur J Immunol* 2009; **39**: 290-300
88 **Scaldaferri F**, Correale C, Gasbarrini A, Danese S. Molecular signaling blockade as a new approach to inhibit leukocyte-endothelial interactions for inflammatory bowel disease treatment. *Cell Adh Migr* 2009; **3**: 296-299
89 **Imaizumi T**, Yoshida H, Satoh K. Regulation of CX3CL1/fractalkine expression in endothelial cells. *J Atheroscler Thromb* 2004; **11**: 15-21
90 **Nishimura M**, Kuboi Y, Muramoto K, Kawano T, Imai T. Chemokines as novel therapeutic targets for inflammatory bowel disease. *Ann N Y Acad Sci* 2009; **1173**: 350-356
91 **Sans M**, Danese S, de la Motte C, de Souza HS, Rivera-Reyes BM, West GA, Phillips M, Katz JA, Fiocchi C. Enhanced recruitment of CX3CR1+ T cells by mucosal endothelial cell-derived fractalkine in inflammatory bowel disease. *Gastroenterology* 2007; **132**: 139-153
92 **Heidemann J**, Ogawa H, Rafiee P, Lügering N, Maaser C, Domschke W, Binion DG, Dwinell MB. Mucosal angiogenesis regulation by CXCR4 and its ligand CXCL12 expressed by human intestinal microvascular endothelial cells. *Am J Physiol Gastrointest Liver Physiol* 2004; **286**: G1059-G1068
93 **Schober A**. Chemokines in vascular dysfunction and remodeling. *Arterioscler Thromb Vasc Biol* 2008; **28**: 1950-1959
94 **Hristov M**, Zernecke A, Schober A, Weber C. Adult progenitor cells in vascular remodeling during atherosclerosis. *Biol Chem* 2008; **389**: 837-844
95 **Garolla A**, D'Incà R, Checchin D, Biagioli A, De Toni L, Nicoletti V, Scarpa M, Bolzonello E, Sturniolo GC, Foresta C. Reduced endothelial progenitor cell number and function in inflammatory bowel disease: a possible link to the pathogenesis. *Am J Gastroenterol* 2009; **104**: 2500-2507
96 **Ikematsu S**, Yano A, Aridome K, Kikuchi M, Kumai H, Nagano H, Okamoto K, Oda M, Sakuma S, Aikou T, Muramatsu H, Kadomatsu K, Muramatsu T. Serum midkine levels are increased in patients with various types of carcinomas. *Br J Cancer* 2000; **83**: 701-706
97 **Muramatsu T**. Midkine and pleiotrophin: two related proteins involved in development, survival, inflammation and tumorigenesis. *J Biochem* 2002; **132**: 359-371
98 **Qi M**, Ikematsu S, Ichihara-Tanaka K, Sakuma S, Muramatsu T, Kadomatsu K. Midkine rescues Wilms' tumor cells from cisplatin-induced apoptosis: regulation of Bcl-2 expression by Midkine. *J Biochem* 2000; **127**: 269-277
99 **Krzystek-Korpacka M**, Neubauer K, Matusiewicz M. Clinical relevance of circulating midkine in ulcerative colitis. *Clin Chem Lab Med* 2009; **47**: 1085-1090
100 **Tsiolakidou G**, Koutroubakis IE, Tzardi M, Kouroumalis EA. Increased expression of VEGF and CD146 in patients with inflammatory bowel disease. *Dig Liver Dis* 2008; **40**: 673-679
101 **Kapsoritakis A**, Sfiridaki A, Maltezos E, Simopoulos K, Giatromanolaki A, Sivridis E, Koukourakis MI. Vascular endothelial growth factor in inflammatory bowel disease. *Int J Colorectal Dis* 2003; **18**: 418-422
102 **Koizumi M**, King N, Lobb R, Benjamin C, Podolsky DK. Expression of vascular adhesion molecules in inflammatory bowel disease. *Gastroenterology* 1992; **103**: 840-847
103 **Sumi Y**, Muramatsu H, Takei Y, Hata K, Ueda M, Muramatsu T. Midkine, a heparin-binding growth factor, promotes growth and glycosaminoglycan synthesis of endothelial cells through its action on smooth muscle cells in an artificial blood vessel model. *J Cell Sci* 2002; **115**: 2659-2667
104 **McDonald SA**, Palmen MJ, Van Rees EP, MacDonald TT. Characterization of the mucosal cell-mediated immune response in IL-2 knockout mice before and after the onset of colitis. *Immunology* 1997; **91**: 73-80
105 **Burns RC**, Rivera-Nieves J, Moskaluk CA, Matsumoto S, Cominelli F, Ley K. Antibody blockade of ICAM-1 and VCAM-1 ameliorates inflammation in the SAMP-1/Yit adoptive transfer model of Crohn's disease in mice. *Gastroenterology* 2001; **121**: 1428-1436
106 **Iijima H**, Neurath MF, Nagaishi T, Glickman JN, Nieuwenhuis EE, Nakajima A, Chen D, Fuss IJ, Utku N, Lewicki DN, Becker C, Gallagher TM, Holmes KV, Blumberg RS. Specific regulation of T helper cell 1-mediated murine colitis by CEACAM1. *J Exp Med* 2004; **199**: 471-482
107 **Oshima T**, Pavlick KP, Laroux FS, Verma SK, Jordan P, Grisham MB, Williams L, Alexander JS. Regulation and distribution of MAdCAM-1 in endothelial cells in vitro. *Am J Physiol Cell Physiol* 2001; **281**: C1096-C1105
108 **Bardin N**, Reumaux D, Geboes K, Colombel JF, Blot-Chabaud M, Sampol J, Duthilleul P, Dignat-George F. Increased expression of CD146, a new marker of the endothelial junction in active inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 16-21
109 **Magro F**, Araujo F, Pereira P, Meireles E, Diniz-Ribeiro M, Velosom FT. Soluble selectins, sICAM, sVCAM, and angiogenic proteins in different activity groups of patients with inflammatory bowel disease. *Dig Dis Sci* 2004; **49**: 1265-1274
110 **Newman W**, Mirabelli CK. The link between inflammatory disease and patterns of leukocyte recruitment. *Expert Opin Investig Drugs* 1998; **7**: 19-25
111 **Sasaki M**, Jordan P, Welbourne T, Minagar A, Joh T, Itoh M, Elrod JW, Alexander JS. Troglitazone, a PPAR-gamma activator prevents endothelial cell adhesion molecule expression and lymphocyte adhesion mediated by TNF-alpha. *BMC Physiol* 2005; **5**: 3
112 **Davenpeck KL**, Gauthier TW, Albertine KH, Lefer AM. Role of P-selectin in microvascular leukocyte-endothelial interaction in splanchnic ischemia-reperfusion. *Am J Physiol* 1994; **267**: H622-H630
113 **Kobayashi M**, Fukuda M, Nakayama J. Role of sulfated O-glycans expressed by high endothelial venule-like vessels in pathogenesis of chronic inflammatory gastrointestinal diseases. *Biol Pharm Bull* 2009; **32**: 774-779
114 **Rivera-Nieves J**, Burcin TL, Olson TS, Morris MA, McDuffie M, Cominelli F, Ley K. Critical role of endothelial P-selectin glycoprotein ligand 1 in chronic murine ileitis. *J Exp Med* 2006; **203**: 907-917
115 **Fägerstam JP**, Whiss PA. Higher platelet P-selectin in male patients with inflammatory bowel disease compared to healthy males. *World J Gastroenterol* 2006; **12**: 1270-1272
116 **Rho YH**, Chung CP, Oeser A, Solus J, Asanuma Y, Sokka T, Pincus T, Raggi P, Gebretsadik T, Shintani A, Stein CM. Inflammatory mediators and premature coronary atherosclerosis in rheumatoid arthritis. *Arthritis Rheum* 2009; **61**: 1580-1585
117 **Zittermann SI**, Issekutz AC. Endothelial growth factors VEGF and bFGF differentially enhance monocyte and neu-

trophil recruitment to inflammation. *J Leukoc Biol* 2006; **80**: 247-257
118 **Kobayashi M**, Hoshino H, Masumoto J, Fukushima M, Suzawa K, Kageyama S, Suzuki M, Ohtani H, Fukuda M, Nakayama J. GlcNAc6ST-1-mediated decoration of MAdCAM-1 protein with L-selectin ligand carbohydrates directs disease activity of ulcerative colitis. *Inflamm Bowel Dis* 2009; **15**: 697-706
119 **Briskin M**, Winsor-Hines D, Shyjan A, Cochran N, Bloom S, Wilson J, McEvoy LM, Butcher EC, Kassam N, Mackay CR, Newman W, Ringler DJ. Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue. *Am J Pathol* 1997; **151**: 97-110
120 **Burgio VL**, Fais S, Boirivant M, Perrone A, Pallone F. Peripheral monocyte and naive T-cell recruitment and activation in Crohn's disease. *Gastroenterology* 1995; **109**: 1029-1038
121 **Mizushima T**, Sasaki M, Ando T, Wada T, Tanaka M, Okamoto Y, Ebi M, Hirata Y, Murakami K, Mizoshita T, Shimura T, Kubota E, Ogasawara N, Tanida S, Kataoka H, Kamiya T, Alexander JS, Joh T. Blockage of angiotensin II type 1 receptor regulates TNF-alpha-induced MAdCAM-1 expression via inhibition of NF-kappaB translocation to the nucleus and ameliorates colitis. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G255-G266
122 **Alexander JS**, Ando T. Density-dependent control of MAdCAM-1 and chronic inflammation. Focus on "Mechanisms of MAdCAM-1 gene expression in human intestinal microvascular endothelial cells". *Am J Physiol Cell Physiol* 2005; **288**: C243-C244
123 **Ando T**, Jordan P, Wang Y, Itoh M, Joh T, Sasaki M, Elrod JW, Carpenter A, Jennings MH, Minagar A, Alexander JS. MAdCAM-1 expression and regulation in murine colonic endothelial cells in vitro. *Inflamm Bowel Dis* 2005; **11**: 258-264
124 **Lu M**, Perez VL, Ma N, Miyamoto K, Peng HB, Liao JK, Adamis AP. VEGF increases retinal vascular ICAM-1 expression in vivo. *Invest Ophthalmol Vis Sci* 1999; **40**: 1808-1812
125 **Goebel S**, Huang M, Davis WC, Jennings M, Siahaan TJ, Alexander JS, Kevil CG. VEGF-A stimulation of leukocyte adhesion to colonic microvascular endothelium: implications for inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G648-G654
126 **Ostanin DV**, Bao J, Koboziev I, Gray L, Robinson-Jackson SA, Kosloski-Davidson M, Price VH, Grisham MB. T cell transfer model of chronic colitis: concepts, considerations, and tricks of the trade. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G135-G146
127 **Binion DG**, Heidemann J, Li MS, Nelson VM, Otterson MF, Rafiee P. Vascular cell adhesion molecule-1 expression in human intestinal microvascular endothelial cells is regulated by PI 3-kinase/Akt/MAPK/NF-kappaB: inhibitory role of curcumin. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G259-G268
128 **Kim I**, Moon SO, Kim SH, Kim HJ, Koh YS, Koh GY. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. *J Biol Chem* 2001; **276**: 7614-7620
129 **Sans M**, Fuster D, Vázquez A, Setoain FJ, Piera C, Piqué JM, Panés J. 123Iodine-labelled anti-VCAM-1 antibody scintigraphy in the assessment of experimental colitis. *Eur J Gastroenterol Hepatol* 2001; **13**: 31-38
130 **Soriano A**, Salas A, Salas A, Sans M, Gironella M, Elena M, Anderson DC, Piqué JM, Panés J. VCAM-1, but not ICAM-1 or MAdCAM-1, immunoblockade ameliorates DSS-induced colitis in mice. *Lab Invest* 2000; **80**: 1541-1551
131 **Rijcken E**, Mennigen RB, Schaefer SD, Laukoetter MG, Anthoni C, Spiegel HU, Bruewer M, Senninger N, Krieglstein CF. PECAM-1 (CD 31) mediates transendothelial leukocyte migration in experimental colitis. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G446-G452
132 **Elshal MF**, Khan SS, Raghavachari N, Takahashi Y, Barb J, Bailey JJ, Munson PJ, Solomon MA, Danner RL, McCoy JP Jr. A unique population of effector memory lymphocytes identified by CD146 having a distinct immunophenotypic and genomic profile. *BMC Immunol* 2007; **8**: 29
133 **Elshal MF**, Khan SS, Takahashi Y, Solomon MA, McCoy JP Jr. CD146 (Mel-CAM), an adhesion marker of endothelial cells, is a novel marker of lymphocyte subset activation in normal peripheral blood. *Blood* 2005; **106**: 2923-2924
134 **Covas DT**, Panepucci RA, Fontes AM, Silva WA Jr, Orellana MD, Freitas MC, Neder L, Santos AR, Peres LC, Jamur MC, Zago MA. Multipotent mesenchymal stromal cells obtained from diverse human tissues share functional properties and gene-expression profile with CD146+ perivascular cells and fibroblasts. *Exp Hematol* 2008; **36**: 642-654
135 **Bardin N**, Blot-Chabaud M, Despoix N, Kebir A, Harhouri K, Arsanto JP, Espinosa L, Perrin P, Robert S, Vely F, Sabatier F, Le Bivic A, Kaplanski G, Sampol J, Dignat-George F. CD146 and its soluble form regulate monocyte transendothelial migration. *Arterioscler Thromb Vasc Biol* 2009; **29**: 746-753
136 **Tabibian JH**, Roth BE. Local thrombolysis: a newer approach to treating inflammatory bowel disease-related thromboembolism. *J Clin Gastroenterol* 2009; **43**: 391-398
137 **Hatoum OA**, Spinelli KS, Abu-Hajir M, Attila T, Franco J, Otterson MF, Telford GL, Binion DG. Mesenteric venous thrombosis in inflammatory bowel disease. *J Clin Gastroenterol* 2005; **39**: 27-31
138 **Bernstein C**N, Sargent M, Vos HL, Rosendaal FR. Mutations in clotting factors and inflammatory bowel disease. *Am J Gastroenterol* 2007; **102**: 338-343
139 **Dardik R**, Loscalzo J, Eskaraev R, Inbal A. Molecular mechanisms underlying the proangiogenic effect of factor XIII. *Arterioscler Thromb Vasc Biol* 2005; **25**: 526-532
140 **Vrij AA**, Rijken J, van Wersch JW, Stockbrügger RW. Differential behavior of coagulation factor XIII in patients with inflammatory bowel disease and in patients with giant cell arteritis. *Haemostasis* 1999; **29**: 326-335
141 **Danese S**, Katz JA, Saibeni S, Papa A, Gasbarrini A, Vecchi M, Fiocchi C. Activated platelets are the source of elevated levels of soluble CD40 ligand in the circulation of inflammatory bowel disease patients. *Gut* 2003; **52**: 1435-1441
142 **Danese S**, de la Motte C, Sturm A, Vogel JD, West GA, Strong SA, Katz JA, Fiocchi C. Platelets trigger a CD40-dependent inflammatory response in the microvasculature of inflammatory bowel disease patients. *Gastroenterology* 2003; **124**: 1249-1264
143 **Vogel JD**, West GA, Danese S, De La Motte C, Phillips MH, Strong SA, Willis J, Fiocchi C. CD40-mediated immune-nonimmune cell interactions induce mucosal fibroblast chemokines leading to T-cell transmigration. *Gastroenterology* 2004; **126**: 63-80
144 **Srirajaskanthan R**, Winter M, Muller AF. Venous thrombosis in inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2005; **17**: 697-700
145 **Hathaway CA**, Appleyard CB, Percy WH, Williams JL. Experimental colitis increases blood-brain barrier permeability in rabbits. *Am J Physiol* 1999; **276**: G1174-G1180
146 **Koutroubakis IE**, Xidakis C, Karmiris K, Sfiridaki A, Kandidaki E, Kouroumalis EA. Serum angiogenin in inflammatory bowel disease. *Dig Dis Sci* 2004; **49**: 1758-1762
147 **Im E**, Choi YJ, Kim CH, Fiocchi C, Pothoulakis C, Rhee SH. The angiogenic effect of probiotic Bacillus polyfermenticus on human intestinal microvascular endothelial cells is mediated by IL-8. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G999-G1008
148 **Roessner A**, Kuester D, Malfertheiner P, Schneider-Stock R. Oxidative stress in ulcerative colitis-associated carcinogenesis. *Pathol Res Pract* 2008; **204**: 511-524

149 **Tolstanova G**, Khomenko T, Deng X, Chen L, Tarnawski A, Ahluwalia A, Szabo S, Sandor Z. Neutralizing anti-vascular endothelial growth factor (VEGF) antibody reduces severity of experimental ulcerative colitis in rats: direct evidence for the pathogenic role of VEGF. *J Pharmacol Exp Ther* 2009; **328**: 749-757

150 **Alexander J**. Extracellular matrix, junctional integrity and matrix metalloproteinase interactions. *J Anat* 2002; **200**: 525

151 **Allport JR**, Muller WA, Luscinskas FW. Monocytes induce reversible focal changes in vascular endothelial cadherin complex during transendothelial migration under flow. *J Cell Biol* 2000; **148**: 203-216

152 **Weber C**, Fraemohs L, Dejana E. The role of junctional adhesion molecules in vascular inflammation. *Nat Rev Immunol* 2007; **7**: 467-477

153 **Meijer MJ**, Mieremet-Ooms MA, van der Zon AM, van Duijn W, van Hogezand RA, Sier CF, Hommes DW, Lamers CB, Verspaget HW. Increased mucosal matrix metalloproteinase-1, -2, -3 and -9 activity in patients with inflammatory bowel disease and the relation with Crohn's disease phenotype. *Dig Liver Dis* 2007; **39**: 733-739

154 **Mosnier JF**, Jarry A, Bou-Hanna C, Denis MG, Merlin D, Laboisse CL. ADAM15 upregulation and interaction with multiple binding partners in inflammatory bowel disease. *Lab Invest* 2006; **86**: 1064-1073

155 **Alexander JS**, Zhu Y, Elrod JW, Alexander B, Coe L, Kalogeris TJ, Fuseler J. Reciprocal regulation of endothelial substrate adhesion and barrier function. *Microcirculation* 2001; **8**: 389-401

156 **Carden D**, Xiao F, Moak C, Willis BH, Robinson-Jackson S, Alexander S. Neutrophil elastase promotes lung microvascular injury and proteolysis of endothelial cadherins. *Am J Physiol* 1998; **275**: H385-H392

157 **Orlova VV**, Economopoulou M, Lupu F, Santoso S, Chavakis T. Junctional adhesion molecule-C regulates vascular endothelial permeability by modulating VE-cadherin-mediated cell-cell contacts. *J Exp Med* 2006; **203**: 2703-2714

158 **Spalinger J**, Patriquin H, Miron MC, Marx G, Herzog D, Dubois J, Dubinsky M, Seidman EG. Doppler US in patients with crohn disease: vessel density in the diseased bowel reflects disease activity. *Radiology* 2000; **217**: 787-791

159 **Danese S**, Sans M, de la Motte C, Graziani C, West G, Phillips MH, Pola R, Rutella S, Willis J, Gasbarrini A, Fiocchi C. Angiogenesis as a novel component of inflammatory bowel disease pathogenesis. *Gastroenterology* 2006; **130**: 2060-2073

160 **Danese S**, Papa A, Saibeni S, Repici A, Malesci A, Vecchi M. Inflammation and coagulation in inflammatory bowel disease: The clot thickens. *Am J Gastroenterol* 2007; **102**: 174-186

161 **Wakefield AJ**, Sankey EA, Dhillon AP, Sawyerr AM, More L, Sim R, Pittilo RM, Rowles PM, Hudson M, Lewis AA. Granulomatous vasculitis in Crohn's disease. *Gastroenterology* 1991; **100**: 1279-1287

162 **Ginnan R**, Guikema BJ, Halligan KE, Singer HA, Jourd'heuil D. Regulation of smooth muscle by inducible nitric oxide synthase and NADPH oxidase in vascular proliferative diseases. *Free Radic Biol Med* 2008; **44**: 1232-1245

163 **Ganta VC**, Cromer W, Mills GL, Traylor J, Jennings M, Daley S, Clark B, Mathis JM, Bernas M, Boktor M, Jordan P, Witte M, Alexander JS. Angiopoietin-2 in experimental colitis. *Inflamm Bowel Dis* 2010; **16**: 1029-1039

164 **Morris GP**, Beck PL, Herridge MS, Depew WT, Szewczuk MR, Wallace JL. Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterology* 1989; **96**: 795-803

165 **Peterson TC**, Cleary CE, Shaw AM, Malatjalian DA, Veldhuyzen van Zanten SJ. Therapeutic role for bismuth compounds in TNBS-induced colitis in the rat. *Dig Dis Sci* 2000; **45**: 466-473

166 **Chidlow JH Jr**, Langston W, Greer JJ, Ostanin D, Abdelbaqi M, Houghton J, Senthilkumar A, Shukla D, Mazar AP, Grisham MB, Kevil CG. Differential angiogenic regulation of experimental colitis. *Am J Pathol* 2006; **169**: 2014-2030

167 **Scaldaferri F**, Vetrano S, Sans M, Arena V, Straface G, Stigliano E, Repici A, Sturm A, Malesci A, Panes J, Yla-Herttuala S, Fiocchi C, Danese S. VEGF-A links angiogenesis and inflammation in inflammatory bowel disease pathogenesis. *Gastroenterology* 2009; **136**: 585-95.e5

168 **Kenet G**, Wardi J, Avni Y, Aeed H, Shirin H, Zaidel L, Hershkoviz R, Bruck R. Amelioration of experimental colitis by thalidomide. *Isr Med Assoc J* 2001; **3**: 644-648

169 **Maconi G**, Sampietro GM, Russo A, Bollani S, Cristaldi M, Parente F, Dottorini F, Bianchi Porro G. The vascularity of internal fistulae in Crohn's disease: an in vivo power Doppler ultrasonography assessment. *Gut* 2002; **50**: 496-500

170 **Wallace JL**, Le T, Carter L, Appleyard CB, Beck PL. Hapten-induced chronic colitis in the rat: alternatives to trinitrobenzene sulfonic acid. *J Pharmacol Toxicol Methods* 1995; **33**: 237-239

171 **Funayama Y**, Sasaki I, Naito H, Fukushima K, Matsuno S, Masuda T. Remodeling of vascular wall in Crohn's disease. *Dig Dis Sci* 1999; **44**: 2319-2323

172 **Angerson WJ**, Allison MC, Baxter JN, Russell RI. Neoterminal ileal blood flow after ileocolonic resection for Crohn's disease. *Gut* 1993; **34**: 1531-1534

173 **Olsson AK**, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. *Nat Rev Mol Cell Biol* 2006; **7**: 359-371

174 **Usui T**, Ishida S, Yamashiro K, Kaji Y, Poulaki V, Moore J, Moore T, Amano S, Horikawa Y, Dartt D, Golding M, Shima DT, Adamis AP. VEGF164(165) as the pathological isoform: differential leukocyte and endothelial responses through VEGFR1 and VEGFR2. *Invest Ophthalmol Vis Sci* 2004; **45**: 368-374

175 **Bousvaros A**, Leichtner A, Zurakowski D, Kwon J, Law T, Keough K, Fishman S. Elevated serum vascular endothelial growth factor in children and young adults with Crohn's disease. *Dig Dis Sci* 1999; **44**: 424-430

176 **Ozawa CR**, Banfi A, Glazer NL, Thurston G, Springer ML, Kraft PE, McDonald DM, Blau HM. Microenvironmental VEGF concentration, not total dose, determines a threshold between normal and aberrant angiogenesis. *J Clin Invest* 2004; **113**: 516-527

177 **Danese S**, Sans M, Spencer DM, Beck I, Doñate F, Plunkett ML, de la Motte C, Redline R, Shaw DE, Levine AD, Mazar AP, Fiocchi C. Angiogenesis blockade as a new therapeutic approach to experimental colitis. *Gut* 2007; **56**: 855-862

178 **Appleton I**, Brown NJ, Willis D, Colville-Nash PR, Alam C, Brown JR, Willoughby DA. The role of vascular endothelial growth factor in a murine chronic granulomatous tissue air pouch model of angiogenesis. *J Pathol* 1996; **180**: 90-94

179 **Edelman JL**, Castro MR, Wen Y. Correlation of VEGF expression by leukocytes with the growth and regression of blood vessels in the rat cornea. *Invest Ophthalmol Vis Sci* 1999; **40**: 1112-1123

180 **Petersen W**, Tsokos M, Pufe T. Expression of VEGF121 and VEGF165 in hypertrophic chondrocytes of the human growth plate and epiphyseal cartilage. *J Anat* 2002; **201**: 153-157

181 **Lu J**, Kasama T, Kobayashi K, Yoda Y, Shiozawa F, Hanyuda M, Negishi M, Ide H, Adachi M. Vascular endothelial growth factor expression and regulation of murine collagen-induced arthritis. *J Immunol* 2000; **164**: 5922-5927

182 **Kevil CG**, De Benedetti A, Payne DK, Coe LL, Laroux FS, Alexander JS. Translational regulation of vascular permeability factor by eukaryotic initiation factor 4E: implications for tumor angiogenesis. *Int J Cancer* 1996; **65**: 785-790

183 **Griga T**, Gutzeit A, Sommerkamp C, May B. Increased production of vascular endothelial growth factor by peripheral blood mononuclear cells in patients with inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 1999; **11**: 175-179

184 **Birmingham JM**, Busik JV, Hansen-Smith FM, Fenton JI.

Novel mechanism for obesity-induced colon cancer progression. *Carcinogenesis* 2009; **30**: 690-697
185 **Kusumanto YH**, Dam WA, Hospers GA, Meijer C, Mulder NH. Platelets and granulocytes, in particular the neutrophils, form important compartments for circulating vascular endothelial growth factor. *Angiogenesis* 2003; **6**: 283-287
186 **Kucharzik T**, Walsh SV, Chen J, Parkos CA, Nusrat A. Neutrophil transmigration in inflammatory bowel disease is associated with differential expression of epithelial intercellular junction proteins. *Am J Pathol* 2001; **159**: 2001-2009
187 **Perminow G**, Reikvam DH, Lyckander LG, Brandtzaeg P, Vatn MH, Carlsen HS. Increased number and activation of colonic macrophages in pediatric patients with untreated Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 1368-1378
188 **Upile T**, Jerjes W, Kafas P, Harini S, Singh SU, Guyer M, Bentley M, Sudhoff H, Hopper C. Salivary VEGF: a non-invasive angiogenic and lymphangiogenic proxy in head and neck cancer prognostication. *Int Arch Med* 2009; **2**: 12
189 **Krzystek-Korpacka M**, Neubauer K, Matusiewicz M. Platelet-derived growth factor-BB reflects clinical, inflammatory and angiogenic disease activity and oxidative stress in inflammatory bowel disease. *Clin Biochem* 2009; **42**: 1602-1609
190 **Del Zotto B**, Mumolo G, Pronio AM, Montesani C, Tersigni R, Boirivant M. TGF-beta1 production in inflammatory bowel disease: differing production patterns in Crohn's disease and ulcerative colitis. *Clin Exp Immunol* 2003; **134**: 120-126
191 **Beck PL**, Podolsky DK. Growth factors in inflammatory bowel disease. *Inflamm Bowel Dis* 1999; **5**: 44-60
192 **Ma Y**, Guan Q, Bai A, Weiss CR, Hillman CL, Ma A, Zhou G, Qing G, Peng Z. Targeting TGF-beta1 by employing a vaccine ameliorates fibrosis in a mouse model of chronic colitis. *Inflamm Bowel Dis* 2010; **16**: 1040-1050
193 **Rieder F**, Fiocchi C. Intestinal fibrosis in IBD--a dynamic, multifactorial process. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 228-235
194 **Zeisberg EM**, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, Chandraker A, Yuan X, Pu WT, Roberts AB, Neilson EG, Sayegh MH, Izumo S, Kalluri R. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. *Nat Med* 2007; **13**: 952-961
195 **Sundberg C**, Ivarsson M, Gerdin B, Rubin K. Pericytes as collagen-producing cells in excessive dermal scarring. *Lab Invest* 1996; **74**: 452-466
196 **Bousvaros A**, Zurakowski D, Fishman SJ, Keough K, Law T, Sun C, Leichtner AM. Serum basic fibroblast growth factor in pediatric Crohn's disease. Implications for wound healing. *Dig Dis Sci* 1997; **42**: 378-386
197 **Mannaioni PF**, Di Bello MG, Masini E. Platelets and inflammation: role of platelet-derived growth factor, adhesion molecules and histamine. *Inflamm Res* 1997; **46**: 4-18
198 **Di Lorenzo A**, Fernández-Hernando C, Cirino G, Sessa WC. Akt1 is critical for acute inflammation and histamine-mediated vascular leakage. *Proc Natl Acad Sci USA* 2009; **106**: 14552-14557
199 **Dulic-Sills A**, Blunden MJ, Mawdsley J, Bastin AJ, McAuley D, Griffiths M, Rampton DS, Yaqoob MM, Macey MG, Agrawal SG. New flow cytometric technique for the evaluation of circulating endothelial progenitor cell levels in various disease groups. *J Immunol Methods* 2006; **316**: 107-115
200 **Jakobsson** L, Bentley K, Gerhardt H. VEGFRs and Notch: a dynamic collaboration in vascular patterning. *Biochem Soc Trans* 2009; **37**: 1233-1236
201 **Gerhardt H**, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, Betsholtz C. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *J Cell Biol* 2003; **161**: 1163-1177
202 **Imhof BA**, Aurrand-Lions M. Angiogenesis and inflammation face off. *Nat Med* 2006; **12**: 171-172
203 **Fiedler U**, Augustin HG. Angiopoietins: a link between angiogenesis and inflammation. *Trends Immunol* 2006; **27**: 552-558
204 **Fiedler U**, Scharpfenecker M, Koidl S, Hegen A, Grunow V, Schmidt JM, Kriz W, Thurston G, Augustin HG. The Tie-2 ligand angiopoietin-2 is stored in and rapidly released upon stimulation from endothelial cell Weibel-Palade bodies. *Blood* 2004; **103**: 4150-4156
205 **Fujiyama S**, Matsubara H, Nozawa Y, Maruyama K, Mori Y, Tsutsumi Y, Masaki H, Uchiyama Y, Koyama Y, Nose A, Iba O, Tateishi E, Ogata N, Jyo N, Higashiyama S, Iwasaka T. Angiotensin AT(1) and AT(2) receptors differentially regulate angiopoietin-2 and vascular endothelial growth factor expression and angiogenesis by modulating heparin binding-epidermal growth factor (EGF)-mediated EGF receptor transactivation. *Circ Res* 2001; **88**: 22-29
206 **Chen YH**, Wu HL, Chen CK, Huang YH, Yang BC, Wu LW. Angiostatin antagonizes the action of VEGF-A in human endothelial cells via two distinct pathways. *Biochem Biophys Res Commun* 2003; **310**: 804-810
207 **Sandor Z**, Deng XM, Khomenko T, Tarnawski AS, Szabo S. Altered angiogenic balance in ulcerative colitis: a key to impaired healing? *Biochem Biophys Res Commun* 2006; **350**: 147-150
208 **Cornelius LA**, Nehring LC, Harding E, Bolanowski M, Welgus HG, Kobayashi DK, Pierce RA, Shapiro SD. Matrix metalloproteinases generate angiostatin: effects on neovascularization. *J Immunol* 1998; **161**: 6845-6852
209 **Folkman J**. Antiangiogenesis in cancer therapy--endostatin and its mechanisms of action. *Exp Cell Res* 2006; **312**: 594-607
210 **Deng X**, Tolstanova G, Khomenko T, Chen L, Tarnawski A, Szabo S, Sandor Z. Mesalamine restores angiogenic balance in experimental ulcerative colitis by reducing expression of endostatin and angiostatin: novel molecular mechanism for therapeutic action of mesalamine. *J Pharmacol Exp Ther* 2009; **331**: 1071-1078
211 **Ambati BK**, Nozaki M, Singh N, Takeda A, Jani PD, Suthar T, Albuquerque RJ, Richter E, Sakurai E, Newcomb MT, Kleinman ME, Caldwell RB, Lin Q, Ogura Y, Orecchia A, Samuelson DA, Agnew DW, St Leger J, Green WR, Mahasreshti PJ, Curiel DT, Kwan D, Marsh H, Ikeda S, Leiper LJ, Collinson JM, Bogdanovich S, Khurana TS, Shibuya M, Baldwin ME, Ferrara N, Gerber HP, De Falco S, Witta J, Baffi JZ, Raisler BJ, Ambati J. Corneal avascularity is due to soluble VEGF receptor-1. *Nature* 2006; **443**: 993-997
212 **Ambati BK**, Patterson E, Jani P, Jenkins C, Higgins E, Singh N, Suthar T, Vira N, Smith K, Caldwell R. Soluble vascular endothelial growth factor receptor-1 contributes to the corneal antiangiogenic barrier. *Br J Ophthalmol* 2007; **91**: 505-508
213 **Albuquerque RJ**, Hayashi T, Cho WG, Kleinman ME, Dridi S, Takeda A, Baffi JZ, Yamada K, Kaneko H, Green MG, Chappell J, Wilting J, Weich HA, Yamagami S, Amano S, Mizuki N, Alexander JS, Peterson ML, Brekken RA, Hirashima M, Capoor S, Usui T, Ambati BK, Ambati J. Alternatively spliced vascular endothelial growth factor receptor-2 is an essential endogenous inhibitor of lymphatic vessel growth. *Nat Med* 2009; **15**: 1023-1030
214 **Bates DO**, Cui TG, Doughty JM, Winkler M, Sugiono M, Shields JD, Peat D, Gillatt D, Harper SJ. VEGF165b, an inhibitory splice variant of vascular endothelial growth factor, is down-regulated in renal cell carcinoma. *Cancer Res* 2002; **62**: 4123-4131
215 **Perrin RM**, Konopatskaya O, Qiu Y, Harper S, Bates DO, Churchill AJ. Diabetic retinopathy is associated with a switch in splicing from anti- to pro-angiogenic isoforms of vascular endothelial growth factor. *Diabetologia* 2005; **48**: 2422-2427
216 **Varey AH**, Rennel ES, Qiu Y, Bevan HS, Perrin RM, Raffy S, Dixon AR, Paraskeva C, Zaccheo O, Hassan AB, Harper SJ, Bates DO. VEGF 165 b, an antiangiogenic VEGF-A isoform, binds and inhibits bevacizumab treatment in experimental colorectal carcinoma: balance of pro- and antiangiogenic

VEGF-A isoforms has implications for therapy. *Br J Cancer* 2008; **98**: 1366-1379
217 **Nowak DG**, Woolard J, Amin EM, Konopatskaya O, Saleem MA, Churchill AJ, Ladomery MR, Harper SJ, Bates DO. Expression of pro- and anti-angiogenic isoforms of VEGF is differentially regulated by splicing and growth factors. *J Cell Sci* 2008; **121**: 3487-3495
218 **Leong TT**, Fearon U, Veale DJ. Angiogenesis in psoriasis and psoriatic arthritis: clues to disease pathogenesis. *Curr Rheumatol Rep* 2005; **7**: 325-329
219 **Veale DJ**, Fearon U. Inhibition of angiogenic pathways in rheumatoid arthritis: potential for therapeutic targeting. *Best Pract Res Clin Rheumatol* 2006; **20**: 941-947
220 **Cordiali-Fei P**, Trento E, D'Agosto G, Bordignon V, Mussi A, Ardigó M, Mastroianni A, Vento A, Solivetti F, Berardesca E, Ensoli F. Effective therapy with anti-TNF-alpha in patients with psoriatic arthritis is associated with decreased levels of metalloproteinases and angiogenic cytokines in the sera and skin lesions. *Ann N Y Acad Sci* 2007; **1110**: 578-589
221 **Siegmund B**. Targeted therapies in inflammatory bowel disease. *Dig Dis* 2009; **27**: 465-469
222 **Rafiee P**, Heidemann J, Ogawa H, Johnson NA, Fisher PJ, Li MS, Otterson MF, Johnson CP, Binion DG. Cyclosporin A differentially inhibits multiple steps in VEGF induced angiogenesis in human microvascular endothelial cells through altered intracellular signaling. *Cell Commun Signal* 2004; **2**: 3
223 **Carvalho AT**, Souza H, Carneiro AJ, Castelo-Branco M, Madi K, Schanaider A, Silv F, Pereira Junior FA, Pereira MG, Tortori C, Dines I, Carvalho J, Rocha E, Elia C. Therapeutic and prophylactic thalidomide in TNBS-induced colitis: synergistic effects on TNF-alpha, IL-12 and VEGF production. *World J Gastroenterol* 2007; **13**: 2166-2173
224 **Ogawa H**, Rafiee P, Fisher PJ, Johnson NA, Otterson MF, Binion DG. Butyrate modulates gene and protein expression in human intestinal endothelial cells. *Biochem Biophys Res Commun* 2003; **309**: 512-519
225 **Kruh J**, Defer N, Tichonicky L. [Molecular and cellular action of butyrate] *C R Seances Soc Biol Fil* 1992; **186**: 12-25
226 **Ogawa H**, Rafiee P, Fisher PJ, Johnson NA, Otterson MF, Binion DG. Sodium butyrate inhibits angiogenesis of human intestinal microvascular endothelial cells through COX-2 inhibition. *FEBS Lett* 2003; **554**: 88-94
227 **Rafiee P**, Stein DJ, Nelson VM, Otterson MF, Shaker R, Binion DG. Thalidomide inhibits inflammatory and angiogenic activation of human intestinal microvascular endothelial cells (HIMEC). *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G167-G176
228 **Rafiee P**, Johnson CP, Li MS, Ogawa H, Heidemann J, Fisher PJ, Lamirand TH, Otterson MF, Wilson KT, Binion DG. Cyclosporine A enhances leukocyte binding by human intestinal microvascular endothelial cells through inhibition of p38 MAPK and iNOS. Paradoxical proinflammatory effect on the microvascular endothelium. *J Biol Chem* 2002; **277**: 35605-35615
229 **Choi PM**, Zelig MP. Similarity of colorectal cancer in Crohn's disease and ulcerative colitis: implications for carcinogenesis and prevention. *Gut* 1994; **35**: 950-954
230 **Karlén P**, Löfberg R, Broström O, Leijonmarck CE, Hellers G, Persson PG. Increased risk of cancer in ulcerative colitis: a population-based cohort study. *Am J Gastroenterol* 1999; **94**: 1047-1052
231 **Gyde SN**, Prior P, Allan RN, Stevens A, Jewell DP, Truelove SC, Lofberg R, Brostrom O, Hellers G. Colorectal cancer in ulcerative colitis: a cohort study of primary referrals from three centres. *Gut* 1988; **29**: 206-217
232 **Wagner AD**, Arnold D, Grothey AA, Haerting J, Unverzagt S. Anti-angiogenic therapies for metastatic colorectal cancer. *Cochrane Database Syst Rev* 2009; CD005392
233 **Sonoshita M**, Takaku K, Oshima M, Sugihara K, Taketo MM. Cyclooxygenase-2 expression in fibroblasts and endothelial cells of intestinal polyps. *Cancer Res* 2002; **62**: 6846-6849
234 **Oshima M**, Dinchuk JE, Kargman SL, Oshima H, Hancock B, Kwong E, Trzaskos JM, Evans JF, Taketo MM. Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). *Cell* 1996; **87**: 803-809
235 **Oshima M**, Taketo MM. COX selectivity and animal models for colon cancer. *Curr Pharm Des* 2002; **8**: 1021-1034
236 **Paiotti AP**, Miszputen SJ, Oshima CT, de Oliveira Costa H, Ribeiro DA, Franco M. Effect of COX-2 inhibitor after TNBS-induced colitis in Wistar rats. *J Mol Histol* 2009; **40**: 317-324
237 **Binion DG**, Otterson MF, Rafiee P. Curcumin inhibits VEGF-mediated angiogenesis in human intestinal microvascular endothelial cells through COX-2 and MAPK inhibition. *Gut* 2008; **57**: 1509-1517
238 **Klenke FM**, Gebhard MM, Ewerbeck V, Abdollahi A, Huber PE, Sckell A. The selective Cox-2 inhibitor Celecoxib suppresses angiogenesis and growth of secondary bone tumors: an intravital microscopy study in mice. *BMC Cancer* 2006; **6**: 9
239 **Chan AT**. Can aspirin prevent colorectal cancer? *Lancet* 2007; **369**: 1577-1578
240 **Chan AT**, Ogino S, Fuchs CS. Aspirin and the risk of colorectal cancer in relation to the expression of COX-2. N *Engl J Med* 2007; **356**: 2131-2142
241 **Park YS**. [COX-2 inhibitors in inflammatory bowel disease: friends or foes?] *Korean J Gastroenterol* 2007; **50**: 350-355
242 **Wu AW**, Gu J, Li ZF, Ji JF, Xu GW. COX-2 expression and tumor angiogenesis in colorectal cancer. *World J Gastroenterol* 2004; **10**: 2323-2326
243 **Ishikawa TO**, Herschman HR. Tumor formation in a mouse model of colitis-associated colon cancer does not require COX-1 or COX-2 expression. *Carcinogenesis* 2010; **31**: 729-736
244 **Toomey DP**, Murphy JF, Conlon KC. COX-2, VEGF and tumour angiogenesis. *Surgeon* 2009; **7**: 174-180

S- Editor Wang JL **L- Editor** Cant MR **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.594

World J Gastroenterol 2011 February 7; 17(5): 594-608
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

TOPIC HIGHLIGHT

Silvio Danese, MD, PhD, Head, *Series Editor*

Haemostatic system in inflammatory bowel diseases: New players in gut inflammation

Franco Scaldaferri, Stefano Lancellotti, Marco Pizzoferrato, Raimondo De Cristofaro

Franco Scaldaferri, Marco Pizzoferrato, Department of Internal Medicine and Medical Specialties, Gastroenterology Division, Catholic University of Rome, 00168 Rome, Italy
Stefano Lancellotti, Raimondo De Cristofaro, Department of Internal Medicine and Medical Specialties, Haemostasis Research Center, Catholic University of Rome, 00168 Rome, Italy
Author contributions: All authors performed the literature revision and writing; Scaldaferri F and De Cristofaro R also revised and edited the paper.
Supported by Italian Ministry of University, No. PRIN-2007; and Catholic University School of Medicine, No. Linea D1-2009
Correspondence to: Raimondo De Cristofaro, MD, Professor, Department of Internal Medicine and Medical Specialties, Haemostasis Research Center, Catholic University of Rome, Largo Gemelli 8, 00168 Rome, Italy. rdecristofaro@rm.unicatt.it
Telephone: +39-6-30154438 Fax: +39-6-30155915
Received: January 25, 2010 Revised: March 25, 2010
Accepted: April 1, 2010
Published online: February 7, 2011

Abstract

Inflammation and coagulation constantly influence each other and are constantly in balance. Emerging evidence supports this statement in acute inflammatory diseases, such as sepsis, but it also seems to be very important in chronic inflammatory settings, such as inflammatory bowel disease (IBD). Patients with Crohn's disease and ulcerative colitis have an increased risk of thromboembolic events, and several abnormalities concerning coagulation components occur in the endothelial cells of intestinal vessels, where most severe inflammatory abnormalities occur. The aims of this review are to update and classify the type of coagulation system abnormalities in IBD, and analyze the strict and delicate balance between coagulation and inflammation at the mucosal level. Recent studies on possible therapeutic applications arising from investigations on coagulation abnormalities associated with IBD pathogenesis will also be briefly presented and critically reviewed.

Key words: Activated protein C; Coagulation; Crohn's disease; Inflammation; Inflammatory bowel disease; Platelets; Ulcerative colitis

Peer reviewer: Kenji Okajima, Professor, PhD, MD, Department of Translational Medical Science Research, Nagoya City University Graduate School of Medical Sciences, Kawassumi 1, Mizuho-cho, Mizuho-ku, Nagoya 467-8601, Japan

Scaldaferri F, Lancellotti S, Pizzoferrato M, De Cristofaro R. Haemostatic system in inflammatory bowel diseases: New players in gut inflammation. *World J Gastroenterol* 2011; 17(5): 594-608 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/594.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.594

INTRODUCTION

Inflammation and coagulation are two crucial systems in mammals. They constantly influence each other and are constantly in balance. In particular, inflammatory processes can promote coagulation which, in turn, can also sustain inflammation. The inter-dependence of the two processes is confirmed by clinical settings where the inherited or acquired deficiency of natural anticoagulants is associated with an increase in inflammatory processes[1].

This observation is particularly relevant in acute inflammatory diseases, such as sepsis[1], but it also seems to be very important in chronic inflammatory conditions, such as inflammatory bowel disease (IBD).

Patients with Crohn's disease (CD) and ulcerative colitis (UC) have an increased risk of thromboembolic events[2], which appears to be more frequent when IBD is in an active phase[3-5] and is affecting the whole colon[3,6,7]. However, it is worth noting that, in a large study, one-third of thromboembolic complications occurred during

disease quiescence, supporting the hypothesis of a greater prothrombotic tendency in IBD, independent of disease activity[3].

The incidence of thromboembolic events in patients with IBD has been reported to be 1%-8%[4,8,9]. Patients with IBD have a 3-fold increased risk for deep vein thrombosis and pulmonary embolism compared with the general population[8,10,11]. In addition, IBD patients experience more thromboembolic events at a younger age than the general population or patients affected by rheumatoid arthritis or celiac disease[4,8].

Finally, indirect evidence that vascular thrombosis may be involved in the pathogenesis of IBD was provided by an epidemiological study performed on a large cohort of subjects with hemophilia or von Willebrand's disease[3,12]. In this population, in which more than 9000 patients were included (6000 patients with hemophilia and more than 3000 with von Willebrand's disease), IBD occurred less frequently than expected, and it was suggested that inherited hemorrhagic disorders might be protective against IBD[3].

Most available reports tried to explain the increased thromboembolic risk in IBD by analyzing different components of the coagulation cascade, such as serological/phenotypical markers and genetic pro-thrombotic mutations/polymorphisms. Several studies exist on major pro-thrombotic genetic predispositions and IBD. Most published data demonstrate that there is no difference in the prevalence of Factor V Leiden between IBD patients and healthy controls[3,13-15], as well as PT gene G20210A mutation[3,15-17]. Polymorphisms of Methylenetetrahydrofolate reductase, the enzyme involved in the re-methylation pathway of homocysteine metabolism, have been found to have discordant results in IBD patients compared to controls[3,15,18]. Other studies looking at the prevalence of Antithrombin Ⅲ deficiency[17,19], Protein C[20] and Protein S deficiencies[21] in IBD have been contradictory or equivocal[22,23], suggesting that these factors, although possibly related to IBD pathogenesis, are not genetically related to IBD[23]. Finally, the inherited Val34Leu factor XⅢ polymorphism, which is protective against thrombosis, has been evaluated in IBD patients[3,24]. Available data demonstrated no significant difference in the prevalence of this polymorphism in IBD patients with respect to the general population[25].

Taken together, the information on genetic factors does not explain the greater risk of venous thrombosis in CD and UC[26,27]. On the contrary, a pathogenesis-oriented approach suggests that the coagulation abnormalities occurring in IBD are very likely the result of the biological and biochemical effects exerted by the activation of the inflammatory machinery (cells, cytokines, *etc.*) in these disorders. Furthermore, activation of the coagulation cascade can in turn sustain activation of inflammatory reactions, promoting the vicious circle between chronic inflammation and thrombosis.

In this review, we will firstly describe single quantitative abnormalities of coagulation factors observed in IBD and cellular components closely involved in the coagulation pathway. We will then describe the mechanisms by which these abnormalities interfere with intestinal mucosa homeostasis. Finally, the possible therapeutic implications emerging from the unraveling of coagulation abnormalities associated with IBD pathogenesis will also be briefly presented and critically reviewed

QUANTITATIVE ALTERATIONS OF HEMOSTATIC FACTORS IN IBD

Coagulation is a complex system, which can be schematically divided into different pathways, referred to as "intrinsic", "extrinsic" and "common" pathways[28]. An important role is also played by the fibrinolysis system, which controls clot dissolution, and the family of serine protease inhibitors, which inhibits many coagulation enzymes. We will use this classification to better summarize findings concerning the linkage between the coagulation system and inflammation associated with IBD.

The extrinsic pathway

The extrinsic pathway is initiated by tissue damage that exposes tissue factor (TF) to blood, causing the formation of TF/FⅦa, which circulates at low levels in the bloodstream. It is thought that high factor Ⅶa activity is associated with an increased risk of ischemic myocardial events in men over 40 years of age[8,29] It is the main determinant of the laboratory assay referred to as prothrombin time (PT). No definitive data are available on changes of the extrinsic pathway in IBD. Most of the data available report no significant difference in PT among active UC and/or CD and control patients[3,8,30]. Other studies reported different findings showing that PT values and platelet levels are predictors of CD activity index in female patients[2]. Levels of factor Ⅶa seem to be higher in active IBD compared to controls[3,31,32]. This finding suggests the existence of a pro-thrombotic tendency in IBD patients, arising from activation of the extrinsic pathway.

The intrinsic pathway

Activation of the "intrinsic" pathway of coagulation leads to formation of factor Xa (FXa)[28]. This process stems from previous activation of Factor Ⅸ and FⅧ, with formation of the tenase complex, that is FⅧa-factor Ⅸa on the membrane of platelets and endothelial cells[28]. As FⅧ strongly accelerates the formation of FXa, recent studies showed that inherited high levels of FⅧ (> 140%) can be considered a risk factor for venous thromboembolic disorders[33]. No significant difference in APTT value or FⅧ level was observed in IBD patients compared to controls[8,34]. However, higher APTT levels were found in other reports, although this finding may be the expression of mere consumption of some coagulation factors upon their activation[2]. Other investigations found that factor Ⅺa and ⅫⅠa levels, were higher in active IBD patients compared to patients in the quiescent phase[8,35]. This finding may be in agreement with studies showing that higher levels of FⅪa and FⅫa may be considered coagulation

markers associated with increased risk for thromboembolic disorders[36-38].

The common pathway

FXa thus occupies a central position in the coagulation cascade as a convergence point between the intrinsic and extrinsic pathway. In fact, in the presence of its cofactor FVa, FXa converts prothrombin to thrombin[28]. The common pathway is considered the main determinant of both PT and APTT assays. In observational studies, FX a and FVa levels were significantly elevated in active IBD patients compared to those in patients with disease remission[3,8,39]. The protease-mediated stimulus for inflammatory reactions, particularly for FXa and thrombin, is mediated by cleavage of membrane cleavable receptors and will be discussed below.

The thrombin-generating system

Markers of the thrombin generating system directly involve zymogen prothrombin but also other side-products of prothrombin cleavage such as prothrombin fragment 1+2 (F_{1+2}) and the thrombin-antithrombin complex (TAT). Prothrombin levels in active IBD patients were significantly higher than those in patients with inactive disease or control patients[8,30,32,39-41]. The same observation was also made for F_{1+2} and TAT, suggesting that thrombin generation might be an early event in IBD[8,40,42,43].

Factor XIII

Coagulation factor XIII is a plasma transglutaminase involved in the crosslinking of fibrin, the last step of the coagulation cascade and a connective tissue factor contributing to the wound healing process. It circulates as a heterotetrameric molecule consisting of two identical proenzyme subunits (factor XIIIA) and two carrier protein subunits (factor XIIIS).

A study by Chiarantini *et al*[30] reported decreased factor XIII (FXIII) levels, especially in acute phases of the disease, and deposits of FXIII have been detected in both affected and macroscopically normal bowel mucosa[30,41,43-45]. Those results were confirmed by several other reports, comparing IBD patients to control subjects as well as patients affected by diverticulitis and rheumatoid subjects[46,47]. As anticipated above for other coagulation factors, this finding does not play an etiopathogenetic role in IBD but may represent only the result of chronic consumption of this enzyme associated to deposition of fibrin at the level of inflamed vessels in enteric mucosa.

The system of natural coagulation inhibitors

The system of coagulation inhibitors is composed of a family of proteins, globally referred to as serpins, a typical example of which is antithrombin (AT), and by the protein C pathway, in which a series of different proteins (such as protein C and protein S) and membrane receptors [thrombomodulin (TM), endothelial protein C receptor (EPCR)] are involved. AT is the physiological inhibitor of thrombin, factor Xa, FIXa, FXIa and FXIIa. There is a growing body of evidence that AT is not only an inhibitor of blood coagulation, but it is also able, when present at high concentrations, to reduce the inflammatory responses of endothelial and other cells[48-50]. Thus, AT was shown to reduce the mortality of patients with severe sepsis in a recent clinical trial[49]. AT-induced attenuation of inflammatory responses might be linked to endothelial production of prostacyclin and inhibition of leukocyte and endothelial cell expression of pro-inflammatory mediators *via* suppression of nuclear factor (NF)-κB activation[48,50,51]. Furthermore, AT prevents water-immersion restraint stress-induced gastric mucosal injury in rats by promoting the endothelial release of PGI_2[52]. In addition, non-uniform information exists on the quantitative expression of these components in IBD. Overall, it seems that no differences in the levels of protein S[8], protein C[3,53] and ATIII[34,41] exist among IBD patients compared to controls. However, in studies comparing the levels of these molecules in the active *vs* inactive form of the disease or in controls, lower levels of protein C, ATIII and protein S were observed[21,30,34,53,54]. In a single study, higher levels of protein S and C in IBD patients compared to controls were also reported[30]. These conflicting results indicate that changes in systemic levels of these inhibitors do not necessarily reflect the local loss of inhibition of coagulation occurring within enteric mucosa in IBD. Hence, a new approach has tried to correlate an enhanced production of thrombin in IBD with a possible loss of function in natural anticoagulants mostly occurring on the endothelium of enteric mucosa. This issue will be addressed in the section below.

The fibrinolytic system

In normal hemostasis, the fibrinolytic system allows removal of a fibrin clot when the damaged vessel wall is restored. Activation and regulation of fibrinolysis occurs by multiple proteins and results in the generation of plasmin. Plasminogen may be activated by tissue plasminogen activator (tPA) or urokinase plasminogen activator (uPA). The latter binds to a cellular receptor [urokinase plasminogen activator receptor (uPAR)] resulting in enhanced activation of cell-bound plasminogen and its main role is the induction of pericellular proteolysis[55]. tPA is the most potent activator of plasminogen in plasma and the main regulator of fibrinolysis. After stimulation, tPA is locally released into the circulation from the endothelial cells where it is produced. tPA-mediated plasminogen activation is facilitated by a fibrin surface, which restricts fibrinolysis to the site of thrombus formation[56]. Moreover, once bound to fibrin, tPA is protected from inhibition by plasminogen activator inhibitor 1 (PAI-1), its principal inhibitor in plasma[57]. The level of PAI-1 in blood usually exceeds that of tPA; thus, in general, no active tPA circulates in plasma[58].

α_2-Antiplasmin is the primary physiological inhibitor of plasmin, as it can very rapidly inhibit plasmin in plasma[59]. However, plasmin is partly protected from α_2-antiplasmin inhibitory activity when the enzyme is bound to fibrin[60]. During thrombus formation, α_2-antiplasmin is cross-linked to fibrin by factor XIIIa, facilitating local inhibition of fibrinolysis[61].

Another important player in the fibrinolytic system is

thrombin-activatable fibrinolysis inhibitor (TAFI), which directly connects coagulation and fibrinolysis. It is activated by thrombin, but its activation is over 1000-fold enhanced by the thrombin-TM complex. Activated TAFI removes carboxyl-terminal lysine residues from partially degraded fibrin. Consequently, the binding of plasminogen and tPA to fibrin clots is decreased, which attenuates clot lysis[62].

Reduced activity of the fibrinolytic system has been described in IBD[63-65] Indeed, a reduction in activators (such as tPA) and an increase in inhibitors (such as PAI and TAFI) of the fibrinolytic system have been described in IBD patients[40,63-66]. This condition would favor prothrombotic mechanisms.

Cellular elements involved in the coagulation pathway

The haemostatic system is composed not only of soluble proteins and enzymes but also of different cell types. Platelets and endothelial cells play a central role in the maintenance of a physiological balance between pro- and anti-coagulant mechanisms. Moreover, accumulating evidence indicates that platelets and endothelial cells, besides their well-known haemostatic functions, play a role in inflammation and its resolution mechanisms[67].

Platelets

Platelets can release a number of mediators of the inflammatory response, including cytokines, chemokines, nitric oxide (NO) and eicosanoids. Furthermore, they interact with polymorphonuclear cells (PMN) and monocytes, regulating their extravasation and recruitment at sites of inflammation. Along these lines, platelets have the enzymatic machinery to synthesize both pro- and anti-inflammatory eicosanoids. As an example, platelets contain 2-lipoxygenase (12-LO), a key enzyme in the biosynthesis of the lipoxins (LXs), arachidonic acid metabolites with potent anti-inflammatory properties[68]. LX formation during platelet/PMN interactions occurs *in vivo* and represents a main stop signal of inflammation[69]. Thus, a sustained inflammatory response, as it occurs in IBD may originate by both increased formation of pro-inflammatory mediators, and reduction in counter-regulatory signals.

Platelet integrin GPIbα is a ligand of P-selectin, a transmembrane adhesion molecule present on endothelial cells, and supports platelet rolling on activated endothelium. P-Selectin binding to P-Selectin Glycoprotein Ligand 1 (PSGL-1) stimulates the release of microparticles bearing tissue factor on their surface by leukocytes[70]. P-selectin is split as a soluble form (sP-selectin) that stimulates expression and exposition on the monocyte surface of further tissue factor[71]. Microparticles, together with sPselectin, are considered the main factors responsible for the high procoagulant status of blood in inflammation[72]. During adhesion to endothelium, platelets release pro-inflammatory cytokine CD40-ligand (CD40L) (CD154, gp39) that can stimulate the endothelium, stabilize platelet aggregates, their binding to blood cells and vascular wall cells and promote stable thrombus formation[73]. CD40L is expressed on activated platelets and also on immune system cells activated during inflammation (activated $CD4^+$ T cells, basophils, and mast cells)[74]. This factor is a transmembrane protein related to tumor necrosis factor (TNF)-α. The inducible CD40L on platelets binds to the CD40 receptor on endothelial cells and on monocytes, macrophages, and smooth muscle cells (SMC)[74]. The CD40/CD40L interaction plays an important role in inflammation and atherothrombosis[73]. Through binding to the ligand, CD40 induces the inflammatory response independent of cytokines. The pro-inflammatory activity of CD40L also occurs on platelets and other cells by stimulation of the expression of chemokines [monocyte chemotactic protein 1 (MCP-1)], interleukins (IL-6, IL-8), pro-inflammatory adhesive molecules [vascular cell adhesive molecule-1 (VCAM-1)], intracellular adhesive molecules (ICAM-1, CD54), and P-/E-selectins.

Notably, the activity of CD40L induces the expression of tissue factor, which, as mentioned above, is the major inducer of blood coagulation, and suppresses the expression of TM, which is a thrombin cofactor in activation of the protein C anticoagulant system[75]. Upon binding of CD40L to the CD40 receptor, intracellular signaling results in activation of the transcriptional factor NF-κB and its translocation into the nucleus. This event induces the expression of new molecules of CD40L and CD40. The interaction of CD40 expressed by endothelial cells with CD40L exposed on activated platelets stimulates the synthesis of a powerful pro-inflammatory mediator, platelet activating factor (PAF), which induces platelet aggregation with leukocytes and also contributes to remodeling of vessels, stimulating neo-angiogenesis[76].

Platelet activation is associated with the metalloprotease-mediated split of a soluble fragment of the CD40-ligand (sCD40L)[76]. Soluble CD40L was shown to promote blood coagulation by two mechanisms: induction of tissue factor expression on monocytes and activation of platelets *via* interaction with integrin αⅡb/β3. The sCD40L binding to integrin αⅡb/β3 activates platelets at high shear stress and stabilizes arterial thrombi[74]. Increased levels of CD40L on platelets and sCD40L in circulating blood were found in clinical settings characterized by thrombosis associated with inflammation, such as unstable angina, myocardial infarction and other cardiovascular diseases[77,78].

Thus, a realistic scenario shows that platelet activation during inflammation and expression of adhesive proteins, P-selectin and integrins, leads to their aggregation with leukocytes and the release of contents of intracellular granules. In conclusion, platelet-platelet and platelet-leukocyte aggregates produce a cell surface, which provides activation of both blood coagulation and inflammation.

The association between active IBD and thrombocytosis was first recognized in 1968 and it became clear that patients with IBD also have increased numbers of circulating platelet aggregates and activated platelets compared with healthy controls[79-81]. More recently, it was demonstrated in studies from different groups that supranormal platelet-leukocyte aggregates are frequently present in patients with IBD compared with both healthy and inflammatory controls[82]. In other studies, significant changes in platelet volume were also observed[83,84]. In particular, an

increase in platelet count[83] and a statistically significant decrease in MPV was noted in patients with colitis compared with healthy controls. Moreover, MPV of active colitis patients was significantly lower than that of the inactive phase of the disease. It is, however, difficult to correlate this finding with the functional alterations that could be responsible for the platelet-mediated thrombotic mechanisms summarized above. It may be hypothesized that in IBD the reduction in MPV may be associated with a peripheral platelet activation responsible for an exalted formation of platelet-platelet, platelet-PMN and platelet-endothelium adducts. This process would mainly involve younger platelets which have a bigger volume. Thus, the overall reduction of MPV could reflect the relative prevalence in circulating blood of less reactive platelets, which are older and smaller[85]. In a very recent study, an enhanced expression of CD40/CD40L in intestinal epithelial cells was demonstrated[86]. In particular, endoscopy biopsies taken from CD and UC patients showed a positive immunofluorescence staining for CD40 in intestinal epithelial cells of inflamed ileal or colonic mucosa, while no staining was observed in uninvolved intestinal segments[86]. These findings provide, for the first time, direct evidence for the epithelial expression and modulation of CD40 in IBD-affected mucosa and indicate its involvement in the pro-inflammatory and platelet-activating function of inflamed intestinal cells.

Finally, another paper from our group showed that *in vitro* activated platelets directly increase CD40L expression by intestinal endothelial cells, leading them to interact with other immune cells and sustain intestinal chronic inflammation. This pathway has been proposed as a new mechanism of chronic inflammation, as a result of the complex interplay among different cell types in the intestinal mucosa[87].

Endothelium

In a normal artery, endothelium creates a non-thrombogenic surface that acts as a selectively permeable barrier. Endothelium plays a key role in response to vascular injury, regulating leukocyte adhesion, platelet activation and adhesion and blood coagulation. Endothelium expresses and responds to multiple active substances, including cell adhesive molecules, cytokines and chemokines, to accomplish these functions[88,89]. Injury to a vessel wall results in the triggering and propagation of inflammatory and coagulation events. The cell adhesion molecules (CAM) are expressed onto the surface of activated endothelial cells and attach leukocytes and platelets. Adhesive proteins provide for the binding and spreading of leukocytes, their rolling, and their further transmigration across endothelium. There are three major classes of CAM: selectins, the immunoglobulin superfamily CAM and integrins. Some integrins in turn can be receptors of CAM and the endothelial adhesion molecule, von Willebrand factor (VWF), which binds platelets.

Weibel-Palade bodies in endothelial cells and platelet α-granules contain and release platelet P-selectin (CD62P, GMP140) responsible for adhesion of leukocytes, their rolling, and for stabilization of platelet aggregates[90,91]. The lectin-containing N-terminal domain of P-selectin binds to PSGL-1 on monocytes, neutrophils and platelets[91,92].

E-selectin (CD62E) is another molecule exposed on the endothelial cell surface that can bind to PSGL-1 in response to mechanical injury and inflammatory mediators as IL-1β, TNF-α, bacterial toxins and oxidants[89]. P- and E-selectin mediate rolling of activated and quiescent platelets on activated endothelium similar to the mechanism of leukocyte rolling[93].

The immunoglobulin superfamily CAM includes ICAM-1 (CD54), ICAM-2 and VCAM-1, which are expressed by many cell types including endothelial and SMC. In response to vascular injury, these cells upregulate expression of ICAM-1 and VCAM-1[89], engaged in leukocyte adhesion. Adhesion of platelets to injured endothelium is controlled by VWF, a multimeric protein, whose molecular weight ranges from 0.5 to 20 million Da[94] and is stored and released from Weibel-Palade bodies in endothelial cells[95]. Hence, VWF is considered a marker of endothelial injury. The VWF molecule contains domains responsible for binding blood coagulation factor Ⅷ and platelet integrins such as glycoprotein transmembrane complexes GPIb/Ⅸ/Ⅴ and integrin αⅡb3 (GPⅡb/Ⅲa), as well as collagen[94]. VWF binds subendothelial collagens and after immobilization attaches to platelets *via* the membrane complex GPIbα-Ⅸ-Ⅴ[96]. VWF may be involved in the pathogenesis of acute thrombotic occlusion of stenosed arteries, where high shear stress promotes the formation of "stretched" VWF conformers, which are suitable for binding to platelets and subendothelial components[88]. P-Selectin could serve as an anchor site for the ultra large VWF multimers on the surface of activated endothelium, to facilitate their cleavage at the Tyr1605-Met1606 peptide bond by the disintegrin and metalloproteinase with thrombospondin motif-13 (ADAMTS-13)[97]. Microvascular dysfunction has been clearly demonstrated in IBD patients and involved several aspects of endothelium biochemical physiology[98,99]. In particular, such dysfunction involves an alteration in nitrogen and reactive oxygen species balance, where the microvascular endothelium fails to generate ·NO, a potent vasodilator and anti-aggregating agent, forming instead elevated levels of superoxide anion[54]. However, the mechanism responsible for the loss of endothelial nitrogen oxide in IBD gut microvessels also involves additional biochemical pathways. Previous studies showed an acquired deficient transcription of nitric oxide synthase 2 (NOS2) in chronically inflamed IBD endothelium[100]. Furthermore, more recently, it was demonstrated that decreased production of nitrogen oxide in IBD endothelial cells can also arise from the induction by many inflammatory cytokines (IL2, TNF-α) of the enzyme arginase (isoform Ⅰ and Ⅱ)[101]. This enzyme converts L-Arg into urea and L-ornithine, precursors for polyamines and L-proline compounds, which are vital to tissue homeostasis and wound repair[102]. Arginase Ⅰ and Ⅱ compete with inducible NOS (iNOS, NOS2), the most relevant inducible pathway for the production of ·NO, for L-Arg, which is their common substrate in endothelial cells[103]. Thus, an

increased arginase activity in IBD may contribute to inhibit the production of a potent antithrombotic agent such as nitric oxide. The increased production of reactive oxygen species in inflamed endothelium may also contribute to oxidative stress in VWF molecules, which become unresponsive to proteolysis by ADAMTS-13 and the accumulation of ultra large VWF multimers[104]. The latter are the most haemostatically active forms of VWF and, favoring platelet adhesion and aggregation, may contribute to microvascular thrombosis in IBD.

To conclude, endothelium plays an essential role in inflammation due to its central "gatekeeper" function, which controls the quality and quantity of leukocytes that transmigrate from the vasculature into the interstitial space, regulates vascular tone and promotes platelet adhesion and aggregation. The latter function directly affects the haemostatic system and may clearly favor thrombotic phenomena. Several papers reviewed over the last few years suggest an activated status of endothelium in the course of IBD[98,99].

A COHERENT SCENARIO FOR UNBALANCED HAEMOSTASIS IN IBD

At this point a question arises as to whether the haemostatic and inflammatory alterations briefly described in the above paragraphs could be functionally linked in a coherent framework.

Globally, the coagulation system in IBD patients seems to sustain pro-thrombotic mechanisms, involving both soluble factors and cells, such as platelets, endothelium and leukocytes. This conclusion is supported by results obtained from new laboratory assays. The conventional and global coagulation tests such as PT and APTT both have low sensitivity and specificity and do not contain sufficient amounts of TM or glycosaminoglycans. Thus, these assays do not automatically reflect the coagulation reactions and their inhibition as they occur *in vivo*[105,106]. In contrast, the latest generation of methods that monitor the tissue factor induced thrombin generation in the presence of TM are credited as better laboratory tools to represent the balance of pro- and anti-coagulant forces operating in plasma[105-108]. In a recent study from Saibeni *et al*[105] endogenous thrombin potential, a parameter of the thrombin generation curve, was significantly higher in IBD patients than controls only when the test was performed in the presence of TM. This new assay strongly suggests that in IBD, as anticipated above, the increased generation of thrombin is mainly linked to a partial loss of function of natural anticoagulant pathways, and particularly of the TM-PC system.

Thus, systemic coagulation alterations in IBD may be recognized using more sophisticated techniques, which better reflect the *in vivo* setting. Furthermore, pathogenic considerations suggest that the coagulation imbalance in IBD could be particularly relevant in the vasculature of enteric mucosa, where inflammation shows the majority of destroying effects.

THE INFLAMMATION-COAGULATION INTERPLAY WITHIN THE INTESTINAL MILIEU

In addition to the demonstration that coagulation abnormalities and thromboembolic complications are clinically relevant events in IBD, they have been shown to exert effects at the mucosal level, where a coagulative imbalance exists[3].

In fact, one of the earliest abnormalities in CD mucosa is the presence of platelet thrombi cross-linked with fibrin in the mucosal microvasculature[109]. This feature, however, is not specific for CD and can be found in other idiopathic IBD[110]. The involvement of the microcirculation in IBD pathogenesis is underlined by the analysis of a segment of the small and/or large bowel during active IBD which reveals vasodilatation, venocongestion, edema, infiltration of large inflammatory cells and ulcerations[111]. This picture is the result of an unregulated intestinal inflammation with a consequent abnormal immune response and production of inflammatory cytokines, which, in turn, sustain the activation of the microvascular endothelium and subsequent recruitment of more leukocytes into the intestinal wall. This uncontrolled inflammatory response produces dramatic alterations in gut microvascular function which contributes greatly to perpetuating the inflammatory damage observed in IBD[98,112].

Coagulation factors mainly interact with local endothelium, although this interaction is potently conditioned by many features of the mucosal immunity.

The principal link between endothelium and the coagulation cascade is determined by Protease-activated receptors (PARs)[113]. PARs, and in particular PAR-1 and PAR-2, are cellular receptors activated after proteolytic cleavage by enzymes[114], mainly thrombin and activated factor X. Only a single study reports over-expression of PAR1 in patients with IBD[115], and there are no data available on animal models addressing its role in IBD. The expression of PAR2, which is greatly increased in patients with UC and CD[116-118], and the functional consequences of its activation in animal models are more widely documented.

Next to expression in the intestine, PAR expression in enteric neurons might be highly relevant for IBD because PARs can mediate gut inflammation *via* neurogenic mechanisms. Interestingly, PAR activation on submucosal and myenteric neurons causes severe edema in rat models. Moreover, the local activation of PAR2 but not PAR1 in the gut causes colitis through a neurogenic mechanism[116]. Taken together, these results point towards PAR2 expression/cleavage as a cardinal factor in IBD.

The APC-TM system is the natural pathway by which the pro-inflammatory activity of PAR-1 and 2 signaling is contra-balanced. In the following section major findings on how thrombin, factor X and APC contribute to IBD, will be shown and briefly discussed.

Thrombin

Once thrombin is sequentially activated through the in-

trinsic and extrinsic pathway, it not only amplifies the coagulant process but it can also favor inflammation induced by other stimuli, either through ischemia (consequent upon thrombosis), indirectly through the generation of downstream mediators or directly *via* signals through protease-activated receptors (PAR)[119].

Thrombin activates PARs, thereby establishing a link between activation of coagulation and pathophysiology of IBD. Indeed, thrombin signals through PAR1, PAR3 (in mouse) and PAR4, while tissue factor (TF)/FⅦa activates PAR2, and FXa activates PAR1 and PAR2[116,120].

In addition to promoting platelet activation, thrombin exerts influence over monocytes, macrophages[121] and neutrophils in processes related to tissue repair at the site of injury[122,123]. Thrombin also interacts through an equilibrium high affinity binding with the N-terminus of GpIbα of platelets and endothelial cells[124,125]. Notably, on platelet membrane, binding of thrombin to GpIb, accelerates cleavage of PAR1 by the enzyme[126]. Thrombin also affects endothelial cells through various pathways including NF-κB, early growth response factor-1 and GATA binding proteins[127]. Thrombin signaling might result in post-transcriptional changes, including calcium influx, cytoskeletal reorganization, and release of soluble mediators, growth factors, and matrix metalloproteinases. In addition, thrombin signaling results in changes in downstream gene transcription, for example increasing the expression of genes involved in cell proliferation, inflammation, leukocyte adhesion, vasomotor tone, and hemostasis[128-130].

Factor Xa

Borensztajn *et al*[116] suggested that Factor Xa signaling through PAR2 contributes to the progression of IBD and fibro-proliferative responses. Because FXa is a well-known PAR2 agonist, FXa-induced PAR2 activation is gaining attention in intestinal pathology. Accordingly, in a variety of endothelial *in vitro* systems, FXa induces an array of pro-inflammatory responses and the deposition of connective tissue growth factor[116,131,132]. It also leads to the activation of NF-kB, and the release of IL-6, IL-8, and MCP-1 on endothelial cells as well as fibroblasts[120,133]. Moreover, on endothelial cells, FXa induces the expression of E-selectin and both intracellular ICAM-1 and VCAM-1, resulting in leukocyte adhesion[116,134,135]. In synergy with tumor necrosis factor, FXa induces TF expression *via* inhibition of its negative regulators IkBa and A20[116,136]. Most of these responses are mediated *via* PAR2 activation, although some studies showed minor involvement of PAR1[136,137].

Although the potential pro-inflammatory role of FXa on epithelial cells of the gastrointestinal tract is not fully investigated, studies on Hela cells showing that FXa induces activation of the pro-inflammatory transcription factor NF-κB, suggest that it plays an important role[116,138]. Finally, FXa also affects immune cells inducing the production of IL-2 by lymphocytes[139]. Evidence that FX a may mediate inflammatory responses *in vivo* has come from several studies. In particular, Cirino *et al*[140] demonstrated that FXa induces the formation of edema when injected subcutaneously in a rat paw inflammation model, *via* local recruitment of mast cells.

The PC pathway

Traditionally described as a major anti-coagulant system, the protein C (PC) pathway, consisting of TM, the EPCR and activated PC (APC), is gaining increasing attention as an important regulator of microvascular inflammation, and in particular intestinal inflammation observed in IBD[141]. The anticoagulant function of the PC pathway has been reviewed extensively[142-144]. The main components of the PC system are the cell membrane receptor for PC, referred to as EPCR, the integral membrane glycoprotein TM, and two vitamin K-dependent plasma proteins, the zymogen PC, and the cofactor protein S. Upon cleavage of a dodecapeptide from the N-terminus of the light chain of PC by the thrombin-TM complex, the zymogen PC is activated to PC. Protein C *per se* is a poor substrate for thrombin. Allosteric binding of free thrombin to TM enhances, by several orders of magnitude, the thrombin-PC interaction and subsequent conversion of PC zymogen into its proteolytically active form, APC. The rate of PC activation by the TM-thrombin complex is further enhanced when the substrate PC zymogen is bound to its receptor, EPCR, which is able to reduce the K_m value of the catalytic interaction with thrombin. The extent of *in vivo* PC activation is therefore greatly linked to the bioavailability of PC, thrombin and, critically, by the density of TM and EPCR molecules expressed on endothelial cells. With the exception of disseminated intravascular coagulation, consumptive coagulopathy and defective biosynthesis, thrombin bioavailability in first approximation reflects the intensity of coagulation activation. Within such limits, it was shown that thrombin formation and generation of APC are strongly correlated[145]. APC is formed mainly within the microcirculation, where endothelial cells express high levels of TM. As a consequence, due to the very small intravascular volume, the concentration of TM may be > 100 nmol/L, greatly exceeding the K_d value of the thrombin-TM interaction. Under these conditions, any amount of formed thrombin is rapidly and completely bound to TM. At variance with this situation, expression of TM is much lower in the endothelium of larger arteries and veins. Notably, EPCR expression is inversely related to that of TM, as it is more abundant in large vessel endothelium than in microcapillary beds. Thus, the efficiency and extent of APC formation differs considerably between different organs. The anticoagulant activity of the PC pathway includes the limited proteolysis by APC of the activated forms of coagulation factors V and Ⅷ (FVa and FⅧa), thereby limiting active thrombin generation. Protein S, in turn, cooperates with APC in inactivating FVa and FⅧa, exerting an accelerating effect[146]. The current model of APC suppression of excessive thrombin generation assumes that the EPCR-bound pool of endothelial cell-associated APC plays a more important role for FV inactivation than the circulating plasma pool of APC. In part, this may be explained by the fact that binding of APC to cell surfaces is

mediated only by EPCR, implying that the site of EPCR expression largely dictates the site of the anticoagulant function of APC. This model is fully consistent with the finding that FVa is highly susceptible to proteolytic degradation by APC when it is associated with the endothelial cell surface. FVa is instead refractory to APC cleavage in the platelet-associated prothrombinase complex[147]. Other mechanisms can potentially limit the anticoagulant activity of APC on, or close to platelets, i.e. inhibition of APC by the vitronectin-plasminogen activator inhibitor-1 complex, secondary to local release of plasminogen activator inhibitor-1 from activated platelets, and the inhibition of protein S activity by platelet factor 4 released from platelet α-granules[148-150]. Notably, platelet factor 4 can inhibit the anticoagulant function of APC alone, but not its ability to cleave and activate PAR-1. Thus, the interaction of platelet factor 4 may potentially redirect APC function toward anti-inflammatory and cytoprotective signaling pathways.

Overall, the relatively poor ability of APC to suppress thrombin generation in forming platelet aggregates might support effective and localized platelet-dependent hemostasis while sustaining the systemic anticoagulant potential. Thus, the APC activity in the presence of platelets may be considered another example of the compartmentalized haemostatic system.

Recently, it was demonstrated that surface-immobilized PC supports in a GPIbα- and apolipoprotein E receptor 2-dependent manner the adhesion and aggregation of platelets under flow conditions[151]. Thus, the ability of zymogen to engage these receptors raises the question as to whether PC immobilization occurs *in vivo* and whether changes in PC plasma levels are associated with altered platelet adhesion and aggregation.

Protein S circulates in blood in complex with a carrier protein, C4b-binding protein[152]. The level of C4b-binding protein increases in clinical settings characterized by inflammation. Hence, the amount of bound protein S increases, causing a decrease of free protein S concentration[152]. It is known that the anticoagulant function of protein S is exerted by its free form. Thus, systemic inflammatory conditions may represent a risk factor for protein-S dependent thrombotic disorders[152]. Finally, protein S exerts both APC-dependent and aPC-independent anticoagulant effects. The APC-dependent mechanism, involving the cofactor function of protein S for the acceleration of APC-mediated degradation of factors Ⅷ and Ⅴ, is likely the physiologically dominant pathway. The APC-independent anticoagulant activity of protein S is attained by stimulating the inhibition of tissue factor (TF) by tissue factor pathway inhibitor (TFPI)[153]. The latter blocks the intermediate complex of TF-FⅦa-FXa, thereby preventing substrate exchange of already activated FXa for new FX. Protein S enhances the inhibitory interaction of TFPI with the TF initiation complex, and thereby limits the extent of thrombin generation in plasma. This APC-independent anticoagulant activity of protein S is most pronounced at low TF levels. Due to the anticoagulant effects described above, severe protein S deficiency is associated with severe thrombotic disorders.

The relevance of the PC system for the prevention of atherothrombotic diseases is further corroborated by studies in animals. Some interesting aspects of *in vivo* PC activation were unraveled by analyzing the role of EPCR in the response of mice to an inflammatory challenge with lipopolysaccharide (LPS)[154]. In these studies, mice lacking EPCR showed substantially enhanced activation of coagulation, concomitantly with reduced APC formation attributable to the absence of EPCR. Yet, the plasma APC levels in LPS-challenged EPCR-deficient mice were almost identical to that measured in wild-type animals. The authors of this study then showed that in wild-type mice a large fraction (approximately 40%) of APC did not enter the systemic circulation but remained bound to endothelial cell-associated EPCR at its site of activation. It is this sequestered APC pool that is completely missing in EPCR deficient mice, and its absence apparently accounts for all the pro-coagulant and pro-inflammatory effects of EPCR deficiency in mice[154].

Recent studies have shown that the anti-inflammatory effect of APC, at least in part, is mediated through the EPCR-dependent proteolysis of PAR-1 in endothelial cells[155,156]. This finding seems paradoxical, as it is known that the cleavage of PAR-1 by thrombin elicits potent prothrombotic and pro-inflammatory responses[157]. It is well known that thrombin is mainly responsible for the activation of PC in the presence of TM and that the enzyme also cleaves PAR-1 with a high catalytic efficiency, which is 3-4 orders of magnitude higher than that of APC. This finding raises the question as to how APC in the presence of thrombin is able to produce physiologically significant cleavage of PAR1 associated with protective signaling events[158], as is presented in Figure 1. Notably, both APC and PC bind to EPCR with a similar equilibrium constant, so that it can be hypothesized that thrombin can increase the local concentrations of EPCR-bound APC[159]. This phenomenon may induce channeling of the protease directly into the signaling pathway.

In a very elegant study, Rezaie and coworkers demonstrated that the critical receptors required for both protein C activation (TM and EPCR) and APC cellular signaling (EPCR and PAR-1) pathways colocalize in the membrane lipid rafts of endothelial cells. The co-localization of EPCR and PAR-1 in lipid rafts of endothelial cells is a fundamental requirement for the cellular signaling activity of APC, which leads to anti-inflammatory and anti-apoptotic cellular effects, such as phosphorylation of mitogen-activated protein kinase[160] and suppression of NFkB expression[161].

TM co-localization with these receptors on the same membrane microdomain can also recruit thrombin to activate the EPCR-bound protein C, therefore eliciting PAR-1 signaling events that are involved in the APC protective pathways[159] linked to dissociation of caveolin subunits (Figure 1). These findings explain how thrombin effectively channels endogenous APC to the protective signaling pathways, through cleaving the same receptor of thrombin.

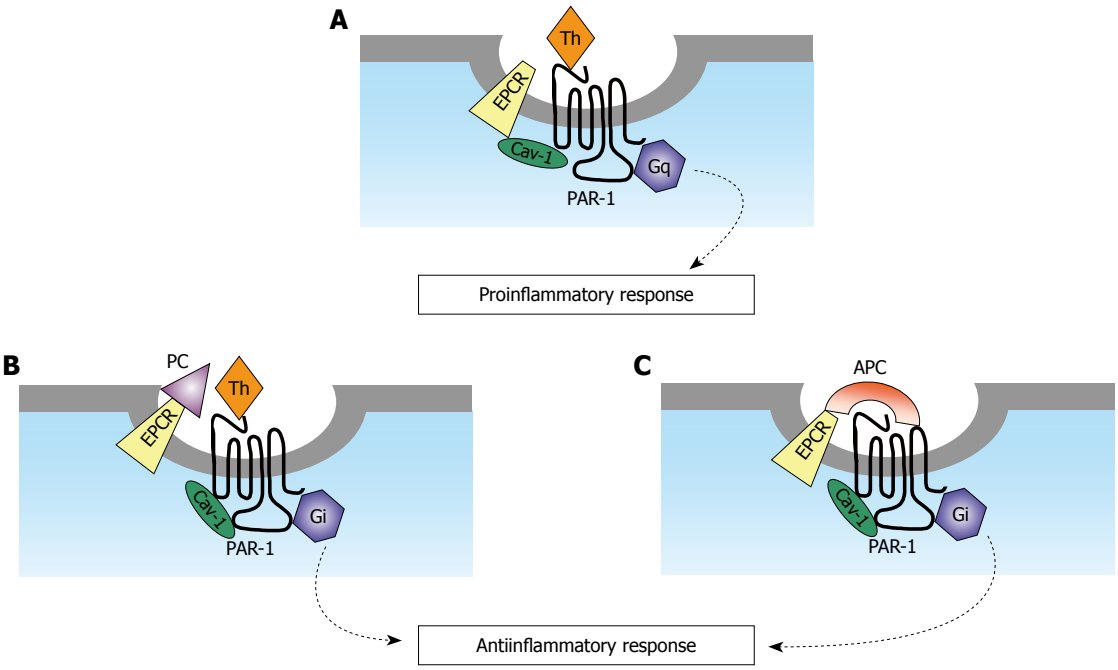


Figure 1 Models of protease-activated receptor-1 cleavage and activation by either activated protein C or thrombin when endothelial protein C receptor is occupied by its ligand protein C. A: The unoccupied endothelial protein C receptor (EPCR) is associated with caveolin-1 (Cav-1) within lipid rafts of endothelial cells. Upon thrombin cleavage of protease-activated receptor (PAR)-1, a pro-inflammatory signal is generated through G12/13 and Gq under these conditions; B: The occupancy of EPCR by protein C (PC) results in dissociation of EPCR from Cav-1. This process is linked with the coupling of PAR-1 to Gi. Thrombin cleavage of PAR-1 initiates an antiinflammatory response under these conditions; C: The same as (B) except that the EPCR and PAR-1 dependent protective signaling response is mediated by activated protein C (APC) (adapted from[159]).

The APC pathway in IBD

TM and EPCR expression is diminished in the colonic mucosal microvasculature of IBD patients[3,162-164], but is increased in their sera, suggesting increased shedding of TM and EPCR from cells. Inflammatory cytokines also down-regulate TM and EPCR by inhibiting transcription on cultured intestinal endothelial cells (HIMEC)[162]. These changes in TM and EPCR expression would be expected to affect the conversion of protein C in its activated form, which, in addition to its anticoagulant properties, also has potent anti-inflammatory activity[165], as described above.

Restoring the function of the PC pathway has anti-inflammatory effects on HIMEC, by decreasing pro-inflammatory cytokines secretion as well as adhesion molecules induced by TNF-α stimulation[144,162,166]. Furthermore, restoration of APC by supplementation reduces stress-induced gastric mucosal injury in rats by inhibiting the decrease in gastric mucosal blood flow through attenuation of the activated neutrophil-induced endothelial cell injury *via* inhibition of TNF-α production[167].

Overall, it can be concluded that a homeostatic balance exists between thrombin and APC in coagulation and inflammation. In particular, activated thrombin promotes the generation of APC and the two molecules influence the extent of both fibrin (clot) formation and the inflammatory response. This mechanism is mediated mainly by the cleavage of PAR-1 by either APC or thrombin on endothelial cells. Through binding to EPCR, APC would reverse the pro-inflammatory effects of thrombin on the same PAR[127,168].

THERAPEUTIC PERSPECTIVES FOR COAGULATION ABNORMALITIES IN IBD

Based on the reported findings from several studies, one can conclude that the PC pathway is strategically located at the crossroads between coagulation and inflammation, where it exerts entirely unexpected roles in the damage that occurs in chronic inflammatory conditions[169]. Unraveling the pathogenic role of the PC pathway offers a very promising tool in the therapeutic arsenal against IBD as well as many other chronic inflammatory diseases. Inflammation most likely mediates systemic hypercoagulability through various cytokines, which can affect the coagulation cascade at numerous points as well as platelet quantity and function. Unfortunately, perhaps due to this diversity of prothrombotic abnormalities that can exist in IBD patients and their likely multifactorial etiology, no specific therapy has ever been proposed in any clinical randomized trial to correct the cytokine-linked pro-inflammatory unbalance and pro-thrombotic phenomena occurring in IBD. Notably, unfractioned (UFH) and low-molecular-weight heparins (LMWHs), apart from their known anticoagulant/antithrombotic activities, display a broad spectrum of immune modulating and anti-inflammatory properties, such as modulation of cytokine production, T-lymphocyte cytotoxic activity[170] and inhibition of leukocyte adhesion, activation and trafficking[171]. Based on these features, these molecules have been proposed for the treatment of IBD. Some open studies suggested the efficacy of UFH[172,173] and LMWHs[174] for the treatment of active

UC. Conversely, large controlled studies using UFH and LMWHs did not show a clear efficacy[175-178]. Moreover, a recent meta-analysis by Shen *et al*[179] indicated no significant additive benefit for heparins in the treatment of active UC. However, all studies included in the meta-analysis were very heterogeneous about their clinical, methodological and pharmacological features.

These studies, not only considered different definitions for response and remission, but also used different heparins, with theoretically very different anti-inflammatory activities[179]. For these reasons, it is still difficult to set the real value of this therapeutic approach in IBD.

Recently, experimental data on animal models of IBD suggested efficacy of LMWHs, when selectively delivered in the site of disease, compared to the other route of administration. The multimatrix oral formulation MMX releasing parnaparin sodium at three different doses was evaluated in a clinical trial in patients with mild-to-moderate UC activity[180]. This study, carried out on ten UC patients, showed no relevant side effects, including either interference with haemostatic parameters or increased bleeding. After treatment, seven patients were in clinical remission and only one achieved endoscopic healing. However, in a recent meta-analysis it was found that there is no evidence to support the use of UFH or LMWH for the treatment of active UC. In this study no further trials examining these drugs in patients with UC were warranted, except perhaps a trial of UFH in patients with mild disease[181]. Furthermore, it has to be outlined that any benefit found using heparins in this clinical setting should be weighed against a possible increased risk of rectal bleeding, especially in patients with active UC.

In conclusion, a direct therapeutic approach for controlling inflammation-driven imbalances in the coagulation system in IBD patients is not yet available. The growing body of evidence concerning the molecular and cellular perturbations in this setting should be unraveled to promote a more efficacious, pathogenesis-oriented therapy for these disorders.

CONCLUSION

Overall, this paper was designed to underline the delicate and unstable equilibrium, at the mucosal level, between inflammation and coagulation. This complex equilibrium actively participates in the pathogenesis of several inflammatory disorders, in particular IBD.

Although the majority of available reports have looked at alterations in single coagulation components in IBD patients, no clear evidence of single alterations have been demonstrated to be crucial in IBD development. On the contrary, it is evident that several factors, with diverse relevance, are involved in maintaining chronic inflammation as well as a pro-coagulant profile in IBD. These factors include mainly classical coagulation components as well as inhibitors, and also cells, such as endothelium and platelets, which interact extensively at the mucosal level. Pathways that seem to play a major role in IBD pathogenesis are the APC, thrombin and Factor Xa pathways. The delicate balance between these pathways, affecting different mucosal cell types, is responsible for controlling endothelium, leukocyte activation and trafficking, cytokines and chemokines secretion as well as the coagulation cascade. Despite a better understanding of the interaction between coagulation and inflammation, very few drugs targeting the coagulation pathways are available or under evaluation for clinical purposes. In conclusion, further studies are required to better characterize the relationship between coagulation and inflammation in different IBD patients and to identify good therapeutic targets.

REFERENCES

1 **Esmon CT**. The interactions between inflammation and coagulation. *Br J Haematol* 2005; **131**: 417-430
2 **Shen J**, Ran ZH, Zhang Y, Cai Q, Yin HM, Zhou XT, Xiao SD. Biomarkers of altered coagulation and fibrinolysis as measures of disease activity in active inflammatory bowel disease: a gender-stratified, cohort analysis. *Thromb Res* 2009; **123**: 604-611
3 **Danese S**, Papa A, Saibeni S, Repici A, Malesci A, Vecchi M. Inflammation and coagulation in inflammatory bowel disease: The clot thickens. *Am J Gastroenterol* 2007; **102**: 174-186
4 **Miehsler W**, Reinisch W, Valic E, Osterode W, Tillinger W, Feichtenschlager T, Grisar J, Machold K, Scholz S, Vogelsang H, Novacek G. Is inflammatory bowel disease an independent and disease specific risk factor for thromboembolism? *Gut* 2004; **53**: 542-548
5 **Spina L**, Saibeni S, Battaglioli T, Peyvandi F, de Franchis R, Vecchi M. Thrombosis in inflammatory bowel diseases: role of inherited thrombophilia. *Am J Gastroenterol* 2005; **100**: 2036-2041
6 **Novotny DA**, Rubin RJ, Slezak FA, Porter JA. Arterial thromboembolic complications of inflammatory bowel disease. Report of three cases. *Dis Colon Rectum* 1992; **35**: 193-196
7 **Oldenburg B**, Van Tuyl BA, van der Griend R, Fijnheer R, van Berge Henegouwen GP. Risk factors for thromboembolic complications in inflammatory bowel disease: the role of hyperhomocysteinaemia. *Dig Dis Sci* 2005; **50**: 235-240
8 **Kume K**, Yamasaki M, Tashiro M, Yoshikawa I, Otsuki M. Activations of coagulation and fibrinolysis secondary to bowel inflammation in patients with ulcerative colitis. *Intern Med* 2007; **46**: 1323-1329
9 **Talbot RW**, Heppell J, Dozois RR, Beart RW Jr. Vascular complications of inflammatory bowel disease. *Mayo Clin Proc* 1986; **61**: 140-145
10 **Bernstein CN**, Wajda A, Blanchard JF. The incidence of arterial thromboembolic diseases in inflammatory bowel disease: a population-based study. *Clin Gastroenterol Hepatol* 2008; **6**: 41-45
11 **Bernstein CN**, Blanchard JF, Houston DS, Wajda A. The incidence of deep venous thrombosis and pulmonary embolism among patients with inflammatory bowel disease: a population-based cohort study. *Thromb Haemost* 2001; **85**: 430-434
12 **Thompson NP**, Wakefield AJ, Pounder RE. Inherited disorders of coagulation appear to protect against inflammatory bowel disease. *Gastroenterology* 1995; **108**: 1011-1015
13 **Turri D**, Rosselli M, Simioni P, Tormene D, Grimaudo S, Martorana G, Siragusa S, Mariani G, Cottone M. Factor V Leiden and prothrombin gene mutation in inflammatory bowel disease in a Mediterranean area. *Dig Liver Dis* 2001; **33**: 559-562
14 **Vecchi M**, Sacchi E, Saibeni S, Meucci G, Tagliabue L, Duca F, De Franchis R. Inflammatory bowel diseases are not associated with major hereditary conditions predisposing to thrombosis. *Dig Dis Sci* 2000; **45**: 1465-1469

15 **Papa A**, De Stefano V, Gasbarrini A, Chiusolo P, Cianci R, Casorelli I, Paciaroni K, Cammarota G, Leone G, Gasbarrini G. Prevalence of factor V Leiden and the G20210A prothrombin-gene mutation in inflammatory bowel disease. *Blood Coagul Fibrinolysis* 2000; **11**: 499-503
16 **Papa A**, Danese S, Grillo A, Gasbarrini G, Gasbarrini A. Review article: inherited thrombophilia in inflammatory bowel disease. *Am J Gastroenterol* 2003; **98**: 1247-1251
17 **Jackson LM**, O'Gorman PJ, O'Connell J, Cronin CC, Cotter KP, Shanahan F. Thrombosis in inflammatory bowel disease: clinical setting, procoagulant profile and factor V Leiden. *QJM* 1997; **90**: 183-188
18 **Yilmaz S**, Bayan K, Tüzün Y, Batun S, Altintaş A. A comprehensive analysis of 12 thrombophilic mutations and related parameters in patients with inflammatory bowel disease: data from Turkey. *J Thromb Thrombolysis* 2006; **22**: 205-212
19 **Lake AM**, Stauffer JQ, Stuart MJ. Hemostatic alterations in inflammatory bowel disease: response to therapy. *Am J Dig Dis* 1978; **23**: 897-902
20 **Korsten S**, Reis HE. [Acquired protein C deficiency in ulcerative colitis. The cause of thromboembolic complications] *Dtsch Med Wochenschr* 1992; **117**: 419-424
21 **Aadland E**, Odegaard OR, Røseth A, Try K. Free protein S deficiency in patients with chronic inflammatory bowel disease. *Scand J Gastroenterol* 1992; **27**: 957-960
22 **Wyshock E**, Caldwell M, Crowley JP. Deep venous thrombosis, inflammatory bowel disease, and protein S deficiency. *Am J Clin Pathol* 1988; **90**: 633-635
23 **Sundaram KK**, Cotton R, Hart P, Jones L, Gould SR. Laboratory findings associated with thrombophilia are not more common in inflammatory bowel disease. *Clin Lab Haematol* 2000; **22**: 243-245
24 **Franco RF**, Reitsma PH, Lourenço D, Maffei FH, Morelli V, Tavella MH, Araújo AG, Piccinato CE, Zago MA. Factor XIII Val34Leu is a genetic factor involved in the etiology of venous thrombosis. *Thromb Haemost* 1999; **81**: 676-679
25 **Saibeni S**, Vecchi M, Faioni EM, Franchi F, Rondonotti E, Borsi G, de Franchis R. Val34Leu factor XIII polymorphism in Italian patients with inflammatory bowel disease. *Dig Liver Dis* 2003; **35**: 32-36
26 **Bernstein CN**, Sargent M, Vos HL, Rosendaal FR. Mutations in clotting factors and inflammatory bowel disease. *Am J Gastroenterol* 2007; **102**: 338-343
27 **Guédon C**, Le Cam-Duchez V, Lalaude O, Ménard JF, Lerebours E, Borg JY. Prothrombotic inherited abnormalities other than factor V Leiden mutation do not play a role in venous thrombosis in inflammatory bowel disease. *Am J Gastroenterol* 2001; **96**: 1448-1454
28 **Esmon CT**. Regulation of blood coagulation. *Biochim Biophys Acta* 2000; **1477**: 349-360
29 **Pottinger BE**, Read RC, Paleolog EM, Higgins PG, Pearson JD. von Willebrand factor is an acute phase reactant in man. *Thromb Res* 1989; **53**: 387-394
30 **Chiarantini E**, Valanzano R, Liotta AA, Cellai AP, Fedi S, Ilari I, Prisco D, Tonelli F, Abbate R. Hemostatic abnormalities in inflammatory bowel disease. *Thromb Res* 1996; **82**: 137-146
31 **Hudson M**, Chitolie A, Hutton RA, Smith MS, Pounder RE, Wakefield AJ. Thrombotic vascular risk factors in inflammatory bowel disease. *Gut* 1996; **38**: 733-737
32 **Appleyard CB**, Williams JL, Hathaway CA, Percy WH. Temporal patterns of colonic blood flow and tissue damage in an animal model of colitis. *Dig Dis Sci* 1999; **44**: 431-438
33 **Schambeck CM**, Grossmann R, Zonnur S, Berger M, Teuchert K, Spahn A, Walter U. High factor VIII (FVIII) levels in venous thromboembolism: role of unbound FVIII. *Thromb Haemost* 2004; **92**: 42-46
34 **Yurekli BP**, Aksoy DY, Aybar M, Egesel T, Gurgey A, Hascelik G, Kirazli S, Haznedaroglu IC, Arslan S. The search for a common thrombophilic state during the active state of inflammatory bowel disease. *J Clin Gastroenterol* 2006; **40**: 809-813
35 **Payzin B**, Adakan FY, Yalçin HC, Cetinkaya GS, Berkmen S, Eraslan S, Unsal B. Natural coagulation inhibitory proteins and activated protein C resistance in Turkish patients with inflammatory bowel disease. *Turk J Gastroenterol* 2006; **17**: 183-190
36 **Schousboe I**. Pharmacological regulation of factor XII activation may be a new target to control pathological coagulation. *Biochem Pharmacol* 2008; **75**: 1007-1013
37 **Grundt H**, Nilsen DW, Hetland Ø, Valente E, Fagertun HE. Activated factor 12 (FXIIa) predicts recurrent coronary events after an acute myocardial infarction. *Am Heart J* 2004; **147**: 260-266
38 **Yamashita A**, Nishihira K, Kitazawa T, Yoshihashi K, Soeda T, Esaki K, Imamura T, Hattori K, Asada Y. Factor XI contributes to thrombus propagation on injured neointima of the rabbit iliac artery. *J Thromb Haemost* 2006; **4**: 1496-1501
39 **Zezos P**, Papaioannou G, Nikolaidis N, Vasiliadis T, Giouleme O, Evgenidis N. Elevated plasma von Willebrand factor levels in patients with active ulcerative colitis reflect endothelial perturbation due to systemic inflammation. *World J Gastroenterol* 2005; **11**: 7639-7645
40 **Weber P**, Husemann S, Vielhaber H, Zimmer KP, Nowak-Göttl U. Coagulation and fibrinolysis in children, adolescents, and young adults with inflammatory bowel disease. *J Pediatr Gastroenterol Nutr* 1999; **28**: 418-422
41 **van Bodegraven AA**, Schoorl M, Linskens RK, Bartels PC, Tuynman HA. Persistent activation of coagulation and fibrinolysis after treatment of active ulcerative colitis. *Eur J Gastroenterol Hepatol* 2002; **14**: 413-418
42 **Zezos P**, Papaioannou G, Nikolaidis N, Patsiaoura K, Vassiliadis T, Mpoumponaris A, Giouleme O, Evgenidis N. Elevated markers of thrombin generation and fibrinolysis in patients with active and quiescent ulcerative colitis. *Med Sci Monit* 2009; **15**: CR563-CR572
43 **Hayat M**, Ariëns RA, Moayyedi P, Grant PJ, O'Mahony S. Coagulation factor XIII and markers of thrombin generation and fibrinolysis in patients with inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2002; **14**: 249-256
44 **Stadnicki A**, Kloczko J, Nowak A, Sierka E, Sliwiński Z. Factor XIII subunits in relation to some other hemostatic parameters in ulcerative colitis. *Am J Gastroenterol* 1991; **86**: 690-693
45 **Hudson M**, Wakefield AJ, Hutton RA, Sankey EA, Dhillon AP, More L, Sim R, Pounder RE. Factor XIIIA subunit and Crohn's disease. *Gut* 1993; **34**: 75-79
46 **Vrij AA**, Rijken J, van Wersch JW, Stockbrügger RW. Differential behavior of coagulation factor XIII in patients with inflammatory bowel disease and in patients with giant cell arteritis. *Haemostasis* 1999; **29**: 326-335
47 **Chamouard P**, Grunebaum L, Wiesel ML, Sibilia J, Coumaros G, Wittersheim C, Baumann R, Cazenave JP. Significance of diminished factor XIII in Crohn's disease. *Am J Gastroenterol* 1998; **93**: 610-614
48 **Uchiba M**, Okajima K, Murakami K. Effects of various doses of antithrombin III on endotoxin-induced endothelial cell injury and coagulation abnormalities in rats. *Thromb Res* 1998; **89**: 233-241
49 **Warren BL**, Eid A, Singer P, Pillay SS, Carl P, Novak I, Chalupa P, Atherstone A, Pénzes I, Kübler A, Knaub S, Keinecke HO, Heinrichs H, Schindel F, Juers M, Bone RC, Opal SM. Caring for the critically ill patient. High-dose antithrombin III in severe sepsis: a randomized controlled trial. *JAMA* 2001; **286**: 1869-1878
50 **Uchiba M**, Okajima K, Kaun C, Wojta J, Binder BR. Inhibition of the endothelial cell activation by antithrombin in vitro. *Thromb Haemost* 2004; **92**: 1420-1427
51 **Oelschläger C**, Römisch J, Staubitz A, Stauss H, Leithäuser B, Tillmanns H, Hölschermann H. Antithrombin III inhibits nuclear factor kappaB activation in human monocytes and vascular endothelial cells. *Blood* 2002; **99**: 4015-4020
52 **Isobe H**, Okajima K, Liu W, Harada N. Antithrombin pre-

vents stress-induced gastric mucosal injury by increasing the gastric prostacyclin level in rats. *J Lab Clin Med* 1999; **133**: 557-565
53 **Ghosh S**, Mackie MJ, McVerry BA, Galloway M, Ellis A, McKay J. Chronic inflammatory bowel disease, deep-venous thrombosis and antithrombin activity. *Acta Haematol* 1983; **70**: 50-53
54 **Hatoum OA**, Binion DG, Otterson MF, Gutterman DD. Acquired microvascular dysfunction in inflammatory bowel disease: Loss of nitric oxide-mediated vasodilation. *Gastroenterology* 2003; **125**: 58-69
55 **Lijnen HR**. Plasmin and matrix metalloproteinases in vascular remodeling. *Thromb Haemost* 2001; **86**: 324-333
56 **Hoylaerts M**, Rijken DC, Lijnen HR, Collen D. Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. *J Biol Chem* 1982; **257**: 2912-2919
57 **Chmielewska J**, Rånby M, Wiman B. Kinetics of the inhibition of plasminogen activators by the plasminogen-activator inhibitor. Evidence for 'second-site' interactions. *Biochem J* 1988; **251**: 327-332
58 **Rijken DC**, Lijnen HR. New insights into the molecular mechanisms of the fibrinolytic system. *J Thromb Haemost* 2009; **7**: 4-13
59 **Collen D**. Identification and some properties of a new fast-reacting plasmin inhibitor in human plasma. *Eur J Biochem* 1976; **69**: 209-216
60 **Rákóczi I**, Wiman B, Collen D. On the biological significance of the specific interaction between fibrin, plasminogen and antiplasmin. *Biochim Biophys Acta* 1978; **540**: 295-300
61 **Sakata Y**, Aoki N. Significance of cross-linking of alpha 2-plasmin inhibitor to fibrin in inhibition of fibrinolysis and in hemostasis. *J Clin Invest* 1982; **69**: 536-542
62 **Mosnier LO**, Bouma BN. Regulation of fibrinolysis by thrombin activatable fibrinolysis inhibitor, an unstable carboxypeptidase B that unites the pathways of coagulation and fibrinolysis. *Arterioscler Thromb Vasc Biol* 2006; **26**: 2445-2453
63 **Saibeni S**, Bottasso B, Spina L, Bajetta M, Danese S, Gasbarrini A, de Franchis R, Vecchi M. Assessment of thrombin-activatable fibrinolysis inhibitor (TAFI) plasma levels in inflammatory bowel diseases. *Am J Gastroenterol* 2004; **99**: 1966-1970
64 **Gris JC**, Schved JF, Raffanel C, Dubois A, Aguilar-Martinez P, Arnaud A, Sanchez N, Sarlat C, Balmès JL. Impaired fibrinolytic capacity in patients with inflammatory bowel disease. *Thromb Haemost* 1990; **63**: 472-475
65 **de Jong E**, Porte RJ, Knot EA, Verheijen JH, Dees J. Disturbed fibrinolysis in patients with inflammatory bowel disease. A study in blood plasma, colon mucosa, and faeces. *Gut* 1989; **30**: 188-194
66 **Koutroubakis IE**, Sfiridaki A, Tsiolakidou G, Coucoutsi C, Theodoropoulou A, Kouroumalis EA. Plasma thrombin-activatable fibrinolysis inhibitor and plasminogen activator inhibitor-1 levels in inflammatory bowel disease. *Eur J Gastroenterol Hepatol* 2008; **20**: 912-916
67 **von Hundelshausen P**, Weber C. Platelets as immune cells: bridging inflammation and cardiovascular disease. *Circ Res* 2007; **100**: 27-40
68 **Fiore S**, Serhan CN. Formation of lipoxins and leukotrienes during receptor-mediated interactions of human platelets and recombinant human granulocyte/macrophage colony-stimulating factor-primed neutrophils. *J Exp Med* 1990; **172**: 1451-1457
69 **Gangemi S**, Luciotti G, D'Urbano E, Mallamace A, Santoro D, Bellinghieri G, Davi G, Romano M. Physical exercise increases urinary excretion of lipoxin A4 and related compounds. *J Appl Physiol* 2003; **94**: 2237-2240
70 **Breitenstein A**, Camici GG, Tanner FC. Tissue factor: beyond coagulation in the cardiovascular system. *Clin Sci* (Lond) 2009; **118**: 159-172
71 **Celi A**, Pellegrini G, Lorenzet R, De Blasi A, Ready N, Furie BC, Furie B. P-selectin induces the expression of tissue factor on monocytes. *Proc Natl Acad Sci USA* 1994; **91**: 8767-8771
72 **Cambien B**, Wagner DD. A new role in hemostasis for the adhesion receptor P-selectin. *Trends Mol Med* 2004; **10**: 179-186
73 **Davì G**, Patrono C. Platelet activation and atherothrombosis. *N Engl J Med* 2007; **357**: 2482-2494
74 **André P**, Prasad KS, Denis CV, He M, Papalia JM, Hynes RO, Phillips DR, Wagner DD. CD40L stabilizes arterial thrombi by a beta3 integrin--dependent mechanism. *Nat Med* 2002; **8**: 247-252
75 **Schönbeck U**, Libby P. The CD40/CD154 receptor/ligand dyad. *Cell Mol Life Sci* 2001; **58**: 4-43
76 **Russo S**, Bussolati B, Deambrosis I, Mariano F, Camussi G. Platelet-activating factor mediates CD40-dependent angiogenesis and endothelial-smooth muscle cell interaction. *J Immunol* 2003; **171**: 5489-5497
77 **Anand SX**, Viles-Gonzalez JF, Badimon JJ, Cavusoglu E, Marmur JD. Membrane-associated CD40L and sCD40L in atherothrombotic disease. *Thromb Haemost* 2003; **90**: 377-384
78 **Lutgens E**, Lievens D, Beckers L, Wijnands E, Soehnlein O, Zernecke A, Seijkens T, Engel D, Cleutjens J, Keller AM, Naik SH, Boon L, Oufella HA, Mallat Z, Ahonen CL, Noelle RJ, de Winther MP, Daemen MJ, Biessen EA, Weber C. Deficient CD40-TRAF6 signaling in leukocytes prevents atherosclerosis by skewing the immune response toward an antiinflammatory profile. *J Exp Med* 2010; **207**: 391-404
79 **Morowitz DA**, Allen LW, Kirsner JB. Thrombocytosis in chronic inflammatory bowel disease. *Ann Intern Med* 1968; **68**: 1013-1021
80 **Webberley MJ**, Hart MT, Melikian V. Thromboembolism in inflammatory bowel disease: role of platelets. *Gut* 1993; **34**: 247-251
81 **Collins CE**, Cahill MR, Newland AC, Rampton DS. Platelets circulate in an activated state in inflammatory bowel disease. *Gastroenterology* 1994; **106**: 840-845
82 **Irving PM**, Macey MG, Shah U, Webb L, Langmead L, Rampton DS. Formation of platelet-leukocyte aggregates in inflammatory bowel disease. *Inflamm Bowel Dis* 2004; **10**: 361-372
83 **Larsen TB**, Nielsen JN, Fredholm L, Lund ED, Brandslund I, Munkholm P, Hey H. Platelets and anticoagulant capacity in patients with inflammatory bowel disease. *Pathophysiol Haemost Thromb* 2002; **32**: 92-96
84 **Yüksel O**, Helvaci K, Başar O, Köklü S, Caner S, Helvaci N, Abayli E, Altiparmak E. An overlooked indicator of disease activity in ulcerative colitis: mean platelet volume. *Platelets* 2009; **20**: 277-281
85 **Vizioli L**, Muscari S, Muscari A. The relationship of mean platelet volume with the risk and prognosis of cardiovascular diseases. *Int J Clin Pract* 2009; **63**: 1509-1515
86 **Borcherding F**, Nitschke M, Hundorfean G, Rupp J, von Smolinski D, Bieber K, van Kooten C, Lehnert H, Fellermann K, Büning J. The CD40-CD40L pathway contributes to the proinflammatory function of intestinal epithelial cells in inflammatory bowel disease. *Am J Pathol* 2010; **176**: 1816-1827
87 **Danese S**, Scaldaferri F, Papa A, Pola R, Gasbarrini A, Sgambato A, Cittadini A. CD40L-positive platelets induce CD40L expression de novo in endothelial cells: adding a loop to microvascular inflammation. *Arterioscler Thromb Vasc Biol* 2004; **24**: e162
88 **Ruggeri ZM**. Platelets in atherothrombosis. *Nat Med* 2002; **8**: 1227-1234
89 **Davis C**, Fischer J, Ley K, Sarembock IJ. The role of inflammation in vascular injury and repair. *J Thromb Haemost* 2003; **1**: 1699-1709
90 **Vestweber D**, Blanks JE. Mechanisms that regulate the function of the selectins and their ligands. *Physiol Rev* 1999; **79**: 181-213
91 **Vandendries ER**, Furie BC, Furie B. Role of P-selectin and PSGL-1 in coagulation and thrombosis. *Thromb Haemost* 2004; **92**: 459-466

92 **Berndt MC**, Shen Y, Dopheide SM, Gardiner EE, Andrews RK. The vascular biology of the glycoprotein Ib-IX-V complex. *Thromb Haemost* 2001; **86**: 178-188
93 **Wagner DD**, Burger PC. Platelets in inflammation and thrombosis. *Arterioscler Thromb Vasc Biol* 2003; **23**: 2131-2137
94 **Sadler JE**. Biochemistry and genetics of von Willebrand factor. *Annu Rev Biochem* 1998; **67**: 395-424
95 **Huang RH**, Wang Y, Roth R, Yu X, Purvis AR, Heuser JE, Egelman EH, Sadler JE. Assembly of Weibel-Palade body-like tubules from N-terminal domains of von Willebrand factor. *Proc Natl Acad Sci USA* 2008; **105**: 482-487
96 **Huizinga EG**, Tsuji S, Romijn RA, Schiphorst ME, de Groot PG, Sixma JJ, Gros P. Structures of glycoprotein Ibalpha and its complex with von Willebrand factor A1 domain. *Science* 2002; **297**: 1176-1179
97 **Padilla A**, Moake JL, Bernardo A, Ball C, Wang Y, Arya M, Nolasco L, Turner N, Berndt MC, Anvari B, López JA, Dong JF. P-selectin anchors newly released ultralarge von Willebrand factor multimers to the endothelial cell surface. *Blood* 2004; **103**: 2150-2156
98 **Papa A**, Scaldaferri F, Danese S, Guglielmo S, Roberto I, Bonizzi M, Mocci G, Felice C, Ricci C, Andrisani G, Fedeli G, Gasbarrini G, Gasbarrini A. Vascular involvement in inflammatory bowel disease: pathogenesis and clinical aspects. *Dig Dis* 2008; **26**: 149-155
99 **Danese S**, Sans M, de la Motte C, Graziani C, West G, Phillips MH, Pola R, Rutella S, Willis J, Gasbarrini A, Fiocchi C. Angiogenesis as a novel component of inflammatory bowel disease pathogenesis. *Gastroenterology* 2006; **130**: 2060-2073
100 **Rafiee P**, Johnson CP, Li MS, Ogawa H, Heidemann J, Fisher PJ, Lamirand TH, Otterson MF, Wilson KT, Binion DG. Cyclosporine A enhances leukocyte binding by human intestinal microvascular endothelial cells through inhibition of p38 MAPK and iNOS. Paradoxical proinflammatory effect on the microvascular endothelium. *J Biol Chem* 2002; **277**: 35605-35615
101 **Horowitz S**, Binion DG, Nelson VM, Kanaa Y, Javadi P, Lazarova Z, Andrekopoulos C, Kalyanaraman B, Otterson MF, Rafiee P. Increased arginase activity and endothelial dysfunction in human inflammatory bowel disease. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1323-G1336
102 **Gobert AP**, Cheng Y, Akhtar M, Mersey BD, Blumberg DR, Cross RK, Chaturvedi R, Drachenberg CB, Boucher JL, Hacker A, Casero RA Jr, Wilson KT. Protective role of arginase in a mouse model of colitis. *J Immunol* 2004; **173**: 2109-2117
103 **Buga GM**, Singh R, Pervin S, Rogers NE, Schmitz DA, Jenkinson CP, Cederbaum SD, Ignarro LJ. Arginase activity in endothelial cells: inhibition by NG-hydroxy-L-arginine during high-output NO production. *Am J Physiol* 1996; **271**: H1988-H1998
104 **Lancellotti S**, De Filippis V, Pozzi N, Peyvandi F, Palla R, Rocca B, Rutella S, Pitocco D, Mannucci PM, De Cristofaro R. Formation of methionine sulfoxide by peroxynitrite at position 1606 of von Willebrand factor inhibits its cleavage by ADAMTS-13: A new prothrombotic mechanism in diseases associated with oxidative stress. *Free Radic Biol Med* 2010; **48**: 446-456
105 **Saibeni S**, Saladino V, Chantarangkul V, Villa F, Bruno S, Vecchi M, de Franchis R, Sei C, Tripodi A. Increased thrombin generation in inflammatory bowel diseases. *Thromb Res* 2010; **125**: 278-282
106 **Tripodi A**, Chantarangkul V, Mannucci PM. Acquired coagulation disorders: revisited using global coagulation/anticoagulation testing. *Br J Haematol* 2009; **147**: 77-82
107 **Hemker HC**, Al Dieri R, De Smedt E, Béguin S. Thrombin generation, a function test of the haemostatic-thrombotic system. *Thromb Haemost* 2006; **96**: 553-561
108 **Tripodi A**, Salerno F, Chantarangkul V, Clerici M, Cazzaniga M, Primignani M, Mannuccio Mannucci P. Evidence of normal thrombin generation in cirrhosis despite abnormal conventional coagulation tests. *Hepatology* 2005; **41**: 553-558
109 **Sankey EA**, Dhillon AP, Anthony A, Wakefield AJ, Sim R, More L, Hudson M, Sawyerr AM, Pounder RE. Early mucosal changes in Crohn's disease. *Gut* 1993; **34**: 375-381
110 **More L**, Sim R, Hudson M, Dhillon AP, Pounder R, Wakefield AJ. Immunohistochemical study of tissue factor expression in normal intestine and idiopathic inflammatory bowel disease. *J Clin Pathol* 1993; **46**: 703-708
111 **Kirsner JB**, Shorter RG. Inflammatory Bowel Disease. Baltimore: Williams & Wilkins, 1995
112 **Arfors KE**, Rutili G, Svensjö E. Microvascular transport of macromolecules in normal and inflammatory conditions. *Acta Physiol Scand Suppl* 1979; **463**: 93-103
113 **Cunningham MA**, Rondeau E, Chen X, Coughlin SR, Holdsworth SR, Tipping PG. Protease-activated receptor 1 mediates thrombin-dependent, cell-mediated renal inflammation in crescentic glomerulonephritis. *J Exp Med* 2000; **191**: 455-462
114 **Howell DC**, Laurent GJ, Chambers RC. Role of thrombin and its major cellular receptor, protease-activated receptor-1, in pulmonary fibrosis. *Biochem Soc Trans* 2002; **30**: 211-216
115 **Cenac N**, Cellars L, Steinhoff M, Andrade-Gordon P, Hollenberg MD, Wallace JL, Fiorucci S, Vergnolle N. Proteinase-activated receptor-1 is an anti-inflammatory signal for colitis mediated by a type 2 immune response. *Inflamm Bowel Dis* 2005; **11**: 792-798
116 **Borensztajn K**, Peppelenbosch MP, Spek CA. Coagulation factor Xa signaling: the link between coagulation and inflammatory bowel disease? *Trends Pharmacol Sci* 2009; **30**: 8-16
117 **Vergnolle N**, Cellars L, Mencarelli A, Rizzo G, Swaminathan S, Beck P, Steinhoff M, Andrade-Gordon P, Bunnett NW, Hollenberg MD, Wallace JL, Cirino G, Fiorucci S. A role for proteinase-activated receptor-1 in inflammatory bowel diseases. *J Clin Invest* 2004; **114**: 1444-1456
118 **Kim JA**, Choi SC, Yun KJ, Kim DK, Han MK, Seo GS, Yeom JJ, Kim TH, Nah YH, Lee YM. Expression of protease-activated receptor 2 in ulcerative colitis. *Inflamm Bowel Dis* 2003; **9**: 224-229
119 **Chen D**, Dorling A. Critical roles for thrombin in acute and chronic inflammation. *J Thromb Haemost* 2009; **7** Suppl 1: 122-126
120 **Ruf W**, Dorfleutner A, Riewald M. Specificity of coagulation factor signaling. *J Thromb Haemost* 2003; **1**: 1495-1503
121 **Bar-Shavit R**, Kahn AJ, Mann KG, Wilner GD. Identification of a thrombin sequence with growth factor activity on macrophages. *Proc Natl Acad Sci USA* 1986; **83**: 976-980
122 **Dutt T**, Toh CH. The Yin-Yang of thrombin and activated protein C. *Br J Haematol* 2008; **140**: 505-515
123 **Frenette PS**, Mayadas TN, Rayburn H, Hynes RO, Wagner DD. Susceptibility to infection and altered hematopoiesis in mice deficient in both P- and E-selectins. *Cell* 1996; **84**: 563-574
124 **De Cristofaro R**, De Candia E, Landolfi R, Rutella S, Hall SW. Structural and functional mapping of the thrombin domain involved in the binding to the platelet glycoprotein Ib. *Biochemistry* 2001; **40**: 13268-13273
125 **Sabo TM**, Maurer MC. Biophysical investigation of GpIbalpha binding to thrombin anion binding exosite II. *Biochemistry* 2009; **48**: 7110-7122
126 **De Candia E**, Hall SW, Rutella S, Landolfi R, Andrews RK, De Cristofaro R. Binding of thrombin to glycoprotein Ib accelerates the hydrolysis of Par-1 on intact platelets. *J Biol Chem* 2001; **276**: 4692-4698
127 **Minami T**, Sugiyama A, Wu SQ, Abid R, Kodama T, Aird WC. Thrombin and phenotypic modulation of the endothelium. *Arterioscler Thromb Vasc Biol* 2004; **24**: 41-53
128 **Tesfamariam B**, Allen GT, Normandin D, Antonaccio MJ. Involvement of the "tethered ligand" receptor in thrombin-induced endothelium-mediated relaxations. *Am J Physiol* 1993; **265**: H1744-H1749
129 **Hamilton JR**, Moffatt JD, Frauman AG, Cocks TM. Protease-activated receptor (PAR) 1 but not PAR2 or PAR4 mediates endothelium-dependent relaxation to thrombin and trypsin

in human pulmonary arteries. *J Cardiovasc Pharmacol* 2001; **38**: 108-119
130 **Wu SQ**, Aird WC. Thrombin, TNF-alpha, and LPS exert overlapping but nonidentical effects on gene expression in endothelial cells and vascular smooth muscle cells. *Am J Physiol Heart Circ Physiol* 2005; **289**: H873-H885
131 **Blanc-Brude OP**, Archer F, Leoni P, Derian C, Bolsover S, Laurent GJ, Chambers RC. Factor Xa stimulates fibroblast procollagen production, proliferation, and calcium signaling via PAR1 activation. *Exp Cell Res* 2005; **304**: 16-27
132 **Riewald M**, Ruf W. Mechanistic coupling of protease signaling and initiation of coagulation by tissue factor. *Proc Natl Acad Sci USA* 2001; **98**: 7742-7747
133 **Daubie V**, Cauwenberghs S, Senden NH, Pochet R, Lindhout T, Buurman WA, Heemskerk JW. Factor Xa and thrombin evoke additive calcium and proinflammatory responses in endothelial cells subjected to coagulation. *Biochim Biophys Acta* 2006; **1763**: 860-869
134 **Seymour ML**, Binion DG, Compton SJ, Hollenberg MD, MacNaughton WK. Expression of proteinase-activated receptor 2 on human primary gastrointestinal myofibroblasts and stimulation of prostaglandin synthesis. *Can J Physiol Pharmacol* 2005; **83**: 605-616
135 **Senden NH**, Jeunhomme TM, Heemskerk JW, Wagenvoord R, van't Veer C, Hemker HC, Buurman WA. Factor Xa induces cytokine production and expression of adhesion molecules by human umbilical vein endothelial cells. *J Immunol* 1998; **161:** 4318-4324
136 **Hezi-Yamit A**, Wong PW, Bien-Ly N, Komuves LG, Prasad KS, Phillips DR, Sinha U. Synergistic induction of tissue factor by coagulation factor Xa and TNF: evidence for involvement of negative regulatory signaling cascades. *Proc Natl Acad Sci USA* 2005; **102**: 12077-12082
137 **McLean K**, Schirm S, Johns A, Morser J, Light DR. FXa-induced responses in vascular wall cells are PAR-mediated and inhibited by ZK-807834. *Thromb Res* 2001; **103**: 281-297
138 **Riewald M**, Kravchenko VV, Petrovan RJ, O'Brien PJ, Brass LF, Ulevitch RJ, Ruf W. Gene induction by coagulation factor Xa is mediated by activation of protease-activated receptor 1. *Blood* 2001; **97**: 3109-3116
139 **Altieri DC**, Stamnes SJ. Protease-dependent T cell activation: ligation of effector cell protease receptor-1 (EPR-1) stimulates lymphocyte proliferation. *Cell Immunol* 1994; **155**: 372-383
140 **Cirino G**, Cicala C, Bucci M, Sorrentino L, Ambrosini G, De-Dominicis G, Altieri DC. Factor Xa as an interface between coagulation and inflammation. Molecular mimicry of factor Xa association with effector cell protease receptor-1 induces acute inflammation in vivo. *J Clin Invest* 1997; **99**: 2446-2451
141 **Lust M**, Vulcano M, Danese S. The protein C pathway in inflammatory bowel disease: the missing link between inflammation and coagulation. *Trends Mol Med* 2008; **14**: 237-244
142 **Dahlbäck B**, Villoutreix BO. Regulation of blood coagulation by the protein C anticoagulant pathway: novel insights into structure-function relationships and molecular recognition. *Arterioscler Thromb Vasc Biol* 2005; **25**: 1311-1320
143 **Esmon CT**. Basic mechanisms and pathogenesis of venous thrombosis. *Blood Rev* 2009; **23**: 225-229
144 **Castellino FJ**, Ploplis VA. The protein C pathway and pathologic processes. *J Thromb Haemost* 2009; **7** Suppl 1: 140-145
145 **Bauer KA**, Kass BL, Beeler DL, Rosenberg RD. Detection of protein C activation in humans. *J Clin Invest* 1984; **74**: 2033-2041
146 **Castoldi E**, Hackeng TM. Regulation of coagulation by protein S. *Curr Opin Hematol* 2008; **15**: 529-536
147 **Magdeleyns EJ**, Keuren JF, Curvers J. Factor Va, bound to microparticles released during platelet storage, is resistant to inactivation by activated protein C. *Transfusion* 2007; **47**: 1880-1888
148 **Rezaie AR**. Vitronectin functions as a cofactor for rapid inhibition of activated protein C by plasminogen activator inhibitor-1. Implications for the mechanism of profibrinolytic action of activated protein C. *J Biol Chem* 2001; **276**: 15567-15570
149 **Komissarov AA**, Andreasen PA, Declerck PJ, Kamikubo Y, Zhou A, Gruber A. Redirection of the reaction between activated protein C and a serpin to the substrate pathway. *Thromb Res* 2008; **122**: 397-404
150 **Preston RJ**, Tran S, Johnson JA, Ainle FN, Harmon S, White B, Smith OP, Jenkins PV, Dahlbäck B, O'Donnell JS. Platelet factor 4 impairs the anticoagulant activity of activated protein C. *J Biol Chem* 2009; **284**: 5869-5875
151 **White TC**, Berny MA, Tucker EI, Urbanus RT, de Groot PG, Fernández JA, Griffin JH, Gruber A, McCarty OJ. Protein C supports platelet binding and activation under flow: role of glycoprotein Ib and apolipoprotein E receptor 2. *J Thromb Haemost* 2008; **6**: 995-1002
152 **Dahlbäck B**. The tale of protein S and C4b-binding protein, a story of affection. *Thromb Haemost* 2007; **98**: 90-96
153 **Heeb MJ**, Prashun D, Griffin JH, Bouma BN. Plasma protein S contains zinc essential for efficient activated protein C-independent anticoagulant activity and binding to factor Xa, but not for efficient binding to tissue factor pathway inhibitor. *FASEB J* 2009; **23**: 2244-2253
154 **Zheng X**, Li W, Gu JM, Qu D, Ferrell GL, Esmon NL, Esmon CT. Effects of membrane and soluble EPCR on the hemostatic balance and endotoxemia in mice. *Blood* 2007; **109**: 1003-1009
155 **Mosnier LO**, Griffin JH. Inhibition of staurosporine-induced apoptosis of endothelial cells by activated protein C requires protease-activated receptor-1 and endothelial cell protein C receptor. *Biochem J* 2003; **373**: 65-70
156 **Cheng T**, Liu D, Griffin JH, Fernández JA, Castellino F, Rosen ED, Fukudome K, Zlokovic BV. Activated protein C blocks p53-mediated apoptosis in ischemic human brain endothelium and is neuroprotective. *Nat Med* 2003; **9**: 338-342
157 **Coughlin SR**. Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J Thromb Haemost* 2005; **3**: 1800-1814
158 **Ludeman MJ**, Kataoka H, Srinivasan Y, Esmon NL, Esmon CT, Coughlin SR. PAR1 cleavage and signaling in response to activated protein C and thrombin. *J Biol Chem* 2005; **280**: 13122-13128
159 **Bae JS**, Yang L, Rezaie AR. Receptors of the protein C activation and activated protein C signaling pathways are colocalized in lipid rafts of endothelial cells. *Proc Natl Acad Sci USA* 2007; **104**: 2867-2872
160 **Riewald M**, Petrovan RJ, Donner A, Mueller BM, Ruf W. Activation of endothelial cell protease activated receptor 1 by the protein C pathway. *Science* 2002; **296**: 1880-1882
161 **Joyce DE**, Gelbert L, Ciaccia A, DeHoff B, Grinnell BW. Gene expression profile of antithrombotic protein c defines new mechanisms modulating inflammation and apoptosis. *J Biol Chem* 2001; **276**: 11199-11203
162 **Scaldaferri F**, Sans M, Vetrano S, Graziani C, De Cristofaro R, Gerlitz B, Repici A, Arena V, Malesci A, Panes J, Grinnell BW, Danese S. Crucial role of the protein C pathway in governing microvascular inflammation in inflammatory bowel disease. *J Clin Invest* 2007; **117**: 1951-1960
163 **Boehme MW**, Autschbach F, Zuna I, Scherbaum WA, Stange E, Raeth U, Sieg A, Stremmel W. Elevated serum levels and reduced immunohistochemical expression of thrombomodulin in active ulcerative colitis. *Gastroenterology* 1997; **113**: 107-117
164 **Diamant M**, Tushuizen ME, Sturk A, Nieuwland R. Cellular microparticles: new players in the field of vascular disease? *Eur J Clin Invest* 2004; **34**: 392-401
165 **Van de Wouwer M**, Collen D, Conway EM. Thrombomodulin-protein C-EPCR system: integrated to regulate coagulation and inflammation. *Arterioscler Thromb Vasc Biol* 2004; **24**: 1374-1383
166 **Faioni EM**, Ferrero S, Fontana G, Gianelli U, Ciulla MM, Vecchi M, Saibeni S, Biguzzi E, Cordani N, Franchi F, Bosari S, Cattaneo M. Expression of endothelial protein C recep-

tor and thrombomodulin in the intestinal tissue of patients with inflammatory bowel disease. *Crit Care Med* 2004; **32**: S266-S270
167 **Isobe H**, Okajima K, Harada N, Liu W, Okabe H. Activated protein C reduces stress-induced gastric mucosal injury in rats by inhibiting the endothelial cell injury. *J Thromb Haemost* 2004; **2**: 313-320
168 **Bae JS**, Rezaie AR. Thrombin and activated protein C inhibit the expression of secretory group IIA phospholipase A(2) in the TNF-alpha-activated endothelial cells by EPCR and PAR-1 dependent mechanisms. *Thromb Res* 2010; **125**: e9-e15
169 **Danese S**, Vetrano S, Zhang L, Poplis VA, Castellino FJ. The protein C pathway in tissue inflammation and injury: pathogenic role and therapeutic implications. *Blood* 2010; **115**: 1121-1130
170 **Elsayed E**, Becker RC. The impact of heparin compounds on cellular inflammatory responses: a construct for future investigation and pharmaceutical development. *J Thromb Thrombolysis* 2003; **15**: 11-18
171 **Papa A**, Danese S, Gasbarrini A, Gasbarrini G. Review article: potential therapeutic applications and mechanisms of action of heparin in inflammatory bowel disease. *Aliment Pharmacol Ther* 2000; **14**: 1403-1409
172 **Folwaczny C**, Wiebecke B, Loeschke K. Unfractioned heparin in the therapy of patients with highly active inflammatory bowel disease. *Am J Gastroenterol* 1999; **94**: 1551-1555
173 **Ang YS**, Mahmud N, White B, Byrne M, Kelly A, Lawler M, McDonald GS, Smith OP, Keeling PW. Randomized comparison of unfractionated heparin with corticosteroids in severe active inflammatory bowel disease. *Aliment Pharmacol Ther* 2000; **14**: 1015-1022
174 **Dotan I**, Hallak A, Arber N, Santo M, Alexandrowitz A, Knaani Y, Hershkoviz R, Brazowski E, Halpern Z. Low-dose low-molecular weight heparin (enoxaparin) is effective as adjuvant treatment in active ulcerative colitis: an open trial. *Dig Dis Sci* 2001; **46**: 2239-2244
175 **Panés J**, Esteve M, Cabré E, Hinojosa J, Andreu M, Sans M, Fernandez-Bañares F, Feu F, Gassull MA, Piqué JM. Comparison of heparin and steroids in the treatment of moderate and severe ulcerative colitis. *Gastroenterology* 2000; **119**: 903-908
176 **Bloom S**, Kiilerich S, Lassen MR, Forbes A, Leiper K, Langholz E, Irvine EJ, O'Morain C, Lowson D, Orm S. Low molecular weight heparin (tinzaparin) vs. placebo in the treatment of mild to moderately active ulcerative colitis. *Aliment Pharmacol Ther* 2004; **19**: 871-878
177 **Zezos P**, Papaioannou G, Nikolaidis N, Patsiaoura K, Papageorgiou A, Vassiliadis T, Giouleme O, Evgenidis N. Low-molecular-weight heparin (enoxaparin) as adjuvant therapy in the treatment of active ulcerative colitis: a randomized, controlled, comparative study. *Aliment Pharmacol Ther* 2006; **23**: 1443-1453
178 **de Bièvre MA**, Vrij AA, Schoon EJ, Dijkstra G, de Jong AE, Oberndorff-Klein Woolthuis AH, Hemker HC, Stockbrügger RW. Randomized, placebo-controlled trial of low molecular weight heparin in active ulcerative colitis. *Inflamm Bowel Dis* 2007; **13**: 753-758
179 **Shen J**, Ran ZH, Tong JL, Xiao SD. Meta-analysis: The utility and safety of heparin in the treatment of active ulcerative colitis. *Aliment Pharmacol Ther* 2007; **26**: 653-663
180 **Pastorelli L**, Saibeni S, Spina L, Signorelli C, Celasco G, de Franchis R, Vecchi M. Oral, colonic-release low-molecular-weight heparin: an initial open study of Parnaparin-MMX for the treatment of mild-to-moderate left-sided ulcerative colitis. *Aliment Pharmacol Ther* 2008; **28**: 581-588
181 **Chande N**, McDonald JW, Macdonald JK. Unfractionated or low-molecular weight heparin for induction of remission in ulcerative colitis. *Cochrane Database Syst Rev* 2008; CD006774

S- Editor Tian L **L- Editor** Webster JR **E- Editor** Lin YP

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.609

World J Gastroenterol 2011 February 7; 17(5): 609-617
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Bacteriocinogeny in experimental pigs treated with indomethacin and *Escherichia coli Nissle*

Jan Bures, David Smajs, Jaroslav Kvetina, Miroslav Förstl, Jan Smarda, Darina Kohoutova, Martin Kunes, Jiri Cyrany, Ilja Tacheci, Stanislav Rejchrt, Jirina Lesna, Viktor Vorisek, Marcela Kopacova

Jan Bures, Darina Kohoutova, Jiri Cyrany, Ilja Tacheci, Stanislav Rejchrt, Marcela Kopacova, 2nd Department of Medicine, Charles University in Praha, Faculty of Medicine at Hradec Kralove, University Teaching Hospital, 500 05 Hradec Kralove, Czech Republic
David Smajs, Jan Smarda, Department of Biology, Masaryk University, Faculty of Medicine, 625 00 Brno, Czech Republic
Jaroslav Kvetina, Martin Kunes, Institute of Experimental Biopharmaceutics, Joint Research Centre of Czech Academy of Sciences and PRO.MED.CS Praha a.s., 500 02 Hradec Kralove, Czech Republic
Miroslav Förstl, Jirina Lesna, Institute of Clinical Microbiology, Charles University in Praha, Faculty of Medicine at Hradec Kralove, University Teaching Hospital, 500 05 Hradec Kralove, Czech Republic
Viktor Vorisek, Institute of Clinical Biochemistry and Diagnostics, Charles University in Praha, Faculty of Medicine at Hradec Kralove, University Teaching Hospital, 500 05 Hradec Kralove, Czech Republic
Author contributions: All authors contributed equally to this work.
Supported by Research project MZO 00179906 from the Ministry of Health of the Czech Republic, by institutional support from the Czech Republic (MSM0021622415) and by research grants GAČR 305/080535 and NS9665-4/2008 (Ministry of Health of the Czech Republic)
Correspondence to: Jan Bures, MD, PhD, Professor, 2nd Department of Medicine, Charles University in Praha, Faculty of Medicine at Hradec Kralove, University Teaching Hospital, 500 05 Hradec Kralove, Czech Republic. bures@lfhk.cuni.cz
Telephone: +420-495-834240 Fax: +420-495-834785
Received: August 3, 2010 Revised: September 25, 2010
Accepted: October 2, 2010
Published online: February 7, 2011

Abstract

AIM: To evaluate bacteriocinogeny in short-term high-dose indomethacin administration with or without probiotic *Escherichia coli Nissle 1917* (EcN) in experimental pigs.

METHODS: Twenty-four pigs entered the study: Group A (controls), Group B (probiotics alone), Group C (indomethacin alone) and Group D (probiotics and indomethacin). EcN (3.5×10^{10} bacteria/d for 14 d) and/or indomethacin (15 mg/kg per day for 10 d) were administrated orally. Anal smears before and smears from the small and large intestine were taken from all animals. Bacteriocin production was determined with 6 different indicator strains; all strains were polymerase chain reaction tested for the presence of 29 individual bacteriocin-encoding determinants.

RESULTS: The general microbiota profile was rather uniform in all animals but there was a broad diversity in coliform bacteria (parallel genotypes A, B1, B2 and D found). In total, 637 bacterial strains were tested, mostly *Escherichia coli* (*E. coli*). There was a higher incidence of non-*E. coli* strains among samples taken from the jejunum and ileum compared to that of the colon and rectum indicating predominance of *E. coli* strains in the large intestine. Bacteriocinogeny was found in 24/77 (31%) before and in 155/560 (28%) isolated bacteria at the end of the study. Altogether, 13 individual bacteriocin types (out of 29 tested) were identified among investigated strains. Incidence of four *E. coli* genotypes was equally distributed in all groups of *E. coli* strains, with majority of genotype A (ranging from 81% to 88%). The following types of bacteriocins were most commonly revealed: colicins Ia/Ib (44%), microcin V (18%), colicin E1 (16%) and microcin H47 (6%). There was a difference in bacteriocinogeny between control group A (52/149, 35%) and groups with treatment at the end of the study: B: 31/122 (25%, $P = 0.120$); C: 43/155 (28%, $P = 0.222$); D: 29/134 (22%, $P = 0.020$). There was a significantly lower prevalence of colicin Ib, microcins H47 and V (probiotics group, $P < 0.001$), colicin E1 and microcin H47 (indomethacin group, $P < 0.001$) and microcins H47 and V (probiotics and indomethacin group, $P = 0.025$) compared to controls. *Escherichia fergusonii* (*E. fergusonii*) was identi-

fied in 6 animals (6/11 isolates from the rectum). One strain was non-colicinogenic, while all other strains of *E. fergusonii* solely produced colicin E1. All animals started and remained methanogenic despite the fact that EcN is a substantial hydrogen producer. There was an increase in breath methane (after the treatment) in 5/6 pigs from the indomethacin group (C).

CONCLUSION: EcN did not exert long-term liveability in the porcine intestine. All experimental pigs remained methanogenic. Indomethacin and EcN administered together might produce the worst impact on bacteriocinogeny.

Key words: Bacteriocinogeny; *Escherichia coli Nissle 1917*; Experimental pigs; Indomethacin

Peer reviewer: Javier San Martín, Chief, Gastroenterology and Endoscopy, Sanatorio Cantegril, Av. Roosevelt y P 13, Punta del Este 20100, Uruguay

Bures J, Smajs D, Kvetina J, Förstl M, Smarda J, Kohoutova D, Kunes M, Cyrany J, Tacheci I, Rejchrt S, Lesna J, Vorisek V, Kopacova M. Bacteriocinogeny in experimental pigs treated with indomethacin and *Escherichia coli Nissle*. *World J Gastroenterol* 2011; 17(5): 609-617 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/609.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.609

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) represent the group of most commonly used drugs worldwide. NSAIDs may cause severe injury to all parts of the gastrointestinal tract. The pathogenesis of NSAID-induced entero- and colopathy is more multifactorial and complex than formerly assumed but is not yet fully understood. A combination of local and systemic effects plays an important role in pathogenesis. NSAID-induced entero- and colopathy is a stepwise process involving direct mucosal toxicity, mitochondrial damage, breakdown of intercellular integrity, enterohepatic recirculation and neutrophil activation by luminal contents including bacteria. Unlike upper gastrointestinal toxicity, cyclo-oxygenase-mediated mechanisms are probably less important[1-3]. Intestinal bacteria play a significant role in the pathogenesis of NSAID-induced entero- and colopathy. In experimental studies, NSAIDs cannot induce enteropathy in germ-free rats[4].

Probiotic bacteria are live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host[5]. Probiotics likely function through enhancement of the barrier function of the gut, immunomodulation, and competitive adherence to the mucus and epithelium[6]. Probiotic bacteria may exert a systemic anti-inflammatory effect[7] and modulate apoptosis[8]. Probiotics have been suggested for amelioration or prevention of various diseases including antibiotic-associated diarrhoea, irritable bowel syndrome and inflammatory bowel disease. Further possible beneficial effects are being studied (including anti-cancer potential, lowering of serum cholesterol levels and blood pressure reduction, *etc.*)[9-11]. It has been hypothesised that probiotic bacteria might reduce the adverse effects of NSAIDs on the small and large intestine. However, initial studies provided controversial results, both with ameliorating and deteriorating outcomes[12-15]. NSAID-induced small intestinal injury is Toll-like receptor 4 dependent[14]. Probiotic *Escherichia coli Nissle 1917* (EcN) might ameliorate experimental colitis (induced by dextran sodium sulphate) *via* Toll-like receptor 2 and 4 pathways[16,17].

Colicins and microcins, members of the bacteriocin family, are produced by bacteriocinogenic strains of *Escherichia coli* (*E. coli*) and some related species of *Enterobacteriaceae*. They are toxic to susceptible bacterial strains of the same family[18-20]. However, some bacteriocins also exert an inhibitory effect on eukaryotic cells, including observed antineoplastic action *in vitro* and *in vivo*[21-25]. Bacteriocins might induce apoptosis[26] as some regulators of apoptosis (e.g. Bcl family with pro- and anti-apoptotic members) share similar structures with pore-forming colicins[27]. The possible role of bacteriocins was also investigated in clinical studies on bacillary dysentery[28], inflammatory bowel disease[29] and colorectal cancer[30]. Bacteriocins might have a dual role: they may act as both antibiotics and probiotics[31]. One of the most commonly used probiotic bacterial strains, EcN, is a producer of microcins H47 and M[32-34].

The aim of this study was to evaluate bacteriocinogeny in short-term high-dose indomethacin administration with or without probiotic bacteria EcN in an experimental porcine model. A small adult pig can be used in experiments as a representative of an omnivore due to its relatively similar gastrointestinal functions in comparison with man[35-38].

MATERIALS AND METHODS

Ethics

The Project was approved by the Institutional Review Board of Animal Care Committee of the Institute of Experimental Biopharmaceutics, Academy of Sciences of the Czech Republic, Record Number 1492006. Animals were held and treated in accordance with the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes[39].

Animals

Twenty-four healthy mature (4-5 mo old) female pigs (*Sus scrofa* f. *domestica*, hybrids of Czech White and Landrace breeds) weighing 33.0 ± 1.7 kg, were included in our study. The animals were divided into four groups: Group A (controls, 6 animals), Group B (probiotics alone, n = 6), Group C (indomethacin alone, n = 6) and Group D (probiotics and indomethacin, n = 6). All animals were fed twice a day (standard assorted food A1 of equal amounts).

Drug and probiotic bacteria administration

EcN (3.5×10^{10} live bacteria/d for 14 d) and/or indomethacin (Indomethacin suppositories, Berlin-Chemie, Germany; 15 mg/kg per day for 10 d) were administrated as one-shot dietary bolus to hungry pigs.

Autopsy

Twenty-four hours after the last drug and/or probiotic bacteria administration (groups B to D) or after 14 d of stabling (Group A), the pigs were sacrificed (after 24 h of fasting) by means of pharmacological euthanasia (iv administration of embutramide, mebezonium iodide and tetracaine hydrochloride - T61, Intervet International BV, Boxmeer, the Netherlands; dose of 2 mL per kg) and exsanguinated. Immediate autopsy was performed and smears for bacterial cultures were taken.

Bacterial culture, isolation and identification

Before the experiment anal smears were taken from all animals. At autopsy, smears from mucosa of the jejunum, ileum, caecum, transverse colon and rectum were taken from each animal and immediately inserted into a transport liver-enriched broth. Standard primary cultures were inoculated on blood and MacConkey agars (24 h at 37℃), followed by standard clone isolation. Up to 9 different colonies of coliform bacteria were isolated from each sample (on blood, MacConkey and deoxycholate agars). Particular bacteria were precisely identified by the Vitek2 system (BioMérieux, Marcy l'Etoile, France). All bacterial strains were frozen in cryotube vials at minus 90℃ until bacteriocin genotyping.

Analysis of bacteriocin production

The bacteriocin production of all strains was tested in parallel on 4 different agar plates containing (1) TY medium; (2) nutrient broth; (3) TY medium supplemented with mitomycin C; and (4) TY medium supplemented with trypsin. The TY medium consisted of yeast extract (Hi-Media, Mumbai, India) 5 g/L, tryptone (Hi-Media) 8 g/L, sodium chloride 5 g/L; the TY agar consisted of a base layer (1.5%, w/v, solid agar) and a top layer (0.7%, w/v, soft agar). A Difco™ nutrient broth (Difco Laboratories, Sparks, MD) 8 g/L, NaCl 5 g/L, was used for production of relatively unenriched 1.5% (w/v) agar plates. Mitomycin C (0.01%, w/v) and trypsin (0.1%, w/v) were used for induction of colicin production and for protease sensitivity tests, respectively. The previously described set of *E. coli* indicator strains including *E. coli* K12-Row, C6 (φ), B1, P400, and S40 was used to identify the producer strains together with *Shigella sonnei* 17 indicator[40,41]. To test bacteriocin production, the agar plates were inoculated by needle stab and the plates were incubated at 37℃ for 48 h. The tested macrocolonies were then killed with chloroform vapours and each plate was then overlaid with a thin layer of soft agar containing 10^7 cells/mL of an indicator strain and the plates were incubated at 37℃ overnight. All investigated *E. coli* strains were tested on four parallel plates against 6 indicator strains stated.

Table 1 DNA primers used for polymerase chain reaction detection of colicin encoding genes

Bacteriocin type	Primer name	5'-sequence-3'	Length of PCR product (nt)
A	ColA-F	CGTGGGGAAAAGTCATCATC	
	ColA-R	GCTTTGCTCTTTCCTGATGC	475
B	colicinB-F	AAGAAAATGACGAGAAGACG	
	colicinB-R	GAAAGACCAAAGGCTATAAGG	493
D	ColD-F	CTGGACTGCTGCTGGTGATA	
	ColD-R	GAAGGTGCGCCTACTACTGC	420
E1	colicinE1-F	TGTGGCATCGGGCGAGAATA	
	colicinE1-R	CTGCTTCCTGAAAAGCCTTTT	650
E2	ColE2-F	TGATGCTGCTGCAAAAGAG	
	ColE2-R	TTCAAAGCGTTCCCTACCAC	409
E3	ColE3-F	TAAGCAGGCTGCATTTGATG	
	ColE3-R	TCGGATCTGGACCTTTCAAC	413
E4	ColE4-F	GAAGGCTGCATTTGATGCT	
	ColE4-R	CGGATCCGGACCTTTAATTT	409
E5	ColE3-F	TAAGCAGGCTGCATTTGATG	
	ColE5-R	TTGAATTCTCGAATCGTCCA	430
E6	ColE6-F	ACCGAACGTCCAGGTGTT	
	ColE6-R	TTTAGCCTGTCGCTCCTGAT	399
E7	ColE7-F	GCATTCTGCCATCTGAAAT	
	ColE7-R	CTTCTGCCCACTTTCTTTCG	431
E8	ColE3-F	TAAGCAGGCTGCATTTGATG	
	ColE8-R	GACTGATTGGCTTGTCGTGA	449
E9	ColE3-F	TAAGCAGGCTGCATTTGATG	
	ColE9-R	GACTTTTCTCCCTCCGACCT	418
Ia	ColIa-F	GCATGCAAATGACGCTCTTA	
	ColIa-R	GAGGACGCCAGTTCTCTGTC	473
Ib	ColIb-F	AACGAGTGGGTCGATGATTC	
	ColIb-R	CCTTTTCTGCGCTCGTATTC	464
Js	ColJs-F	TCAAAATGTTTGGGCTCCTC	
	ColJs-R	TAATCTGCCCTGTCCCACTG	254
K	ColK-F	CAGAGGTCGCTGAACATGAA	
	ColK-R	TCCGCTAAATCCTGAGCAAT	469
M	ColM-F	GCTTACCACTTCGCAAAACC	
	ColM-R	GAGCGACTCTCCGATAATGC	429
N	ColN-F	AGCTTGGCGAGTATCTTGGA	
	ColN-R	CAACACAGCCCCGAATAAAC	401
S4	ColS4-F	TATATGGCCCAACTGCTGGT	
	ColS4-R	CGTAAGGACGGACACCTGTT	456
U	ColU-F	TGATTGCTGCGAGAAAAATG	
	ColU-R	TCTGACAGCCTCTCCCTGTT	485
Y	ColY-F	GCAGGCAGAAAAGAACAAGG	
	ColY-R	CGGACGTTATTTGCCTTCAT	477
5	Col5-F	CATTGGCAAAAGCGAAATCT	
	Col5-R	TGCAACTCTGGAAACAATCG	443
10	Col10-F	GGTTACCGGATTTCCTGGAT	
	Col10-R	TTCTAGATGCTTGGCCCACT	448

PCR: Polymerase chain reaction.

Identification of individual colicin types

All investigated strains were tested with colony polymerase chain reaction (PCR). A bacterial colony was resuspended in 100 μL of sterile water and 1 μL of this suspension was added to the PCR reaction. Individual colicin types (colicins A, D, E2-E9, Ia, Ib, Js, K, M, N, S4, U, Y, 5 and 10) were detected using PCR with primers designed using the Primer3 program[42]. The list of primer pairs and the corresponding length of PCR products are listed in Table 1. Control bacterial producers stemmed from our stock and comprised *E. coli* BZB2101pColA - CA31, BZB2102 pColB - K260, BZB2103 pColD -

Table 2 Bacteriocinogeny of particular strains isolated at the end of experiment

Parameter	Small intestine			Colon and rectum		
	Bacterio-cinogeny	Types of bacteriocin producers (No. of strains)	No. of unique bacteriocin producers	Bacterio-cinogeny	Types of bacteriocin producers (No. of strains)	No. of unique bacteriocin producers
Group A	22/55 (40%)	E1 (1); E1, Ia, V (1); E1, V (2); Ia (2); Ia, B, K, M, H47 (2); Ia, H47, V (2); Ia, V (6); Ib (3); J25 (1); S4, U (1); S4, V (1)	11	30/94 (32%)	B (1); B, H47, Ib, K, M (2); B, M (1); C7, E1, Ib, V (1); E1 (1); E1, Ia (1); E1, Ia, V (1); E1, V (2); E7 (1); H47, Ia, V (1); H47, S4 (1); H47, V (2); Ia (6); Ia, V (4); Ib (3); M (1); S4, V (1)	17
Group B	11/43 (26%)	E1 (7); Ia (2); Ia, V, H47 (1); Ib (1)	4	20/79 (25%)	B, H47, K, M, Ia (1); B, M (1); E1 (4); E1, V (1); Ia (11); Ia, V (1); Ib (1)	7
Group C	17/58 (29%)	B, M, V (1); Ia (1); Ia, V (7); Ib (6); S4 (2)	5	26/97 (27%)	B, Ia, (1); E1, Ib (1); J25, Ia (1); Ia (5); Ia, E7, V (1); Ia, H47 (1); Ia, M (1); Ia, V (8); Ib (7)	9
Group D	9/45 (20%)	E1 (3); E1, Ia (1); E1, Ia, V (1); E1, Ib (1); Ia (1); Ia, V (1); Ib (1)	7	20/89 (22%)	E1 (4); E1, Ia (1); E1, Ia, V (1); E1, Ib (2); H47, V (1); Ia (7); Ia, V (1); Ib (2); Ib, V (1)	9

Small intestine: Bacterial strains isolated from mucosa of the jejunum and ileum; Colon and rectum: Bacterial strains isolated from mucosa of the caecum, transverse colon and rectum; Group A: Control animals with no treatment; Group B: Probiotics alone (see text for details); Group C: Indomethacin alone (see text for details); Group D: Probiotics and indomethacin (see text for details); Bacteriocinogeny: Number of bacteriocinogenic strains out of all tested; Types: Particular bacteriocin types found in single isolates; M: Colicin M, not for microcin M.

CA23, BZB2107 pColE4 - CT9, BZB2108 pColE5 - 099, BZB2150 pColE6 - CT14, BZB2120 pColE7 - K317, BZB2279 pColIa - CA53, BZB2202 ColIb - P9, BZB2116 pColK - K235, PAP1 pCol101M - BZBNC22, BZB2123 pColN - 284, *E.* 189BM pColE2 - P9, *E. coli* 385/80 pColE1, pColV, *E. coli* 185M4 pColE3 - CA38, *E. coli* W3110 pColE8, W3110 pColE9, *E. coli* K-12 pColS4, *Shigella boydii* M592 (serovar 8) pColU, *E. coli* K339 pColY, *Sh. sonnei* pColJs, *E. coli* pCol5, *E. coli* pCol10, *E. coli* 449/82 pColX (microcin B17), *E. coli* 313/66 pColG (microcin H47), *E. coli* 363/79 pColV (microcin V), *E. coli* TOP10F' pDS601 (microcin C7), *E. coli* D55/1 (microcin J25), and *E. coli* B1239 (microcin L). Sequentially related colicin genes (colicins E2-E9, Ia-Ib, U-Y, and 5-10, respectively) often yielded PCR products with primer pairs of related colicin types and therefore all these PCR products were sequenced. The PCR detection primers for colicins B and E1 and for 6 microcin types including B17, C7, H47, J25, L, and V, were taken from Gordon *et al*[43]. The phylogenetic group of each *E. coli* strain was determined using the triplex PCR protocol according to Clermont *et al*[44]. Sequence analysis was performed using Lasergene software (DNASTAR, Inc., Madison, WI, USA).

Hydrogen and methane breath testing

Hydrogen and methane breath tests were performed before and the morning following completion of the treatment, carried out under general anaesthesia in spontaneously breathing animals. Alveolar air was aspirated by means of percutaneous puncture of the trachea. Immediate measurement of hydrogen and methane was accomplished in triplicate by means of gas chromatography (Microlyzer DP Plus Quintron, Milwaukee, WI, USA). Results were expressed as parts per million (ppm).

Statistical analysis

Data were statistically analysed with χ^2 with Yates correction and by Mann-Whitney rank sum test. Statistical software was used for this analysis (SigmaStat version 3.1, Jandel Co., Erkrath, Germany).

RESULTS

The general microbiota profile was rather uniform in all animals but there was a broad diversity in coliform bacteria (parallel genotypes A, B1, B2 and D found). In total, 637 bacterial strains were tested, mostly *E. coli*. The remaining isolates comprised *Salmonella enterica* ssp *Arizonae* (21 isolates), *Pasteurella aerogenes* (20), *Escherichia fergusonii* (*E. fergusonii*) (11), *Aeromonas hydrophila/caviae* (9), *Klebsiella pneumoniae* (8), *Enterobacter cloacae* (4), *Morganella morganii* (4), *Citrobacter braakii* (2), *Citrobacter youngae* (2), *Citrobacter freundii* (1), *Acinetobacter lwoffii* (1) and *Pseudomonas aeruginosa* (1). There was a higher incidence of non-*E. coli* strains among samples taken from the jejunum and ileum compared to that of the colon and rectum indicating predominance of *E. coli* strains in the large intestine (data not shown).

Bacteriocinogeny was found in 24/77 (31%) before and in 155/560 (28%) isolated bacteria at the end of the study. Altogether, 13 individual bacteriocin types (out of 29 tested) were identified among investigated strains. Incidence of four *E. coli* genotypes was equally distributed in all groups of *E. coli* strains, with majority of genotype A (ranging from 81% to 88%). The following types of bacteriocins were most commonly revealed: colicins Ia/Ib (44%), microcin V (18%), colicin E1 (16%) and microcin H47 (6%). There was a difference in bacteriocinogeny between control group A (52/149, 35%) and groups with treatment at the end of the study: B: 31/122 (25%, P = 0.120); C: 43/155 (28%, P = 0.222); D: 29/134 (22%, P = 0.020). See Table 2 for details. There was a significantly lower prevalence of colicin Ib, microcins H47 and V (probiotics group, $P < 0.001$), colicin E1 and microcin H47 (indomethacin group, $P < 0.001$) and microcins H47 and V (probiotics and indomethacin group, P = 0.025) com-

Table 3 Analysis of porcine alveolar breath for hydrogen and methane (in ppm - parts per million) before and after the treatment

Group	Hydrogen before	Hydrogen after	Statistical significance	Methane before	Methane after	Statistical significance
A	N/A	3.50 ± 2.81	N/A	N/A	69.33 ± 56.64	N/A
B	6.0 ± 2.82	2.0 ± 0	NS	106.50 ± 94.05	80.00 ± 48.02	NS
C	1.17 ± 0.41	5.0 ± 3.29	NS	34.67 ± 25.65	66.17 ± 38.83	NS
D	2.0 ± 1.16	6.0 ± 6.0	NS	60.75 ± 34.77	62.00 ± 27.71	NS

Group A (controls with no treatment, n = 6), Group B (probiotics alone, n = 6), Group C (indomethacin alone, n = 6) and Group D (probiotics plus indomethacin, n = 6). N/A: Not applicable; NS: Not significant.

pared to controls (Table 2). *E. fergusonii* was identified in 6 animals (6/11 isolates from the rectum). One strain was non-colicinogenic, while all other strains of *E. fergusonii* solely produced colicin E1.

Data on porcine alveolar breath analysis of hydrogen and methane are given in Table 3. All animals started and remained methanogenic. Differences between groups were not statistically significant. There was an increase in breath methane (after the treatment) in 5/6 pigs from the indomethacin group (C).

DISCUSSION

Probiotic bacteria might act in three different ways: they are able to modulate the host's defence mechanisms, they have a direct impact on other micro-organisms and finally probiotic effects may be based on actions affecting microbial products like toxins, host products (e.g. bile salts) and food ingredients[45].

Our hypothesis for this study was that (1) indomethacin would suppress bacteriocin production of *Enterobacteriaceae*; (2) probiotic bacteria EcN would colonise the porcine gastrointestinal tract permanently; (3) they would protect intestinal microbiota from suppressive action of indomethacin; and (4) EcN would convert the starting methanogenic phenotype of pigs to a hydrogenic one. Surprisingly, most of our presumptions were not proved.

There is no simple way to explain this. The first question that should be addressed is a possible role of human probiotic bacteria in the porcine gastrointestinal tract. It is necessary to consider whether human probiotics can be also assumed to act as probiotic microbiota for domestic pigs. Criteria for probiotics of human origin were proposed[46], however, potential probiotic bacteria isolated from porcine faeces are usually tested *in vitro* to be active against two or three common porcine pathogens only[47-50].

Genotype B2 and production of microcin H47 were considered as markers of EcN in our study. None of our 637 isolates comprised these bacteria. Viability and sufficient amount of bacteria were ensured before their administration in our project. According to our results, it is unlikely that EcN could exert long-term viability in the porcine intestinal tract. Other swine studies by several authors[51-54] were able to identify intestinal colonisation by EcN in pigs and piglets but not by all of them[55]. There is no final proof of long-term colonisation of the gastrointestinal tract by EcN in healthy humans. In an interesting study by Schierack *et al*[56], probiotic *Enterococcus faecium* supplementation showed no significant effect on the numbers and diversity of *Enterobacteriaceae* species, or on the total counts, diversity and distribution of virulence gene-positive *E. coli* strains in healthy domestic pigs.

Aspirin and some NSAIDs, including indomethacin, influence intestinal bacteria[57-60]. Indomethacin might exert some impact on intestinal microbiota in our study, as there was an increase in breath methane after the treatment in 5/6 pigs from the indomethacin group. Another interesting result from our current study showed a marked lower prevalence of colicin Ib, microcins H47 and V (probiotics group), colicin E1 and microcin H47 (indomethacin group) and microcins H47 and V (EcN and indomethacin group) compared to controls. We interpret this difference as a sign of adverse effects of probiotics and/or indomethacin on porcine microbiota. Bacteriocinogeny in controls (35%) was higher compared to the indomethacin (28%), probiotic (25%) and indomethacin and probiotic groups (22%). This evident trend did not reach statistical significance for the probiotic group (B) and indomethacin group (C). However, there was a statistically significant difference between controls and indomethacin and probiotic group (D). We can assume that indomethacin and EcN comprise the worst impact on bacteriocinogeny in the porcine gastrointestinal tract. This would be consistent with other studies showing that other probiotics might deteriorate NSAID-induced injury to the intestine[13].

Composition of food, especially supplements with prebiotics, might influence the probiotic effect of intestinal bacteria[61,62]. This factor is unlikely to play an important role in our study. All animals received identical assorted food with cereals, animal fat, soya oil and a mix of supplements (lysine, threonine, methionine, lactic acid).

In our current study, the microbiota profile was rather uniform in all animals due to identical breed and feed. However, there was a broad diversity in coliform bacteria; the main four genotypes A, B1, B2 and D were identified in parallel. Similar diversity was also found in other porcine studies, with prevailing group A[56,63]. Dixit *et al*[63] showed that differences among individual pigs accounted for 6% of the observed genetic diversity, whilst 27% of the genetic variation could be explained by clonal composition differences among gut regions (isolates obtained from the duodenum, ileum, colon and faeces of 8 pigs). Finally, the absence of virulence genes in these com-

mensals indicates that they may be suitable as a probiotic consortium, particularly if they also display increased adherence to enterocytes and antagonistic activity against pathogenic strains of *E. coli*[63].

E. fergusonii was identified as a new species of *Enterobacteriaceae* in 1985[64]. This is considered to be an opportunistic pathogen of farm animals including domestic pigs[65]. We identified *E. fergusonii* in 6 animals, all pigs were healthy without any sign of infective disease. Interestingly 10/11 isolated bacteria solely produced colicin E1. Colicins produced by *E. fergusonii* strains closely resemble colicins encoded by *E. coli*[66]. In a previous series of human isolates, only 6/50 (12%) strains were bacteriocinogenic, 3 of which produced colicin E1[67].

In humans, all intestinal hydrogen and methane are produced by so called "hydrogenic and methanogenic" bacteria[68-70]. However, most authors do not usually specify which particular bacteria constitute these producers. Hydrogen is produced by bacterial fermentation of saccharides in the intestinal lumen. Concurrently, hydrogen is consumed by other intestinal bacteria to synthesise methane, acetate and hydrogen sulphide. Methane is synthesised solely by bacteria in the intestine (four mols of hydrogen and one mol of carbon dioxide create one mol of methane and water). This reaction reduces the volume of gas that would otherwise be present in the colon[71-76]. The question of intestinal methane producers has not been definitely solved yet. We hypothesised that common coliform bacteria could also synthesise methane[77], however, this assumption was not proved by our further studies[78,79]. McKay *et al*[80] found that several anaerobes (*Bacteroides*, *Clostridium* and others) produced hydrogen but rarely methane. Hydrogen is also produced by *Enterobacteriaceae*[81]. In adult Caucasians, only 30%-50% of people produce methane while hydrogen is produced by 90%-98% of people[69]. Kien *et al*[82] found low breath hydrogen and higher methane in piglets (even in a subgroup supplemented with lactulose). In our current study, all animals revealed a solely methanogenic phenotype (by the analysis of their alveolar breath). This fact could be explained as they came from an identical breed and received the same assorted food. All animals remained methanogenic despite the fact that EcN is a substantial hydrogen producer[77]. This further supports our finding that EcN *1917* did not have major impact on porcine intestinal microbiota.

In conclusion, it is unlikely that probiotic EcN could exert long-term liveability in the porcine intestine. All experimental pigs remained methanogenic, despite the fact that EcN is a substantial hydrogen producer. The indomethacin and probiotic group had a significantly lower rate of bacteriocinogeny compared to controls with no treatment. These control pigs revealed higher bacteriocinogeny with simultaneous production of up to five different bacteriocins per single strain. Indomethacin and probiotics administered together might provide the worst impact on bacteriocinogeny in the porcine gastrointestinal tract.

COMMENTS

Background

Non-steroidal anti-inflammatory drugs (NSAIDs) represent the group of most commonly used drugs worldwide. NSAIDs may cause severe injury to all parts of the gastrointestinal tract. The pathogenesis of NSAID-induced entero- and colopathy is more multifactorial and complex than formerly assumed but has still not been fully understood. A combination of local and systemic effects plays an important role in pathogenesis. NSAID-induced entero- and colopathy is a stepwise process involving direct mucosal toxicity, mitochondrial damage, breakdown of intercellular integrity, enterohepatic recirculation and neutrophil activation by luminal contents including bacteria. Unlike upper gastrointestinal toxicity, cyclo-oxygenase-mediated mechanisms are probably less important. Intestinal bacteria play a significant role in the pathogenesis of NSAID-induced entero- and colopathy. In experimental studies, NSAIDs cannot induce enteropathy in germ-free rats.

Research frontiers

Probiotic bacteria are live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host. Probiotics likely function through enhancement of the barrier function of the gut, immunomodulation, and competitive adherence to the mucus and epithelium. Probiotic bacteria might exert a systemic anti-inflammatory effect and modulate apoptosis. Probiotics have been suggested for amelioration or prevention of various diseases including antibiotic-associated diarrhoea, irritable bowel syndrome and inflammatory bowel disease. Further possible beneficial effects are being studied (including anti-cancer potential, lowering serum cholesterol levels and blood pressure reduction, *etc.*). It has been hypothesised that probiotic bacteria might reduce the adverse effects of NSAIDs on the small and large intestine. However, initial studies provided controversial results, both with ameliorating and deteriorating outcomes.

Innovations and breakthroughs

Based on the current study, it is unlikely that probiotic *Escherichia coli Nissle 1917* (EcN) could exert long-term liveability in the porcine intestine. Genotype B2 and production of microcin H47 were considered as markers of EcN in the study. The authors did not find such bacteria among any of the 637 isolates. All experimental pigs remained methanogenic, despite the fact that EcN is a substantial hydrogen producer. The indomethacin and probiotic group had a significantly lower rate of bacteriocinogeny compared to controls with no treatment. These control pigs revealed higher bacteriocinogeny with simultaneous production of up to five different bacteriocins per single strain. Indomethacin and probiotics administered together might produce the worst impact on bacteriocinogeny in the porcine gastrointestinal tract.

Applications

Bacteriocins might induce apoptosis as some regulators of apoptosis (e.g. Bcl family with pro- and anti-apoptotic members) share similar structures with pore-forming colicins. Bacteriocins might have a dual role: they may act as both antibiotics and probiotics. One of the most commonly used probiotic bacterial strains, EcN, is a producer of microcins H47 and M.

Terminology

Colicins and microcins, members of the bacteriocin family, are produced by bacteriocinogenic strains of *Escherichia coli* and some related species of *Enterobacteriaceae*. They are toxic to susceptible bacterial strains of the same family. However, some bacteriocins also exert an inhibitory effect on eukaryotic cells, including observed antineoplastic action *in vitro* and *in vivo*.

Peer review

This is an innovative manuscript in basic research, which adequately addresses the ethics of the experiment. Its presentation is accurate but very complex in the reading and interpretation of many variables.

REFERENCES

1 **Hawkey CJ**. NSAIDs, coxibs, and the intestine. *J Cardiovasc Pharmacol* 2006; **47** Suppl 1: S72-S75
2 **Fortun PJ**, Hawkey CJ. Nonsteroidal antiinflammatory drugs and the small intestine. *Curr Opin Gastroenterol* 2007; **23**: 134-141
3 **Wong Kee Song LM**, Marcon NE. NSAIDs: Adverse effects

on the distal small bowel and colon. 2010. Available from: URL: http://www.uptodate.com/patients/content/topic.do?topicKey=~icYPTJgCSGCuc
4 **Robert A**, Asano T. Resistance of germfree rats to indomethacin-induced intestinal lesions. *Prostaglandins* 1977; **14**: 333-341
5 Health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. October 2001. Available from: URL: http://www.who.int/foodsafety/publications/fs_management/en/probiotics.pdf
6 **Ohland CL**, Macnaughton WK. Probiotic bacteria and intestinal epithelial barrier function. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G807-G819
7 **Arribas B**, Rodríguez-Cabezas ME, Camuesco D, Comalada M, Bailón E, Utrilla P, Nieto A, Concha A, Zarzuelo A, Gálvez J. A probiotic strain of Escherichia coli, Nissle 1917, given orally exerts local and systemic anti-inflammatory effects in lipopolysaccharide-induced sepsis in mice. *Br J Pharmacol* 2009; **157**: 1024-1033
8 **Carol M**, Borruel N, Antolin M, Llopis M, Casellas F, Guarner F, Malagelada JR. Modulation of apoptosis in intestinal lymphocytes by a probiotic bacteria in Crohn's disease. *J Leukoc Biol* 2006; **79**: 917-922
9 **Brady LJ**, Gallaher DD, Busta FF. The role of probiotic cultures in the prevention of colon cancer. *J Nutr* 2000; **130**: 410S-414S
10 **Sanders ME**. Considerations for use of probiotic bacteria to modulate human health. *J Nutr* 2000; **130**: 384S-390S
11 **Wollowski I**, Rechkemmer G, Pool-Zobel BL. Protective role of probiotics and prebiotics in colon cancer. *Am J Clin Nutr* 2001; **73**: 451S-455S
12 **Gotteland M**, Cruchet S, Verbeke S. Effect of Lactobacillus ingestion on the gastrointestinal mucosal barrier alterations induced by indometacin in humans. *Aliment Pharmacol Ther* 2001; **15**: 11-17
13 **Kamil R**, Geier MS, Butler RN, Howarth GS. Lactobacillus rhamnosus GG exacerbates intestinal ulceration in a model of indomethacin-induced enteropathy. *Dig Dis Sci* 2007; **52**: 1247-1252
14 **Watanabe T**, Nishio H, Tanigawa T, Yamagami H, Okazaki H, Watanabe K, Tominaga K, Fujiwara Y, Oshitani N, Asahara T, Nomoto K, Higuchi K, Takeuchi K, Arakawa T. Probiotic Lactobacillus casei strain Shirota prevents indomethacin-induced small intestinal injury: involvement of lactic acid. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G506-G513
15 **Montalto M**, Gallo A, Curigliano V, D'Onofrio F, Santoro L, Covino M, Dalvai S, Gasbarrini A, Gasbarrini G. Clinical trial: the effects of a probiotic mixture on non-steroidal anti-inflammatory drug enteropathy - a randomized, double-blind, cross-over, placebo-controlled study. *Aliment Pharmacol Ther* 2010; **32**: 209-214
16 **Sturm A**, Rilling K, Baumgart DC, Gargas K, Abou-Ghazalé T, Raupach B, Eckert J, Schumann RR, Enders C, Sonnenborn U, Wiedenmann B, Dignass AU. Escherichia coli Nissle 1917 distinctively modulates T-cell cycling and expansion via toll-like receptor 2 signaling. *Infect Immun* 2005; **73**: 1452-1465
17 **Grabig A**, Paclik D, Guzy C, Dankof A, Baumgart DC, Erckenbrecht J, Raupach B, Sonnenborn U, Eckert J, Schumann RR, Wiedenmann B, Dignass AU, Sturm A. Escherichia coli strain Nissle 1917 ameliorates experimental colitis via toll-like receptor 2- and toll-like receptor 4-dependent pathways. *Infect Immun* 2006; **74**: 4075-4082
18 **Hardy KG**. Colicinogeny and related phenomena. *Bacteriol Rev* 1975; **39**: 464-515
19 **Smarda J**, Smajs D. Colicins--exocellular lethal proteins of Escherichia coli. *Folia Microbiol* (Praha) 1998; **43**: 563-582
20 **Riley MA**, Wertz JE. Bacteriocin diversity: ecological and evolutionary perspectives. *Biochimie* 2002; **84**: 357-364
21 **Farkas-Himsley H**, Cheung R. Bacterial proteinaceous products (bacteriocins) as cytotoxic agents of neoplasia. *Cancer Res* 1976; **36**: 3561-3567
22 **Smarda J**, Keprtová J. Cytotoxic effects of colicins E1-E5 and K on hamster fibroblasts. *Folia Microbiol* (Praha) 1987; **32**: 133-136
23 **Farkas-Himsley H**, Hill R, Rosen B, Arab S, Lingwood CA. The bacterial colicin active against tumor cells in vitro and in vivo is verotoxin 1. *Proc Natl Acad Sci USA* 1995; **92**: 6996-7000
24 **Smarda J**, Fialová M, Smarda J Jr. Cytotoxic effects of colicins E1 and E3 on v-myb-transformed chicken monoblasts. *Folia Biol* (Praha) 2001; **47**: 11-13
25 **Chumchalová J**, Smarda J. Human tumor cells are selectively inhibited by colicins. Folia Microbiol (Praha) 2003; 48: 111-115
26 **Farkas-Himsley H**, Zhang YS, Yuan M, Musclow CE. Partially purified bacteriocin kills malignant cells by apoptosis: programmed cell death. *Cell Mol Biol* (Noisy-le-grand) 1992; **38**: 643-651
27 **Anderluh G**, Lakey JH. Disparate proteins use similar architectures to damage membranes. *Trends Biochem Sci* 2008; **33**: 482-490
28 **Bures J**, Horák V, Duben J. Importance of colicinogeny for the course of acute bacillary dysentery. *Zentralbl Bakteriol Orig A* 1979; **245**: 469-475
29 **Bures J**, Horák V, Buresová E, Fixa B, Komárková O, Hartmann M. Colicinogeny in chronic inflammatory bowel disease. *Scand J Gastroenterol* 1986; **21**: 819-823
30 **Bures J**, Horák V, Fixa B, Komárková O, Zaydlar K, Lonský V, Masurka V. Colicinogeny in colorectal cancer. *Neoplasma* 1986; **33**: 233-237
31 **Gillor O**, Etzion A, Riley MA. The dual role of bacteriocins as anti- and probiotics. *Appl Microbiol Biotechnol* 2008; **81**: 591-606
32 **Patzer SI**, Baquero MR, Bravo D, Moreno F, Hantke K. The colicin G, H and X determinants encode microcins M and H47, which might utilize the catecholate siderophore receptors FepA, Cir, Fiu and IroN. *Microbiology* 2003; **149**: 2557-2570
33 **Grozdanov L**, Raasch C, Schulze J, Sonnenborn U, Gottschalk G, Hacker J, Dobrindt U. Analysis of the genome structure of the nonpathogenic probiotic Escherichia coli strain Nissle 1917. *J Bacteriol* 2004; **186**: 5432-5441
34 **Vassiliadis G**, Destoumieux-Garzón D, Lombard C, Rebuffat S, Peduzzi J. Isolation and characterization of two members of the siderophore-microcin family, microcins M and H47. *Antimicrob Agents Chemother* 2010; **54**: 288-297
35 **Kararli TT**. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm Drug Dispos* 1995; **16**: 351-380
36 **Bures J**, Kopácová M, Kvetina J, Osterreicher J, Sinkorová Z, Svoboda Z, Tachecí I, Filip S, Spelda S, Kunes M, Rejchrt S. Different solutions used for submucosal injection influenced early healing of gastric endoscopic mucosal resection in a preclinical study in experimental pigs. *Surg Endosc* 2009; **23**: 2094-2101
37 **Tachecí I**, Kvetina J, Bures J, Osterreicher J, Kunes M, Pejchal J, Rejchrt S, Spelda S, Kopácová M. Wireless capsule endoscopy in enteropathy induced by nonsteroidal anti-inflammatory drugs in pigs. *Dig Dis Sci* 2010; **55**: 2471-2477
38 **Kopácová M**, Tachecí I, Kvetina J, Bures J, Kunes M, Spelda S, Tycová V, Svoboda Z, Rejchrt S. Wireless video capsule enteroscopy in preclinical studies: methodical design of its applicability in experimental pigs. *Dig Dis Sci* 2010; **55**: 626-630
39 **Council of Europe**. European convention for the protection of vertebrate animals used for experimental and other scientific purposes. Strasbourg: Council of Europe, 1986
40 **Smarda J**, Obdrzálek V. Incidence of colicinogenic strains among human Escherichia coli. *J Basic Microbiol* 2001; **41**: 367-374
41 **Smarda J**, Smajs D, Horynová S. Incidence of lysogenic, colicinogenic and siderophore-producing strains among human

non-pathogenic Escherichia coli. *Folia Microbiol* (Praha) 2006; **51**: 387-391
42 **Rosen S**, Skaletsky HJ. Primer3 on the WWW for general users and for biologist programmers. In: Krawetz S, Misener S, editors. Bioinformatics Methods and Protocols: Methods in Molecular Biology. Totowa: Humana Press, 2000: 365-386
43 **Gordon DM**, O'Brien CL. Bacteriocin diversity and the frequency of multiple bacteriocin production in Escherichia coli. *Microbiology* 2006; **152**: 3239-3244
44 **Clermont O**, Bonacorsi S, Bingen E. Rapid and simple determination of the Escherichia coli phylogenetic group. *Appl Environ Microbiol* 2000; **66**: 4555-4558
45 **Oelschlaeger TA**. Mechanisms of probiotic actions - A review. *Int J Med Microbiol* 2010; **300**: 57-62
46 **Dunne C**, O'Mahony L, Murphy L, Thornton G, Morrissey D, O'Halloran S, Feeney M, Flynn S, Fitzgerald G, Daly C, Kiely B, O'Sullivan GC, Shanahan F, Collins JK. In vitro selection criteria for probiotic bacteria of human origin: correlation with in vivo findings. *Am J Clin Nutr* 2001; **73**: 386S-392S
47 **Fairbrother JM**, Nadeau E, Gyles CL. Escherichia coli in postweaning diarrhea in pigs: an update on bacterial types, pathogenesis, and prevention strategies. *Anim Health Res Rev* 2005; **6**: 17-39
48 **Lata J**, Juránková J, Doubek J, Příbramská V, Frič P, Dítě P, Kolář M, Scheer P, Kosáková D. Labelling and content evaluation of commercial veterinary probiotics. *Acta Vet Brno* 2006; **75**: 139-144
49 **Setia A**, Bhandari SK, House JD, Nyachoti CM, Krause DO. Development and in vitro evaluation of an Escherichia coli probiotic able to inhibit the growth of pathogenic Escherichia coli K88. *J Anim Sci* 2009; **87**: 2005-2012
50 **Yun JH**, Lee KB, Sung YK, Kim EB, Lee HG, Choi YJ. Isolation and characterization of potential probiotic lactobacilli from pig feces. *J Basic Microbiol* 2009; **49**: 220-226
51 **Duncker SC**, Lorentz A, Schroeder B, Breves G, Bischoff SC. Effect of orally administered probiotic E. coli strain Nissle 1917 on intestinal mucosal immune cells of healthy young pigs. *Vet Immunol Immunopathol* 2006; **111**: 239-250
52 **Kleta S**, Steinrück H, Breves G, Duncker S, Laturnus C, Wieler LH, Schierack P. Detection and distribution of probiotic Escherichia coli Nissle 1917 clones in swine herds in Germany. *J Appl Microbiol* 2006; **101**: 1357-1366
53 **Schroeder B**, Duncker S, Barth S, Bauerfeind R, Gruber AD, Deppenmeier S, Breves G. Preventive effects of the probiotic Escherichia coli strain Nissle 1917 on acute secretory diarrhea in a pig model of intestinal infection. *Dig Dis Sci* 2006; **51**: 724-731
54 **Barth S**, Duncker S, Hempe J, Breves G, Baljer G, Bauerfeind R. Escherichia coli Nissle 1917 for probiotic use in piglets: evidence for intestinal colonization. *J Appl Microbiol* 2009; **107**: 1697-1710
55 **Schultz M**, Watzl S, Oelschlaeger TA, Rath HC, Göttl C, Lehn N, Schölmerich J, Linde HJ. Green fluorescent protein for detection of the probiotic microorganism Escherichia coli strain Nissle 1917 (EcN) in vivo. *J Microbiol Methods* 2005; **61**: 389-398
56 **Schierack P**, Walk N, Reiter K, Weyrauch KD, Wieler LH. Composition of intestinal Enterobacteriaceae populations of healthy domestic pigs. *Microbiology* 2007; **153**: 3830-3837
57 **Unmack MA**, Hansen MB, Grondahl ML, Olsen JE, Christensen P, Skadhauge E. Effects of indomethacin on Salmonella typhimurium- and cholera toxin-induced fluid accumulation in the porcine small intestine. *J Vet Med A Physiol Pathol Clin Med* 2001; **48**: 153-163
58 **Dalby AB**, Frank DN, St Amand AL, Bendele AM, Pace NR. Culture-independent analysis of indomethacin-induced alterations in the rat gastrointestinal microbiota. *Appl Environ Microbiol* 2006; **72**: 6707-6715
59 **Chang-Ying Y**, Yi L, Jun-Cheng Z, Dan Z. Inhibitory effect of copper complex of indomethacin on bacteria studied by microcalorimetry. *Biol Trace Elem Res* 2008; **122**: 82-88
60 **Upreti RK**, Kannan A, Pant AB. Experimental impact of aspirin exposure on rat intestinal bacteria, epithelial cells and cell line. *Hum Exp Toxicol* 2010; **29**: 833-843
61 **Bomba A**, Nemcová R, Gancarčíková S, Herich R, Guba P, Mudronová D. Improvement of the probiotic effect of microorganisms by their combination with maltodextrins, fructooligosaccharides and polyunsaturated fatty acids. *Br J Nutr* 2002; **88** Suppl 1: S95-S99
62 **Deng ZY**, Zhang JW, Li J, Fan YW, Cao SW, Huang RL, Yin YL, Zhong HY, Li TJ. Effect of polysaccharides of cassiae seeds on the intestinal microflora of piglets. *Asia Pac J Clin Nutr* 2007; **16** Suppl 1: 143-147
63 **Dixit SM**, Gordon DM, Wu XY, Chapman T, Kailasapathy K, Chin JJ. Diversity analysis of commensal porcine Escherichia coli - associations between genotypes and habitat in the porcine gastrointestinal tract. *Microbiology* 2004; **150**: 1735-1740
64 **Farmer JJ 3rd**, Fanning GR, Davis BR, O'Hara CM, Riddle C, Hickman-Brenner FW, Asbury MA, Lowery VA 3rd, Brenner DJ. Escherichia fergusonii and Enterobacter taylorae, two new species of Enterobacteriaceae isolated from clinical specimens. *J Clin Microbiol* 1985; **21**: 77-81
65 **Wragg P**, La Ragione RM, Best A, Reichel R, Anjum MF, Mafura M, Woodward MJ. Characterisation of Escherichia fergusonii isolates from farm animals using an Escherichia coli virulence gene array and tissue culture adherence assays. *Res Vet Sci* 2009; **86**: 27-35
66 **Smajs D**, Karpathy SE, Smarda J, Weinstock GM. Colicins produced by the Escherichia fergusonii strains closely resemble colicins encoded by Escherichia coli. *FEMS Microbiol Lett* 2002; **208**: 259-262
67 **Smarda J**, Smajs D, Lhotová H. Three recently acknowledged Escherichia species strikingly differ in the incidence of bacteriocinogenic and lysogenic strains. *J Basic Microbiol* 2002; **42**: 429-433
68 **Christl SU**, Murgatroyd PR, Gibson GR, Cummings JH. Production, metabolism, and excretion of hydrogen in the large intestine. *Gastroenterology* 1992; **102**: 1269-1277
69 **Hamilton LH**. Breath tests and gastroenterology. 2nd ed. Milwaukee: QuinTron Instrument Company, 1998
70 **Gasbarrini A**, Corazza GR, Gasbarrini G, Montalto M, Di Stefano M, Basilisco G, Parodi A, Usai-Satta P, Vernia P, Anania C, Astegiano M, Barbara G, Benini L, Bonazzi P, Capurso G, Certo M, Colecchia A, Cuoco L, Di Sario A, Festi D, Lauritano C, Miceli E, Nardone G, Perri F, Portincasa P, Risicato R, Sorge M, Tursi A. Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference. *Aliment Pharmacol Ther* 2009; **29** Suppl 1: 1-49
71 **Strocchi A**, Levitt MD. Factors affecting hydrogen production and consumption by human fecal flora. The critical roles of hydrogen tension and methanogenesis. *J Clin Invest* 1992; **89**: 1304-1311
72 **Strocchi A**, Levitt MD. Maintaining intestinal H2 balance: credit the colonic bacteria. *Gastroenterology* 1992; **102**: 1424-1426
73 **Strocchi A**, Ellis CJ, Levitt MD. Use of metabolic inhibitors to study H2 consumption by human feces: evidence for a pathway other than methanogenesis and sulfate reduction. *J Lab Clin Med* 1993; **121**: 320-327
74 **Strocchi A**, Ellis CJ, Furne JK, Levitt MD. Study of constancy of hydrogen-consuming flora of human colon. *Dig Dis Sci* 1994; **39**: 494-497
75 **Strocchi A**, Furne J, Ellis C, Levitt MD. Methanogens outcompete sulphate reducing bacteria for H2 in the human colon. *Gut* 1994; **35**: 1098-1101
76 **Koetse HA**, Vonk RJ, Pasterkamp S, Pal J, de Bruijn S, Stellaard F. Variations in colonic H2 and CO2 production as a cause of inadequate diagnosis of carbohydrate maldigestion

in breath tests. *Scand J Gastroenterol* 2000; **35**: 607-611
77 **Bures J**, Cyrany J, Lesna J, Kopacova M, Vorisek V, Palicka V, Rejchrt S. Hydrogen breath tests: hydrogenic and methanogenic bacteria are common Enterobacteriaceae strains. *Gut* 2008; **57** Suppl 2: A223
78 **Cyrany J**, Kvetina J, Kunes M, Förstl M, Moravkova M, Lesna J, Bartos V, Kopacova M, Rejchrt S, Bures J. Non-steroidal anti-inflammatory drugs and probiotics influence on porcine colonic bacteria evaluated by hydrogen production in vitro - a pilot study. *Gut* 2009; **58** Suppl 2: A257-A258
79 **Bures J**, Cyrany J, Kohoutova D, Förstl M, Rejchrt S, Kvetina J, Vorisek V, Kopacova M. Small intestinal bacterial overgrowth syndrome. *World J Gastroenterol* 2010; **16**: 2978-2990
80 **McKay LF**, Holbrook WP, Eastwood MA. Methane and hydrogen production by human intestinal anaerobic bacteria. *Acta Pathol Microbiol Immunol Scand B* 1982; **90**: 257-260
81 **Forbes BA**, Sahm DF, Weissfeld AS. Bailey & Scott's diagnostic microbiology. 12th ed. St. Louis: Mosby Inc., 2007
82 **Kien CL**, Murray RD, Qualman SJ, Marcon M. Lactulose feeding in piglets: a model for persistent diarrhea and colitis induced by severe sugar malabsorption. *Dig Dis Sci* 1999; **44**: 1476-1484

S- Editor Sun H **L- Editor** O'Neill M **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.618

World J Gastroenterol 2011 February 7; 17(5): 618-624
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Analysis of the urinary peptidome associated with *Helicobacter pylori* infection

Di Xiao, Fan-Liang Meng, Li-Hua He, Yi-Xin Gu, Jian-Zhong Zhang

Di Xiao, Fan-Liang Meng, Li-Hua He, Yi-Xin Gu, Jian-Zhong Zhang, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing 102206, China

Author contributions: Zhang JZ and Xiao D designed the study, acquired the data and drafted the manuscript; Meng FL, He LH and Gu YX recruited volunteers, collected and analyzed urine samples, and performed 13C-urea breath test; all authors read and approved the final manuscript.

Supported by The National Science and Technology Pillar Program of the Ministry of Science and Technology of the People's Republic of China during the Eleventh Five-Year plan period, No. 2007BAID4B02

Correspondence to: Jian-Zhong Zhang, Professor, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, PO Box 5, Changping District, Beijing 102206, China. zhangjianzhong@icdc.cn
Telephone: +86-10-58900754 Fax: +86-10-58900707

Received: August 14, 2010 Revised: September 29, 2010
Accepted: October 6, 2010
Published online: February 7, 2011

Abstract

AIM: To investigate the relationship between urinary peptide changes and *Helicobacter pylori* (*H. pylori*) infection using urinary peptidome profiling.

METHODS: The study was performed in volunteers (n = 137) who gave informed consent. Urinary peptides were enriched by magnetic beads based weak cation exchange chromatography and spectrums acquired by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS). ClinProTools bioinformatics software was used for statistical analysis and the recognition of peptide patterns. The marker peptides were identified by LTQ Obitrap XL tandem MS.

RESULTS: Approximately 50 proteins or peptides which loaded onto the magnetic beads were detected by MALDI-TOF MS. By optimizing the parameters of the model, the Genetic Algorithm model had good recognition capability (97%) and positive predictive value (94%). Based on the model, 2 markers with molecular masses of 6788 and 1912 Da were found that differentiated between *H. pylori* positive and negative volunteers. The m/z 1912 sequence was parsed as SKQFTSSTSYNRGDSTF. The peptide was identified as isoform 1 of the fibrinogen α chain precursor, whose concentration in urine was markedly higher in *H. pylori* infected volunteers than in *H. pylori* non-infected ones.

CONCLUSION: The appearance of urinary fibrinogen degradation products is caused by an active *H. pylori*-induced process.

Key words: Urinary peptidome profiling; MB-matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; *Helicobacter pylori*; Fibrinogen degradation products

Peer reviewer: David J McGee, PhD, Associate Professor, Department of Microbiology and Immunology, Louisiana State University Health Sciences Center-Shreveport, 1501 Kings Highway, Shreveport, LA 71130, United States

Xiao D, Meng FL, He LH, Gu YX, Zhang JZ. Analysis of the urinary peptidome associated with *Helicobacter pylori* infection. *World J Gastroenterol* 2011; 17(5): 618-624 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/618.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.618

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a Gram-negative, microaerophilic bacterium adapted for survival in the human stomach, where it can cause chronic gastritis and peptic ulcer disease and is an important risk factor that may lead

to gastric cancer. Great progress has been made in understanding *H. pylori* pathogenicity since its discovery 25 years ago. *H. pylori* infection has been proposed as a risk factor not only for gastrointestinal diseases but also for cardiovascular diseases such as peripheral arterial disease[1] and atherosclerosis[2]. In addition, some studies have shown that *H. pylori* infection is associated with Henoch-Schonlein purpura[3,4] and membranous nephropathy[5,6]. Purpura nephritis is one of the serious complications of Henoch-Schonlein purpura[7]. As a result of its long delitescence, rapid growth of drug resistance and the ease of infection, *H. pylori* infection has become a prominent chronic digestive system disease.

Recent progress in proteomic analysis and strategies for the identification of clinically useful biomarkers in biological fluids has shown that urine can be an excellent non-invasive reservoir[8-10]. By virtue of its noninvasiveness and the availability of specimens, peptidome profiling of human urine is now becoming an important method for detecting novel disease-associated markers[11,12]. Bruker Daltonics provides the mass spectrometry (MS)-based ClinProt™ system solution for preparation, measurement and visualization of peptides and proteins in body fluid[13]. The Profiling Kit MB-WCX (Magnetic Beads based Weak Cation Exchange Chromatography) was developed for the enrichment of proteins and peptides from biological samples based on cation exchange chromatography prior to matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) analysis. Successful applications and reproducibility of the MB-WCX beads using serum, plasma and urine samples was demonstrated in various studies[14-16]. Nanoliquid chromatography coupled to micro-electrospray ionization tandem MS (ESI-MS/MS) has become a powerful tool for identification and quantification in peptide analysis due to its higher sensitivity[17,18]. In this study, we analyzed the urine peptidome profiles of *H. pylori* infected and non-infected volunteers by the ClinProt™ system, followed by MALDI-TOF MS, and we identified the biomarkers using Aquity nano-ultra-performance liquid chromatography coupled to a Thermo LTQ Orbitrap high resolution/high accuracy ultra-performance liquid chromatography (UPLC)-ESI-MS/MS.

MATERIALS AND METHODS

Protein/peptide marker discovery in urine

Urine specimen collection: Urine samples were collected from healthy volunteers (n = 137, 70 male, 67 female) who did not have cardiovascular diseases and had received a health checkup 3 mo prior to the study, and gave written informed consent before participation. The volunteers received ^{13}C-urea breath tests to determine whether they were infected with *H. pylori,* and their midstream urine was collected the following morning[19]. Urine samples were kept at a low temperature with ice and were transferred to the laboratory within 2 h, centrifuged at 3000 g for 20 min, aliquotted and stored at -80℃ until use.

Urinary peptide enrichment

The urine samples were thawed at room temperature for 30 min, adjusted to pH 7, and centrifuged again. Urinary peptides were separated using MB-WCX kit (Bruker Daltonics, Bremen, Germany; particle size < 1 μm; mean pore size, 40 nm; specific surface area, 100 cm^2/g). The magnetic beads were mixed thoroughly on a vortex device for 1 min, then a 30 μL urine sample was diluted in 60 μL MB-WCX binding solution, and 10 μL WCX beads were added. After thorough stirring, sample mixtures were incubated for 1 min at room temperature. The tube was placed into the magnetic separator and the beads at the wall of the tube were collected for 1 min. The supernatant was removed by using a pipette. Wash buffer (100 μL) was added to the tube, which was moved back and forth in the magnetic separator 10 times. The beads were collected at the tube wall for 1 min and the supernatant was removed carefully using a pipette. Elution buffer (5 μL) was added and the beads dissolved at the tube wall by pipetting up and down intensively 10 times. The beads were collected at the tube wall for 2 min and the clear supernatant was transferred into a fresh tube. Stabilization buffer (5 μL) was added to the eluate.

MALDI-TOF data acquisition

Sample solution (1 μL) was dropped onto an AnchorChip™ 600-μm target (Bruker Daltonics) and dried. Next, 1 μL of freshly prepared α-cyano-4-hydroxycinnamic acid [0.4 mg/mL matrix solution in ethanol/acetone (2:1, v/v)] was added onto the sample and crystallized. MALDI-TOF MS analysis of the peptidome profile was performed using an autoflex™ instrument (Bruker Daltonics), equipped with a N_2 laser (λ = 377 nm), with the ion source voltage as follows: source 1, 120 kV; ion source 2, 18.6 kV; lens 7.6 kV. The pulsed ion extraction delay was 320 ns and operated in positive ion linear mode (LP-ClinProt) with a total of 450 shots (30 shots at each of 15 different spot positions) per sample. All signals with a signal-to-noise ratio > 3 in a m/z range of 1000-10 000 Da were collected with the AutoXecute tool of the flexControl™ acquisition software (version 3.0; Bruker Daltonics). Mass calibration was performed with the standard calibration mixture of peptides and proteins (CPS, preparation method in the MB-WCX operation manual, MW range 1000-10 000 Da).

Statistical data analysis

The spectra were analyzed statistically using Clin-Prot™ (version 2.2 β; Bruker Daltonics) bioinformatics software. Parameters were as follows: peak definition: signal to noise ratio > 3; statistical analysis: Wilcoxon/Kruskal-Wallis; area normalization: against total ion count; integration: end point level; mass recalibration: maximal peak shift of 500 ppm; sort mode: t-test P-value/analysis of variance (ANOVA). The spectra from 90 samples (40 in the *H. pylori* infected group and 50 in *H. pylori* non-infected group) were used to build models and 47 samples (23 in the *H. pylori* infection group and 24 in the *H. pylori*

non-infected group) were used in model verification by the Genetic Algorithm (GA), Quick classifier, and Supervised Neural mathematical algorithms. The parameters k-nearest neighbor classification (KNN), maximal number of generations (MNG) were optimized and the best model was determined. The performance of the models was evaluated by recognition capability (RC) and positive predictive value (PPV): RC = TP/*n* where TP is the number of true positives (correctly classified) in a data set and *n* is the number of samples in a data set and PPV = TP/(TP + FP) where FP is the number of false positives (misclassified). The best model (RC and PPV values are a maximum one of 1) was implemented to determine the marker peptides. The *P*-value of the Anderson-Darling test (PAD) which can give information about the normal distribution: < 1 not normally distributed, > 1 normally distributed, the *P*-value of the *t*-test (2 classes) or ANOVA test (> 2 classes) (PTTA, preferable for normal distributed data) or the *P*-value of the Wilcoxon test (2 classes) or Kruskal-Wallis test (> 2 classes) (PWKW, preferable for abnormally distributed data) was used to confirm significant differences. If the PWKW or PTTA value was < 0.05, the protein/peptide was confirmed to be significantly different.

Identification of significant peptides by nano UPLC-ESI-MS/MS

UPLC: The peptides from urine samples (the differential peptides are relatively abundant) were eluted from the magnetic beads and were analyzed by nano-UPLC-ESI-MS/MS using a nano Aquity UPLC (Waters Corporation, Milford, USA) coupled to a LTQ-Orbitrap mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Samples of 5 μL (the sample was diluted by 2 times) were loaded on a C18 precolumn (Symmetry®C18, 5 μm, 180 μm × 20 mm, nanoAcquity™Column) at 15 μL/min in 5% acetonitrile (Sigma-Aldrich, St Louis, MO, USA), 0.05% trifluoroacetic acid (Sigma-Aldrich) for 3 min. The precolumn was switched online with the analytical column (Symmetry®C18, 3.5 μm, 75 μm × 150 mm, nanoAcquity™ Column) equilibrated in 95% solvent A (5% acetonitrile, 0.1% formic acid; Sigma-Aldrich) and 5% solvent B (95% acetonitrile, 1.2% formic acid). Peptides were eluted using a 5% to 80% gradient of solvent B over 60 min at a flow rate of 400 nL/min.

UPLC-MS/MS and data analysis

The LTQ Orbitrap XL mass spectrometer was operated in the data-dependent mode to switch automatically between MS and MS/MS acquisition. Full-scan survey MS spectra with 2 microscans (m/z 400-2000) were acquired with the Orbitrap with a mass resolution of 100 000 at m/z 400, followed by 10 sequential LTQ-MS/MS scans. Dynamic exclusion was used with 2 repeat counts, 10 s repeat duration and 60 s exclusion duration. For MS/MS, charge state 1 was rejected and precursor ions were activated using 25% normalized collision energy at the default activation q of 0.25. The mass spectra were searched against the human International Protein Index (IPI) database (IPI human v3.45 fasta with 71 983 entries) using Bioworks software (Version 3.3.1; Thermo Electron Co.) based on the SEQUEST algorithm. To reduce false positive identification results, a decoy database containing the reverse sequences was appended to the database. The parameters for the SEQUEST search were as follows: no enzyme, the variable modification was oxidation of methionine, peptide tolerance, 10 ppm, MS/MS tolerance, 1.0 Da. Positive protein identification was accepted for a peptide with Xcorr of greater than or equal to 3.20 for triply and 2.86 for doubly charged ions, and all with ΔCn ⩾ 0.1, peptide probability ⩽ 2e-3.

Table 1 Comparison of results for the classification models

Model	Algorithms	KNN	MNG	Max. peaks	RC (%)	PPV (%)
1	GA	5	60	7	90.5	83.0
2	GA	3	60	10	91.3	85.1
3	GA	7	60	15	96.5	93.6
4	GA	3	60	20	93.3	91.5
5	GA	5	60	25	93.3	87.2
6	SNN			25	78.5	63.8
7	QC			25	78.8	66.0

Model 3 was the best. GA: Genetic Algorithm; QC: Quickclassifier; SNN: Supervised Neural Network; KNN: k-nearest neighbor classification; MNG: Maximal number of generations; RC: Recognition capability; PPV: Positive predictive value.

RESULTS

Urinary peptidome profiling

^{13}C-urea breath tests showed that 74 volunteers were *H. pylori* negative and 63 volunteers were *H. pylori* positive (delta over baseline > 4). About 50 peaks with signal-to-noise ratios greater than 5 were detected between m/z 1000 and 10 000 in urine from the volunteers (Figure 1). The average intensities of peaks for the negative group and positive group are shown in Figure 2A, and the complete spectra from both the healthy group and the *H. pylori*-infected group are shown in Figure 2C.

Statistical data analysis and classification

When parameter KNN = 7, MNG = 60 and Max.peaks = 15, the GA model was the best fit: RC = 96.5%, PPV = 93.6% (Table 1). All the data PAD were < 1, so the data were abnormally distributed and PWKW was used to confirm marker peptides. Two markers that differentiated between the *H. pylori* non-infected group and the *H. pylori* infected group (PWKW < 0.05) with molecular masses of 6788 and 1912 Da were found in urine (Table 2). The content of these peptides in urine was markedly higher in *H. pylori* infected volunteers than in non-infected subjects (Figure 2B and D).

Identification of peptides

The peptides from urine were separated using nano-UPLC. Product-ion-spectra of the doubly charged mol-

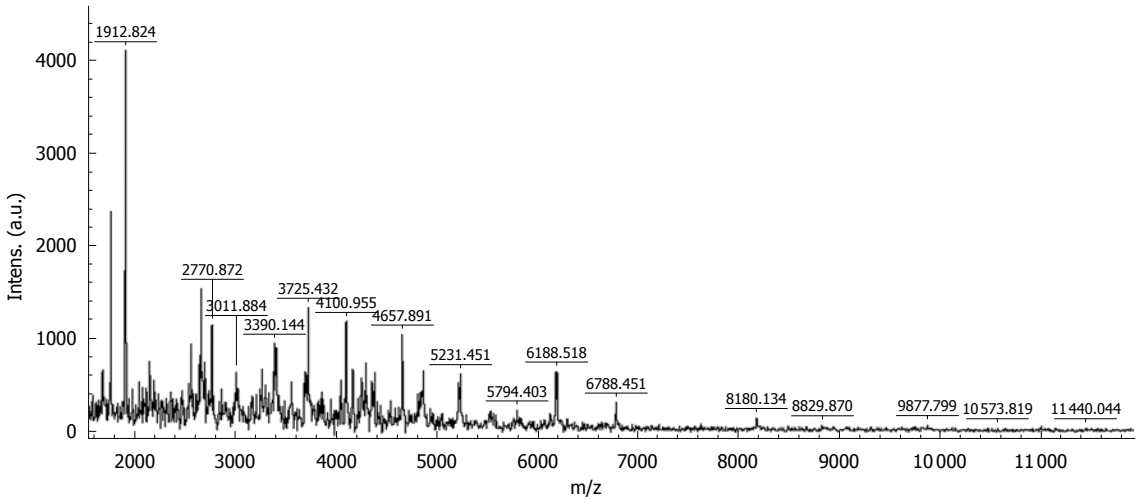


Figure 1 The mass spectrum of peptides in urine ranging between 1000 and 10 000 Da.

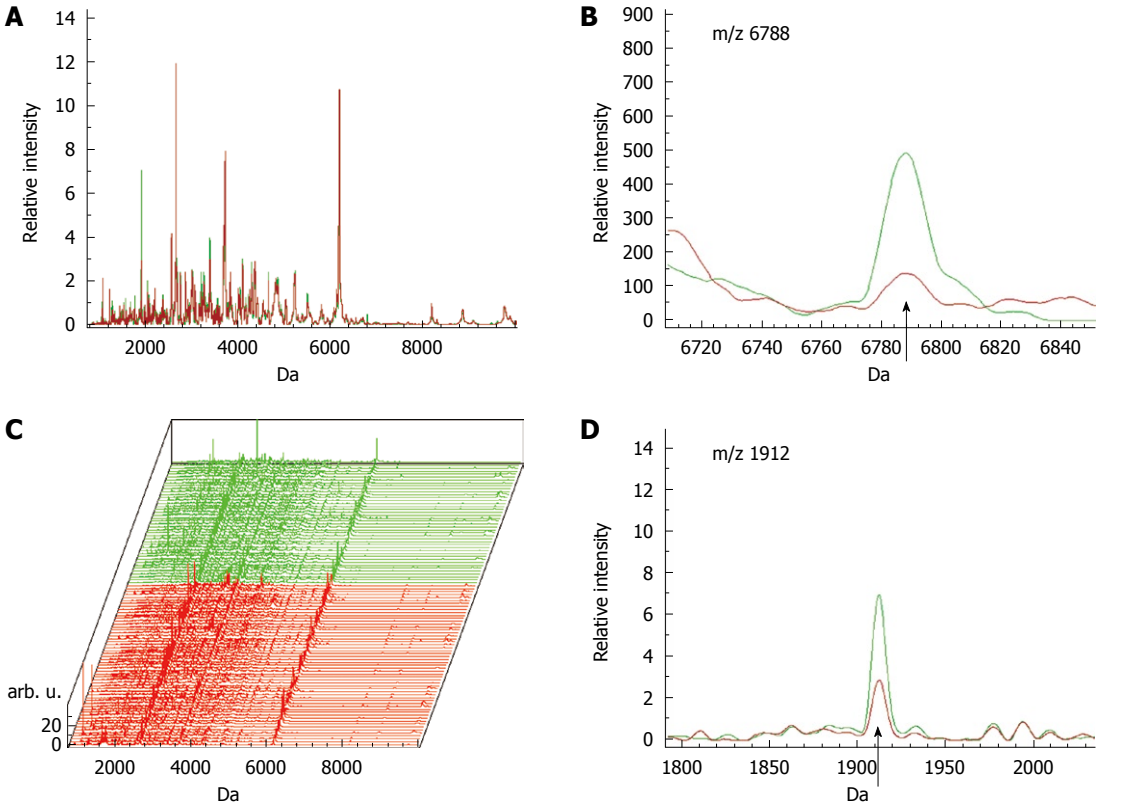


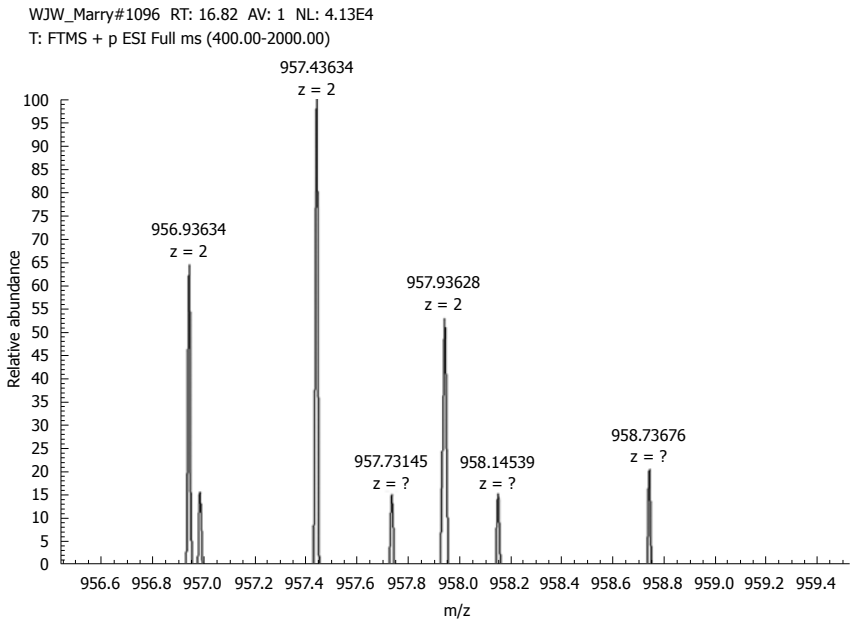
Figure 2 Differentially expressed low-mass peptides in human urine. A: The average intensities of matrix-assisted laser desorption/ionization time-of-flight peaks for the healthy group (red line), and the *H. pylori*-infected group (green line); B, D: The enlarged picture m/z 6788 and m/z 1912, respectively, the healthy group (red line) and the *H. pylori*-infected group (green line); C: The complete spectra from both the healthy group (red line) and the *H. pylori*-infected group (green line).

ecule m/z 957.436 for the 1912 Da peak was recorded with the linear ion trap (Figure 3A) and the sequence was parsed as SKQFTSSTSYNRGDSTF following MS/MS (Figure 3B). The sequence was identified as isoform 1 of fibrinogen α chain precursor (AC: IPI00021885) using the IPI database with Xcorr 3.201 (doubly charged ion), ΔCn = 0.267, P = 1.10E-04 and MS/MS tolerance 0.26 Da. Unfortunately, the m/z 6788 peak sequence was not identified. Because it was possible that the peptide m/z 1912 was from *H. pylori*, the sequence was searched against all the species in the NCBInr. The fibrinogen was identified again as a fragment of human fibrinogen [gi|4503689|ref|NP_000499.1| fibrinogen, α polypeptide isoform α-E preproprotein (Homo sapiens)].

DISCUSSION

Urine is an especially attractive medium for biomarker

A



B

	AA	B+	B2+	Y+	Y2+	
1	S	88.0	44.5	-	-	17
2	K	216.1	108.6	1825.8	913.4	16
3	Q	344.2	172.6	1697.7	849.4	15
4	F	491.3	246.1	1569.7	785.3	14
5	T	592.3	296.7	1422.6	711.8	13
6	S	679.3	340.2	1321.6	661.3	12
7	S	766.4	383.7	1234.5	617.8	11
8	T	867.4	434.2	1147.5	574.3	10
9	S	954.5	477.7	1046.5	523.7	9
10	Y	1117.5	559.3	959.4	480.2	8
11	N	1231.6	616.3	796.4	398.7	7
12	R	1387.7	694.3	682.3	341.7	6
13	G	1444.7	722.8	526.2	263.6	5
14	D	1559.7	780.4	469.2	235.1	4
15	S	1646.7	823.9	354.2	177.6	3
16	T	1747.8	874.4	267.1	134.1	2
17	F	-	-	166.1	83.5	1

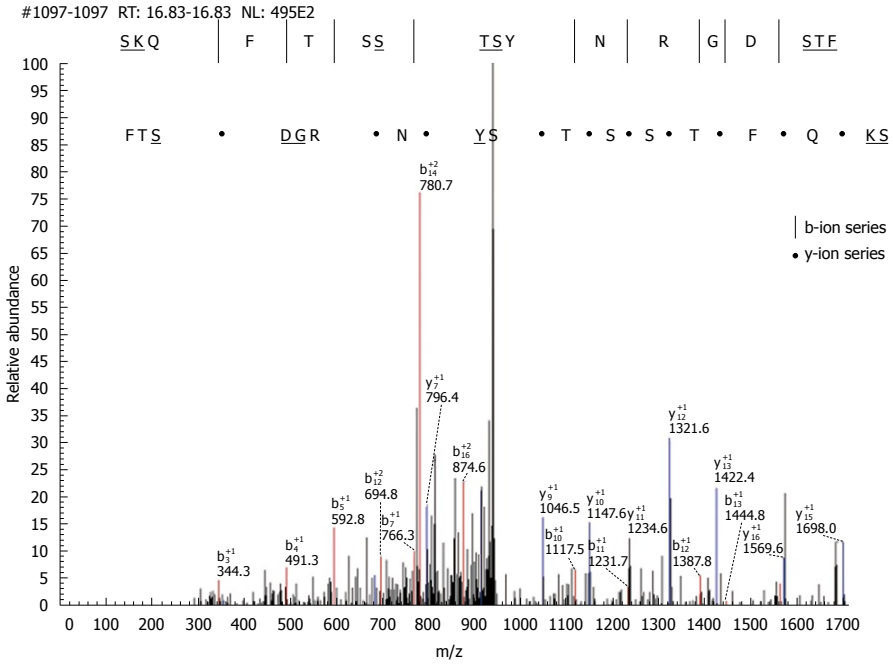


Figure 3 Protein identified by mass spectrometry/mass spectrometry. A: The enlarged picture of m/z 1912 (two charges 957.4); B: The b and y ions spectra used to identify the m/z 1912 as the fragment SKQFTSSTSYNRGDSTF. The underlined amino acids represent b or y ions of amino acids that cannot be found in the spectra. The underlined values represent the peaks where amino acids match with the b, y-ion peak.

Table 2 Statistical information for marker peptides of *Helicobacter Pylori* negative and positive groups

Index	Mass	DAve	PTTA	PWKW	PAD
56	6787.91	8.11	0.0000242	< 0.000001	0.00000418
9	1911.86	39.52	0.00847	0.00545	< 0.000001
23	3210.11	16.1	0.538	0.195	< 0.000001
27	3688.78	14.99	0.538	0.195	< 0.000001

DAve: Difference between the maximal and the minimal average peak area/intensity of all classes; PTTA: *P*-value of *t*-test (2 classes) or ANOVA test (> 2 classes); PWKW: *P*-value of Wilcoxon test (2 classes) or Kruskal-Wallis test (> 2 classes); PAD: *P*-value of Anderson-Darling test.

analysis, because urine can be obtained in large quantities using noninvasive procedures, and ample material is available for analysis and assessment of reproducibility. In addition, repeated sampling from the same individual is simple, facilitating longitudinal studies. Urine generally contains proteins and peptides of lower molecular mass (< 30 kDa) that are highly soluble. These features facilitate analysis of such polypeptides in their natural state, without any need for additional manipulation. Urinary polypeptides are stable and generally do not undergo significant proteolysis for several hours after collection[20,21]. Urine has been known, or at least has been suspected, to

reflect pathological changes for centuries. Even early pathological changes are thought to be associated with disease-specific changes in the urinary proteome[22]. In this study, we found 2 specific factors in human urine that were associated with *H. pylori* infection by urinary peptidome profiling. Urinary fibrinogen degradation products (FDP) increased with *H. pylori* infection.

Fibrinogen is a major plasma protein (340 kDa) that consists of pairs of 3 different polypeptide chains, α, β, and γ, joined by disulfide bonds to form a symmetric dimeric structure. The NH_2-terminal regions of all 6 chains form the central E-domain[23]. Fibrinogen is directly involved in the clotting process as a clotting factor and is synthesized in hepatocytes[24]. In addition, fibrinogen has a variety of other functions, such as a mediated platelet aggregation response[25]. Many studies have found that an elevated level of plasma fibrinogen is an important risk factor for cardiovascular and cerebrovascular thrombotic diseases[26,27] and renal failure[28].

Fibrinogen can be digested either by plasmin or thrombin. When fibrinogen is cleaved by plasmin, it releases 2 D fragments (the COOH termini of the α, β, and γ chains), one E fragment (the NH_2 termini of the α, β, and γ chains), and several smaller fragments including a small peptide, β 1-42 (the NH_2 terminus of the β-chain). Cleavage by thrombin releases the two fibrinopeptides A and B (FpA and FpB) from the NH_2 termini of the α and β chains, respectively, while exposed polymerization sites form electrostatic bonds between the E-domain of one molecule and the D-domain of an adjacent one. Factor XIIIa, a transglutaminase, then introduces γ-glutamyl-ε-amino-lysine isopeptide cross-links between D domains of adjacent fibrin monomers, generating a stable polymer known as fibrin. Then, fibrin can be broken down by plasmin cleavage into the 3-stranded coils found between the D and E domains, yielding a D dimer, D fragment, and fibrin E fragment (which lacks the fibrinopeptides A and B) and smaller fragments[29]. FDP, such as D-dimer, E-fragment and α, β-chain, have been widely studied in cardiovascular disease and cancer-related research fields[30,31]. The m/z 1912 peptide is a fragment of an FDP (site 580-596). Our study shows that the peptide m/z 1912 in urine was significantly increased in patients with *H. pylori* infection.

The normal glomerular basement membrane has a filtration function, and the average pore size is 5.5 nm. Therefore, under normal circumstances, some small molecular weight proteins can filter through tiny pores in the glomerular membrane. Because of endocytosis, the major proteins are normally reabsorbed when they pass through the proximal tubule, so there is low protein content in urine, a random urinary protein of 0-80 mg/L. Although there are many kinds of fibrinogen degradation fragments, large fragments are retained by the glomerulus or are taken up by the renal tubule, therefore only small peptides are normally seen in the urine. In this study, the peptides or proteins below 10 kDa in the urine were captured by weak cation beads, so only the marker peptides 1912 and 6788 were detected, while the fragments of FDP that exceeded 10 kDa were not captured.

The reasons why *H. pylori* infection results in an FDP increase in urine are not clear. Our preliminary studies have shown that *H. pylori* will lead to human gastric adenocarcinoma epithelial cell calreticulin phosphorylation, and dephosphorylation of its calcium-binding protein (nucleobindin-2), which affects cell calcium ion channels[32]. Fibrinogen achieves its biological functions by being degraded by plasmin or thrombin. The activities of plasmin and thrombin are regulated or progressively activated by calcium ions; therefore, the changes in the calcium ion channels will affect the fibrinolytic system. In short, the changes in FDP in urine are important for gaining a comprehensive understanding of the pathogenesis of *H. pylori*.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) infection has been proposed as a risk factor not only for gastrointestinal diseases but also for cardiovascular diseases and nephropathy. The pathogenic mechanisms of *H. pylori* are not yet clear since its discovery 25 years ago.

Research frontiers

The peptidome has been widely used in finding biomarkers with the development of mass spectrometry (MS). As it can be obtained in large quantities using noninvasive procedures, urine is an especially attractive medium for biomarker analysis. In this study, the authors analyzed the urine peptidome profiles of *H. pylori* infected and non-infected volunteers using the ClinProt™ system, followed by matrix-assisted laser desorption/ionization time-of-flight MS, and identified the marker peptides using liquid chromatography coupled to MS.

Innovations and breakthroughs

Cardiovascular diseases and nephropathy have been reported which associated with *H. pylori* infection. To date, the pathogenic mechanism is not clear. This study suggests that the appearance of urinary fibrinogen degradation products is caused by an active *H. pylori*-induced process. The results of this study are important to further the comprehensive understanding of the pathogenesis of *H. pylori*.

Applications

This study suggests that fibrinogen degradation products are associated with *H. pylori* infection. This result can help researchers in this field further understand the potential mechanism associated with *H. pylori* infection and cardiovascular diseases and nephropathy, and provide important information for prevention and control of *H. pylori*-related diseases.

Peer review

It would be of great interest in future experiments to investigate whether the urine fibrinogen peptide correlates with serum fibrinogen levels and *H. pylori* infection, as serum fibrinogen levels have been investigated in *H. pylori* infection in many studies.

REFERENCES

1 **Sawayama Y**, Hamada M, Otaguro S, Maeda S, Ohnishi H, Fujimoto Y, Taira Y, Hayashi J. Chronic Helicobacter pylori infection is associated with peripheral arterial disease. *J Infect Chemother* 2008; **14**: 250-254

2 **Longo-Mbenza B**, Nkondi Nsenga J, Vangu Ngoma D. Prevention of the metabolic syndrome insulin resistance and the atherosclerotic diseases in Africans infected by Helicobacter pylori infection and treated by antibiotics. *Int J Cardiol* 2007; **121**: 229-238

3 **Hoshino C**. Adult onset Schönlein-Henoch purpura associated with Helicobacter pylori infection. *Intern Med* 2009; **48**: 847-851

4 **Mytinger JR**, Patterson JW, Thibault ES, Webb J, Saulsbury FT. Henoch-Schönlein purpura associated with Helicobacter

pylori infection in a child. *Pediatr Dermatol* 2008; **25**: 630-632
5 **Nagashima R**, Maeda K, Yuda F, Kudo K, Saitoh M, Takahashi T. Helicobacter pylori antigen in the glomeruli of patients with membranous nephropathy. *Virchows Arch* 1997; **431**: 235-239
6 **Sugimoto T**, Furukawa T, Maeda T, Somura M, Uzu T, Kashiwagi A. Marked reduction of proteinuria after eradication of gastric Helicobacter pylori infection in a patient with membranous nephropathy: coincidental or associated? *Intern Med* 2007; **46**: 1483-1484
7 **Lau KK**, Suzuki H, Novak J, Wyatt RJ. Pathogenesis of Henoch-Schönlein purpura nephritis. *Pediatr Nephrol* 2010; **25**: 179
8 **Fliser D**, Novak J, Thongboonkerd V, Argilés A, Jankowski V, Girolami MA, Jankowski J, Mischak H. Advances in urinary proteome analysis and biomarker discovery. *J Am Soc Nephrol* 2007; **18**: 1057-1071
9 **Ward DG**, Nyangoma S, Joy H, Hamilton E, Wei W, Tselepis C, Steven N, Wakelam MJ, Johnson PJ, Ismail T, Martin A. Proteomic profiling of urine for the detection of colon cancer. *Proteome Sci* 2008; **6**: 19
10 **Caubet C**, Lacroix C, Decramer S, Drube J, Ehrich JH, Mischak H, Bascands JL, Schanstra JP. Advances in urinary proteome analysis and biomarker discovery in pediatric renal disease. *Pediatr Nephrol* 2010; **25**: 27-35
11 **Hortin GL**, Meilinger B, Drake SK. Size-selective extraction of peptides from urine for mass spectrometric analysis. *Clin Chem* 2004; **50**: 1092-1095
12 **Haubitz M**, Wittke S, Weissinger EM, Walden M, Rupprecht HD, Floege J, Haller H, Mischak H. Urine protein patterns can serve as diagnostic tools in patients with IgA nephropathy. *Kidney Int* 2005; **67**: 2313-2320
13 **Ketterlinus R**, Hsieh SY, Teng SH, Lee H, Pusch W. Fishing for biomarkers: analyzing mass spectrometry data with the new ClinProTools software. *Biotechniques* 2005; **Suppl**: 37-40
14 **Fiedler GM**, Baumann S, Leichtle A, Oltmann A, Kase J, Thiery J, Ceglarek U. Standardized peptidome profiling of human urine by magnetic bead separation and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Clin Chem* 2007; **53**: 421-428
15 **Baumann S**, Ceglarek U, Fiedler GM, Lembcke J, Leichtle A, Thiery J. Standardized approach to proteome profiling of human serum based on magnetic bead separation and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. *Clin Chem* 2005; **51**: 973-980
16 **Shin S**, Cazares L, Schneider H, Mitchell S, Laronga C, Semmes OJ, Perry RR, Drake RR. Serum biomarkers to differentiate benign and malignant mammographic lesions. J Am Coll Surg 2007; **204**: 1065-1071; discussion 1071-1073
17 **Noga M**, Sucharski F, Suder P, Silberring J. A practical guide to nano-LC troubleshooting. *J Sep Sci* 2007; **30**: 2179-2189
18 **Hernández-Borges J**, Aturki Z, Rocco A, Fanali S. Recent applications in nanoliquid chromatography. *J Sep Sci* 2007; **30**: 1589-1610
19 **Thongboonkerd V**. Practical points in urinary proteomics. *J Proteome Res* 2007; **6**: 3881-3890
20 **Kumar S**, Tsai CJ, Nussinov R. Temperature range of thermodynamic stability for the native state of reversible two-state proteins. *Biochemistry* 2003; **42**: 4864-4873
21 **Pieper R**, Gatlin CL, McGrath AM, Makusky AJ, Mondal M, Seonarain M, Field E, Schatz CR, Estock MA, Ahmed N, Anderson NG, Steiner S. Characterization of the human urinary proteome: a method for high-resolution display of urinary proteins on two-dimensional electrophoresis gels with a yield of nearly 1400 distinct protein spots. *Proteomics* 2004; **4**: 1159-1174
22 **Mischak H**, Kaiser T, Walden M, Hillmann M, Wittke S, Herrmann A, Knueppel S, Haller H, Fliser D. Proteomic analysis for the assessment of diabetic renal damage in humans. *Clin Sci* (Lond) 2004; **107**: 485-495
23 **Mosesson MW**. Fibrinogen and fibrin structure and functions. *J Thromb Haemost* 2005; **3**: 1894-1904
24 **Vu D**, Neerman-Arbez M. Molecular mechanisms accounting for fibrinogen deficiency: from large deletions to intracellular retention of misfolded proteins. *J Thromb Haemost* 2007; **5** Suppl 1: 125-131
25 **Keller MA**, Martinez J, Baradet TC, Nagaswami C, Chernysh IN, Borowski MK, Surrey S, Weisel JW. Fibrinogen Philadelphia, a hypodysfibrinogenemia characterized by abnormal polymerization and fibrinogen hypercatabolism due to gamma S378P mutation. *Blood* 2005; **105**: 3162-3168
26 **Shlipak MG**, Ix JH, Bibbins-Domingo K, Lin F, Whooley MA. Biomarkers to predict recurrent cardiovascular disease: the Heart and Soul Study. *Am J Med* 2008; **121**: 50-57
27 **Zoccali C**, Mallamaci F, Tripepi G, Cutrupi S, Parlongo S, Malatino LS, Bonanno G, Rapisarda F, Fatuzzo P, Seminara G, Stancanelli B, Nicocia G, Buemi M. Fibrinogen, mortality and incident cardiovascular complications in end-stage renal failure. *J Intern Med* 2003; **254**: 132-139
28 **Muntner P**, He J, Astor BC, Folsom AR, Coresh J. Traditional and nontraditional risk factors predict coronary heart disease in chronic kidney disease: results from the atherosclerosis risk in communities study. *J Am Soc Nephrol* 2005; **16**: 529-538
29 **Bootle-Wilbraham CA**, Tazzyman S, Marshall JM, Lewis CE. Fibrinogen E-fragment inhibits the migration and tubule formation of human dermal microvascular endothelial cells in vitro. *Cancer Res* 2000; **60**: 4719-4724
30 **Akakura N**, Hoogland C, Takada YK, Saegusa J, Ye X, Liu FT, Cheung AT, Takada Y. The COOH-terminal globular domain of fibrinogen gamma chain suppresses angiogenesis and tumor growth. *Cancer Res* 2006; **66**: 9691-9697
31 **Kwon HC**, Oh SY, Lee S, Kim SH, Han JY, Koh RY, Kim MC, Kim HJ. Plasma levels of prothrombin fragment F1+2, D-dimer and prothrombin time correlate with clinical stage and lymph node metastasis in operable gastric cancer patients. *Jpn J Clin Oncol* 2008; **38**: 2-7
32 **Xiao D**, Zhao F, Song YY, Meng FL, He LH, Zhang HF, Zhang JZ. Influence of Helicobacter pylori CagA on Ca2+-associated phosphorylated proteins of AGS cells. *Shijie Huaren Xiaohua Zazhi* 2008; **16**: 3610-3615

S- Editor Sun H **L- Editor** Cant MR **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.625

World J Gastroenterol 2011 February 7; 17(5): 625-632
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

ORIGINAL ARTICLE

Influence of CXCR4/SDF-1 axis on E-cadherin/β-catenin complex expression in HT29 colon cancer cells

Lin Wang, Cui-Ling Li, Lei Wang, Wen-Bin Yu, Hai-Peng Yin, Guang-Yong Zhang, Li-Feng Zhang, Sheng Li, San-Yuan Hu

Lin Wang, Lei Wang, Wen-Bin Yu, Guang-Yong Zhang, Li-Feng Zhang, San-Yuan Hu, Department of General Surgery, Qilu Hospital, Shandong University, Jinan 250012, Shandong Province, China
Cui-Ling Li, Hai-Peng Yin, Key Laboratory for Modern Medicine and Technology of Shandong Province, Institute of Basic Medicines, Shandong Academy of Medical Sciences, Jinan 250062, Shandong Province, China
Sheng Li, Department of Hepatobiliary Surgery, Shandong Tumor Hospital, Jinan 250117, Shandong Province, China
Author contributions: Hu SY and Li S designed the study; Li CL, Yin HP, Zhang LF and Wang L performed the experiments; Wang L, Yu WB and Zhang GY analyzed the data; Wang L wrote the paper.
Supported by National Natural Science Foundation of China, No. 30571712 and 30810403081
Correspondence to: San-Yuan Hu, MD, Department of General Surgery, Qilu Hospital, Shandong University, Jinan 250012, Shandong Province, China. 33943219@qq.com
Telephone: +86-531-82169441 Fax: +86-531-82169441
Received: October 15, 2010 Revised: January 11, 2011
Accepted: January 18, 2011
Published online: February 7, 2011

Abstract

AIM: To study the influence of CXCR4/stromal cell-derived factor-1 (SDF-1) axis on E-cadherin/β-catenin complex expression in HT29 colon cancer cells and its underlying mechanisms.

METHODS: Effect of SDF-1 on E-cadherin/β-catenin expression was detected by immunocytochemistry. E-cadherin and β-catenin mRNA expression levels were measured by reverse transcriptase-polymerase chain reaction. SDF-1-induced phosphorylation of phosphatidylinositol 3-kinase (PI3K)/AKT and β-catenin was detected by Western blotting.

RESULTS: The E-cadherin and β-catenin mRNA expression levels in HT29 cells were lower 48 h after incubated with SDF-1 at the concentrations of 20 and 40 ng/mL ($P < 0.05$). SDF-1-induced significant phosphorylation of PI3K/AKT and β-catenin. AMD3100 and LY294002 inhibited the phosphorylation of PI3K/AKT and β-catenin.

CONCLUSION: SDF-1 down-regulates the E-cadherin/β-catenin complex expression in HT29 cells by decreasing mRNA synthesis and increasing β-catenin phosphorylation.

Key words: CXCR4; Stromal cell-derived factor-1; E-cadherin; β-catenin; Phosphatidylinositol 3-kinase/AKT; Colon cancer

Peer reviewer: Julio Mayol, MD, PhD, Department of Digestive surgery, Hospital Clinico San Carlos, MARTIN-LAGOS S/n, Madrid, 28040, Spain

Wang L, Li CL, Wang L, Yu WB, Yin HP, Zhang GY, Zhang LF, Li S, Hu SY. Influence of CXCR4/SDF-1 axis on E-cadherin/β-catenin complex expression in HT29 colon cancer cells. *World J Gastroenterol* 2011; 17(5): 625-632 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/625.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.625

INTRODUCTION

Colorectal cancer (CRC) is one of the most common cancers and the second leading cause of cancer-related death in the Western world. Death usually results from its uncontrolled metastasis. Although the 5-year survival rate approaches 90% for patients with local CRC, it has decreased to 19% for patients with distant metastasis[1]. The metastatic process of CRC consists of a series of individ-

ual steps, which are required to establish the diagnosis of metastatic lesions[2,3]. A number of molecules have been implicated in the metastatic process of CRC.

Chemokines are a group of chemoattractant cytokines that mediate several cellular functions. Stromal cell-derived factor-1 (SDF-1) is expressed in stromal cells, including fibroblasts and endothelial cells[4,5], and interacts specifically with the seven-transmembrane, G protein-coupled receptor CXCR4[6]. Recent studies showed that chemotaxis effect of CXCR4/SDF-1 axis is related with lymph node and liver metastasis of CRC[7-10]. Although there is evidence that the CXCR4/SDF-1 signaling pathway is involved in the metastatic process of CRC, the precise molecular mechanism underlying SDF-1-induced chemotaxis effect has not been completely elucidated.

E-cadherin, a transmembrane glycoprotein located at the adheren junction, mediates calcium-dependent cell-cell adhesion[11,12]. C terminus of E-cadherin is linked to α-catenin and actin cytoskeleton through the association with β-catenin. Strong cell-cell interactions result in a tight cell cluster as a community, and constrain cells from moving away. It has been shown that dysregulation of E-cadherin/β-catenin complex expression is responsible for the invasion and metastasis of CRC[13,14], indicating that the CXCR4/SDF-1 axis is correlated with E-cadherin/β-catenin complex expression in invasion and metastasis of CRC.

This study was to observe whether SDF-1 can alter E-cadherin/β-catenin expression in HT29 colon cancer cell line. In addition, the E-cadherin/β-catenin mRNA expression level was measured and the phosphorylation of phosphatidylinositol 3-kinase (PI3K)/AKT and β-catenin was examined to provide insights into the mechanism underlying the change in E-cadherin/β-catenin expression.

MATERIALS AND METHODS

Reagents

Antibodies against E-cadherin and β-catenin, and p-β-catenin antibody (Ser33/37) and p-AKT antibody (Ser473) were purchased from Cell Signaling Technology (Beverly, MA, USA). Peroxidase-conjugated goat anti-rabbit IgG and peroxidase-conjugated goat anti-mouse IgG were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). SDF-1 was bought from Pepro Tech Inc. (Rocky Hill, NJ, USA). AMD3100 was purchased from Sigma Aldrich, USA and LY29400 was bought from Beyotime, China.

Cell culture

Human colon cancer HT29 cell line was obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). Tumor cells were cultured in RPMI-1640 (Invitrogen product), supplemented with 10% newborn calf serum (Shanghai Mafa Corporation), 100 U/mL penicillin, and 100 mg/mL streptomycin in a humidified incubator containing 5% CO_2 and 95% air at 37℃.

Cell proliferation assay

Exponentially growing HT29 cells were seeded in 96-well plates in RPMI-1640 containing 3% newborn calf serum at a density of 2×10^4 cells/well. After 24 h, either PBS or AMD3100 (100 ng/mL) was added and incubated for 2 h. SDF-1 was added into three wells daily at different concentrations (10, 20 and 40 ng/mL). MTT assay (Amersham Biosciences, USA) was performed after 24, 48 and 72 h. Absorbance was measured at 570 and 630 nm (630 nm as the reference wave length). The results were expressed as a mean of three wells in each group. Proliferation rate of HT29 cells was calculated by the absorbance of experimental groups divided by that of the control group. The results were expressed as a mean of three individual experiments.

Cell chemotaxis and migration assay

Migration of HT29 cells was assessed in a HTS transwell-24 system (Corning, Acton, MA, USA) with 8-μm membrane pores. After rehydration for 2 h, RPMI-1640 and different SDF-1 concentrations (10, 20 and 40 ng/mL) were added into the lower chamber (0.5 mL per well) and 2×10^4 cells treated with PBS or AMD3100 (100 ng/mL) were added into the upper chamber 30 min before assay. After incubated at 37℃ for 24 h, Matrigel and cells on the upper side of the membrane were wiped off with PBS-rinsed cotton swabs and invading cells migrated to the lower surface of the membrane were photographed and counted under an inverted light microscope at 100 × magnification. Six random fields were counted for each well. Migration of HT29 cells was assayed in triplicate.

Immunocytochemistry

To observe the effect of SDF-1 on E-cadherin/β-catenin complex expression, HT29 cells were seeded in 24-well plates with glass slides at a density of 2×10^5 cells/well in RPMI-1640 medium in the absence of serum. After incubated overnight, either PBS or CXCR4 antagonist AMD3100 (100 ng/mL) was added and incubated for 2 h. Then, SDF-1 was added at different concentrations (10, 20 and 40 ng/mL). Glass slides were collected after incubated for 24 and 48 h with SDF-1. Cells were washed thrice with PBS prior to fixation with 4% formaldehyde in PBS. Then, the cells were covered with 3% H_2O_2-methanol for 30 min at room temperature to inactivate the endogenous peroxidase. A methanol permeabilisation step was needed for β-catenin, during which cells were covered with ice-cold 100% methanol for 10 min in a freezer. After rinsed thrice with PBS, the cells were covered with 5% normal goat serum for 30 min at room temperature to block the non-specific binding. Primary rabbit anti-E-cadherin antibodies and mouse anti-β-catenin antibodies were applied to the slides and incubated overnight at 4℃. Slides were washed three times with PBS. Secondary antibodies were applied for 1 h at room temperature. Finally, the slides were stained with 0.025% diaminobenzidine tetrahydrochloride containing 4% H_2O_2 for 1-20 min, counterstained with hematoxylin

for an appropriate period of time, and analyzed under a light microscope. The level of nonspecific background staining was established for each measurement using control cells processed in the same way without exposure to primary antibodies.

Reverse transcriptase-polymerase chain reaction analysis

HT29 cells (1×10^6) treated as in immunocytochemistry were collected and washed three times with 1 × PBS. Total RNA was extracted using trizol reagents and quantified by ND-1000 UV-visible spectrophotometry. cDNA was synthesized from 1 μg of total RNA using Revert Aid™ M-MuLV reverse transcriptase. Reaction mixture contained 4 μL of 5 × reaction buffer, 2 μL of 10 mmol/L dNTP mix, 1 μg of Oligo(dT)18, 1 μg of total RNA, 0.5 μL of ribonuclease inhibitor, and 200 U Aid™ M-MuLV reverse transcriptase in a total volume of 20 μL. PCR contained 2.5 μL of 10 × PCR buffer, 1.5 μL of 25 mmol/L $MgCl_2$, 0.5 μL of 10 mmol/L dNTP mix, 0.8 umol/L β-catenin primer, 0.04 μmol/L GAPDH primer (for β-catenin) and 0.8 μmol/L E-cadherin primer, 0.1 μmol/L β-actin primer (for E-cadherin), 3 μL of cDNA and 25 U *Taq* polymerase in a total volume of 25 μL. The sequences of gene-specific primers are 5'-TTTGCGTGAGCAGGGTGC-3' (forward) and 5'-GCTGCATATGTCGCCACACC-3' (reverse) for β-catenin, 5'-CCACCCATGGCAAATTCCATGGCA-3' (forward) and 5'-TCTAGACGGCAGGTCAGGTCCACC-3' (reverse) for GAPDH, 5'-GATTCTGCTGCTCTTGCTGT-3' (forward) and 5'-CCTGGTCTTTGTCTGACTCTG-3' (reverse) for E-cadherin, 5'-CCTTCCTGGGCATGGAGTCCT-3' (forward) and 5'-GGAGCAATGATCTTGATCTT-3' (reverse) for β-actin, respectively. The PCR conditions for β-catenin were as follows: denaturing at 94℃ for 5 min, followed by 35 cycles at 94℃ for 30 s, at 63℃ for 30 s, at 72℃ for 1 min, and a final extension at 72℃ for 7 min. The PCR conditions for E-cadherin were as follows: annealing at 58℃ for 5 min, followed by 35 cycles at 94℃ for 30 s, at 63℃ for 30 s, at 72℃ for 1 min, and a final extension at 72℃ for 7 min. The PCR products were separated on a 2% agarose gel in 1 × TAE, visualized with ethidium bromide staining by BIO-RAD Gel Dos1000, and quantified using the Molecular Analyst software version 1.5 using GAPDH as an internal control for β-catenin, and β-actin as an internal control for E-cadherin. The hue value was calculated as the ratio of each group and the internal control group. Results were expressed as the mean value of three individual experiments.

Western blotting

To study the effect of SDF-1 on phosphorylation of various signaling proteins, HT29 cells were treated with 20 ng/mL SDF-1 for different periods of time (from 1 min to 2 d) or with different concentrations of SDF-1 (5-100 ng/mL) for 30 min after overnight starvation of growth factors. Cell lysates were analyzed for the presence of phosphorylated AKT and β-catenin by phospho-specific antibodies to the specific phosphorylation sites of AKT (Ser473) and β-catenin (Ser33/37). For experiments using inhibitors, HT29 cells were pretreated with AMD3100 (100 ng/mL) or PI3K/AKT inhibitor LY294002 (20 μmol/L) for 2 h, followed by stimulation with 20 ng/mL SDF-1. After each treatment, 4×10^6 HT29 cells were collected and washed three times with 1 × PBS. Cytoplasm and membrane extracts were acquired according to the instruction datasheet of Pierce Biotechnology Corporation and quantified by Bradford protein assay. Different extraction proteins were separated by SDS-PAGE and transferred onto the PVDF membrane. Membranes were blocked with 5% BSA for 2 h at room temperature, incubated overnight at 4℃ with primary antibodies (p-β-catenin 1:1000, p-AKT 1:1000 and β-actin 1:400) and washed three times prior to incubation with HRP-conjugated secondary antibodies (peroxidase conjugated goat anti rabbit IgG 1:10 000) for 1 h at room temperature. Protein expression was visualized by chemiluminescence and quantified using the multigauge software. β-actin was used as a loading control. Data were shown as the ratio of phosphorylation and loading control. Western blotting assay was performed in triplicate.

Statistical analysis

All data were analyzed using the SPSS 16.0 and expressed as mean ± SD. Statistical significance of differences was determined by Student's *t*-test in two groups and one-way ANOVA among multiple groups. $P < 0.05$ was considered statistically significant.

RESULTS

SDF-1 enhanced viability of HT29 colon cancer cells

The viability of HT29 cells in any experiment groups was not different from that in control group 48 h after incubated with SDF-1. The cells grew much faster with a proliferation rate of 129% and 135%, respectively ($P < 0.05$) 72 h after incubated with SDF-1 at the concentrations of 20 and 40 ng/mL. AMD3100 plus SDF-1 inhibited the cell growth. AMD3100 alone had no effect on cell proliferation.

SDF-1 promoted migration of HT29 colon cancer cells

MTT assay revealed no significant difference in viability of HT29 colon cancer cells in any experiment groups within 24 h after incubated with SDF-1. Therefore, the migration of HT29 colon cancer cells was assayed 24 h after incubated with SDF-1 to exclude the influence of cell viability. The migration ability of HT29 cells was significantly greater in experiment groups than in control group 24 h after incubated with SDF-1 at the concentration of 10 ng/mL (149 ± 13.3 *vs* 92.3 ± 12.4, $P = 0.041$), 20 ng/mL (161 ± 13.5 *vs* 92.3 ± 12.4, $P = 0.023$), and 40 g/mL (187.5 ± 14 *vs* 92.3 ± 12.4, $P < 0.001$). AMD3100 plus SDF-1 inhibited

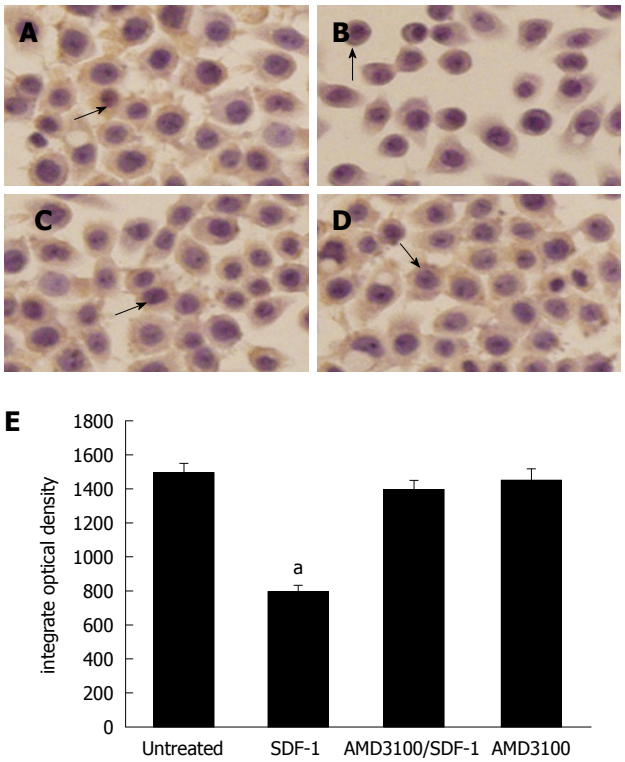


Figure 1 Effect of stromal cell-derived factor-1 (20 ng/mL) on E-cadherin expression (× 400). A: E-cadherin expression in HT29 cells; B: Significantly lower E-cadherin expression level 48 h after incubated with stromal cell-derived factor-1 (SDF-1) ($P < 0.05$); C: AMD3100- inhibited E-cadherin expression; D: No effect of AMD3100 alone on E-cadherin expression. Arrows mean the expression of E-cadherin; Bars indicate mean ± SD of six random fields. [a]$P < 0.05$.

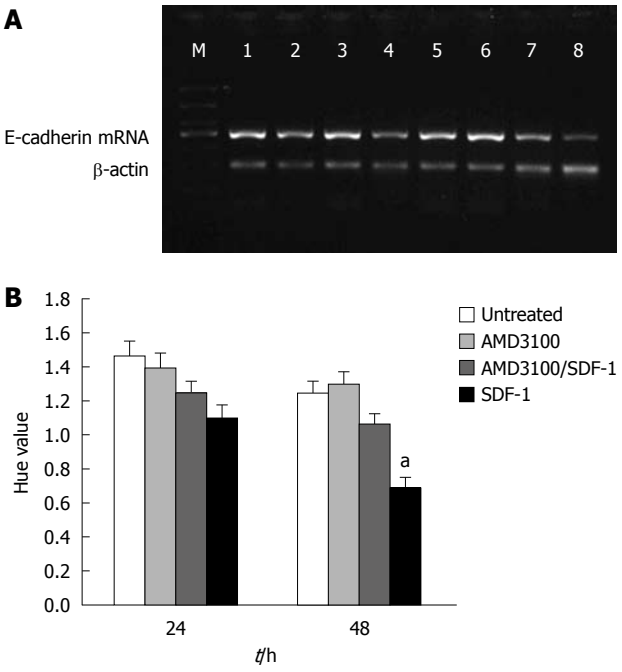


Figure 2 Effect of stromal cell-derived factor-1 (20 ng/mL) on E-cadherin mRNA expression after 24 h (A) and 48 h (B) in different groups. Bars represent mean ± SD of three individual experiments. [a]$P < 0.05$. SDF-1: Stromal cell-derived factor-1.

the migration of HT29 cells. AMD3100 alone had no effect on cell migration.

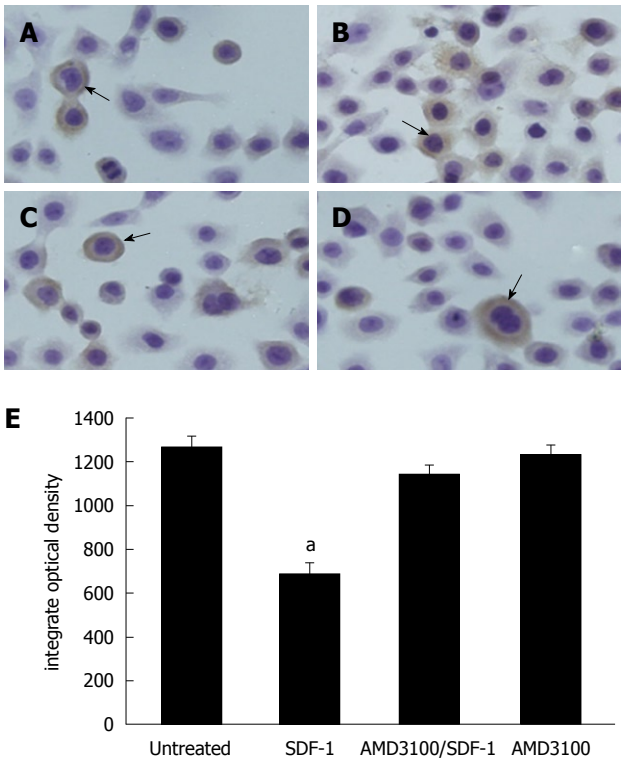


Figure 3 Effect of stromal cell-derived factor-1 (20 ng/mL) on β-catenin expression after 48 h (× 400). A: β-catenin expression in HT29 cells; B: Significantly lower β-catenin expression level 48 h after incubated with stromal cell-derived factor-1 (SDF-1) ($P = 0.031$); C: AMD310-inhibited β-catenin expression; D: No effect of AMD3100 alone on β-catenin expression. Arrows mean the expression of β-catenin; Bars indicate mean ± SD of six random fields. [a]$P < 0.05$.

SDF-1 down-regulated activation of CXCR4 and E-cadherin expression at protein and mRNA levels

Immunocytochemistry assay showed that E-cadherin was significantly expressed in HT29 cells (Figure 1A). No change was found in E-cadherin expression 24 h after incubated with SDF-1. The E-cadherin expression level was significantly lower 48 h after incubated with SDF-1 at the concentrations of 20 and 40 ng/mL ($P < 0.05$, Figure 1B). HT29 cells treated with AMD3100 prior to administration of SDF-1 did not decrease the E-cadherin expression level. However, HT29 cells treated SDF-1 decreased the E-cadherin expression level (Figure 1C). AMD3100 alone had no effect on the E-cadherin expression (Figure 1D). The changes of E-cadherin expression in HT29 cells are demonstrated in Figure 1E.

Reverse transcriptase-polymerase chain reaction (RT-PCR) analysis demonstrated that the E-cadherin mRNA expression level was lower 48 h after incubated with SDF-1 at the concentrations of 20 and 40 ng/mL ($P < 0.05$). AMD3100 plus SDF-1 inhibited the E-cadherin mRNA expression. AMD3100 alone had no influence on E-cadherin mRNA expression (Figure 2A and B).

SDF-1 down-regulated β-catenin expression at protein and mRNA levels

β-catenin was strongly stained in HT29 cells not incubated with SDF-1 (Figure 3A). The β-catenin expression

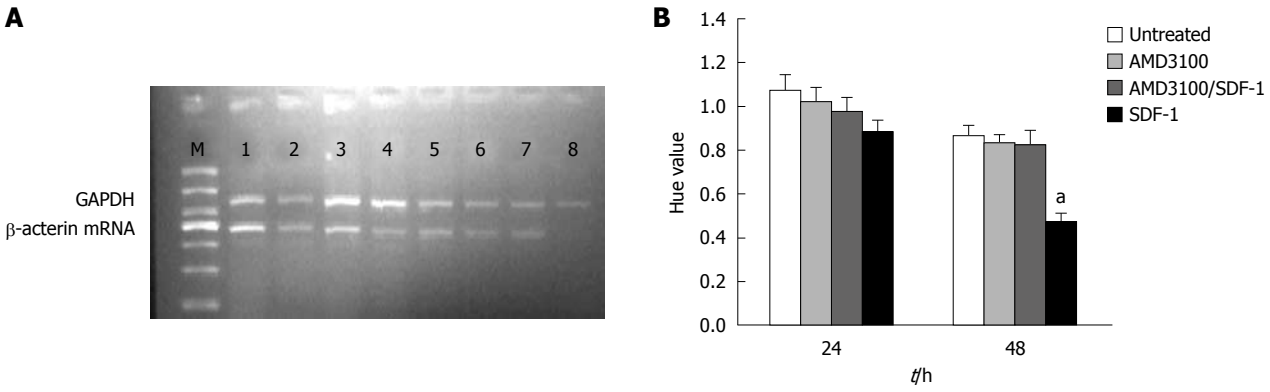


Figure 4 Effect of stromal cell-derived factor-1 (20 ng/mL) on β-catenin mRNA expression after 24 h (A) and 48 h (B) in different groups. Bars represent mean ± SD of three individual experiments. $^{a}P < 0.05$. SDF-1: Stromal cell-derived factor-1.

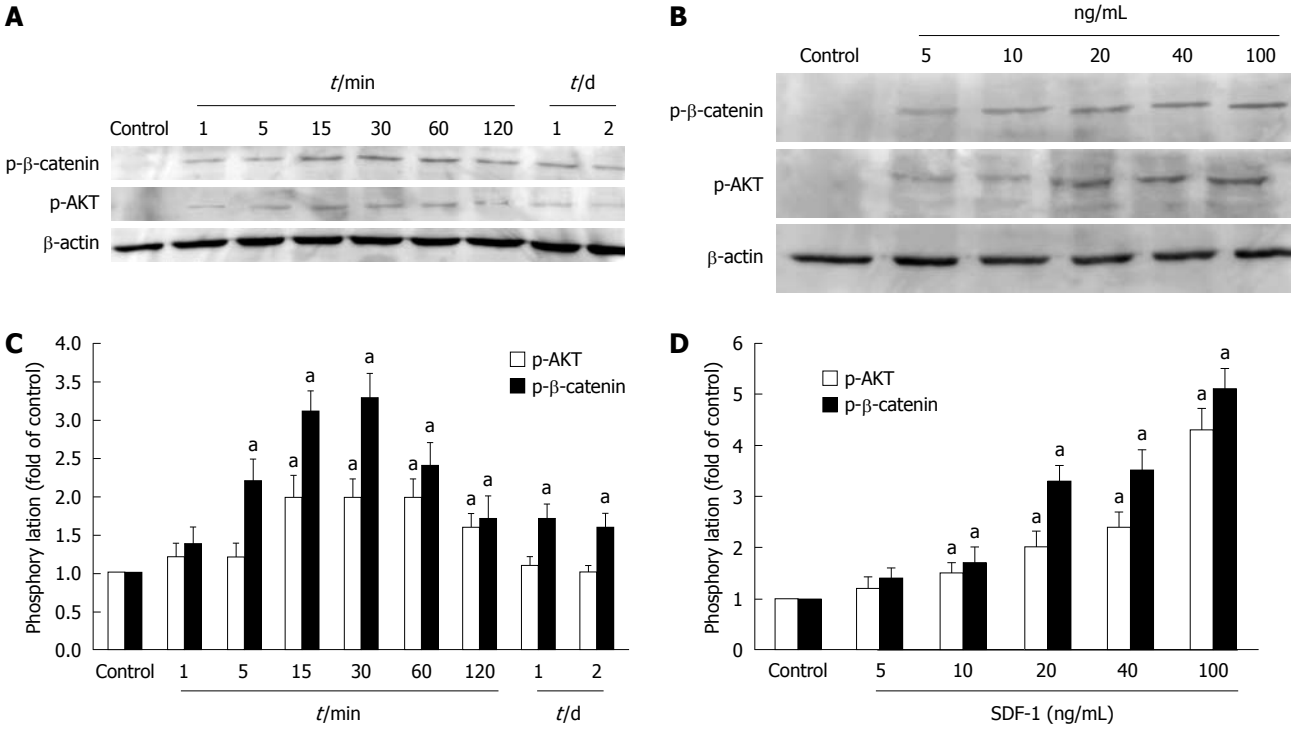


Figure 5 Effect of stromal cell-derived factor-1 on the phosphorylation of PI3K/AKT and β-catenin. A: Phosphorylation of PI3K/AKT and β-catenin after incubated with stromal cell-derived factor (SDF) at different periods of time (1, 5, 10, 15, 30, 60, 120 min and on days 1 and 2); B: Phosphorylation of PI3K/AKT and β-catenin after incubated with SDF-1 at different concentrations (5, 10, 20, 40 and 100 ng/mL) for 30 min; C and D: SDF-1 increases phosphorylation of PI3K/AKT and β-catenin in HT29 cells in a time- and dose-dependent manner. Bars represent mean ± SD of triplicate experiments. $^{a}P < 0.05$.

level was lower 24 and 48 h after incubated with SDF-1 at the concentrations of 20 and 40 ng/mL ($P < 0.05$, Figure 3B). The β-catenin expression level was slightly lower in AMD3100- treated HT29 cells (Figure 3C). AMD3100 alone had no influence on β-catenin expression (Figure 3D). The changes of β-catenin expression in HT29 cells are demonstrated in Figure 3E.

RT-PCR analysis demonstrated that the β-catenin mRNA expression level was lower 24 and 48 h after incubated with SDF-1 at the concentrations of 20 and 40 ng/mL ($P < 0.05$, Figure 4A and B). AMD3100 plus SDF-1 inhibited the β-catenin mRNA expression. AMD3100 alone did not influence the β-catenin mRNA expression.

Involement of phosphorylation of PI3K/AKT and β-catenin in SDF-1-induced down-regulation of E-cadherin/β-catenin expression

The phosphorylation of PI3K/AKT and β-catenin was detected to observe whether phosphorylation is involved in down-regulation of E-cadherin/β-catenin complex expression, which demonstrated that SDF-1 increased the phosphorylation of AKT and β-catenin. The β-catenin was evidently activated at 5 min and the AKT was significantly phosphorylated at 15 min after incubated with SDF-1. The phosphorylation of β-catenin reached its peak 30 min after incubated with SDF-1. Then, β-catenin was evidently phosphorylated for 2 d (Figure 5A and C). The

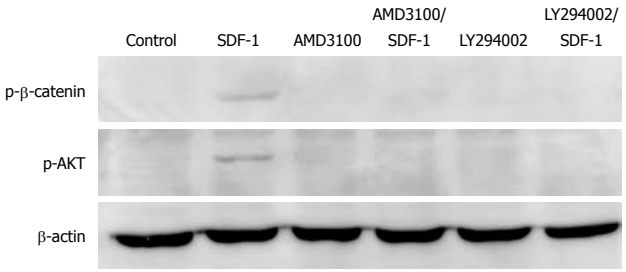


Figure 6 AMD3100 and LY294002 inhibit stromal cell-derived factor-1-induced phosphorylation of PI3K/AKT and β-catenin while AMD3100 or LY294002 alone has no effect on phosphorylation of PI3K/AKT and β-catenin. SDF-1: Stromal cell-derived factor-1.

phosphorylation of AKT reached its peak at 15-60 min after incubated with SDF-1, remained there for 2 h, and then slowly declined. To determine the dose-dependent effect of SDF-1 on the phosphorylation of PI3K/AKT and β-catenin, HT29 cells were treated with SDF-1 at different concentrations (0, 5, 10, 20, 40 and 100 ng/mL) for 30 min. Then, cell lysates were analyzed for the phosphorylation of PI3K/AKT and β-catenin. Administration of SDF-1 for 30 min increased the phosphorylation of PI3K/AKT and β-catenin in a dose-dependent manner. PI3K/AKT and β-catenin were phosphorylated after incubated with SDF-1 at the concentration of 5 ng/mL and reached its peak after incubated with SDF-1 at the concentration of 100 ng/mL (Figure 5B and D).

Inhibition of PI3K/AKT prevented phosphorylation of SDF-1-induced β-catenin

To investigate the relation between PI3K/AKT and β-catenin, AMD3100 and LY294002 were used to inhibit the effect of SDF-1 and the signal transmission through PI3K/AKT, which showed that AMD3100 inhibited the phosphorylation of PI3K/AKT and β-catenin (Figure 6). Further study demonstrated that administration of LY294002 prior to SDF-1 also prevented the phosphorylation of PI3K/AKT and β-catenin (Figure 6), suggesting that β-catenin is phosphorylated *via* the PI3K/AKT, and may be the downstream signaling molecule of PI3K/AKT.

DISCUSSION

Although the CXCR4/SDF-1 biological axis contributes to organ-selective metastasis of tumors[15-17], the mechanism underlying the effect of chemotaxis remains unclear. In this study, the relation between CXCR4/SDF-1 axis and E-cadherin/β-catenin complex expression was observed. Experiment on HT29 colon cancer cell line was performed because it shows a high expression level of CXCR4[18]. SDF-1 promoted the proliferation of HT29 cells, thereby contributing to primary tumor formation and down-regulated the E-cadherin/β-catenin expression by reducing the mRNA expression levels and decomposing their complex formation by phosphorylating the PI3K/AKT and β-catenin. AMD3100 and LY294002 blocked the two processes mediated by SDF-1, suggesting that they may be effective anti-metastatic agents, at least against CRC.

It was reported that CXCR4/SDF-1 axis-induced chemotaxis effect plays a role in the invasion of CRC cells[16,17]. A number of molecules, such as vascular endothelial growth factor, matrix metalloproteinase (MMP)9, and MMP2, have been implicated in SDF-1-induced CRC invasion[19]. E-cadherin is not only an adhesion molecule but also a tumor suppressor as well as the most important epithelial marker[20]. In most cancers of epithelial origin, E-cadherin mediates cell-cell adhesion. Loss of E-cadherin-mediated cell-cell adhesion is implicated in tumor invasion and metastasis[21,22]. Recent studies showed that Krüppel-like factor 4 inhibits epithelial to mesenchymal transition by regulating E-cadherin gene expression[23]. In our study, the E-cadherin expression level at protein and mRNA levels was lower 48 h after incubated with SDF-1 at the concentrations of 20 and 40 ng/mL, suggesting that E-cadherin is involved in SDF-1-induced chemotaxis effect. In this study, the molecular mechanism underlying SDF-1-induced chemotaxis effect was studied. The E-cadherin mRNA expression level was lower after incubated with SDF-1, which may account for the down-regulation of E-cadherin expression.

Tumor invasion is a complex process and loss of E-cadherin-mediated cell adhesion is not sufficient to confer an invasive phenotype to tumor cells. Cell migration requires precise control, which is altered or lost when tumors become invasive and metastatic. It has been shown that decreased β-catenin expression is often related with the absent or reduced E-cadherin, which contributes to the development of several cervical carcinoma cell lines[24,25]. A recent study revealed that β-catenin membrane/cytosolic expression level is significantly lower in primary tumors than in corresponding matched metastases, suggesting that the low β-catenin expression level may be a prognostic factor for the occurrence of metastasis and a worse outcome[26]. In this study, the β-catenin protein and mRNA expression levels were significantly lower 48 h after incubated with SDF-1 at the concentrations of 20 and 40 ng/mL, suggesting that β-catenin is involved in SDF-1-induced chemotaxis effect. Changes in β-catenin may also lead to the degradation of E-cadherin/β-catenin complex and alter cytoskeleton, thus promoting cell migration.

Growth factors, such as epidermal growth factor, induce β-catenin and plakoglobin phosphorylation, resulting in breakage of E-cadherin binding to actin cytoskeleton and contact disassembly[26]. In this study, the phosphorylation of β-catenin was increased after incubated with SDF-1, indicating that SDF-1 induces phosphorylation of β-catenin. Phosphorylation of β-catenin reduces β-catenin and may lead to decomposition of E-cadherin/β-catenin complex. G protein-coupled receptor activation results in PI3K and downstream AKT activation[27]. In this study, SDF-1- induced phosphorylation of PI3K/AKT and β-catenin in a time- and dose-dependent manner. Finally,

whether SDF-1 induces β-catenin phosphorylation *via* PI3K/AKT was also studied. Given that both AMD3100 and LY294002 inhibited the phosphorylation of PI3K/AKT and β-catenin, β-catenin may be the downstream signaling molecule of PI3K/AKT, indicating that the phosphorylation of β-catenin may account for the down-regulation of β-catenin, and the breakage of E-cadherin/β-catenin complex. The phosphorylation of β-catenin may exert its effect *via* the PI3K/AKT pathway.

In conclusion, down-regulation of E-cadherin/β-catenin complex expression is involved in SDF-1-induced chemotaxis. The decreased E-cadherin mRNA expression and the down-regulated β-catenin expression may account for the down-regulation of E-cadherin. CXCR4/SDF-1 axis stimulates the phosphorylation of PI3K/AKT and β-catenin. The down-regulation of β-catenin may be induced by the decreased β-catenin mRNA expression and the increased phosphorylation of PI3K/AKT. PI3K inhibitor LY294002 inhibits the SDF-1-induced phosphorylation of PI3K/AKT and β-catenin. β-catenin may be the downstream signaling molecule of PI3K/AKT.

ACKNOWLEDGMENTS

The authors thank Cui-Ling Li and Hai-Peng Yin for their excellent technical support in this study.

COMMENTS

Background

CXCR4/stromal cell-derived factor-1 (SDF-1) axis-mediated chemotaxis effect is crucial in organ-selective metastasis. E-cadherin/β-catenin complex plays an important role in colorectal tumorigenesis. Relatively little is known about the role of E-cadherin/β-catenin in CXCR4/SDF-1 axis-mediated chemotaxis effect.

Research frontiers

Recent studies demonstrated that the CXCR4/SDF-1 signaling pathway is involved in the metastatic process of colorectal cancer (CRC). In addition, inhibiting the interaction of SDF-1 and CXCR4 with CXCR4 antagonist AMD3100 prevents the chemotactic migration of CRC cells. However, the underlying molecular mechanism has not been elucidated.

Innovations and breakthroughs

Down-regulation of E-cadherin/β-catenin complex is involved in SDF-1-induced chemotaxis effect in HT29 colon cancer cells. Moreover, decreased mRNA synthesis and increased β-catenin phosphorylation may down-regulate E-cadherin/β-catenin. To the best of our knowledge, this is the first study that analyzes the relation between CXCR4/SDF-1 axis and E-cadherin/β-catenin complex.

Applications

This study demonstrated the relation between E-cadherin/β-catenin complex and SDF-1-induced chemotaxis effect in HT29 colon cancer cells, thus providing a possible molecular mechanism underlying the SDF-1-induced chemotaxis effect.

Terminology

SDF-1, also know as CXCL12, belongs to the CXC chemokine family and interacts specifically with the seven-transmembrane, G protein-coupled receptor CXCR4. E-cadherin is not only an adhesion molecule but also a tumor suppressor as well as the most important epithelial marker. β-catenin is a key component of adheren junctions, necessary for homophilic cell-cell adhesion. In addition to the membrane-associated pool, β-catenin plays a role in cell-signaling and gene transcription.

Peer review

This is a well designed study, which may explain the development of colorectal metastasis *in vitro*.

REFERENCES

1 **Jemal A**, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E, Feuer EJ, Thun MJ. Cancer statistics, 2004. *CA Cancer J Clin* 2004; **54**: 8-29
2 **Folkman J**. The role of angiogenesis in tumor growth. *Semin Cancer Biol* 1992; **3**: 65-71
3 **Chambers AF**, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. *Nat Rev Cancer* 2002; **2**: 563-572
4 **Salvucci O**, Yao L, Villalba S, Sajewicz A, Pittaluga S, Tosato G. Regulation of endothelial cell branching morphogenesis by endogenous chemokine stromal-derived factor-1. *Blood* 2002; **99**: 2703-2711
5 **Müller A**, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verástegui E, Zlotnik A. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001; **410**: 50-56
6 **Federsppiel B**, Melhado IG, Duncan AM, Delaney A, Schappert K, Clark-Lewis I, Jirik FR. Molecular cloning of the cDNA and chromosomal localization of the gene for a putative seven-transmembrane segment (7-TMS) receptor isolated from human spleen. *Genomics* 1993; **16**: 707-712
7 **Matsusue R**, Kubo H, Hisamori S, Okoshi K, Takagi H, Hida K, Nakano K, Itami A, Kawada K, Nagayama S, Sakai Y. Hepatic stellate cells promote liver metastasis of colon cancer cells by the action of SDF-1/CXCR4 axis. *Ann Surg Oncol* 2009; **16**: 2645-2653
8 **Kim J**, Takeuchi H, Lam ST, Turner RR, Wang HJ, Kuo C, Foshag L, Bilchik AJ, Hoon DS. Chemokine receptor CXCR4 expression in colorectal cancer patients increases the risk for recurrence and for poor survival. *J Clin Oncol* 2005; **23**: 2744-2753
9 **Schimanski CC**, Schwald S, Simiantonaki N, Jayasinghe C, Gönner U, Wilsberg V, Junginger T, Berger MR, Galle PR, Moehler M. Effect of chemokine receptors CXCR4 and CCR7 on the metastatic behavior of human colorectal cancer. *Clin Cancer Res* 2005; **11**: 1743-1750
10 **Zeelenberg IS**, Ruuls-Van Stalle L, Roos E. The chemokine receptor CXCR4 is required for outgrowth of colon carcinoma micrometastases. *Cancer Res* 2003; **63**: 3833-3839
11 **Gumbiner BM**. Regulation of cadherin-mediated adhesion in morphogenesis. *Nat Rev Mol Cell Biol* 2005; **6**: 622-634
12 **Brembeck FH**, Rosário M, Birchmeier W. Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. *Curr Opin Genet Dev* 2006; **16**: 51-59
13 **Li JN**, Li X, Qian JM, Lu XQ, Yang H. [Effects of K-ras gene mutation on colon cancer cell line Caco-2 metastasis by regulating E-cadherin/beta-catenin/p120 protein complex formation and RhoA protein activity]. *Zhongguo Yixue Kexueyuan Xuebao* 2010; **32**: 46-50
14 **Choi HN**, Kim KR, Lee JH, Park HS, Jang KY, Chung MJ, Hwang SE, Yu HC, Moon WS. Serum response factor enhances liver metastasis of colorectal carcinoma via alteration of the E-cadherin/beta-catenin complex. *Oncol Rep* 2009; **21**: 57-63
15 **Tan CT**, Chu CY, Lu YC, Chang CC, Lin BR, Wu HH, Liu HL, Cha ST, Prakash E, Ko JY, Kuo ML. CXCL12/CXCR4 promotes laryngeal and hypopharyngeal squamous cell carcinoma metastasis through MMP-13-dependent invasion via the ERK1/2/AP-1 pathway. *Carcinogenesis* 2008; **29**: 1519-1527
16 **Kajiyama H**, Shibata K, Terauchi M, Ino K, Nawa A, Kikkawa F. Involvement of SDF-1alpha/CXCR4 axis in the enhanced peritoneal metastasis of epithelial ovarian carcinoma. *Int J Cancer* 2008; **122**: 91-99
17 **Leelawat K**, Leelawat S, Narong S, Hongeng S. Roles of the MEK1/2 and AKT pathways in CXCL12/CXCR4 induced cholangiocarcinoma cell invasion. *World J Gastroenterol* 2007; **13**: 1561-1568
18 **Richard CL**, Tan EY, Blay J. Adenosine upregulates CXCR4 and enhances the proliferative and migratory responses of

human carcinoma cells to CXCL12/SDF-1alpha. *Int J Cancer* 2006; **119**: 2044-2053
19 **Brand S**, Dambacher J, Beigel F, Olszak T, Diebold J, Otte JM, Göke B, Eichhorst ST. CXCR4 and CXCL12 are inversely expressed in colorectal cancer cells and modulate cancer cell migration, invasion and MMP-9 activation. *Exp Cell Res* 2005; **310**: 117-130
20 **Perl AK**, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature* 1998; **392**: 190-193
21 **Sánchez-Tilló E**, Lázaro A, Torrent R, Cuatrecasas M, Vaquero EC, Castells A, Engel P, Postigo A. ZEB1 represses E-cadherin and induces an EMT by recruiting the SWI/SNF chromatin-remodeling protein BRG1. *Oncogene* 2010; **29**: 3490-3500
22 **Lee JM**, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 2006; **172**: 973-981
23 **Yori JL**, Johnson E, Zhou G, Jain MK, Keri RA. Kruppel-like factor 4 inhibits epithelial-to-mesenchymal transition through regulation of E-cadherin gene expression. *J Biol Chem* 2010; **285**: 16854-16863
24 **Denk C**, Hülsken J, Schwarz E. Reduced gene expression of E-cadherin and associated catenins in human cervical carcinoma cell lines. *Cancer Lett* 1997; **120**: 185-193
25 **Gloushankova NA**. Changes in regulation of cell-cell adhesion during tumor transformation. *Biochemistry* (Mosc) 2008; **73**: 742-750
26 **Pancione M**, Forte N, Fucci A, Sabatino L, Febbraro A, Di Blasi A, Daniele B, Parente D, Colantuoni V. Prognostic role of beta-catenin and p53 expression in the metastatic progression of sporadic colorectal cancer. *Hum Pathol* 2010; **41**: 867-876
27 **Zhang W**, Jiang B, Guo Z, Sardet C, Zou B, Lam CS, Li J, He M, Lan HY, Pang R, Hung IF, Tan VP, Wang J, Wong BC. Four-and-a-half LIM protein 2 promotes invasive potential and epithelial-mesenchymal transition in colon cancer. *Carcinogenesis* 2010; **31**: 1220-1229

S- Editor Sun H **L- Editor** Wang XL **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.633

World J Gastroenterol 2011 February 7; 17(5): 633-638
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Natural history of cytomegalovirus infection in a series of patients diagnosed with moderate-severe ulcerative colitis

Valeria Criscuoli, Maria Rosa Rizzuto, Luigi Montalbano, Elena Gallo, Mario Cottone

Valeria Criscuoli, Mario Cottone, Dibimis (Biomedical Department of Internal and Specialist Medicine), "V.Cervello" Hospital Palermo, University of Palermo, Palermo 90143, Italy
Maria Rosa Rizzuto, Elena Gallo, Institute of Pathology. "V.Cervello" Hospital Palermo, Palermo 90143, Italy
Luigi Montalbano, Division of Gastroenterology, "V.Cervello" Hospital Palermo, Palermo 90143, Italy
Author contributions: Criscuoli V and Cottone M planned, conducted the study and drafted the paper; Montalbano L performed proctoscopy and rectal biopsies; Rizzuto MR examined histologic specimens; Gallo E performed PCR assay; all authors approved the final version of the paper.
Correspondence to: Valeria Criscuoli, MD, PhD, Dibimis (Biomedical Department of Internal and Specialist Medicine), "V.Cervello" Hospital Palermo, University of Palermo, Via Imperatore Federico 70, Palermo 90143, Italy. vale.cic@libero.it
Telephone: +39-91-540614 Fax: +39-91-7305218
Received: August 25, 2010 Revised: October 26, 2010
Accepted: November 2, 2010
Published online: February 7, 2011

Abstract

AIM: To evaluate the natural history of human cytomegalovirus (HCMV) infection in a series of 28 ulcerative colitis patients in whom the search for HCMV was positive.

METHODS: A series of 85 patients with moderate-severe ulcerative colitis flare-up were evaluated for a HCMV search by performing a haematoxylin and eosin stain, immunohistochemical assay and nested polymerase chain reaction on rectal biopsies. Among 85 screened patients (19 of whom were steroid resistant/dependant), 28 were positive for HCMV; after remission the patients were followed up clinically and histologically.

RESULTS: Among the 22 patients with complete follow-up, in 8 (36%) patients HCMV-DNA persisted in the intestinal specimens. Among the HCMV positive patients, 4 (50%) experienced at least one moderate-severe flare-up of colitis without evidence of peripheral HCMV. Among the 14 HCMV negative patients, 3 with pouches developed pouchitis and 5 out of 11 (45%) experienced a colitis flare-up.

CONCLUSION: Our preliminary results suggest that HCMV may remain in the colon after an acute colitis flare-up despite remission; it seems that the virus is not responsible for the disease relapse.

Key words: Ulcerative colitis; Cytomegalovirus; Natural history; Polymerase chain reaction; Outcome

Peer reviewer: Dr. Douglas K Rex, MD, FACP, FACG, FASGE, Department of Medicine, Indiana University School of Medicine, 550 N. University Blvd., Indianapolis, IN 46202, United States

Criscuoli V, Rizzuto MR, Montalbano L, Gallo E, Cottone M. Natural history of cytomegalovirus infection in a series of patients diagnosed with moderate-severe ulcerative colitis. *World J Gastroenterol* 2011; 17(5): 633-638 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/633.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.633

INTRODUCTION

The etiology of inflammatory bowel disease (IBD) is still unknown. The most current hypothesis is that IBD derives from an unsuitable and exaggerated immune response to normal mucosal resident bacterial microflora, likely induced by an external agent, in genetically predisposed individuals. Among the external agents, environmental factors unquestionably play a major role in the pathogenesis of IBD. Evidence of associations of bacterial factors derives from both human and animal studies. The virus takes an unclear part in the pathogenesis and disease development, but is probably involved.

In recent years several papers have described the link between viral infection and IBD onset, reactivation or steroid resistance[1-4].

No clear data is available on the natural history of HCMV infection superimposed on IBD. The following questions remain unanswered: (1) does the virus disappear after remission of acute colitis relapse? (2) does the persistence of the virus detected by sensitive assays increase the risk of relapse?

The aim of this study was an attempt to answer the questions raised above and to evaluate the natural history of human cytomegalovirus (HCMV) infection after a severe colitis exacerbation.

MATERIALS AND METHODS

From 1997 to 2007, a prospective study was conducted on 85 patients with ulcerative colitis who were admitted to the Medicine department of V. Cervello Hospital in Palermo due to a severe colitis attack (according to the Truelove-Witts criteria) (Figure 1). All patients were treated with conventional and standardized corticosteroid treatment (1 mg/kg per day) and were endoscopically evaluated with a different approach: from 1997 to 1999 sigmoidoscopy was performed only in steroid resistant patients (17 patients) whereas from 2000 to 2007 sigmoidoscopy was performed in all patients (68 patients) at ward admission, taking systematically rectal biopsies. Therefore a total of 85 patients (Table 1) was investigated for HCMV infection with 3 different techniques.

The rectal biopsies were immediately fixed with 10% buffered formalin for 2 h to obtain tissue fixation; afterwards the preparation was subjected to several processes (lavage, dehydration, clearing, paraffin impregnation and embedding) to prepare sections with a thickness of 3-4 μm.

The histologic specimens were examined using the following techniques: (1) Light microscopy with hematoxylin and eosin (HE) stain in order to document the microscopic disease activity and allow the detection of cytomegalic cells, markers of infected viral cells. Cytomegalic cells, which are 2-or 4-fold larger (25-35 μm) than surrounding cells, contain a basophilic intranuclear inclusion (8-10 μm) eccentrically placed and sometimes surrounded by a clear halo giving it an "owl's eye" appearance, and thickened nuclear membrane, frequently associated with smaller granular intracytoplasmatic inclusions. Intranuclear inclusions were observed in epithelial, endothelial, stromal and smooth muscle cells. A biopsy was regarded as positive by light microscopy for HCMV if a single cell showed intranuclear or cytoplasmic inclusions and cytomegalic characteristics (Figure 2); (2) Immunohistochemical (ICH) procedure for HCMV performed on a paraffin- embedded section with monoclonal mouse antibodies anti-Human CMV (clone BM204) and conjugated to a peroxidase-labeled amino acid polymer by peroxidase-antiperoxidase (PAP) method in order to detect viral proteins. Nuclear or cytoplasmic antigen was identified by the typical brown reaction product of the PAP method; and

Table 1 Clinical characteristics of the series *n* (%)

Patient characteristics	Total	HCMV+	HCMV-
Sex (M/F)	50/35	19/9	31/26
Age at ward admission (yr)			
⩾ 50		17	40
< 50		11	17
Disease extension			
Left sided colitis	39	11 (39)	28 (49)
Subtotal and pancolitis	46	17 (61)	29 (51)
Previous azathioprine treatment	21	11 (39)	10 (17.5)
Previous biologic treatment	9	2 (7)	7 (12)
Active disease at sigmoidoscopy			
Severe	51	16 (57)	35 (61)
Mild/moderate	34	12 (43)	22 (39)
Onset of disease (new diagnosis)	5	4 (14)	1 (2)
Reactivation (established disease)	80	24 (86)	56 (98)

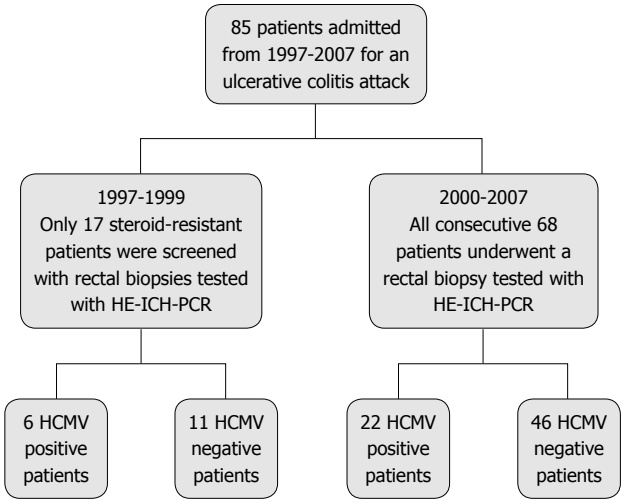


Figure 1 Series studied. HCMV: Human cytomegalovirus; ICH: Immunohistochemical; PCR: Polymerase chain reaction; HE: Hematoxylin and eosin.

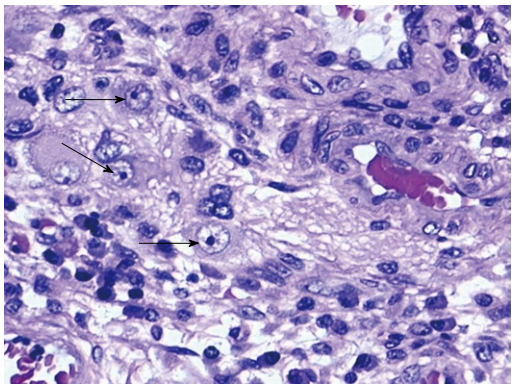


Figure 2 Original reproduction of epithelial cytomegalic cells (arrows) in rectal biopsy (hematoxylin and eosin stain, 40 ×).

(3) Nested polymerase chain reaction (nPCR): (a) DNA extraction**:** DNA was extracted from 10 mm sections of paraffin wax embedded tissues. Five sections were cut with a standard microtome from every paraffin wax block and transferred into a 1.5 mL microtube. To prevent cross

contamination between the samples, the microtome blade was washed with xylene and ethanol after sectioning of each block. DNA extraction was performed using a conventional method. The conventional method consisted of xylene/ethanol dewaxing followed by overnight proteinase k digestion in lysis buffer. The sample was heated at 95℃ for 5 min to inactivate the proteinase K. We checked the quality of samples by PCR for the housekeeping gene b-globin (fragment of 268 bp); and (b) Detection of viral DNA: Nested PCR was used to detect the presence of viral DNA in colon tissues. Two pairs of primers annealed to the gB region of HCMV. Primers used for the first-round product and second-round PCRs are as follows (5' to 3'): first-round primer 1, GAGGACAACGAAATCCTGTTGGGCA; first-round primer 2, GTCGACGGTGGAGATACTGCTGAGG; second-round primer 3, ACCACCGCACTGAGGAATGTCAG, and second-round primer 4, TCAATCATGCGTTTGAAGAGGTA, to obtain a CMV fragment of 100 bp.

PCR reaction mixture contained 0.5 U *Taq* polymerase, 1 × PCR Buffer (50 mmol/L KCl and 10 mmol/L Tris-HCl (pH 8.4), 0.2 mmol/L of each dNTPs, 1.5 mmol/L $MgCl_2$, 10 pmol of each primers and 100-200 ng of extracted DNA. The conditions for the first-round PCR were as follows: denaturation at 94℃ for 5 min, followed by 35 cycles of denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min and extension at 72℃ for 1 min followed by 5 min final extension at 72℃.

The conditions for second-round PCR were as follows: denaturation at 94℃ for 5 min, followed by 25 cycles of denaturation at 94℃ for 1 min, annealing at 55℃ for 1 min and extension at 72℃ for 1 min followed by 5 min final extension at 72℃.

The PCR amplification products were run on 2% agarose gel and stained with ethidium bromide and visualized under ultraviolet light.

HCMV-pp65 antigenemia

All patients were also tested for HCMV-pp65 antigenemia in peripheral leukocytes by CMV-CINAkit (Argene® Biosoft). The samples were considered positive for active HCMV infection using a cut-off value of 5 positive fluorescent nuclei/2 × 10^5 leukocytes[5]; between 1 to 4 positive cells the result was considered questionable and the blood sample was repeated after 48 h to value a possible increase of cell nuclei positivity. If we detected histopathological examination positive we considered the patient HCMV-infected but not surely with active replication; if both tests (histology and pp65 antigenemia) were positive we judged the patients candidates for antiviral treatment due to a probable active replication. However the last decision about antiviral treatment was related to the disease activity without significant improvement of the disease course after treatment for underlying disease.

Sigmoidoscopy was performed in patients reaching clinical remission in order to control endoscopic activity and the presence of HCMV.

Table 2 Results and outcome of the series *n* (%)

	HCMV+ (HE-ICH/PCR)	HCMV-
Total patients	28	57
Steroid dependent/resistant (%)	68	19
Medical remission	22 (78)	51 (89)
Surgical remission	6 (21)	6 (10.5)
Death	3 (10)	0
Persistence of HCMV DNA+ at follow-up	8 (28.5)	0

HCMV: Human cytomegalovirus; ICH: Immunohistochemical; PCR: Polymerase chain reaction; HE: Hematoxylin and eosin.

The patients were followed clinically as outpatients quarterly to evaluate the remission time, the number of relapses, the immunosuppressive therapies and the need for surgery.

We define as remission a combination of clinical parameters (stool frequency ⩽ 3 per day with no bleeding); the term relapse is used to define a flare of symptoms in a patient with established ulcerative colotis (UC) who is in clinical remission, either spontaneously or after medical or surgical treatment.

In the case of clinical relapse, sigmoidoscopy was performed with multiple biopsies and a search for HCMV in rectal biopsies by the 3 methods and peripheral pp65antigenemia was conducted.

Moreover the asymptomatic patients were examined by colonoscopy every year in order to follow up the natural history of HCMV infection regarding histologic persistence, to understand more clearly the possible role of "bystander" or promoter to the disease relapse.

Oral and written informed consent was obtained from each patient before any procedure.

The rate of surgery and clinical relapse rates in the 2 groups of patients (HCMV positive and negative) were compared using χ^2 test.

RESULTS

The median clinical and endoscopic follow-up was 40 mo.

Among the 85 patients evaluated for HCMV infection from 1997 to 2007, in 28 (12 women and 16 men) the intestinal biopsy resulted positive by routine HE/ICH staining and nPCR assay according to the standard methods previously described. The overall prevalence data was 33% (Table 2). There was no association between HCMV and active immunosuppressive or biologic treatment. Ten patients among a total of 28 also displayed positive pp65 antigenemia (35%). Out of 10 positive patients for both histology and pp65 antigenemia, 7 received antiviral treatment (5 with ganciclovir and 2 with foscarnet) because of steroid resistance and clinical disease worsening, achieving remission in 5 patients. In these 5 treated patients HCMV disappeared at the first endoscopic control. Two patients were operated on because of a rapid clinical disease worsening: 1 patient died due to toxic megacolon. Among the 3 patients not treated with antiviral therapy for a contrain-

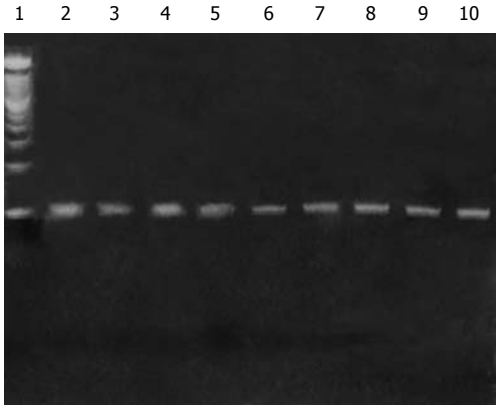


Figure 3 Ethidium bromide-stained 2% agarose gel demonstrating detectable second-round human cytomegalovirus products of nested polymerase chain reaction. Lane 1: Molecular size marker; Lanes 2-9: Positive patient samples; Lane 10: Positive control.

dication, 1 achieved remission with therapy for underlying disease, 2 patients were operated on for intractable disease and 1 of these 2 patients died due to post-operative complications.

Among the 18 patients who were histologically positive but pp65 antigenemia negative, 2 were operated on for intractable disease and 1 died; 16 improved with conventional therapy. Among the overall number of 28 patients who tested positive for HCMV, 6 underwent total colectomy (21%), 2 of them treated with antiviral treatment. Among the 57 patients who were negative for HCMV detection, 6 patients were operated on (10.5%).

Among the 25 surviving patients, 22 remained on follow-up (19 on medical remission and 3 with ileoanal pouch anastomosis) because 3 refused to undertake regular clinical and colonoscopic controls.

The 3 patients operated on underwent endoscopic and histological assessment of the ileoanal pouch during follow-up, showing a moderate grade of pouchitis according to PDAI 1 year after surgery, without detection of HCMV in the pouch.

Eighteen patients achieved clinical remission with medical treatment and 1 patient became steroid-dependent; all were followed up for an average period of 46 mo, all were clinically and endoscopically evaluated in accordance with the methods described above.

During the routine endoscopic and biopsy controls 11/19 patients were negative for HCMV detection both by traditional histology with HE and ICH in the intestinal biopsies and by nPCR assay, whereas 8 were positive for HCMV viral DNA detection by nPCR (Figure 3) and negative on light microscopy (HE and ICH) and on pp65 antigenemia.

Among the 8 patients in which positivity for HCMV-DNA persisted in the intestinal biopsies, 3 were treated with antiviral therapy at first detection of HCMV. Four experienced (50%) an early-moderate colitis flare up (within 3 mo) during the follow-up without detection of pp65 antigenemia in the peripheral leukocytes (Figure 4). A patient achieved remission with conventional steroid

Table 3 Clinical course and outcome of ulcerative colotis with persistent human cytomegalovirus at polymerase chain reaction

Patient No.	Age (yr)/ sex	Immunomodulators at HCMV diagnosis	Disease relapse	Outcome
1	42/F	Aza	No	Remission with 5-asa
2	71/M	No	Yes	Remission with IFX
3	58/M	Aza	Yes	Procto-colectomy
4	74/M	No	Yes	Remission with IFX
5	65/F	No	No	Remission with 5-asa
6	65/M	No	Yes	Remission with steroid
7	45/M	Aza	No	Leukapheresis-IFX
8	28/M	No	No	Remission with Aza

HCMV: Human cytomegalovirus; Aza: Azathioprine; IFX: Infliximab.

treatment, 2 patients became steroid-dependent and were treated with anti-TNF therapy, both achieving remission. The fourth patient, who was steroid-dependent, intolerant to azathioprine and with a contraindication to anti-TNF therapy, underwent a total colectomy due to severity of disease. None were treated with antiviral treatment due to absence of positive antigenemia (Table 3). Among the 11 HCMV negative patients 5 (45%) experienced a reactivation of colitis and achieved remission with steroid treatment.

After remission, 8 patients (among 22) were treated with azathioprine (in 5 of them HCMV-DNA was present when the treatment was started) and 14 patients were treated with mesalazine (3 HCMV-DNA positive).

The χ^2 test comparing surgical intervention among positive HCMV (21%) and negative HCMV (10.5%) patients was not statistically significant ($P = 0.17$, odds ratio 2.32).

The χ^2 test comparing the relapse rate among HCMV positive (50%) and negative (45%) patients was not statistically significant ($P = 0.36$).

DISCUSSION

Our study was an attempt to answer two recurrent questions about the role of HCMV in IBD. The questions are about HCMV disappearance after remission of acute colitis relapse: we have demonstrated that HCMV persists in the colon after recovery of a colitis flare with a HCMV co-infection in a series of UC patients and remains detectable by sensitive methods such as nPCR without histological/ICH virus detection in a minority of patients. From this small series the persistence of virus in the colon of UC patients does not favour disease relapse because the virus persists in a latent state. The absence of pp65 antigenemia during the follow-up relapse and the response to the conventional corticosteroid treatment do not suggest a remarkable role of HCMV in the re-activation. Furthermore, it is unlikely that immunosuppressive treatment favours relapse of UC when HCMV persists in the colon.

To establish a connection between HCMV and UC

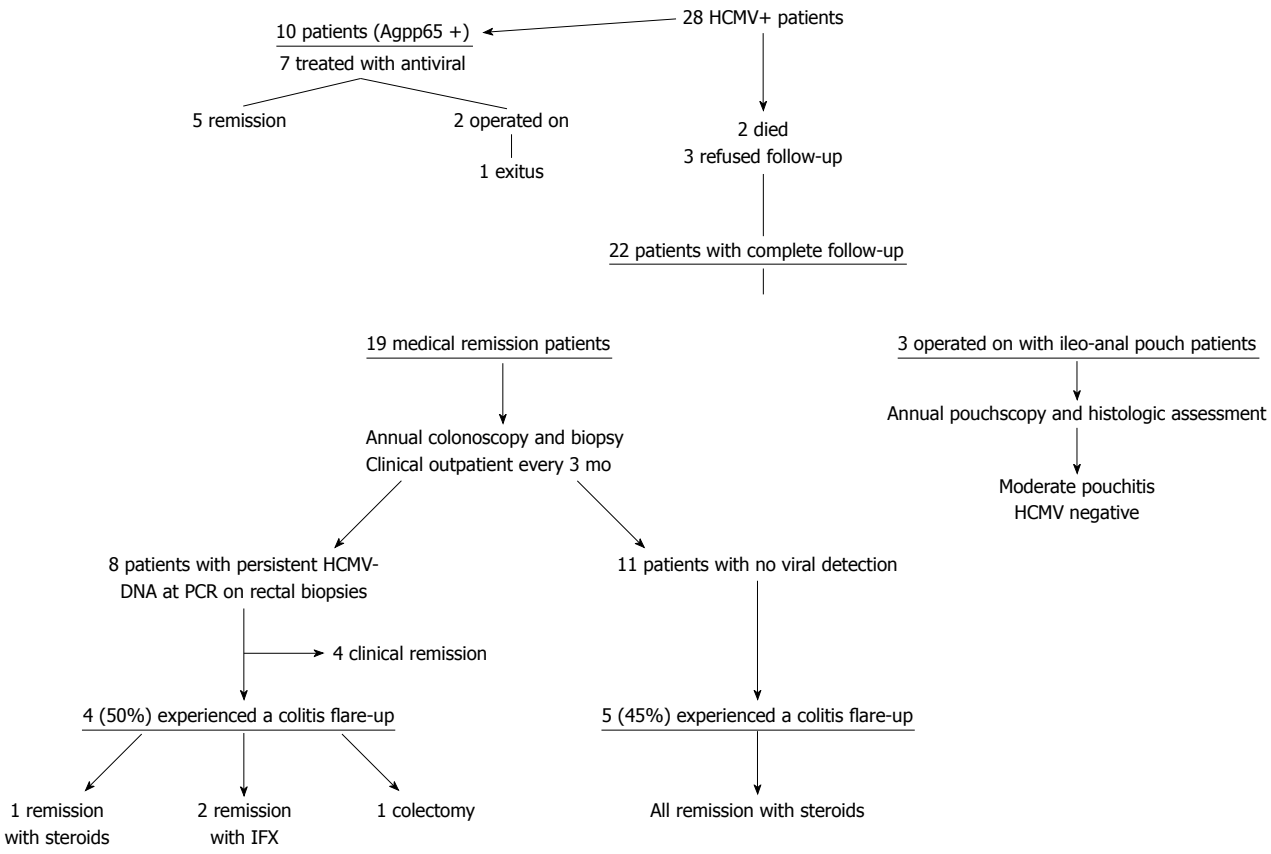


Figure 4 Follow-up human cytomegalovirus+ patients. IFX: Infliximab; HCMV: Human cytomegalovirus; PCR: Polymerase chain reaction.

more reports are needed. The interaction with the immune system plays a key role in the pathogenesis of HCMV disease, and the main determinant is an immunological impairment. As mentioned above, even most primary infections in humans are asymptomatic. No trace of the infection is observable except for seroconversion. In recent years, however, the disease has become more common probably due to the widespread use of immunosuppressants in oncology, in transplantation medicine and in chronic disease[6].

What happens in intestinal tissue after persistence of HCMV-DNA is not known; maybe the colonic epithelial cells harbour the latent virus that became detectable using the highly sensitive PCR assay. The reactivation of virus from latency may depend on a complex interplay of biological factors with the host that, above all in patients with UC, are not clearly understood. Experimental studies suggest that latent CMV infection in the mouse may modulate mucosal immunity altering the susceptibility to gut microbiota without viral reactivation[7].

Dimitroulia *et al*[8] demonstrated that HCMV is frequently detected in IBD patients, showing that the virus genome was detected in intestinal tissue by polymerase chain reaction in 32.9% of the total IBD patients, while the HCMV genome in the blood was detected in 27.1% of these patients. Matsuoka *et al*[9] showed that HCMV is frequently reactivated in a series of active UC seropositive patients, but that reactivation has little effect on the clinical course and that most of the colitis reactivation with positive HCMV responds to conventional immunosuppressive therapies.

Kou *et al*[10] demonstrated that the detected copy number of HCMV-DNA by PCR method is higher in the inflamed colonic tissue than in non inflamed colonic tissue in patients with UC refractory to immunosuppressive therapy. The author strongly supported the hypothesis that HCMV infection is involved in exacerbation of patients with IBD and the early detection of genome in intestinal tissue is important for an eventual change to the therapeutic approach.

We show that patients with HCMV infection were more frequently operated on than those without superimposed HCMV (even though not statistically significant) and this may suggest that the virus is a marker of risk of surgical treatment. This observation is an agreement with Cooper *et al*[11] that showed in 1977 that HCMV infection might be responsible for acute toxic dilatation with increased colonic resection rate.

In patients who have undergone proctocolectomy we did not detect HCMV in the follow-up biopsies of the ileal pouch. Casadesus *et al*[12] detected HCMV in a small series of patients with UC who underwent proctocolectomy and hypothesize that the virus may play an etiological role in pouchitis; other case reports[13,14] demonstrate that HCMV is a rare but possible cause of refractory pouchitis to be considered for antiviral therapy.

In conclusion, our preliminary results suggest that HCMV may remain in the colon despite remission, probably activating a delicate balance with the host immune system in order to avoid its elimination, but the effects are not clear. The variability of genotypes may give an answer to the virulence and cell tropism so that we might be able to understand the different behaviour related to different host conditions.

A multicentre study including a more numerous population (almost 500 patients) with a longer follow-up is warranted to define whether the virus infection causes a disease complication or its presence does not alter the course of disease.

Limitations of the study

A limitation of study is that the assessment is based upon very few patients but it is very difficult to find a large population; possibly a multicentre study would solve this problem.

We performed the nPCR assay that detects only the presence of viral DNA in colon tissues. Maybe a quantitative assay that can distinguish between active and latent disease would give some useful information to correlate the replication of HCMV with bowel disease activity.

ACKNOWLEDGMENTS

The authors thank Professor Florian Mussgnug for correcting the English text and Dr. Giuseppe Malizia for providing the photograph of nested PCR.

COMMENTS

Background

Cytomegalovirus (CMV) infection has been described as a cause of relapse of inflammatory bowel disease (IBD), in particular in ulcerative colotis patients, especially those receiving high-dose corticosteroid therapy. No clear data is available on the natural history of human CMV (HCMV) superimposed on IBD.

Research frontiers

The paper aimed to answer two questions concerning HCMV infection in ulcerative colitis patients.

Innovations and breakthroughs

This paper looks at the role of long-term HCMV persistence in patients with moderate to severe ulcerative colitis together with the likelihood this brings of relapse in patients that both cleared and did not clear the infection.

Applications

This is one of the first studies investigating the persistence of HCMV in colonic tissue of ulcerative patients after an acute flare up of disease.

Terminology

Nested polymerase chain reaction is a molecular assay that detects HCMV DNA using specific primers that amplify the gB region of HCMV. HCMV-pp65 antigenemia is an assay for polymorphonuclear leukocytes.

Peer review

I think there are some useful observations in the study, but some clarifications are needed.

REFERENCES

1 **Berk T**, Gordon SJ, Choi HY, Cooper HS. Cytomegalovirus infection of the colon: a possible role in exacerbations of inflammatory bowel disease. *Am J Gastroenterol* 1985; **80**: 355-360
2 **Orvar K**, Murray J, Carmen G, Conklin J. Cytomegalovirus infection associated with onset of inflammatory bowel disease. *Dig Dis Sci* 1993; **38**: 2307-2310
3 **Loftus EV Jr**, Alexander GL, Carpenter HA. Cytomegalovirus as an exacerbating factor in ulcerative colitis. *J Clin Gastroenterol* 1994; **19**: 306-309
4 **Criscuoli V**, Casà A, Orlando A, Pecoraro G, Oliva L, Traina M, Rizzo A, Cottone M. Severe acute colitis associated with CMV: a prevalence study. *Dig Liver Dis* 2004; **36**: 818-820
5 **Torrús D**, Portilla J, Hernández-Aguado I, Boix V, Plazas J, Gimeno A, Torromé M, Llopis C, Valls V, Sánchez-Payá J. Usefulness of pp65 antigenemia for the early diagnosis of cytomegalovirus disease in patients with AIDS. *Eur J Clin Microbiol Infect Dis* 1999; **18**: 630-635
6 **Baroco AL**, Oldfield EC. Gastrointestinal cytomegalovirus disease in the immunocompromised patient. *Curr Gastroenterol Rep* 2008; **10**: 409-416
7 **Onyeagocha C**, Hossain MS, Kumar A, Jones RM, Roback J, Gewirtz AT. Latent cytomegalovirus infection exacerbates experimental colitis. *Am J Pathol* 2009; **175**: 2034-2042
8 **Dimitroulia E**, Spanakis N, Konstantinidou AE, Legakis NJ, Tsakris A. Frequent detection of cytomegalovirus in the intestine of patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 879-884
9 **Matsuoka K**, Iwao Y, Mori T, Sakuraba A, Yajima T, Hisamatsu T, Okamoto S, Morohoshi Y, Izumiya M, Ichikawa H, Sato T, Inoue N, Ogata H, Hibi T. Cytomegalovirus is frequently reactivated and disappears without antiviral agents in ulcerative colitis patients. *Am J Gastroenterol* 2007; **102**: 331-337
10 **Kou T**, Nakase H, Tamaki H, Kudo T, Nishio A, Chiba T. Cytomegalovirus infection in patients with ulcerative colitis diagnosed by quantitative real-time PCR analysis. *Dig Dis Sci* 2006; **51**: 1052-1055
11 **Cooper HS**, Raffensperger EC, Jonas L, Fitts WT Jr. Cytomegalovirus inclusions in patients with ulcerative colitis and toxic dilation requiring colonic resection. *Gastroenterology* 1977; **72**: 1253-1256
12 **Casadesus D**, Tani T, Wakai T, Maruyama S, Iiai T, Okamoto H, Hatakeyama K. Possible role of human cytomegalovirus in pouchitis after proctocolectomy with ileal pouch-anal anastomosis in patients with ulcerative colitis. *World J Gastroenterol* 2007; **13**: 1085-1089
13 **Muñoz-Juarez M**, Pemberton JH, Sandborn WJ, Tremaine WJ, Dozois RR. Misdiagnosis of specific cytomegalovirus infection of the ileoanal pouch as refractory idiopathic chronic pouchitis: report of two cases. *Dis Colon Rectum* 1999; **42**: 117-120
14 **Moonka D**, Furth EE, MacDermott RP, Lichtenstein GR. Pouchitis associated with primary cytomegalovirus infection. *Am J Gastroenterol* 1998; **93**: 264-266

S- Editor Sun H **L- Editor** O'Neill M **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.639

World J Gastroenterol 2011 February 7; 17(5): 639-645
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Natural history of heartburn: A 10-year population-based study

Linda Bjork Olafsdottir, Hallgrimur Gudjonsson, Heidur Hrund Jonsdottir, Bjarni Thjodleifsson

Linda Bjork Olafsdottir, Hallgrimur Gudjonsson, Bjarni Thjodleifsson, Department of Gastroenterology, Landspitali University Hospitol, 101 Reykjavik, Iceland
Heidur Hrund Jonsdottir, Department of Gastroenterology, The Social Science Research Institute, University of Iceland, 101 Reykjavik, Iceland
Bjarni Thjodleifsson, Department of Gastroenterology, Faculty of Medicine, University of Iceland, 101 Reykjavik, Iceland
Author contributions: Olafsdottir LB, Thjodleifsson B and Gudjosnsson H designed research; Olafsdottir LB performed research; Jonsdottir HH analyzed data; Olafsdottir LB and Thjodleifsson B wrote the paper.
Supported by (in part) The Medical Research Fund of the National Hospital of Iceland; the Medical Research Fund of Wyeth, Iceland; AstraZeneca, Iceland; GlaxoSmithKline, Iceland; and the Icelandic College of Family Physicians
Correspondence to: Linda Bjork Olafsdottir, MSc Pharm, MBA, Department of Gastroenterology, Landspitali University Hospitol, 101 Reykjavik, Iceland. linda04@ru.is
Telephone: +354-8966664 Fax: +354-5200801
Received: July 19, 2010 Revised: November 2, 2010
Accepted: November 9, 2010
Published online: February 7, 2011

Abstract

AIM: To study the natural history and prevalence of heartburn at a 10-year interval, and to study the effect of heartburn on various symptoms and activities.

METHODS: A population-based postal study was carried out. Questionnaires were mailed to the same age- and gender-stratified random sample of the Icelandic population (aged 18-75 years) in 1996 and again in 2006. Subjects were classified with heartburn if they reported heartburn in the preceding year and/or week, based on the definition of heartburn.

RESULTS: Heartburn in the preceding year was reported in 42.8% (1996) and 44.2% (2006) of subjects, with a strong relationship between those who experienced heartburn in both years. Heartburn in the preceding week was diagnosed in 20.8%. There was a significant relationship between heartburn, dyspepsia and irritable bowel syndrome. Individuals with a body mass index (BMI) below or higher than normal weight were more likely to have heartburn. Heartburn caused by food or beverages was reported very often by 20.0% of subjects.

CONCLUSION: Heartburn is a common and chronic condition. Subjects with a BMI below or higher than normal weight are more likely to experience heartburn. Heartburn has a great impact on daily activities, sleep and quality of life.

Key words: Heartburn; Follow-up; Questionnaire study; Epidemiology

Peer reviewer: Joachim Labenz, Associate Professor, Jung-Stilling Hospital, Wichernstr. 40, Siegen 57074, Germany

Olafsdottir LB, Gudjonsson H, Jonsdottir HH, Thjodleifsson B. Natural history of heartburn: A 10-year population-based study. *World J Gastroenterol* 2011; 17(5): 639-645 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/639.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.639

INTRODUCTION

Gastroesophageal reflux disease (GERD) is one of the most prevalent diseases worldwide[1]. GERD is a chronic condition which usually manifests symptomatically, is a great burden for patients, and has significant socioeconomic implications[2]. The prevalence of predominant gastroesophageal reflux symptoms appears to be stable over time[3]. Heartburn is the typical GERD symptom and may be induced by various physiological and pathophysiological mechanisms[4]. Heartburn, coupled with acid regurgitation and odynophagia, are considered to be highly specific for GERD[1].

Functional heartburn is defined as episodic retrosternal burning in the absence of GERD, histopathology-based motility disorders or structural explanations[5]. Heartburn alone has a prevalence of 17%-42% in Western populations[2,3,5-7].

The prevalence of upper gastrointestinal symptoms in the general population is high and symptoms are associated with significant health-care utilization and diminished quality of life[6]. In contrast, the natural history of heartburn has received limited attention and few epidemiological studies have focused on heartburn. Subjects with upper gastrointestinal symptoms are more likely to use prescription medication and are more likely to have seen a physician about symptoms than those with heartburn[6]. There has been more focus on GERD than heartburn.

The aim of this present study was therefore to evaluate the natural history of heartburn in the Icelandic population prospectively over a 10-year period, as well as to evaluate different factors which are affected by heartburn both physically and sociodemographically. A parallel publication based on the same database, focusing on functional dyspepsia (FD), has been published[8] as has another parallel publication regarding irritable bowel syndrome (IBS)[9].

MATERIALS AND METHODS

Participants and setting

In 1996 an epidemiological study of gastrointestinal diseases was carried out in Iceland[10], involving 2000 inhabitants in the range of 18-75 years of age. The individuals were randomly selected from the National Registry of Iceland. Equal distribution of sex and age was secured in each age group. In 2006 we attempted to contact all the same individuals as in 1996 as well as adding 300 new individuals in the 18-27 age group who were also randomly selected from the National Registry. A study questionnaire and explanatory letter were mailed to all eligible individuals at baseline. Reminder letters were mailed at 2, 4 and 7 wk, using the Total Method of Dillman[11]. Individuals who indicated at any point that they did not want to participate in the study were not contacted further.

The questionnaire

The Bowel Disease Questionnaire (BDQ)[12,13] was translated and modified for this study. The questionnaire was designed as a self-report instrument to measure symptoms experienced over the previous year and to collect the subject's past medical history[14].

The Icelandic version of the BDQ questionnaire addresses 47 gastrointestinal symptoms and 32 items that measure past illness, health care use, items on sociodemographic and psychosomatic symptoms, together with a valid measure of non-gastrointestinal (non-GI) somatic complaints ascertained through the Somatic Symptom Checklist (SSC)[15]. The SSC includes questions on 12 non-GI and 5 GI symptoms or illnesses. Individuals are instructed to indicate, on a 5-point scale, how often each symptom has appeared and how bothersome it has been. There were few changes to the later questionnaire (2006) which addressed 51 gastrointestinal symptoms and 33 items that measure past illness, health care use, and sociodemographic and psychosomatic symptoms items. The 2006 Questionnaire furthermore addressed 17 items to identify heartburn and items related to heartburn.

Criteria for identifying heartburn

Subjects were classified with heartburn if they reported heartburn according to the following definition: Heartburn is a burning sensation in the retrosternal area (behind the breastbone). The pain often rises in the upper abdomen and may radiate to the chest.

Transition between disorders from initial and final surveys

A transition model used by Halder *et al*[14] was modified and applied for this study. The responses from the initial (1996) and final (2006) surveys were matched for each subject to examine the changes between disorders at an individual level for the 5 categories (FD, IBS, heartburn, frequent abdominal pain and no symptoms). A 5 × 5 table was used to model these multiple changes and collapsed into 6 groups, as illustrated in Table 1. Those with the most symptoms were prioritized higher. Those who developed more symptoms and those who reported fewer symptoms could be categorized into their respective groups. There were six patterns of symptoms, identified as follows: (1) symptom stability; (2) symptom increase; (3) symptom decrease; (4) symptom onset; (5) became asymptomatic; and (6) none of these symptoms.

Mortality data

For the 2006 survey we identified all deceased individuals with the assistance of the National Registry of Iceland (*Thjodskra*).

Statistical analysis

Tables were constructed to show frequency and percentage. Categorical data were analyzed using the χ^2 test. The type I error protection rate was set at 0.05. The exact *P* is listed in the tables and text. All the research data were imported into SPSS (Statistical Package of Social Science) software.

Ethics

The National Bioethics Committee of Iceland and The Icelandic Data Protection Authority (*Personuvernd*) gave their permission for the research.

RESULTS

Demographic data of involved individuals

In 1996 the response rate was 66.8% (1336/2000). Of the 1336 individuals who participated in 1996, 81 were deceased by 2006, five subjects were unable to answer, mainly because of old age, and 70 could not be traced to a current address. This left 1180 individuals, out of whom 799 responded. Therefore, the response rate in 2006 was 67.7% (799/1180). The mean age of the individuals in

Table 1 Transition among symptom subgroups between the initial and final surveys

FGID in 1996	Proportion of FGID in 2006 based on primary survey disorder (%)				
	FD	IBS	Heartburn	Frequent abdominal pain	No symptoms
FD (n = 111)	52.3[1]	21.6[3]	14.4[3]	1.8[3]	9.9[4]
IBS (n = 152)	25.0[2]	30.3[1]	19.7[3]	4.6[3]	20.4[4]
Heartburn (n = 173)	12.1[2]	12.1[2]	39.3[1]	4.6[3]	31.8[4]
Frequent abdominal pain (n = 39)	12.8[2]	23.1[2]	17.9[2]	15.4[1]	30.8[4]
No symptoms (n = 324)	3.4[5]	9.9[5]	17.3[5]	6.2[5]	63.3[6]

[1]Stable; [2]Increased symptoms; [3]Decreased symptoms; [4]Became asymptomatic; [5]Developed symptoms; [6]Remaining asymptomatic. FGID: Functional gastrointestinal disorder; FD: Functional dyspepsia; IBS: Irritable bowel syndrome.

Table 2 Study population: age and sex distribution

	Population 2006 (%)	Respondents 2006 (%)
Gender		
Male	50.3	42.2
Female	49.7	57.8
Age (yr)		
28-35	19.5	14.52
36-45	24.9	20.40
46-55	22.8	22.15
56-65	15.6	19.52
66-75	10.4	15.14
76-85	6.8	8.26
Total number	173 859	799

1996 was 42 years, in 2006 it was 43 years, and 41 years for non-respondents in 2006. Women were more likely to respond than men in both years. A larger proportion of women than men responded again in 2006 (57.8%) than had responded in 1996, as is common in similar studies. The responders represented the population in all major factors concerning sex and age distribution. The response rate was also higher for older subjects than for younger ones. The age distribution and demographic details of the study cohort are presented in Tables 2 and 3.

Heartburn 10-year follow-up

At the 10-year follow-up, individuals were asked if they had experienced heartburn in the preceding year; 42.8% in 1996 and 44.2% in 2006 reported heartburn. There was a strong relationship between those who experienced heartburn in 2006 and those who reported heartburn in 1996. Two thirds of those who reported heartburn in 1996 also experienced heartburn in 2006. However, one third of those who reported heartburn in 2006 had not experienced it 10 years earlier.

Almost all who were on medication for heartburn reported relief with the medication. Individuals reported acid reflux once a month or more in 11% of cases in 1996 and 10% of cases in 2006.

There was a significant relationship between heartburn and dyspepsia and between heartburn and IBS, both in 1996 and in 2006.

Individuals of normal weight [body mass index (BMI) 18.5-24.9] were less likely to experience heartburn than individuals with a BMI below or higher than normal weight.

Individuals who smoked were not more likely to have heartburn than those who did not smoke. Individual alcohol consumption within the study group changed during the 10-year period of 1996 to 2006. Alcohol consumption was not associated with heartburn.

Transitions among symptom subgroups between the initial and final surveys

As described in the Methods section, the groups in this analysis were defined as mutually exclusive using a symptom hierarchy so that each subject appears in only one category for both the 1996 and 2006 surveys. There was a "no symptoms" category for those who did not meet any of the criteria applied for functional gastrointestinal disorders. Due to the hierarchical classification only a few participants occurred in some categories.

There was a substantial change in numbers in all the categories over time (Table 1). The group "no symptoms" was the most common (63.3%). Of the heartburn group 39.3% were stable and 31.8% reported "no symptoms"; 24.2% reported increased symptoms and 4.6% decreased symptoms. Of the FD group 52.3% remained stable and 9.9% reported "no symptoms" in 2006. Most of the subjects who were in the IBS group, or 30.3% of the total, were stable over the 10-year period; 20.4% reported "no symptoms" in 2006 and 25.0% showed an increase in symptoms over the 10 years. In 2006, 15.4% of the subjects reported stable frequent abdominal pain, 30.8% reported "no symptoms" and 53.8% reported increased symptoms.

The distribution of the 6 transition groups was: 22.3% symptom stability, 12.6% symptom increase, 10.9% symptom decrease, 14.9% developed symptoms, 13.6% became asymptomatic, and 25.7% had no symptoms in either 1996 or 2006.

Heartburn in subjects in 2006

In the 2006 questionnaire individuals were asked additional questions regarding heartburn during the preceding week. Heartburn during the preceding week was reported by 20.8% of the subjects (19.0% male, 22.1% female). Of these, 60.5% reported taking medicine for heartburn. Increasing age was not a significant factor in prevalence of heartburn/reflux disease. Age was, however, a significant factor associated with the use of medication for heartburn (Figure 1). Most subjects took ranitidine or esomeprazole for their symptoms (Figure 2).

Table 3 Sociodemographic characteristics and the development and disappearance of heartburn

	n	Never HB (%)	Lost HB (%)	Retained HB (%)	Developed HB (%)	χ^2	*P*-value
Gender						1.687	0.640
Male	330	40.3	14.5	30.3	14.8		
Female	441	41.5	14.7	26.5	17.2		
Age group (yr)						15.542	< 0.05[a]
66-85	170	54.8	10.6	27.1	10.6		
36-65	488	37.3	16.4	29.3	17.0		
28-35	113	40.7	13.3	24.8	21.2		
BMI (kg/m^2)						21.685	< 0.01[b]
> 30	154	31.8	14.3	37.0	16.9		
> 25 and ≤ 30	314	37.3	14.3	31.5	16.9		
≤ 25	286	49.3	15.0	19.9	15.7		
Level of education						6.156	0.724
> 4 years' further education	225	39.6	12.9	28.9	18.7		
3-4 years' further education	279	41.9	17.6	25.1	15.4		
< 3 years' further education	92	39.1	13.0	33.7	14.1		
No further education	161	41.6	13.0	29.8	15.5		
Employment status						6.276	0.099
Employed	574	39.7	15.5	27.0	17.8		
No employment	189	44.4	12.2	31.7	11.6		
Alcohol						4.503	0.609
≥ 7 drinks per week	43	37.2	9.3	34.9	18.6		
1-6 drinks per week	404	39.1	14.6	28.2	18.1		
No alcohol	309	43.0	15.5	27.5	13.9		
Smoking						8.773	0.187
Smokers, > 15 cigarettes per day	63	34.9	20.6	25.4	19.0		
Smokers, ≤ 15 cigarettes per day	113	31.9	17.7	34.5	15.9		
No smoking	496	43.5	13.7	26.2	16.5		

[a]$P < 0.05$, [b]$P < 0.01$. HB: Heartburn; BMI: Body mass index.

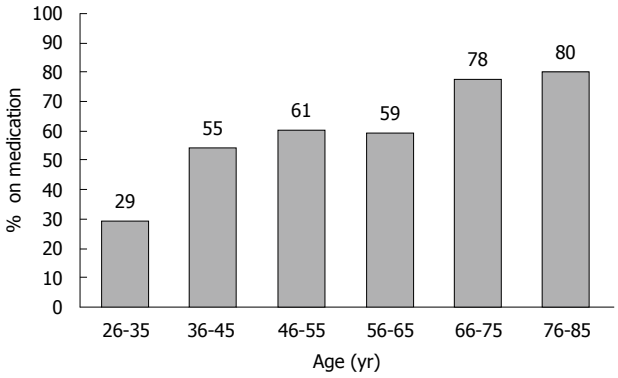


Figure 1 Age and use of medication.

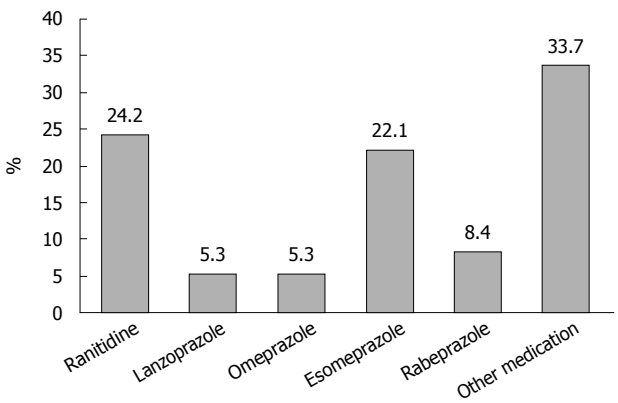


Figure 2 Which medication do you take?

Table 4 Heartburn and relationship to medication, food/beverages and tiredness

Variable	*n*	% of heartburn prior week
On constant medication	30	27.3
Medication only when experiencing symptoms	77	85.6
Tiredness (lethargy)		
Frequent	20	13.2
Sometimes/seldom	73	48.0
Never	59	38.8
Heartburn caused by food and beverages		
Very often	32	20.0
Sometimes/seldom	118	73.8
Never	10	6.3
Increased heartburn caused by specific food		
Very often	35	22.7
Sometimes/seldom	92	59.7
Never	27	17.5

27.3% reported they were on constant medication. Most individuals (85.6%) reported taking medication only when they experienced symptoms (Table 4), although there was some overlap here between groups. Six subjects reported having had an operation for reflux disease.

Tiredness or lethargy was reported as occurring frequently by 13.2% of subjects, reported rarely or seldom by 48%, and reported as never having occurred by 38.8% (Table 4).

Heartburn caused by food or beverages was reported as occurring very often by 20%, 73.8% reported some or

Table 5 Symptoms or activities affected by heartburn (caused by heartburn)

Variable	*n*	% of heartburn prior week
Felt bad		
Frequent	21	13.1
Sometimes/seldom	119	74.4
Never	20	12.5
Less food and beverages consumption		
Frequent	9	5.9
Sometimes/seldom	77	50.3
Never	67	43.8
Less family activities		
Frequent	1	0.6
Sometimes/seldom	32	20.8
Never	121	78.6
Trouble with sleeping		
Frequent	9	5.8
Sometimes/seldom	70	45.2
Never	76	49.0
Felt hopeless, worried or impatient		
Frequent	9	5.8
Sometimes/seldom	42	27.3
Never	103	66.9
Felt worried or scared for their health		
Frequent	5	3.2
Sometimes/seldom	47	30.3
Never	103	66.5
Felt irritable		
Frequent	21	13.6
Sometimes/seldom	80	51.9
Never	53	34.4
Neglect specific food or alcohol		
Frequent	36	23.1
Seldom	66	42.3
Never	54	34.6
Affects their daily activities		
Frequent	3	1.9
Sometimes/seldom	32	20.5
Never	121	77.6
Unable to move (sports, hobbies and outside of home)		
Frequent	3	1.9
Sometimes/seldom	34	21.8
Never	119	73.6

minimal heartburn and 6.3% never. Increased heartburn caused by a specific food was reported as occurring very often by 22.7% and sometimes by 59.7%. A specific food significantly more often provoked considerable heartburn in women than in men (Table 4).

As can be seen in Table 5, heartburn can affect symptoms or activities in many cases. Three out of four heartburn subjects claimed that they felt badly sometimes or seldom. One out of three heartburn subjects felt hopeless, anxious or impatient. And one out of three also reported being worried or scared because of heartburn every week.

Only 1.9% of the subjects reported that heartburn frequently affected their daily activities, whereas one fifth claimed that their daily activities were only sometimes or seldom affected by heartburn. Three out of four subjects reported that heartburn made them irritable. And one out of four heartburn subjects reported that heartburn resulted in less family activities, affected their daily activities and meant they were unable to move in sports, hobbies and outside of home. Half of the heartburn subjects reported trouble with sleeping because of heartburn.

Many heartburn subjects reported less food and beverage consumption and that they avoided specific food or alcohol because of the heartburn.

DISCUSSION

In this study our main focus was on the natural history of heartburn over a 10-year period in an Icelandic population. The only other long-term study, to our knowledge, that has focused on heartburn is a long-term community study in Sweden covering a maximum of 7 years[3]. There are strengths and weaknesses in both studies, but taken together they give a reasonably accurate picture of the natural history of heartburn.

The strength of our study is the use of a stable, homogeneous and well-informed population. The sample was randomly selected from the National Registry of Iceland and represented the nation as a whole in selected age groups. The population of Iceland was around 300 thousand inhabitants at the time of the study and the sample was approximately 1% of the whole population from all around the country. The BDQ, the questionnaire used, assesses the whole range of gastrointestinal functional disorders.

The prevalence of heartburn is high in Iceland. More than two out of five subjects reported heartburn in the preceding year. Half of those reported heartburn in the preceding week. Heartburn was reported as still existing after 10 years for 2 out of 3 subjects in the study. The study by Agréus *et al*[3] showed that the prevalence of predominant gastroesophageal reflux symptoms appears to be stable over time. Results from studies of patients suggest that GERD is a chronic disease in most cases[3,16,17]. One third of subjects who did not report heartburn in 1996 had developed heartburn 10 years later. So even though the total prevalence of heartburn was almost the same in both 1996 and 2006, there was a change among over one third of subjects reporting heartburn.

Heartburn subjects with a BMI either lower than or higher than normal weight were more likely to experience heartburn than subjects with normal weight. A study by Aro *et al*[18] found that reflux symptoms are linked to obesity and specifically that the presence of gastroesophageal reflux symptoms was linked to reflux esophagitis in the obese population. Festi *et al*[19] concluded that it was likely that GERD and obesity are in some way linked and that it was possible to hypothesize that GERD may be a curable condition through the control of body weight. This may also be true for heartburn.

The transition analysis showed a substantial change in numbers in all the categories. The stability of each disease varied. FD subjects were the most stable throughout the 10 years (52.3%). Of the heartburn group 39.3% were stable, as were 30.3% of the IBS group and 15.4% of the frequent abdominal pain group. A quarter of the heartburn group had increased symptoms in 10 years, 4.6% decreased symptoms and one third developed no

symptoms in 10 years. There was a significant relationship between IBS and heartburn as well as FD and heartburn.

Half (45.1%) of the subjects who reported heartburn in the preceding year experienced heartburn in the previous week. Food and beverages play a large part in eliciting heartburn; very often in 20.0% of the cases and sometimes in 73.8% of the cases. Subjects also very often experienced increased heartburn caused by a specific food in 22.7% of the cases. Heartburn did not seem to be the cause for less food and beverage consumption, but one out of five heartburn subjects did avoid a specific food or alcohol because of heartburn. Festi *et al*[19] report that no definitive data exist regarding the role of diet and specific foods or drinks in GERD clinical manifestations[19].

Heartburn is associated with feeling tired (61.2%), feeling bad (87.5%) and with irritation (65.5%). One third felt worried or scared for their health because of heartburn symptoms and one third also felt that heartburn caused them to feel hopeless, worried or impatient (33.1%). Every fifth heartburn subject reported that heartburn affected activities such as daily and family activities, as well as that heartburn caused them to be unable to move normally and therefore affected their participation in sports, hobbies and outdoor activities. This effect of heartburn on normal life and activities may have affected the subjects in the manner of a chronic condition throughout the 10 years of the study, and therefore had a great impact on quality of life. This finding is in line with McDougall *et al*[17] who showed in their study on reflux esophagitis and quality of life that it was not bodily pain and vitality that were impaired, but general health and social function.

Three out of five of all the heartburn subjects in 2006 reported taking medicine for heartburn. Almost all the subjects who were on medication for heartburn reported relief provided by the medication. Age was a significant factor for the use of medication for heartburn. Most subjects took ranitidine or esomeprazole for their symptoms.

Few studies have addressed the impact of nocturnal reflux symptoms in heartburn subjects. A study by Farup *et al*[20] showed that the prevalence of nighttime heartburn in GERD patients under routine care was high, up to 49% for 1 of 3 years. A population-based survey in the United States claimed that the overall prevalence of nocturnal GERD symptoms was 10%, with 74% of subjects with GERD symptoms fitting the criteria for nocturnal GERD[21]. In our study, sleep was frequently affected in 5.8% of cases and 45.2% of heartburn subjects were sometimes or seldom troubled with sleeping in the prior week. These numbers can be expected to be higher for the preceding year, since we asked specifically about the preceding week.

There are some limitations to our study. The subjects were not specifically interviewed or examined to evaluate the possibility of organic disease. However, a 10-year (postal) follow-up went some way towards making an organic cause of symptoms unlikely. Furthermore, since the response rate was 66.8% in 1996 and 67.7% in 2006, a dropout bias cannot be excluded.

In summary, heartburn is a common condition in the population of Iceland. The prevalence is slightly higher than reported elsewhere. Heartburn is a chronic condition, affecting every fifth person every week. Heartburn subjects with a BMI lower or higher than normal weight were more likely to experience heartburn than subjects of normal weight. Heartburn did not seem to result in less food and beverage consumption, but one out of five heartburn subjects did avoid a specific food or alcohol because of the heartburn. Heartburn had a great impact on daily activities and quality of life. Half of the heartburn subjects experienced sleep disturbances because of this condition.

COMMENTS

Background

Heartburn is a signature symptom of gastroesophageal reflux disease (GERD), which is a cluster of symptoms and signs associated with regurgitation of stomach acid up to the pharynx and mouth. Patient-based studies of GERD have shown high prevalence and chronicity, particularly in Western societies. GERD is associated with significant health-care utilization and diminished quality of life. Heartburn, coupled with acid regurgitation and painful swallowing are considered to be highly specific for GERD. Very few epidemiological studies have been performed with regard to heartburn, and only one has been population-based. The natural history of GERD or heartburn has received little attention. The pathophysiology of GERD and heartburn is basically unknown.

Research frontiers

The prevalence of upper gastrointestinal symptoms in the general population is high and symptoms are associated with significant socioeconomic consequences. The prevalence and natural history of heartburn is of importance as well as its association with functional dyspepsia and irritable bowel syndrome, and sociodemographic factors such as body mass index (BMI). The aim of the present study was therefore to evaluate the natural history of heartburn in the Icelandic population prospectively over a 10-year period, as well as to evaluate different factors which are associated with heartburn both physically and sociodemographically.

Innovations and breakthroughs

The prevalence of heartburn is high in Iceland. More than two out of five subjects reported heartburn in the preceding year. Half of those reported heartburn in the preceding week. Heartburn was reported as still existing after 10 years for 2 out of 3 subjects in the study. Heartburn subjects with a BMI either lower or higher than normal weight were more likely to experience heartburn than subjects with normal weight. There was an association between heartburn, functional dyspepsia and irritable bowel syndrome and patients floated over time between these categories. This suggests a common etiopathogenesis of these disorders. The quality of life was diminished due to a variety of factors such as worries, irritability, intolerance to specific foods and sleep disturbance.

Applications

The prevalence and natural history of heartburn and its risk factors are important for management and prognosis. Heartburn can be regarded as a reliable surrogate marker of GERD. This study creates a database for future studies and hopefully stimulates studies in other countries. Secular prevalence trends and international comparison can contribute towards understanding of the pathophysiology of the disease.

Terminology

A 10-year follow-up population-based, questionnaire study of the Icelandic population was performed. The primary aim was to study the prevalence and natural history of heartburn. Subjects were classified as having heartburn if they reported heartburn according to the following definition: Heartburn is a burning sensation in the retrosternal area (behind the breastbone). The pain often rises in the upper abdomen and may radiate to the chest.

Peer review

Heartburn alone has a prevalence of 17%-42% in Western populations and is associated with extensive health care expenses and diminished quality of life. Comparative international population-based studies are needed to document secular trends and to elucidate the reasons for the different prevalence in various countries.

REFERENCES

1 **Armstrong D**, Mönnikes H, Bardhan KD, Stanghellini V. The construction of a new evaluative GERD questionnaire - methods and state of the art. *Digestion* 2004; **70**: 71-78
2 **Kulig M**, Nocon M, Vieth M, Leodolter A, Jaspersen D, Labenz J, Meyer-Sabellek W, Stolte M, Lind T, Malfertheiner P, Willich SN. Risk factors of gastroesophageal reflux disease: methodology and first epidemiological results of the ProGERD study. *J Clin Epidemiol* 2004; **57**: 580-589
3 **Agréus L**, Svärdsudd K, Talley NJ, Jones MP, Tibblin G. Natural history of gastroesophageal reflux disease and functional abdominal disorders: a population-based study. *Am J Gastroenterol* 2001; **96**: 2905-2914
4 **Lee KJ**, Kwon HC, Cheong JY, Cho SW. Demographic, clinical, and psychological characteristics of the heartburn groups classified using the Rome III criteria and factors associated with the responsiveness to proton pump inhibitors in the gastroesophageal reflux disease group. *Digestion* 2009; **79**: 131-136
5 **Drossman DA**. Rome III: The functional gastrointestinal disorders. 3 ed. McLean, VA: Degnon Associates Inc., 2006: 374-381
6 **Frank L**, Kleinman L, Ganoczy D, McQuaid K, Sloan S, Eggleston A, Tougas G, Farup C. Upper gastrointestinal symptoms in North America: prevalence and relationship to healthcare utilization and quality of life. *Dig Dis Sci* 2000; **45**: 809-818
7 **Dent J**, El-Serag HB, Wallander MA, Johansson S. Epidemiology of gastro-oesophageal reflux disease: a systematic review. *Gut* 2005; **54**: 710-717
8 **Olafsdottir LB**, Gudjonsson H, Jonsdottir HH, Thjodleifsson B. Natural history of functional dyspepsia: a 10-year population-based study. *Digestion* 2010; **81**: 53-61
9 **Olafsdottir LB**, Gudjonsson H, Jonsdottir HH, Thjodleifsson B. Stability of the irritable bowel syndrome and subgroups as measured by three diagnostic criteria - a 10-year follow-up study. *Aliment Pharmacol Ther* 2010; **32**: 670-680
10 **Olafsdóttir LB**, Gudjónsson H, Thjódleifsson B. [Epidemiological study of functional bowel disorders in Iceland]. *Laeknabladid* 2005; **91**: 329-333
11 **Dillman DA**. Mail and Telephon Surveys. The total design method. New York, NY: John Wiley & Sons, 1978
12 **Talley NJ**, Phillips SF, Wiltgen CM, Zinsmeister AR, Melton LJ 3rd. Assessment of functional gastrointestinal disease: the bowel disease questionnaire. *Mayo Clin Proc* 1990; **65**: 1456-1479
13 **Talley NJ**, Phillips SF, Melton J 3rd, Wiltgen C, Zinsmeister AR. A patient questionnaire to identify bowel disease. *Ann Intern Med* 1989; **111**: 671-674
14 **Halder SL**, Locke GR 3rd, Schleck CD, Zinsmeister AR, Melton LJ 3rd, Talley NJ. Natural history of functional gastrointestinal disorders: a 12-year longitudinal population-based study. *Gastroenterology* 2007; **133**: 799-807
15 **Attanasio V**, Andrasik F, Blanchard EB, Arena JG. Psychometric properties of the SUNYA revision of the Psychosomatic Symptom Checklist. *J Behav Med* 1984; **7**: 247-257
16 **Kuster E**, Ros E, Toledo-Pimentel V, Pujol A, Bordas JM, Grande L, Pera C. Predictive factors of the long term outcome in gastro-oesophageal reflux disease: six year follow up of 107 patients. *Gut* 1994; **35**: 8-14
17 **McDougall NI**, Johnston BT, Kee F, Collins JS, McFarland RJ, Love AH. Natural history of reflux oesophagitis: a 10 year follow up of its effect on patient symptomatology and quality of life. *Gut* 1996; **38**: 481-486
18 **Aro P**, Ronkainen J, Talley NJ, Storskrubb T, Bolling-Sternevald E, Agréus L. Body mass index and chronic unexplained gastrointestinal symptoms: an adult endoscopic population based study. *Gut* 2005; **54**: 1377-1383
19 **Festi D**, Scaioli E, Baldi F, Vestito A, Pasqui F, Di Biase AR, Colecchia A. Body weight, lifestyle, dietary habits and gastroesophageal reflux disease. *World J Gastroenterol* 2009; **15**: 1690-1701
20 **Nocon M**, Labenz J, Jaspersen D, Leodolter A, Meyer-Sabellek W, Stolte M, Vieth M, Lind T, Malfertheiner P, Willich SN. Nighttime heartburn in patients with gastroesophageal reflux disease under routine care. *Digestion* 2008; **77**: 69-72
21 **Farup C**, Kleinman L, Sloan S, Ganoczy D, Chee E, Lee C, Revicki D. The impact of nocturnal symptoms associated with gastroesophageal reflux disease on health-related quality of life. *Arch Intern Med* 2001; **161**: 45-52

S- Editor Sun H **L- Editor** Logan S **E- Editor** Lin YP

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.646

World J Gastroenterol 2011 February 7; 17(5): 646-650
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Percutaneous aspiration and drainage with adjuvant medical therapy for treatment of hepatic hydatid cysts

Mohammed I Yasawy, Abdelrahman E Mohammed, Sammak Bassam, Mohammed A Karawi, Sohail Shariq

Mohammed I Yasawy, Department of Internal Medicine, Dammam University Hospital, Al-Khobar 31952, Saudi Arabia
Abdelrahman E Mohammed, Consultant Gastroenterologist, Riyadh Military Hospital, Riyadh 11159, Saudi Arabia
Sammak Bassam, Department of Radiology, Riyadh Military Hospital, Riyadh 11159, Saudi Arabia
Mohammed A Karawi, Department of Gastroenterology, Riyadh Military Hospital, Riyadh 11159, Saudi Arabia
Sohail Shariq, Department of Internal Medicine, King Fahd Military Medical Complex, Dhahran 31932, Saudi Arabia
Author contributions: Yasawy MI was in charge of adjuvant medical therapy and wrote the paper; Bassam S was the Senior Radiologist in charge of the radiological intervention; Mohammed AE and Karawi MA were consultants who offered and provided the cases; Shariq S was responsible for the literature search and collected the relevant references.
Correspondence to: Dr. Mohd I Yasawy, Consultant Internist/Gastroenterologist, Associate Professor, Department of Internal Medicine, Dammam University Hospital, PO Box 40143, Al-Khobar 31952, Saudi Arabia. yasawy@hotmail.com
Telephone: +966-3-8966741 Fax: +966-3-8966741
Received: July 23, 2010 Revised: November 11, 2010
Accepted: November 18, 2010
Published online: February 7, 2011

Abstract

AIM: To determine the efficacy and success of percutaneous aspiration irrigation and reaspiration (PAIR) in the management of hepatic hydatidosis.

METHODS: Twenty-six patients with 32 hepatic hydatid cysts had PAIR. Twenty-two patients received at least 2 wk of drug therapy before the procedure was carried out to reduce the risk of recurrence from spillage during the procedure. The procedure was performed under local anesthesia with a 19-gauge 20 cm long needle, the cyst was punctured, cystic content (approximately 30 mL) was aspirated by a 12-14 F pigtail catheter and aspirated fluids were sent for analysis. Once the cyst was almost empty, two-thirds of the net amount of material aspirated was replaced by hypertonic saline and left in the cavity for about 30 min, with the catheter left in place for reaspiration of most of the fluid. When the amount of fluid drained was less than 10 mL per 24 h, the drainage catheter was removed.

RESULTS: All 32 cysts showed evidence of immediate collapse after completion of the procedure, and before discharge from hospital, ultrasound examination showed fluid reaccumulation in all cysts. Serial follow-up showed a progressive decrease in the size and change in the appearance of cysts. To confirm the sterility of these cystic cavities, seven cysts were reaspirated on average 3 mo after the procedure. Investigations revealed no viable scolices.

CONCLUSION: PAIR using hypertonic saline is very effective and safe with proper precautions.

Key words: Percutaneous aspiration irrigation and reaspiration; Hepatic hydatid cyst; Adjuvant medical therapy; Treatment outcome

Peer reviewer: Marco Vivarelli, MD, Assistant Professor, Department of Surgery and Transplantation, University of Bologna, S. Orsola Hospital, Bologna 40123, Italy

Yasawy MI, Mohammed AE, Bassam S, Karawi MA, Shariq S. Percutaneous aspiration and drainage with adjuvant medical therapy for treatment of hepatic hydatid cysts. *World J Gastroenterol* 2011; 17(5): 646-650 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/646.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.646

INTRODUCTION

Until 1980, surgery was the only method of treatment for hepatic hydatidosis. Despite improved surgical techniques

and use of scolicidal compounds, a high incidence of hydatid cyst recurrence and dissemination is still a major problem. Spillage is known to occur at surgery[1,2]. On the other hand, medical therapy is associated with side effects and it is effective only in some cases and need several courses to reach a response with albendazole alone[3], but the outcome is better with combined therapy[4]. In recent years, percutaneous drainage of hepatic hydatid cysts (HHC) has emerged as a minimally invasive, safe therapy, and a potential alternative to surgery. Different methods have been applied with variable success and healing rates[5-7].

In this study, we prospectively assessed the value of percutaneous drainage with adjuvant medical therapy in 26 patients with confirmed 32 HHC over an average follow-up period of 10 years.

The Armed Forces Hospital in Riyadh is a well-known tertiary center in the region and is a 1000-bed hospital with facilities for hepatobiliary and liver transplant services. Percutaneous aspiration irrigation and reaspiration (PAIR) was introduced into the hospital in 1993. Between 1985 and1992 albendazole was used alone in the management of hydatid cysts, and in 1993 praziquantel was added to albendazole as combination therapy.

Previously we reported our first patient with percutaneous drainage of a hydatid cyst of the liver in 1994[8], and our first patient with a lung hydatid cyst with pleural effusion in 1991[9].

MATERIALS AND METHODS

The study involved 26 patients (14 males and 12 females; age range 13-53 years) with 32 HHC (Table 1). Eleven patients were recently diagnosed with HHC and had no previous medical or surgical intervention. Seven patients had cyst recurrence following surgical excision. Eight patients had received long-term medical treatment with albendazole and praziquantel for an average of 24 mo with only a partial response of less than 30% reduction in size of the cysts. Nineteen patients had a solitary cyst and four others had multiple liver cysts. Three patients had extra hepatic disease in the lung (n = 2) and spleen (n = 1). Twenty-six cysts were located in the right lobe of the liver, five in the left, and one in the caudate lobe. All patients were complaining of right upper quadrant pain and/or pressure symptoms and had an abdominal ultrasound (US) examination. The average diameter was 10.2 cm (range, 5.5-18.5 cm). The diagnosis was confirmed by imaging modalities and positive serology (indirect hemagglutination titer > 1:128) in all patients. The cysts were classified by US according to the Gharbi *et al*[10] classification into Type I (n = 4), Type II (n = 8), Type III (n = 2) and Type IV (n = 18) (Table 2). Two of the Type IV cysts had a thin rim at their periphery. Four patients had abnormal liver function tests, 10 had elevated erythrocyte sedimentation rate, and six had high eosinophil count. The 26 patients and their results were evaluated by all relevant staff i.e. surgeons, gastroenterologist and senior radiologist. Management options i.e. a pharmacological approach, surgical intervention and PAIR were explained and all the above patients chose PAIR. Informed consent prior to the procedure was obtained from all patients. In 22 patients, albendazole 400 mg twice daily and praziquantel 50 mg/kg daily was given orally for at least 2 wk prior to and 4 wk after the procedure to all patients to reduce the risk of possible hydatid cyst fluid spillage and dissemination into the peritoneal cavity. The patient fasted overnight. The procedure was performed under heavy sedation (midazolam 5 mg iv and pethadine 50 mg im) with close monitoring to treat any potential complication including anaphylaxis. Under aseptic conditions, a Teflon sheath needle (19 gauge, 20 cm long; Meditech) was introduced percutaneously through the biopsy port of the 3.5 MHz probe into the cyst under US guidance (Aloka SSD 680). The puncture was made through thick normal liver tissue surrounding the cyst and whenever possible the right intercostal route was used to minimize the risk of hydatid fluid spillage into the peritoneum (Figure 1A). Once the cyst is punctured, a small amount of fluid (10-30 mL) was aspirated for cyst decompression followed by insertion of a 12F or 14F (van Sonnenberg sump drainage, Meditech) catheter, the use of such a large catheter was to prevent catheter clogging by membranes and daughter cysts during aspiration. Once the cyst was almost empty, injection of contrast medium under fluoroscopic control was performed to exclude cyst communication with the biliary system. Two-thirds of the aspirated material was replaced by hypertonic saline (23.4%) and left in the cavity for 20-30 min. The fluid was then reaspirated as much as possible and the catheter was left in place to drain by gravity. Immediately after aspiration, examination of fluid to identify scolices, hooklets, pieces of laminated membranes or daughter cysts were performed in the parasitology laboratory. After the procedure, the patients were closely observed for possible com-

Table 1 Location and distribution of the cysts (n)

Type of patient	Site of cyst	Multiple liver cyst or extrahepatic
Prolonged medical therapy with albendazole (8)	Right liver lobe (8) Left liver lobe (1)	Lung cyst (1)
Recovered post surgery (7)	Right liver lobe (4) Left liver lobe (1) Caudate lobe (1)	Multiple cysts (2)
New patients (11)	Right liver lobe (8) Left liver lobe (3)	Multiple cysts (4) Lung cysts (1) Spleen cysts (1)

Table 2 Gharbi classification

Cyst type	No. of cysts
I	4
II	8
III	2
IV	18

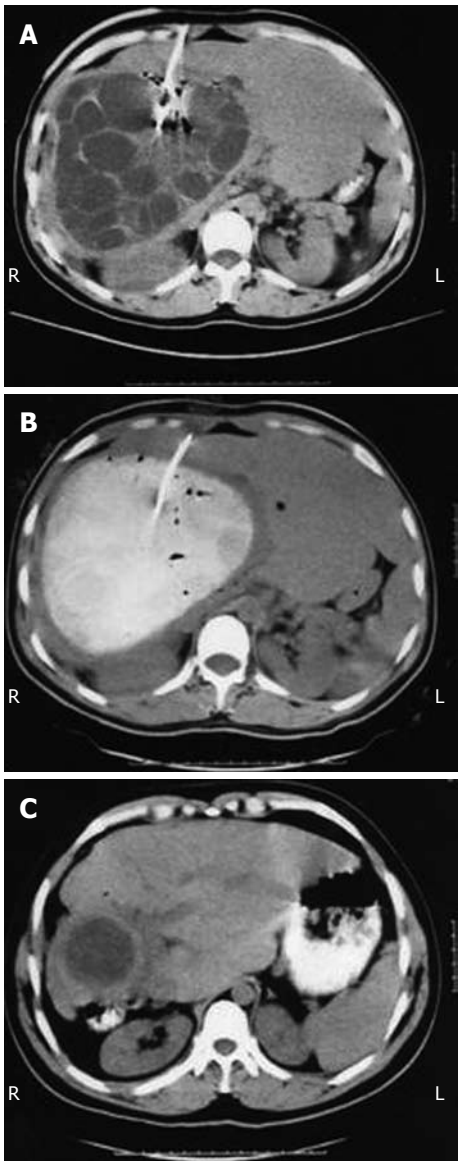


Figure 1 Ultrasound examination. A: Puncture of the hydatid cyst and insertion of a catheter; B: End of percutaneous aspiration irrigation and reaspiration procedure; C: Six months later, aspiration showed only granulation and necrotic tissue without any evidence of hydatidosis.

plications for 48 h. The drainage catheter was left in place for an average period of 3 d (range, 2-6 d) (Figure 1B) and removed when the amount of fluid drained was less than 10 mL/24 h. Follow-up examination in the form of clinical assessment, blood tests, serology and imaging examination with US and/or computed tomography (CT), were performed at 1, 2 and 3 mo after drainage for an average period of 6 mo (range, 2-11 mo), followed by 6-monthly blood tests and yearly imaging for an average period of 10 years.

RESULTS

All 32 cysts were successfully treated by PAIR, with relief of symptoms in all 26 patients with an average hospital stay of 6 d. There was collapse of all cysts immediately after completion of percutaneous drainage and removal of the drainage catheter. Before discharge from hospital, US examination showed fluid reaccumulation in all cysts within an average of 2 d after catheter withdrawal reaching an average size of 59% (range, 48%-74%) compared to the size of the cysts before drainage. Serial follow-up CT and US examination showed a progressive decrease in the size and change in the appearance of the cysts. Two patterns of healing were observed, the first was a predominantly cystic cavity with detached membranes. All the cystic cavities lost their rounded contour appearance suggestive of being under less tension. The second pattern was a predominantly solid mass.

Asymptomatic fluid reaccumulation following drainage and catheter removal happened in nearly all cysts with an average size of 59% (range, 48%-74%) compared to the predrainage cyst size. However, on regular follow-up examinations, a progressive decrease in the residual cavity with two distinctive healing patterns was observed. A cystic residual cavity with internal membranes was predominantly seen in patients with Type Ⅰ-Ⅱ cysts, and a solid mass was predominantly seen in patients with Type Ⅳ cysts. The complex large Type Ⅳ HHC with a predominantly solid component showed better results following drainage, with an overall reduction in size of 51.5% compared to 29% in patients with Type Ⅰ and Ⅱ cysts in whom the cysts had a predominantly fluid component.

To confirm the sterility of the residual cystic cavities, seven out of 32 cysts were reaspirated, three at an average of 3 mo after drainage, and four at an average of 6 mo after drainage. All reaspirated cyst cultures for microorganisms were negative, and microscopy revealed debris of hydatid membranes and hooklets in some cases but no viable scolices (Figure 1C). Serial follow-up serological examination showed a 2-fold elevation in the indirect hemagglutination titer following drainage in 18 patients compared to the titer level before drainage, and it remained elevated at an average follow-up period of 16 mo. No major complications developed during or after the procedure except for a mild anaphylactic reaction which responded very well to immediate treatment (Table 3). Two patients developed urticarial reactions 8 h following drainage, but responded well to antihistamines and steroids. Fever occurred in 10 patients but was mild and transient, and cultures of fluid from the drainage catheters were negative. Minimal right pleural effusion occurred in six patients. The liver cysts in these six patients were rela-

Table 3 Complications post aspiration

Complications	No. of patients
Anaphylactic reaction	1
Urticarial rash	2
Hypernatremia	2
Fever	10
Pleural effusion	6
Total	21

tively large and reached the right hemidiaphragm. However, the pleural effusion was small and resolved completely before the patients were discharged. Two patients developed transient hypernatremia and one patient showed an anaphylactic reaction during the procedure but responded to immediate management. No radiological evidence of reactivation of aspirated cysts was seen during the average of follow-up of 10 years.

DISCUSSION

Surgery is considered as the standard treatment for HHC. However, surgery is not without risks and there is a high incidence of dissemination, infection and recurrence of 2% to 25%, with morbidity of 0.5% to 4%[11-16]. Furthermore, surgery is not advisable in elderly patients with cardiac or pulmonary disease, nor in recurrent cases. Medical treatment alone in the form of mebendazole, and recently albendazole and praziquantel, have been used as an alternative therapy to surgery, but the success rate in terms of a reduction in size of HHC and the change in echotexture has been variable[17-19]. Another prospective randomized study compared albendazole, percutaneous drainage and both modalities combined. These studies showed that cyst size reduction was best achieved by the combined therapy when compared to albendazole or percutaneous drainage alone[20,21]. Percutaneous drainage of HHC was started by Mueller *et al*[22], and since then several series of percutaneous drainage have been published with no single fatality related to the procedure has been reported[23,24]. Reversible anaphylactic shocks, mild to severe allergic reactions, and pleural effusions have been reported in the recent literature[25,26], and any other complications were minor and infrequent. The reason for the pleural effusion is probably due to diaphragmatic irritation by the sudden collapse of the cyst following drainage and/or catheter manipulation during the procedure. However, pleural effusion was discovered incidentally during follow-up and in US examination, and was small and resolved completely before the patients were discharged. Fever was also a common complication, occurring in 10 patients, but was mild and transient, and cultures of fluid from the draining catheter were negative. Two patients developed an urticarial reaction hours following drainage but the patients responded well to antihistamines and steroid therapy. Only one patient developed an anaphylactic reaction which required immediate intubation and management, but there was a full recovery.

Drainage of complex Type Ⅳ cysts have been attempted before. Eighteen cysts in our series belonged to this group, including two patients with a partially calcified wall with multiple daughter cysts, in whom active disease was confirmed by serology and clinical assessment prior to the procedure and microscopy following drainage. It should be remembered that a calcified cyst does not mean always mean an inactive cyst. In our study, a 12F or 14F catheter was used to drain all types of HHC. Such large caliber catheters have not been used before in percutaneous drainage. We used a large catheter in order to minimize clogging of the catheters by membranes and daughter cysts, and to ensure that all the cyst cavities were completely evacuated, though finer catheters might be safer, despite frequent clogging. Future studies will clarify this and many other issues. Follow-up indirect hemagglutination tests were performed in all patients. There was slight elevation of the indirect hemagglutination titer in 18 patients after the procedure and it remained elevated in comparison to the predrainage value during an average follow-up period of 16 mo. This observation has been reported by others[27,28], and we believe that a longer follow-up period is needed for the indirect hemagglutination titer to start decreasing.

Our results have shown that percutaneous drainage of all types of HHC with adjuvant medical therapy is minimally invasive, safe and effective therapy with proper precautions. It can be used as an alternative to surgery, and in some cases is superior to surgery. Further evaluation by means of organized multicenter studies and long-term evaluation will answer questions regarding the use of a larger caliber or fine catheter, types of sedation or anesthesia, duration and requirement of adjuvant medical therapy, possible recurrence and many other unanswered questions.

COMMENTS

Background

Human hydatid disease (Echinococcosis) was recognized by Hippocrates over 2000 years ago. Al Razi and Avicenna made references in 900 AD and 11 200 respectively and was described as liver cysts filled with water. However, it is still seen all over the world and is endemic and common in many countries i.e. Africa, central Asia, the Mediterranean, South America and Middle East and remains a problem for the World Health Organization. It is a slow growing cyst and may produce no symptoms for up to 10 years. In the most common form of the disease (Echinococcosis granulosis) dogs are the definitive host. Humans and sheep are the intermediate victims. Therefore, human hygiene and dogs' sanitation (removing the tapeworm from the dog) are essential issues in the prevention of this disease.

Research frontiers

Any organ and any part of the body could be affected but the most common sites are the liver and lungs. Over the recent decades substantial improvement has been made in the diagnosis and management of hydatid disease, through diagnostic tools such as imaging procedures including ultrasound (US), computed tomography (CT), magnetic resonance imaging and endoscopic retrograde cholangiopancreatography.

Innovations and breakthroughs

Concerning treatment, until recently the only definitive treatment for hydatid disease had been surgery. Different surgical techniques and procedures have been carried out and even in some cases, a liver transplant has been required. Advances in drug therapy has been influenced by the introduction of albendazole and accelerated by addition of praziquantel, but this requires a long period of treatment i.e. up to a year or more, and is not effective for everyone.

Applications

Percutaneous aspiration irrigation and reaspiration (PAIR) under direct US or CT guidance is a real achievement in the management of hydatid disease. The procedure was associated with reversible complications, no mortality, very short hospitalization and minimal cost. All 32 cysts showed evidence of immediate collapse after completion of the procedure. Serial follow-up showed progressive decrease in the size and change in the appearance of the cysts. At 10 years follow-up, the longest follow-up in the literature, there was no evidence of recurrence. Therefore, the authors confirm and believe that PAIR using hypertonic saline with adjuvant medical therapy has encouraging results and, with appropriate precautions, is very safe.

Peer review

The authors retrospectively analyzed a series of 26 patients whose hydatid liver cysts were treated with percutaneous aspiration and hypertonic solution injection. Albendazole was given prophylactically and after the procedure. The study might provide some confirmation of the efficacy of a non-surgical approach to the treatment of liver hydatidosis.

REFERENCES

1 **Acunas B**, Rozanes I, Celik L, Minareci O, Acunas G, Alper A, Ariogul O, Gökmen E. Purely cystic hydatid disease of the liver: treatment with percutaneous aspiration and injection of hypertonic saline. *Radiology* 1992; **182**: 541-543

2 **Giorgio A**, Tarantino L, Francica G, Mariniello N, Aloisio T, Soscia E, Pierri G. Unilocular hydatid liver cysts: treatment with US-guided, double percutaneous aspiration and alcohol injection. *Radiology* 1992; **184**: 705-710

3 **Yasawy MI**, Mohamed AR, Al-Karawi MA. Albendazole in hydatid disease: Results in 22 patients. *Ann Saudi Med* 1992; **12**: 152-156

4 **Mohamed AE**, Yasawy MI, Al Karawi MA. Combined albendazole and praziquantel versus albendazole alone in the treatment of hydatid disease. *Hepatogastroenterology* 1998; **45**: 1690-1694

5 **Gargouri M**, Ben Amor N, Ben Chehida F, Hammou A, Gharbi HA, Ben Cheikh M, Kchouk H, Ayachi K, Golvan JY. Percutaneous treatment of hydatid cysts (Echinococcus granulosus). *Cardiovasc Intervent Radiol* 1990; **13**: 169-173

6 **Filice C**, Pirola F, Brunetti E, Dughetti S, Strosselli M, Foglieni CS. A new therapeutic approach for hydatid liver cysts. Aspiration and alcohol injection under sonographic guidance. *Gastroenterology* 1990; **98**: 1366-1368

7 **Khuroo MS**, Zargar SA, Mahajan R. Echinococcus granulosus cysts in the liver: management with percutaneous drainage. *Radiology* 1991; **180**: 141-145

8 **Al Karawi MA**, Ossa Eidyen, Mohamed Y, et al. Percutaneous management of liver hydatid cyst causing obstructive jaundice. *Saudi Med J* 1994; **15**: 389-391

9 **al Karawi MA**, Mohamed AR, el Tayeb BO, Yasawy MI. Unintentional percutaneous aspiration of a pleural hydatid cyst. *Thorax* 1991; **46**: 859-860

10 **Gharbi HA**, Hassine W, Brauner MW, Dupuch K. Ultrasound examination of the hydatic liver. *Radiology* 1981; **139**: 459-463

11 **Balasegaram M**, Kong LF. Surgical treatment ofhydatid disease of the liver. *Trop Gastroenterol* 1982, **3**: 194-200

12 **Karavias DD**, Vagianos CE, Bouboulis N, Rathosis S, Androulakis J. Improved techniques in the surgical treatment of hepatic hydatidosis. *Surg Gynecol Obstet* 1992; **174**: 176-180

13 **Sayek I**, Yalin R, Sanaç Y. Surgical treatment of hydatid disease of the liver. *Arch Surg* 1980; **115**: 847-850

14 **Ekrami Y**. Surgical treatment of hydatid disease of the liver. *Arch Surg* 1976; **111**: 1350-1352

15 **Ammori BJ**, Jenkins BL, Lim PC, Prasad KR, Pollard SG, Lodge JP. Surgical strategy for cystic diseases of the liver in a western hepatobiliary center. *World J Surg* 2002; **26**: 462-469

16 **Langer JC**, Rose DB, Keystone JS, Taylor BR, Langer B. Diagnosis and management of hydatid disease of the liver. A 15-year North American experience. *Ann Surg* 1984; **199**: 412-417

17 **Teggi A**, Di Vico B, Farinelli S, Lastilla M, Traditi F, De Rosa F. Trattamento medico della idatidosi umana: La nostra esperienza con derivati imidozolici. In: De Rosa F, editor. Proceedings of the 1st International Congress of Imaging Diagnosis of Infectious Diseases. Rome: Monteporzio Cantone, 1990: 318-324

18 **al Karawi MA**, el-Shiekh Mohamed AR, Yasawy MI. Advances in diagnosis and management of hydatid disease. *Hepatogastroenterology* 1990; **37**: 327-331

19 **Yasawy MI**, Alkarawi MA, Mohammed AR. Prospects in medical management of Echinococcus granulosus. *Hepatogastroenterology* 2001; 48: 1467-1470

20 **Khuroo MS**, Dar MY, Yattoo GN, Zargar SA, Javaid G, Khan BA, Boda MI. Percutaneous drainage versus albendazole therapy in hepatic hydatidosis: a prospective, randomized study. *Gastroenterology* 1993; **104**: 1452-1459

21 **Smego RA Jr**, Bhatti S, Khaliq AA, Beg MA. Percutaneous aspiration-injection-reaspiration drainage plus albendazole or mebendazole for hepatic cystic echinococcosis: a meta-analysis. *Clin Infect Dis* 2003; **37**: 1073-1083

22 **Mueller PR**, Dawson SL, Ferrucci JT Jr, Nardi GL. Hepatic echinococcal cyst: successful percutaneous drainage. *Radiology* 1985; **155**: 627-628

23 **Khuroo MS**. Percutaneous drainage of hydatid cysts. *N Engl J Med* 1998; 392: 391-393

24 **Filice C**, Brunetti E, Bruno R, Crippa FG. Percutaneous drainage of echinococcal cysts (PAIR--puncture, aspiration, injection, reaspiration): results of a worldwide survey for assessment of its safety and efficacy. WHO-Informal Working Group on Echinococcosis-Pair Network. *Gut* 2000; **47**: 156-157

25 **Aygün E**, Sahin M, Odev K, Vatansev C, Aksoy F, Paksoy Y, Kartal A, Karahan O. The management of liver hydatid cysts by percutaneous drainage. *Can J Surg* 2001; **44**: 203-209

26 **Khuroo MS**. Hydatid disease: current status and recent advances. *Ann Saudi Med* 2002; **22**: 56-64

27 **Filice C**, Di Perri G, Strosselli M, Pirola F, Brunetti E, Dughetti S, Concia E. Parasitologic findings in percutaneous drainage of human hydatid liver cysts. *J Infect Dis* 1990; **161**: 1290-1295

28 **Khuroo MS**, Wani NA, Javid G, Khan BA, Yattoo GN, Shah AH, Jeelani SG. Percutaneous drainage compared with surgery for hepatic hydatid cysts. *N Engl J Med* 1997; **337**: 881-887

S- Editor Sun H **L- Editor** Cant MR **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.651

World J Gastroenterol 2011 February 7; 17(5): 651-656
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

A population-based case-crossover study of polyethylene glycol use and acute renal failure risk in the elderly

Nam-Kyong Choi, Yoosoo Chang, Sun-Young Jung, Yu-Kyong Choi, Joongyub Lee, Jin-Ho Lee, Ju-Young Kim, Byung-Joo Park

Nam-Kyong Choi, Yoosoo Chang, Ju-Young Kim, Byung-Joo Park, Department of Preventive Medicine, Seoul National University College of Medicine, Seoul 110-799, South Korea
Nam-Kyong Choi, Byung-Joo Park, Medical Research Center, Seoul National University, Seoul 110-799, South Korea
Yoosoo Chang, Department of Occupational Medicine, Health Screening Center, Kangbuk Samsung Hospital, Sungkyunkwan University College of Medicine, Seoul 110-746, South Korea
Sun-Young Jung, National Evidence-Based Healthcare Collaborating Agency, Seoul 110-450, South Korea
Yu-Kyong Choi, Department of Internal Medicine, Gumi Hospital, Gumi 730-909, South Korea
Joongyub Lee, Byung-Joo Park, Medical Research Collaborating Center, Seoul National University Hospital, Seoul National University College of Medicine, Seoul 110-799, South Korea
Jin-Ho Lee, Department of Internal Medicine, Dongguk University Ilsan Hospital, School of Medicine, Dongguk University, Goyang 410-733, South Korea
Ju-Young Kim, Department of Family Medicine, Health Promotion Center, Seoul National University Bundang Hospital, Bundang 463-707, South Korea
Author contributions: Park BJ suggested the study; Choi NK, Chang Y and Jung SY planned and designed the study; Choi NK analyzed the data; Choi NK, Chang Y, Jung SY, Choi YK, Lee J, Lee JH, Kim JY and Park BJ interpreted the results; Choi NK drafted the manuscript; Chang Y, Jung SY, Choi YK, Lee J, Lee JH and Kim JY revised the manuscript; all authors contributed to modifying the manuscript and the final editing of the paper; Park BJ is the guarantor.
Correspondence to: Byung-Joo Park, MD, MPH, PhD, Professor and Chairperson, Department of Preventive Medicine, Seoul National University College of Medicine, 103 Daehakno, Chongno-Gu, Seoul 110-799, South Korea. bjpark@snu.ac.kr
Telephone: +82-2-7408325 Fax: +82-2-7474830
Received: October 5, 2010 Revised: November 3, 2010
Accepted: November 10, 2010
Published online: February 7, 2011

Abstract

AIM: To evaluate the possibility of an association between polyethylene glycol (PEG) and acute renal failure (ARF) in elderly patients using a health insurance claims database.

METHODS: We conducted a population-based case-crossover study using information obtained from Korean Health Insurance Review and Assessment Service (HIRA) claims from January 1, 2005 to December 31, 2005 (Seoul, Korea). The study population consisted of elderly patients who received PEG prior to experiencing their first ARF-related hospitalization from April 1, 2005 to December 31, 2005. For each patient, one case and two control periods were matched. PEG use in a 2- or 4-wk window period prior to hospitalization for ARF was compared with PEG use in two earlier 2- or 4-wk control window periods. Conditional logistic regression analysis was used to estimate odds ratios (ORs) and 95% CI, adjusting for concomitant uses of diuretics, angiotensin converting enzyme inhibitors, non-steroidal anti-inflammatory drugs, antibiotics, anti-cancer drugs, and contrast media.

RESULTS: Within the HIRA database which contained 1093262 elderly patients, 1156 hospitalized ARF cases were identified. Among these cases, PEG was prescribed to 17 (1.5%) patients before hospitalization. The adjusted ORs when applying the 2- and 4-wk window periods were 0.4 (95% CI: 0.03-5.24) and 2.1 (95% CI: 0.16-27.78), respectively.

CONCLUSION: No increased risk of ARF was found in elderly PEG users. However, based on the limited number of study subjects, further analysis should be performed to confirm these results.

Key words: Polyethylene glycol; Acute renal failure; Adverse drug reaction; Health insurance claims database; Case-crossover

Peer reviewer: Venkatesh Shanmugam, MBBS, MS (Gen. Surg.), Dip.NB (Gen. Surg.), FRCS (Glasg.), MD, Specialist Registrar (Trent Deanery), Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3NE, United Kingdom

Choi NK, Chang Y, Jung SY, Choi YK, Lee J, Lee JH, Kim JY, Park BJ. A population-based case-crossover study of polyethylene glycol use and acute renal failure risk in the elderly. *World J Gastroenterol* 2011; 17(5): 651-656 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/651.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.651

INTRODUCTION

Colonoscopy is a common diagnostic or therapeutic procedure. As early detection of colorectal cancer can significantly decrease mortality[1], regular screening is recommended for those aged 50 years or over[2]. The success and accuracy of colonoscopy are largely dependent on appropriate cleansing of the colon[3]. Ideally, a colon preparation would provide safe and rapid cleansing with little or no discomfort to patients[4]. Currently, polyethylene glycol (PEG) bowel preparation and oral sodium phosphate (NaP) are predominantly used as bowel cleansing agents before colonoscopy based on the fact that they are effective and generally well tolerated[5-7]. PEG is a non-digestible, non-absorbable, osmotically-balanced laxative lavage solution that does not cause physiologic changes and can even be administered to patients in poor general condition[8-12].

However, several recent studies have raised concerns about the safety of oral NaP preparations due to their reported association with an increased risk of serious electrolyte disturbances and renal failure[13]. Conversely, although a large volume of PEG produces discomfort to the examinee, it is considered to be a relatively safe agent. Therefore, there has been a tendency to prescribe PEG more than NaP for renally impaired or elderly patients[14]. Recently, several studies have shown that the risk of renal impairment is similar between PEG and NaP users[15,16]. In addition, a case report has raised a possible association between the use of PEG and acute renal failure (ARF)[17]. The patient in that case was a 55-year-old male without pre-existing renal disease who visited the emergency room with severe abdominal pain and frequent diarrhea after ingesting PEG 2 h earlier as pre-treatment for a follow-up colonoscopy. He was diagnosed as having prerenal ARF and improved after intensive fluid administration. Also, a recent cohort study revealed that following colonoscopy, those over 65 years of age without preexisting renal disease were at risk for impaired renal function[16]. Unfortunately, most of the evidence for PEG risk to date has been based on a limited number of hospital patients or the case report mentioned.

There have been no quantitative epidemiological studies analyzing a relationship between PEG preparation and the development of ARF using a national health insurance database. Therefore, this case-crossover study was performed to evaluate the risk of ARF following the use of PEG among elderly patients using information gathered from a Korean national health insurance database.

MATERIALS AND METHODS

Data source

We used the Korean Health Insurance Review and Assessment Service (HIRA) database that contains information on all claims including prescribed medications for approximately all 50 million Koreans[18]. We obtained claims data for elderly patients (age 65 years or older) that had been submitted by healthcare providers based in Seoul between January 1, 2005 and December 31, 2005. Seoul is the capital and largest city of South Korea. A megacity with a population of over 12 million, it is one of the largest cities in the world. The study database contained information on 1 093 262 elderly patients with 11 842 586 prescriptions[19]. This study was exempted from review by the Institutional Review Board of the Seoul National University College of Medicine/Seoul National University Hospital because researchers only accessed a de-identified database which included age, gender, diagnosis, and a list of prescribed drugs.

Study design

We employed a case-crossover approach, using cases at previous time points as their own controls, thereby eliminating time-invariant confounders between subjects through within-subject difference comparisons[20]. In this design, only patients experiencing an event of interest were included and their exposures were measured during case- and control-time windows. Accordingly, the number having medication available in the case period (which is the period immediately before the event of interest) is compared with the number having medication available in the control period (which is a period prior to but of the same length as the case period)[21]. Thus this design eliminates the effects of many potential confounders by keeping characteristics such as age, gender, socioeconomic status, and comorbidity fixed[21,22].

Study subjects

The study population consisted of patients 65 years of age or older who received PEG prior to their first ARF-related hospitalization (ICD-10 code: N17) from April 1, 2005 to December 31, 2005. The index date was defined as the first hospital admission date for ARF. To identify initial ARF admission patients, we excluded those with pre-existing ARF during the preceding 3 mo, from January 1, 2005 to March 30, 2005.

Case and control periods

For each patient, one case and two control periods were matched to increase the study power and improve the precision of the estimates[23]. The time windows, of 2 and 4 wk, were used to determine the periods over which as-

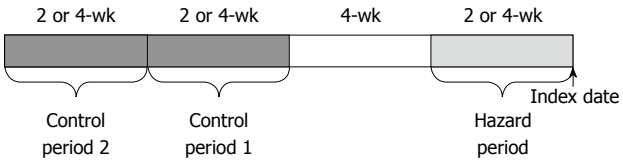


Figure 1 Definition of hazard and control periods in this case-crossover study. Hazard period was defined as the 2- or 4-wk window before the index date. A 4-wk washout period was chosen between the end of the hazard period and the start of the control period. Two consecutive control periods were defined as 2- or 4-wk windows.

sessment of drug exposure occurred. We defined the case period as a 2- or 4-wk window prior to the index date. A 4-wk washout period was chosen between the end of the case period and the start of the control period. Two consecutive control periods were also defined as 2- and 4-wk windows (Figure 1). Accordingly, for each patient, PEG prescription in each window period prior to hospitalization for ARF was compared with PEG prescription in two earlier control-window periods.

Statistical analyses

Descriptive statistics were used to illustrate the characteristics of the first ARF-hospitalized patients by age and gender. For the study population, the distribution of diagnoses on the day of PEG prescription was analyzed. Diagnoses were constructed from records made at the time of PEG prescription and grouped as colorectal cancer (ICD-10 codes: C18-C21, D12), gastric or duodenal ulcer, gastritis or duodenitis, intestinal disorders (K25-K59), renal disease (N03-N20), fibrosis and cirrhosis of the liver (K74), liver cancer (C22), and pancreatic cancer (C25). Conditional logistic regression analysis was used to estimate odds ratios (ORs) and 95% CI. The date of PEG exposure was regarded as the date of PEG prescription in the database. Use of concomitant medications that could induce ARF[24,25] was included and evaluated in the model. Concomitant drugs included diuretics, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), non-steroidal anti-inflammatory drugs (NSAIDs), aminoglycosides, β-lactams, sulfonamides, anti-viral agents, antimycotics, anti-cancer drugs, and contrast media. We assessed PEG prescriptions and uses of concomitant drugs included in the case- or control-window periods. Exposure to PEG and other concomitant drugs was considered as a dichotomous variable in the model (exposed at least once during each specific time window: yes or no). Statistical analysis was performed using the SAS statistical application program (Release 9.1, SAS Institute Inc., Cary, NC, USA).

RESULTS

The total number of elderly patients who had at least one claim for any healthcare service in Seoul between January 1, 2005 and December 31, 2005 was 1 093 262. Their mean age was 71.0 ± 6.1 years and 59.9% were female.

Table 1 Characteristics of elderly patients who had at least one claim for any healthcare service in Seoul between January 1, 2005 and December 31, 2005 and those who were hospitalized for acute renal failure among the population *n* (%)

	Total of elderly patients	Patients hospitalized for ARF
Age (yr)		
mean ± SD	72.0 ± 6.1	75.9 ± 7.3
65-69	472 614 (43.2)	270 (23.4)
70-74	297 348 (27.2)	260 (22.5)
75-79	179 562 (16.4)	272 (23.5)
80-84	96 131 (8.8)	197 (17)
⩾ 85	47 607 (4.4)	157 (13.6)
Sex		
Male	438 795 (40.1)	587 (50.8)
Female	654 497 (59.9)	569 (49.2)
Total	1 093 262 (100)	1156 (100)

ARF: Acute renal failure.

Table 2 Characteristics of elderly patients with polyethylene glycol prescription prior to hospitalization for acute renal failure

	n (%)
Age (yr)	
mean ± SD	70.6 ± 4.6
65-69	8 (47.1)
70-74	5 (29.4)
75-79	3 (17.6)
80-84	1 (5.9)
Sex	
Male	14 (82.4)
Female	3 (17.6)
Diagnoses on the day of PEG prescription	
Colorectal cancer	6 (35.3)
Gastric or duodenal ulcer, gastritis or duodenitis, intestinal disorders	6 (35.3)
Renal disease	3 (17.6)
Fibrosis and cirrhosis of liver	2 (11.8)
Liver cancer	1 (5.9)
Pancreatic cancer	1 (5.9)
Total	17 (100)

PEG: Polyethylene glycol.

Among them, we identified 1156 patients hospitalized for ARF with a mean age of 75.9 ± 7.3 years. Patient sex was split relatively equally with males accounting for 50.8% of cases (Table 1). Among the cases of ARF, 17 (1.5%) had received PEG prior to their hospitalization. Their mean (SD) age was 70.6 (4.6) years and 82.4% (14 cases) of them were male. The most frequent diagnoses on the day of PEG prescription were colorectal cancer (35.3%), gastric or duodenal ulcer, gastritis or duodenitis, or intestinal disorders (35.3%) (Table 2). Using the 2- and 4-wk windows, the crude ORs for ARF were 0.7 (95% CI: 0.07-6.41) and 1.3 (95% CI: 0.22-7.99), respectively. After adjusting for the use of concomitant drugs the adjusted ORs applying the 2- and 4-wk windows were 0.4 (95% CI: 0.03-5.24) and 2.1 (95% CI: 0.16-27.78), respectively (Table 3).

Table 3 Association between polyethylene glycol and the risk of acute renal failure with respect to time-window periods by matched ratio of case and control period

Time-window period	Case period (n = 17)	Control period (n = 34)	Crude OR (95% CI)[1]	Adjusted OR (95% CI)[2]
2 wk				
PEG non-users	16	31	1	1
PEG users	1	3	0.7 (0.07-6.41)	0.4 (0.03-5.24)
4 wk				
PEG non-users	15	31	1	1
PEG users	2	3	1.3 (0.22-7.99)	2.1 (0.16-27.78)

[1]Calculated by conditional logistic regression; [2]Calculated by conditional logistic regression adjusted for use of nephrotoxic drugs (diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, non-steroidal anti-inflammatory drugs, aminoglycoside, β-lactams, anti-viral agents, antimycotics, anti-cancer drugs, and contrast media). PEG: Polyethylene glycol; OR: Odds ratio.

DISCUSSION

This population-based case-crossover study showed that PEG did not increase the risk of ARF in elderly patients. When several different time-window periods were applied, the use of PEG was not associated with ARF risk. This study supports findings of previous reports which have suggested that PEG does not increase the risk of ARF[12,26-29]. Known PEG-associated common adverse effects include volume-related symptoms of abdominal fullness, nausea, and bloating, with minimal discomfort[26]. Also, previous results of a prospective, randomized, multicenter, controlled trial comparing PEG plus ascorbic acid to NaP solution in 352 patients had shown that PEG use was associated with fewer adverse events and no clinically relevant changes in laboratory values[27]. In a study addressing age, 557 patients were stratified into two groups, > 60 years and ≤ 60 years of age, all of whom received either PEG or a cathartic preparation for colonoscopy, barium enema, or elective colon surgery[28]. Patients in the older age group reported significantly fewer cramps ($P < 0.05$) and no differences in overall discomfort compared to their younger PEG counterparts, confirming the generally accepted understanding that PEG is safe and tolerable[12,29].

In our study, we excluded patients admitted with a diagnosis of ARF 3 mo before the study starting date; therefore we could infer that PEG did not increase the risk of ARF among patients without recent worsening of renal function. However, because decreased renal function is extremely common in elderly persons[30], the study population might have asymptomatically decreased renal function. Further studies should be performed to examine the possibility that PEG could worsen existing renal impairment and hasten its progression to ARF. Moreover, although the study results did not show a statistically significant risk for ARF in PEG users, it may be desirable to ensure adequate hydration before, during, and after PEG bowel preparation and provide renal function monitoring before and after colonoscopy in high risk patients.

We applied a case-crossover design optimal for evaluating short-term effects after transient exposures, particularly by removing time-invariant between-subject confounding factors[31]. Results of clinical trials are sometimes difficult to generalize to clinical practice and rarely detect adverse event incidents because they include only small numbers of highly selected patients. Also, the estimates of adverse drug effects derived from observational studies are vulnerable to unmeasured or unknown confounding factors, associated with both the exposure and the outcome[20]. Actually, a previous cohort study which aimed to compare the risk of renal dysfunction related to the use of PEG and NaP mentioned that its results could be affected by potential selection bias[11]. The cohort study was conducted using clinical records of patients undergoing colonoscopy in one hospital. Accordingly, the baseline patient characteristics might have affected which drugs were prescribed and the two groups were not comparable[32,33]. In the present study, using the case-crossover technique, only cases with incident renal failure were considered and their PEG exposures were compared during two different time-windows. Since inherent confounders remain invariant over time, the case-crossover design which is optimal for transient exposures with short-term effects has an advantage in that it can minimize between-subject confounding and assure an optimal sample size[31].

This study has several strengths. Firstly, we evaluated patients from an entire target population of over one million elderly derived from the national health insurance claims database in Seoul, South Korea, rather than use a sample population. Therefore, our results reflect unbiased real world conditions. Nevertheless, we identified only 17 cases of ARF following PEG use. This means that there is little possibility the PEG would increase the risk of ARF. Secondly, this study included elderly patients who are not usually involved in clinical trials or safety studies, but are at high risk of renal failure related to bowel preparations. Thirdly, although we controlled unmeasured confounders which were stable over time by using a case-crossover design, we further adjusted for other medication use which could affect the development of ARF such as diuretics, ACE inhibitors, ARBs, β-blockers, NSAIDs, aminoglycosides, β-lactams, anti-viral agents, antimycotics, anti-cancer drugs, and contrast media[24,25].

However, our results should also be interpreted with caution. Although ARF is generally defined as an abrupt and sustained decline in the glomerular filtration rate (GFR)[34], we defined incident cases of ARF as hospitalization with diagnosis of ARF in the HIRA database. Since the database did not contain laboratory test results such as GFR, a validation study was used to compare the diagnosis derived from the HIRA database with the actual diagnosis in the patients' medical records. The overall positive predictive value of the diagnoses was 81.8% in cases of hospitalized patients[35]. Also, ARF as defined in this study only included symptomatic and serious events requiring hospitalization. We defined the date of PEG exposure as the prescription date of PEG; however, there could be a

difference of several days or more between the date of prescription and actual administration. Nonetheless, the date of PEG administration followed the prescription, so the period from the actual PEG exposure date to ARF hospitalization might in fact be shorter than calculated.

In this study, PEG was found not to be associated with an increased risk of ARF in elderly patients. However, further studies should be conducted to confirm an association or lack thereof.

COMMENTS

Background

Polyethylene glycol (PEG), a commonly used solution for colonoscopy bowel preparation, is regarded as effective and tolerable. Recent reports have cited an increased risk of acute renal failure (ARF) in the elderly. Until now there have been no quantitative population-based epidemiological studies analyzing a possible relationship between PEG and ARF.

Research frontiers

Colonoscopy is a common diagnostic or therapeutic procedure. The success and accuracy of colonoscopy are largely dependent on appropriate cleansing of the colon. Ideally, a colon preparation would provide safe and rapid cleansing with little or no discomfort to patients. Currently, PEG bowel preparation and oral sodium phosphate (NaP) are predominantly used as bowel cleansing agents before colonoscopy based on the fact that they are effective and generally well tolerated.

Innovations and breakthroughs

Several recent studies have raised concerns about the safety of oral NaP preparations due to their reported association with an increased risk of serious electrolyte disturbances and renal failure. There have been no quantitative epidemiological studies analyzing a relationship between PEG preparation and the development of ARF using a national health insurance database. Therefore, this case-crossover study was performed to evaluate the risk of ARF following the use of PEG among elderly patients using information gathered from the Korean national health insurance database.

Applications

No increased risk of ARF was found in elderly PEG users. However, based on the number of study subjects, further analysis should be performed to confirm an association or lack thereof.

Peer review

This is very well constructed paper regarding the possibility of an association between PEG and ARF in elderly patients.

REFERENCES

1 **Rabeneck L**, Paszat LF, Saskin R, Stukel TA. Association between colonoscopy rates and colorectal cancer mortality. *Am J Gastroenterol* 2010; **105**: 1627-1632

2 **Rex DK**, Johnson DA, Lieberman DA, Burt RW, Sonnenberg A. Colorectal cancer prevention 2000: screening recommendations of the American College of Gastroenterology. American College of Gastroenterology. *Am J Gastroenterol* 2000; **95**: 868-877

3 **Occhipinti KE**, Di Palma JA. How to choose the best preparation for colonoscopy. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 279-286

4 **Toledo TK**, DiPalma JA. Review article: colon cleansing preparation for gastrointestinal procedures. *Aliment Pharmacol Ther* 2001; **15**: 605-611

5 **Mathus-Vliegen EM**, Kemble UM. A prospective randomized blinded comparison of sodium phosphate and polyethylene glycol-electrolyte solution for safe bowel cleansing. *Aliment Pharmacol Ther* 2006; **23**: 543-552

6 **Ell C**, Fischbach W, Keller R, Dehe M, Mayer G, Schneider B, Albrecht U, Schuette W. A randomized, blinded, prospective trial to compare the safety and efficacy of three bowel-cleansing solutions for colonoscopy (HSG-01*). *Endoscopy* 2003; **35**: 300-304

7 **Seinelä L**, Pehkonen E, Laasanen T, Ahvenainen J. Bowel preparation for colonoscopy in very old patients: a randomized prospective trial comparing oral sodium phosphate and polyethylene glycol electrolyte lavage solution. *Scand J Gastroenterol* 2003; **38**: 216-220

8 **Davis GR**, Santa Ana CA, Morawski SG, Fordtran JS. Development of a lavage solution associated with minimal water and electrolyte absorption or secretion. *Gastroenterology* 1980; **78**: 991-995

9 **Beck DE**, Harford FJ, DiPalma JA, Brady CE 3rd. Bowel cleansing with polyethylene glycol electrolyte lavage solution. *South Med J* 1985; **78**: 1414-1416

10 **DiPiro JT**, Michael KA, Clark BA, Dickson P, Vallner JJ, Bowden TA Jr, Tedesco FJ. Absorption of polyethylene glycol after administration of a PEG-electrolyte lavage solution. *Clin Pharm* 1986; **5**: 153-155

11 **Nelson DB**, Barkun AN, Block KP, Burdick JS, Ginsberg GG, Greenwald DA, Kelsey PB, Nakao NL, Slivka A, Smith P, Vakil N. Technology Status Evaluation report. Colonoscopy preparations. May 2001. *Gastrointest Endosc* 2001; **54**: 829-832

12 **Dykes C**, Cash BD. Key safety issues of bowel preparations for colonoscopy and importance of adequate hydration. *Gastroenterol Nurs* 2008; **31**: 30-35; quiz 36-37

13 **Lien YH**. Is bowel preparation before colonoscopy a risky business for the kidney? *Nat Clin Pract Nephrol* 2008; **4**: 606-614

14 **Ho JM**, Cavalcanti RB. A shocking bowel preparation: severe electrolyte disturbances after polyethylene glycol-based bowel preparation. *J Am Geriatr Soc* 2009; **57**: 1729-1730

15 **Seol DC**, Hong SN, Kim JH, Sung IK, Park HS, Lee JH, Shim CS. Change in renal function after sodium phosphate preparation for screening colonoscopy. *World J Gastroenterol* 2010; **16**: 2010-2016

16 **Russmann S**, Lamerato L, Marfatia A, Motsko SP, Pezzullo JC, Olds G, Jones JK. Risk of impaired renal function after colonoscopy: a cohort study in patients receiving either oral sodium phosphate or polyethylene glycol. *Am J Gastroenterol* 2007; **102**: 2655-2663

17 **Chun YJ**, Park MK, Kim JS, Kim HK, Cho YS, Chae HS, Han SW, Choi KY, Lee HK. Acute renal failure caused by oral polyethylene glycol ingestion. *Korean J Gastrointest Endosc* 2007; **34**: 161-163

18 **Choi NK**, Hahn S, Park BJ. Increase in mortality rate following coprescription of cisapride and contraindicated drugs. *Ann Pharmacother* 2007; **41**: 667-673

19 **Choi NK**, Chang Y, Choi YK, Hahn S, Park BJ. Signal detection of rosuvastatin compared to other statins: data-mining study using national health insurance claims database. *Pharmacoepidemiol Drug Saf* 2010; **19**: 238-246

20 **Delaney JA**, Suissa S. The case-crossover study design in pharmacoepidemiology. *Stat Methods Med Res* 2009; **18**: 53-65

21 **Gislason GH**, Jacobsen S, Rasmussen JN, Rasmussen S, Buch P, Friberg J, Schramm TK, Abildstrom SZ, KÃ¸ber L, Madsen M, Torp-Pedersen C. Risk of death or reinfarction associated with the use of selective cyclooxygenase-2 inhibitors and nonselective nonsteroidal antiinflammatory drugs after acute myocardial infarction. *Circulation* 2006; **113**: 2906-2913

22 **Hugonnet S**, Villaveces A, Pittet D. Nurse staffing level and nosocomial infections: empirical evaluation of the case-crossover and case-time-control designs. *Am J Epidemiol* 2007; **165**: 1321-1327

23 **Hebert C**, Delaney JA, Hemmelgarn B, Lévesque LE, Suissa S. Benzodiazepines and elderly drivers: a comparison of pharmacoepidemiological study designs. *Pharmacoepidemiol Drug Saf* 2007; **16**: 845-849

24 **Guo X**, Nzerue C. How to prevent, recognize, and treat drug-induced nephrotoxicity. *Cleve Clin J Med* 2002; **69**: 289-290, 293-294, 296-297 passim

25 **Pannu N**, Nadim MK. An overview of drug-induced acute

kidney injury. *Crit Care Med* 2008; **36**: S216-S223
26 **DiPalma JA**, Brady CE 3rd. Colon cleansing for diagnostic and surgical procedures: polyethylene glycol-electrolyte lavage solution. *Am J Gastroenterol* 1989; **84**: 1008-1016
27 **Bitoun A**, Ponchon T, Barthet M, Coffin B, Dugué C, Halphen M. Results of a prospective randomised multicentre controlled trial comparing a new 2-L ascorbic acid plus polyethylene glycol and electrolyte solution vs. sodium phosphate solution in patients undergoing elective colonoscopy. *Aliment Pharmacol Ther* 2006; **24**: 1631-1642
28 **DiPalma JA**, Brady CE 3rd, Pierson WP. Colon cleansing: acceptance by older patients. *Am J Gastroenterol* 1986; **81**: 652-655
29 **Clark LE**, Dipalma JA. Safety issues regarding colonic cleansing for diagnostic and surgical procedures. *Drug Saf* 2004; **27**: 1235-1242
30 **Coresh J**, Astor B. Decreased kidney function in the elderly: clinical and preclinical, neither benign. *Ann Intern Med* 2006; **145**: 299-301
31 **Maclure M**. The case-crossover design: a method for studying transient effects on the risk of acute events. *Am J Epidemiol* 1991; **133**: 144-153
32 **Horwitz J**, Vakil N. Risk of impaired renal function after colonoscopy. *Am J Gastroenterol* 2008; **103**: 1309; author reply 1309-1309; author reply 1310
33 **Zuccaro G Jr**, Connor JT, Schreiber M Jr. Colonoscopy preparation: are our patients at risk? *Am J Gastroenterol* 2007; **102**: 2664-2666
34 **Nissenson AR**. Acute renal failure: definition and pathogenesis. *Kidney Int Suppl* 1998; **66**: S7-S10
35 **Park BJ**, Sung JH, Park KD, Seo SW, Kim SW. Report of the evaluation for validity of discharged diagnoses in Korean Health Insurance database. Seoul: Seoul National University, 2003: 19-52

S- Editor Tian L **L- Editor** Logan S **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.657

World J Gastroenterol 2011 February 7; 17(5): 657-661
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Effects of intestinal mucosal blood flow and motility on intestinal mucosa

Yan-Bin Wang, Jing Liu, Zhao-Xu Yang

Yan-Bin Wang, Zhao-Xu Yang, Department of Gastroenterology, Beijing Tiantan Hospital, Capital Medical University, Beijing 100050, China
Jing Liu, Department of Neurology, Beijing Dianli Hospital, Beijing 100073, China
Author contributions: Wang YB and Yang ZX designed the research; Liu J performed the research; Wang YB wrote the paper.
Correspondence to: Jing Liu, Associate Chief, Department of Neurology, Beijing Dianli Hospital, Beijing 100073, China. maogou0806@sina.com
Telephone: +86-10-67096580 Fax: +86-10-67096567
Received: July 7, 2010 Revised: September 30, 2010
Accepted: October 7, 2010
Published online: February 7, 2011

Abstract

AIM: To investigate the role of intestinal mucosal blood flow (IMBF) and motility in the damage of intestinal mucosal barrier in rats with traumatic brain injury.

METHODS: Sixty-four healthy male Wistar rats were divided randomly into two groups: traumatic brain injury (TBI) group (n = 32), rats with traumatic brain injury; and control group (n = 32), rats with sham-operation. Each group was divided into four subgroups (n = 8) as 6, 12, 24 and 48 h after operation. Intestinal motility was measured by the propulsion ratio of a semi-solid colored marker (carbon-ink). IMBF was measured with the laser-Doppler technique. Endotoxin and D-xylose levels in plasma were measured to evaluate the change of intestinal mucosal barrier function following TBI.

RESULTS: The level of endotoxin was significantly higher in TBI group than in the control group at each time point (0.382 ± 0.014 EU/mL *vs* 0.102 ± 0.007 EU/mL, 0.466 ± 0.018 EU/mL *vs* 0.114 ± 0.021 EU/mL, 0.478 ± 0.029 EU/mL *vs* 0.112 ± 0.018 EU/mL and 0.412 ± 0.036 EU/mL *vs* 0.108 ± 0.011 EU/mL, $P < 0.05$). D-xylose concentrations in plasma in TBI group were significantly higher than in the control group (6.68 ± 2.37 mmol/L *vs* 3.66 ± 1.07 mmol/L, 8.51 ± 2.69 mmol /L *vs* 3.15 ± 0.95 mmol/L, 11.68 ± 3.24 mmol/L *vs* 3.78 ± 1.12 mmol/L and 10.23 ± 2.83 mmol/L *vs* 3.34 ± 1.23 mmol/ L, $P < 0.05$). The IMBF in TBI group was significantly lower than that in the control group (38.5 ± 2.8 PU *vs* 45.6 ± 4.6 PU, 25.2 ± 3.1 PU *vs* 48.2 ± 5.3 PU, 21.5 ± 2.7 PU *vs* 44.9 ± 2.8 PU, 29. 4 ± 3.8 PU *vs* 46.7 ± 3.2 PU) ($P < 0.05$). Significant decelerations of intestinal propulsion ratio in TBI groups were found compared with the control group (0.48% ± 0.06% *vs* 0.62% ± 0.03%, 0.37% ± 0.05% *vs* 0.64% ± 0.01%, 0.39% ± 0.07% *vs* 0.63% ± 0.05% and 0.46% ± 0.03% *vs* 0.65% ± 0.02%) ($P < 0.05$).

CONCLUSION: The intestinal mucosal permeability is increased obviously in TBI rats. Decrease of intestinal motility and IMBF occur early in TBI, both are important pathogenic factors for stress-related damage of the intestinal mucosal barrier in TBI.

Key words: Traumatic brain injury; Intestinal mucosa barrier; Stress; Intestinal mucosa blood flow; Intestinal motility

Peer reviewer: Nageshwar D Reddy, Professor, Asian Institute of Gastroenterology, 6-3-652, Somajiguda, Hyderabad - 500 082, India

Wang YB, Liu J, Yang ZX. Effects of intestinal mucosal blood flow and motility on intestinal mucosa. *World J Gastroenterol* 2011; 17(5): 657-661 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/657.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.657

INTRODUCTION

Multiple system organ dysfunction syndrome (MODS) often occurs following the stress of severe trauma, burn and acute necrotic pancreatitis[1-4]. However, its exact mecha-

nism remains unclear. The gut origin hypothesis suggests that damage of intestinal mucosal barriers as a result of these stress permits bacterial and endotoxin translocation, which triggers systemic immunoinflammatory response to release cytokines and inflammatory mediators. All of these might exacerbate systemic inflammatory response syndrome (SIRS) and MODS. Many patients with severe traumatic brain injury (TBI) often die of MODS[5], but not of the injury itself. So to prevent SIRS and MODS in TBI patients is one of the important factors that affect the prognosis and sequelea.

Our previous studies have found the damage of intestinal mucosal morphology and barrier function following TBI[6]. Although very common, the pathophysiology of this stress-related change is far from understood.

Fortunately, researches over the past decades have provided insight into the potential mechanisms responsible for the pathogenesis of stress-induced gastrointestinal dysfunction. The stressful situation is a multi-factorial disorder involving dysregulation within the brain-gut axis. Upon activation of the brain-gut axis by stress, the release of brain-gut peptides can profoundly affect gastrointestinal physiology and it is frequently associated with gastrointestinal motor, gastrointestinal mucosal blood flow (IMBF), enteric and central nervous system irregularities, and neuroimmune dysregulation[7].

The aim of this study was to further elucidate the effects of TBI on intestinal motility and IMBF, and to explore the putative mechanism of this stress-induced change in the TBI process.

MATERIALS AND METHODS

Animal model of TBI

Sixty-four healthy male Wistar rats, weighing 200-250 g (provided by Experimental Animal Center of Genetics and Developmental Biology Institute, Chinese Academy of Sciences), were randomly assigned to TBI model group (n = 32) and control group (n = 32). Each group was divided into four subgroups as 6, 12, 24 and 48 h after operation (n = 8). Experimental procedures complied with the ethical requirements for animal care.

Establishment of animal models

TBI group (n = 32): RATS with traumatic brain injury by free falling body method[8]. Rats were deprived of food for 12 h prior to experiment, and then was anesthetized with injection of 10% chloral hydrate (0.4 mL/100 g) and fixed on a stereotaxic apparatus. Scalp was cut along the median line and exposed the skull under steriled conditions. At the point of 2.0 mm rearward from the coronal suture and 2.0 mm left to the sagittal suture, open a 3.5 mm diameter bone window and maintain the integrity of the duramater. Then 20 g metal bar was released and fallen freely from 50 cm height to strike the meninges to cause the brain injury.

Control group (n = 32): rats with sham-operation with skull open operation alone and no brain injury.

Determination of endotoxin

One mL blood was collected from portal vein and placed into an apyrogenic tube (containing heparin) immediately. The levels of endotoxin were measured by chromogenic limulus amebocyte lysate test. The test kit was purchased from Shanghai Yihua Clinical Technology Company (Shanghai, China).

Measurement of D-xylose concentrations in plasma

Intestinal permeability was quantified by D-xylose concentrations in plasma. The 5% D-xylose solution of 1.5 mL was administered into the stomach by gastric tube feeding, and blood samples were collected into chilled tubes containing 100 U heparin 1 h later. The blood was centrifuged at 3000 r/min at 4℃ for 10 min. The plasma was stored at -70℃ until assayed. Levels of D-xylose in plasma were measured with D-xylose kit.

Measurement of IMBF

IMBF was measured with Laser Doppler Flowmetry (LDF) equipment (PeriFlux System 5000, Perimed, Sweden). The laser probe was inserted through a small enterotomy at the point that 20 cm from pylorus of the jejunal sac and held in a fixed position in the chamber solution at a distance of 1-2 mm above the mucosa. The measurement was taken as the average flow over a 10-min period following an initial 20-min period of stabilization.

Measurement of intestinal transit

Rats were fasted for 24 h prior to experiment, and 0.5 mL carbon-ink was administered into the stomach by gastric tube feeding. Twenty min later, the rats were killed at each time point, their intestines were removed from the pylorus through the ileocecal junction. The distance of carbon-ink from the pylorus to the most distal point of stain was expressed as migration distance. Results were expressed as propulsion ratio (%) of the migration distance to the total length of the small intestine (the distance between the pylorus and the ileocecal junction).

Statistical analysis

Software SPSS 11.0 was used for the statistical analysis. The data were expressed as mean ± SD. Experimental results were analyzed by unpaired t test and $P < 0.05$ was considered as significant difference.

RESULTS

Serum endotoxin levels

There were significant differences of endotoxin levels between the TBI group and control group at each time point (0.382 ± 0.014 EU/mL *vs* 0.102 ± 0.007 EU/mL, 0.466 ± 0.018 EU/mL *vs* 0.114 ± 0.021 EU/mL, 0.478 ± 0.029 EU/mL *vs* 0.112 ± 0.018 EU/mL and 0.412 ± 0.036 EU/mL *vs* 0.108 ± 0.011 EU/mL, $P < 0.05$, respectively). As shown in Table 1, the endotoxin was significantly increased 6 h after TBI, and reached the peak at 24 h, and then declined at 48 h, but was still higher than that of the control group.

Table 1 Changes of endotoxin in plasma (mean ± SD) (EU/mL)

Groups	6 h	12 h	24 h	48 h
Control	0.102 ± 0.007	0.114 ± 0.021	0.112 ± 0.018	0.108 ± 0.011
TBI	0.382 ± 0.014[a]	0.466 ± 0.018[a]	0.478 ± 0.029[a]	0.412 ± 0.036[a]

[a]$P < 0.05$ *vs* control. TBI: Traumatic brain injury.

Table 2 Changes of D-xylose in plasma (mean ± SD) (mmol/L)

Groups	6 h	12 h	24 h	48 h
Control	3.66 ± 1.07	3.15 ± 0.95	3.78 ± 1.12	3.34 ± 1.23
TBI	6.68 ± 2.37[a]	8.51 ± 2.69[a]	11.68 ± 3.24[a]	10.23 ± 2.83[a]

[a]$P < 0.05$ *vs* control. TBI: Traumatic brain injury.

Table 3 Changes of intestinal mucosal blood flow (mean ± SD) (PU)

Groups	6 h	12 h	24 h	48 h
Control	45.6 ± 4.6	48.2 ± 5.3	44.9 ± 2.8	46.7 ± 3.2
TBI	38.5 ± 2.8	25.2 ± 3.1[a]	21.5 ± 2.7[a]	29.4 ± 3.8[a]

[a]$P < 0.05$ *vs* control. TBI: Traumatic brain injury.

Table 4 Ratio of intestinal propulsion (mean ± SD) (%)

Groups	6 h	12 h	24 h	48 h
Control	0.62 ± 0.03	0.64 ± 0.01	0.63 ± 0.05	0.65 ± 0.02
TBI	0.48 ± 0.06[a]	0.37 ± 0.05[a]	0.39 ± 0.07[a]	0.46 ± 0.03[a]

[a]$P < 0.05$ *vs* control. TBI: Traumatic brain injury.

D-xylose concentrations in plasma

D-xylose concentrations in plasma in TBI rats were significantly higher than in the control group (6.68 ± 2.37 mmol/L *vs* 3.66 ± 1.07 mmol/L, 8.51 ± 2.69 mmol/L *vs* 3.15 ± 0.95 mmol/L, 11.68 ± 3.24 mmol/L *vs* 3.78 ± 1.12 mmol/L and 10.23 ± 2.83 mmol/L *vs* 3.34 ± 1.23 mmol/L, $P < 0.01$, respectively), indicating that the intestinal mucosal barrier was damaged (Table 2).

Changes of IMBF

As shown in Table 3, IMBF was significantly lower in TBI group than that in the control group (38.5 ± 2.8 PU *vs* 45.6 ± 4.6 PU, 25.2 ± 3.1 PU *vs* 48.2 ± 5.3 PU, 21.5 ± 2.7 PU *vs* 44.9 ± 2.8 PU, 29. 4 ± 3.8 PU *vs* 46.7 ± 3.2 PU) ($P < 0.05$). It began to decrease at 6 h, reached the lowest at 24 h, and did not reach the baseline by 48 h.

Changes of intestinal transit

The overall mean ratio of intestinal propulsion under TBI stress was lower than that of the control group (0.48% ± 0.06% *vs* 0.62% ± 0.03%, 0.37% ± 0.05% *vs* 0.64% ± 0.01%, 0.39% ± 0.07% *vs* 0.63% ± 0.05% and 0.46% ± 0.03% *vs* 0.65% ± 0.02%) ($P < 0.05$), indicating that TBI stress could inhibit small intestinal motility (Table 4).

DISCUSSION

Gastrointestinal dysfunction is a common complication of stress. Damage of the gastrointestinal function, especially of the gastrointestinal barrier function, permits translocation of enterogenic bacteria and endotoxins, triggers systemic immunoinflammatory response to release cytokines and inflammatory mediators, which is an important initiator as well as a stimulator for occurrence of SIRS, sepsis and MODS following major stress[9]. The stress including severe trauma, hemorrhagic shock, severe pancreatitis and burn[10,11]. So the gastrointestinal barrier function is one of the important factors that affect the prognosis and sequelea.

Intestinal mucosal barrier function could be evaluated by measuring the permeability of saccharide molecular probe. Lactulose/mannitol and D-xylose have previously been used to assess intestinal mucosal permeability[12-15]. Shi *et al*[16], reported that chronic restraint stress could cause damage of the intestinal barrier function and increased intestinal permeability to D-xylose.

In this study, we used endotoxin and plasma D-xylose to evaluate the intestinal mucosa barrier function. We found that the endotoxin and plasma D-xylose levels in the TBI group were significantly higher than in the control group at 6 h following TBI, and reached its peak at 24 h, and then declined at 48 h, but still markedly higher than that in the control group. All of these demonstrated that TBI stress could be an initiating factor to increase the permeability of intestinal mucosa, suggesting that the intestinal mucosal barrier dysfunction initiated at the early stage of TBI.

At present, the specific pathogenesis and progress of the intestinal mucosal barrier damage still remain unclear. Stress is known to alter ingestive behaviors and associated physiological events such as gastric acid secretion and gastrointestinal motility. Mast cells translate the stress signal that has been transmitted through brain-gut axis to release a wide range of neurotransmitters and proinflammatory mediators, some of them are brain-gut peptides, such as 5-HT, SP, CGRP, CRP, CCK, NO, NE and VIP. Evidences implicated that the brain-gut peptides are involved in these physiological effects which can change the intestinal motility, modulate tight junction proteins and increase the intestinal permeability[7,17]. Animal studies suggest that cholecystokinin (CCK) acts *via* a vagal afferent pathway to decrease gastrointestinal motility[18] and substance P can stimulate a contractile function of smooth muscle[19]. Studies in animal models showed that burn injury and cardiopulmonary bypass markedly down-regulated the expression of occludin and tight junction associated protein ZO-1 in intestinal mucosa of rats. The close correlation between expression of tight junctions and plasma levels of diamine oxidase or *d*-lactate supports the hypothesis that intestinal permeability increases during and after stress events because of decreases in the expression of tight junctions[20,21].

IMBF plays a vital role in intestinal mucosal defense system. Sufficient IMBF brings oxygen and nutrients to the mucosal cells, maintains the normal structure and function of intestinal mucosa and is closely associated with the pathogenesis and healing of intestinal mucosal lesions[22]. Our results revealed that IMBF decreased significantly at the early stage of TBI, and the intestinal mucosal permeability increase occurred at the same time. As intestinal mucosa is very sensitive to the shortage of blood and oxygen, ischemia/reperfusion (I/R) is the main pathogenesis of intestinal mucosal damage. The physiopathology of intestinal mucosal damage by I/R is not fully understood. But, it is believed that cytotoxic substances such as free radicals, nitric oxide, pro-inflammatory cytokines, leukotrienes, serotonin and other related products, play important roles[23,24]. I/R not only damages the intestinal mucosal barrier function but also alters the gastrointestinal motility[25].

It is widely believed that delayed intestinal motility could cause small intestinal bacterial overgrowth (SIBO). Gangarosa[26] demonstrated that intestinal motility served as a normal cleansing mechanism of the intestine. Leveau *et al*[27] noticed a delay in intestinal transit time, appearing as an early event in acute pancreatitis, preceding SIBO, and suggested that impairment in intestinal motility may play a role in the development of SIBO. Tsukada *et al*[28-30] demonstrated that the small intestinal transit was significantly inhibited by restraint stress. Our results revealed that, at the early stage of TBI, the intestinal propulsion ratio decreased significantly as compared with control group ($P < 0.05$). Damage of intestinal mucosal barrier function occurred at the same time, indicating that the inhibition of intestinal motility might be another vital factor of gastrointestinal barrier dysfunction.

The mechanism may be explained by the fact that the prolonged small intestinal transit makes it possible that the small intestinal content remains in the intestinal tract for a long time, preceding SIBO, increasing the chance of bacterial and endotoxin translocation and producing a great deal of gas. The defect of intestinal barrier and the above factors of small intestinal dysfunction may enhance each other.

In summary, the damage of intestinal mucosal barrier function following TBI is caused by multiple factors, the close correlation between decrease of intestinal blood flow and motility and increase of intestinal permeability supports the hypothesis that both of them might play a very important role in the regulation of intestinal epithelial barrier dysfunction during and after TBI. Therefore, maintaining intestinal barrier function is a systematic engineering project. Further research that more precisely characterizes the role of intestinal mucosal blood flow and intestinal motility in these diseases could put new insights into the new therapies for stress-induced injury of intestinal mucosal barrier function.

COMMENTS

Background

Multiple system organ dysfunction syndrome (MODS) often occurs following the stress of traumatic brain injury (TBI). Athough being very common, the pathophysiology of this stress-related change is far from understood. The gut origin hypothesis suggests that damage of intestinal mucosa barriers as a result of these stress permits bacterial and endotoxin translocation, which triggers systemic immunoinflammatory response to release cytokines and inflammatory mediators. All of these might exacerbate the systemic inflammatory response syndrome (SIRS) and MODS.

Research frontiers

Gastrointestinal dysfunction is a common complication of stress. Damage of gastrointestinal function, especially of the gastrointestinal barrier function is an important initiator as well as a stimulator for occurrence of SIRS, sepsis and MODS following major stress, including severe trauma, hemorrhagic shock, severe pancreatitis and burn. Studies in animal models showed that brain-gut axis/ brain-gut peptides are involved in these physiological effects which can change the intestinal motility, modulate tight junction proteins and increase intestinal permeability.

Innovations and breakthroughs

The specific pathogenesis and progress of stress-induced damage of intestinal barrier function remain unclear. But, disruption of the intestinal mucosal protection is certainly involved. Intestinal blood flow plays a vital role in intestinal mucosal defense system and intestinal motility served as a normal cleansing mechanism of the intestine. This study revealed that the intestinal blood flow and motility decreased significantly at the early stage of TBI, and the intestinal mucosal permeability increase occurred at the same time. The results suggested that both might be important pathogenic factors for intestinal barrier function damage during and after TBI.

Applications

Many patients with severe TBI often die of MODS, but not of the injury itself. So to protect the mucosal barrier function at the early stage of TBI will be of significance for reducing the stress-related SIRS and MODS.

Terminology

Brain-gut axis is composed of main regulatory cores in the central nervous system that are connected to peripheral (enteric and autonomic) nervous systems through a series of networks of afferent and efferent nerves. Brain-gut peptide is named because of its distribution in both nervous system and gastrointestinal tract. Intestinal mucosal barrier function include mechanical barrier, chemical barrier, immunologic barrier and biological barrier, any damage of these barriers will damage the intestinal mucosal barrier function.

Peer review

This is a well conducted randomized controlled trial on animal models. The authors presented the results of their study that decreased intestinal blood flow and motility occur early in TBI, which supports the hypothesis that both are important pathogenic factors for increasing the intestinal permeability. So resuming the intestinal blood flow and motility might be a useful method for maintaining intestinal barrier function during and after TBI.

REFERENCES

1 **Ogawa M**. Acute pancreatitis and cytokines: "second attack" by septic complication leads to organ failure. *Pancreas* 1998; **16**: 312-315

2 **Dervenis C**, Smailis D, Hatzitheoklitos E. Bacterial translocation and its prevention in acute pancreatitis. *J Hepatobiliary Pancreat Surg* 2003; **10**: 415-418

3 **Schwarz M**, Thomsen J, Meyer H, Büchler MW, Beger HG. Frequency and time course of pancreatic and extrapancreatic bacterial infection in experimental acute pancreatitis in rats. *Surgery* 2000; **127**: 427-432

4 **Liu ZH**, Peng JS, Li CJ, Yang ZL, Xiang J, Song H, Wu XB, Chen JR, Diao DC. A simple taurocholate-induced model of severe acute pancreatitis in rats. *World J Gastroenterol* 2009; **15**: 5732-5739

5 **Xu XZ**, Qi YC, Li Q, Liao YH. The relationship between the changes in plasma endotoxin, tumor necrosis factor levels and multiple organ dysfunction syndrome in patients with severe brain injury. *Zhongguo Weizhongbing Jijiu Yixue* 2000; **6**: 362-363

6 **Wang YB**, Liang PX, Zhu YQ. The effects of intestinal perfu-

sion and motivity on the intestinal permeability in rats with traumatic brain injury. *Zhonghua Xiaohua Zazhi* 2007; **27**: 103-106
7 **Farhadi A**, Fields JZ, Keshavarzian A. Mucosal mast cells are pivotal elements in inflammatory bowel disease that connect the dots: stress, intestinal hyperpermeability and inflammation. *World J Gastroenterol* 2007; **13**: 3027-3030
8 **Wang YB**, Yang ZX, Zhu YQ. An experimental model to study the stress-ralated changes of intestinal mucosa barrier following serious traumatic brain injury. *Zhongguo Shiyan Dongwu Xuebao* 2007; **15**: 220-222
9 **Wang ZT**, Yao YM, Xiao GX, Sheng ZY. Risk factors of development of gut-derived bacterial translocation in thermally injured rats. *World J Gastroenterol* 2004; **10**: 1619-1624
10 **Söderholm JD**, Perdue MH. Stress and gastrointestinal tract. II. Stress and intestinal barrier function. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G7-G13
11 **MacFie J**. Enteral versus parenteral nutrition: the significance of bacterial translocation and gut-barrier function. *Nutrition* 2000; **16**: 606-611
12 **Doig CJ**, Sutherland LR, Sandham JD, Fick GH, Verhoef M, Meddings JB. Increased intestinal permeability is associated with the development of multiple organ dysfunction syndrome in critically ill ICU patients. *Am J Respir Crit Care Med* 1998; **158**: 444-451
13 **Langkamp-Henken B**, Donovan TB, Pate LM, Maull CD, Kudsk KA. Increased intestinal permeability following blunt and penetrating trauma. *Crit Care Med* 1995; **23**: 660-664
14 **Faries PL**, Simon RJ, Martella AT, Lee MJ, Machiedo GW. Intestinal permeability correlates with severity of injury in trauma patients. *J Trauma* 1998; **44**: 1031-1035; discussion 1035-1036
15 **Li S**, Wu WC, He CY, Han Z, Jin DY, Wang L. Change of intestinal mucosa barrier function in the progress of non-alcoholic steatohepatitis in rats. *World J Gastroenterol* 2008; **14**: 3254-3258
16 **Shi HL**, Cheng YY, Li ST, Wang DL, Yu Z J, Geng ZH, Chen WQ, Feng P. Effect of chronic restraint stress on intestinal barrier function of rats. *Zhongguo Xingwei Yixue Kexue* 2003; **12**: 251-253
17 **Cenac N**, Chin AC, Garcia-Villar R, Salvador-Cartier C, Ferrier L, Vergnolle N, Buret AG, Fioramonti J, Bueno L. PAR2 activation alters colonic paracellular permeability in mice via IFN-gamma-dependent and -independent pathways. *J Physiol* 2004; **558**: 913-925
18 **Raybould HE**, Taché Y. Cholecystokinin inhibits gastric motility and emptying via a capsaicin-sensitive vagal pathway in rats. *Am J Physiol* 1988; **255**: G242-G246
19 **Wang ZJ**, Mei MH, Zhu WY. Gastrointestinal hormones. Beijing: Science Publishing House, 1985: 2-372
20 **Sun YJ**, Chen WM, Zhang TZ, Cao HJ, Zhou J. Effects of cardiopulmonary bypass on tight junction protein expressions in intestinal mucosa of rats. *World J Gastroenterol* 2008; **14**: 5868-5875
21 **Chen CL**, Li YL, Wang P, Sun W, Wang FJ. Role of MLC phosphorylation in intestinal epithelial barrier dysfunction induced by sever burn inury. *Disan Junyi Daxue Xuebao* 2008; **30**: 1434-1437
22 **Kawano S**, Tsuji S. Role of mucosal blood flow: a conceptional review in gastric mucosal injury and protection. *J Gastroenterol Hepatol* 2000; **15** Suppl: D1-D6
23 **Arumugam TV**, Arnold N, Proctor LM, Newman M, Reid RC, Hansford KA, Fairlie DP, Shiels IA, Taylor SM. Comparative protection against rat intestinal reperfusion injury by a new inhibitor of sPLA2, COX-1 and COX-2 selective inhibitors, and an LTC4 receptor antagonist. *Br J Pharmacol* 2003; **140**: 71-80
24 **Cerqueira NF**, Hussni CA, Yoshida WB. Pathophysiology of mesenteric ischemia/reperfusion: a review. *Acta Cir Bras* 2005; **20**: 336-343
25 **Ballabeni V**, Barocelli E, Bertoni S, Impicciatore M. Alterations of intestinal motor responsiveness in a model of mild mesenteric ischemia/reperfusion in rats. *Life Sci* 2002; **71**: 2025-2035
26 **Gangarosa EJ**. Recent developments in diarrheal diseases. *Postgrad Med* 1977; **62**: 113-117
27 **Leveau P**, Wang X, Soltesz V, Ihse I, Andersson R. Alterations in intestinal motility and microflora in experimental acute pancreatitis. *Int J Pancreatol* 1996; **20**: 119-125
28 **Tsukada F**, Sawamura K, Kohno H, Ohkubo Y. Mechanism of inhibition of small intestinal motility by restraint stress differs from that with norepinephrine treatment in rats. *Biol Pharm Bull* 2002; **25**: 122-124
29 **Tsukada F**, Sugawara M, Kohno H, Ohkubo Y. Evaluation of the effects of restraint and footshock stress on small intestinal motility by an improved method using a radionuclide, 51Cr, in the rat. *Biol Pharm Bull* 2001; **24**: 488-490
30 **Tsukada F**, Ohuchi Y, Terunuma T, Sugawara M, Kohno H, Ohkubo Y. Activation of mu-opioid pathway is associated with the canceling effect of footshock stimulus on the restraint stress-induced inhibition of small intestinal motility in rats. *Biol Pharm Bull* 2001; **24**: 1332-1334

S- Editor Sun H **L- Editor** Ma JY **E- Editor** Ma WH

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.662

World J Gastroenterol 2011 February 7; 17(5): 662-665
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Suspended moxibustion relieves chronic visceral hyperalgesia and decreases hypothalamic corticotropin-releasing hormone levels

En-Hua Zhou, Xiao-Mei Wang, Guang-Hong Ding, Huan-Gan Wu, Li Qi, Hui-Rong Liu, Shu-Jing Zhang

En-Hua Zhou, Guang-Hong Ding, Department of Mechanics and Engineering Science, Fudan University, Shanghai 200433, China
En-Hua Zhou, Xiao-Mei Wang, Huan-Gan Wu, Li Qi, Hui-Rong Liu, Shu-Jing Zhang, Key Laboratory of Acupuncture-Moxibustion and Immunological Effects, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, China
Guang-Hong Ding, Shanghai Research Center of Acupuncture and Meridians, Shanghai 200433, China
Author contributions: Zhou EH and Ding GH performed the majority of experiments; Wu HG, Wang XM and Qi L provided vital reagents and analytical tools and were also involved in editing the manuscript; Liu HR and Zhang SJ analyzed the data; Zhou EH and Wu HG designed the study and wrote the manuscript.
Supported by The National Basic Research Program of China (973 program), No. 2009CB522900; the Shanghai Leading Academic Discipline Project, No. S30304; and Shanghai Foundation for Development of Science and Technology, China, No. 08DZ1973000
Correspondence to: Dr. Huan-Gan Wu, Key Laboratory of Acupuncture-Moxibustion and Immunological Effects, Shanghai University of Traditional Chinese Medicine, 650 South WanPing Road, XuHui district, Shanghai 200030, China. wuhuangan@126.com
Telephone: +86-21-64644238 Fax: +86-21-64644238
Received: August 9, 2010 Revised: September 14, 2010
Accepted: September 21, 2010
Published online: February 7, 2011

Abstract

AIM: To evaluate the effect of suspended moxibustion (SM) on rectal sensory thresholds and to analyze the possible mechanisms involved in SM treatment of chronic visceral hypersensitivity (CVH) in rats.

METHODS: SM was administered once daily to 37-d-old CVH rats for 7 d. The two pairs of acupoints (ST25 and ST37, bilateral) were simultaneously treated with SM. Each treatment lasted for 30 min. Rats undergoing treatment with SM were not anesthetized. Untreated CVH rats and normal rats were used as controls. The abdominal withdrawal reflex was determined 30-90 min after the seven treatments. The hypothalamic corticotropin-releasing hormone (CRH) mRNA level was measured using real-time quantitative reverse transcription-polymerase chain reaction.

RESULTS: We found that SM treatment significantly decreased visceral sensitivity to colorectal distention in this rat model. In treated animals, SM also decreased the relative hypothalamic CRH mRNA expression level to control levels.

CONCLUSION: Lower hypothalamic CRH levels may mediate the beneficial effects of SM in this rat irritable bowel syndrome model.

Key words: Chronic visceral hypersensitivity; Corticotropin-releasing hormone; Irritable bowel syndrome; Rat; Suspended moxibustion

Peer reviewer: Ricardo Alberto Cruciani, MD, PhD, Department of Pain Medicine and Palliative Care, Beth Israel Medical Center, First Ave. and 16th Street, New York, NY 10003, United States

Zhou EH, Wang XM, Ding GH, Wu HG, Qi L, Liu HR, Zhang SJ. Suspended moxibustion relieves chronic visceral hyperalgesia and decreases hypothalamic corticotropin-releasing hormone levels. *World J Gastroenterol* 2011; 17(5): 662-665 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/662.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.662

INTRODUCTION

Irritable bowel syndrome (IBS) is a functional disorder

characterized by chronic recurring abdominal pain or discomfort and altered bowel habits[1]. Several recent studies have demonstrated that electro-acupuncture (EA) can decrease chronic visceral hypersensitivity (CVH) in a rat IBS model induced by mechanical colorectal irritation in the postnatal period[2-6]. Previously, we reported that EA in this model can decrease hypothalamic corticotropin-releasing hormone (CRH) levels[7]. CRH, a 41 amino acid peptide produced mainly in the paraventricular nucleus of the hypothalamus, is regarded as a major mediator of the stress response[8]. IBS patients are reported to be hypersensitive to routine stress[9], and stressful life events are known to contribute to the clinical course of IBS[10].

Moxibustion is an alternative or complementary therapy that is also used to treat IBS. Methods of moxibustion include suspended moxibustion (SM, also known as warming moxibustion), scarring moxibustion and herb-partition moxibustion. Previously, we reported that both SM[11] and herb-partition moxibustion[12] could decrease CVH in a rat IBS model induced by mechanical colorectal irritation in the postnatal period. Moreover, we found that rats were relaxed or asleep during SM, indicating that the procedure was not stressful to the animals[11]. Therefore, in the present study, we focused on whether SM could decrease hypothalamic CRH levels in rats.

MATERIALS AND METHODS

Animals

We used a rat model of CVH[13] induced by mechanical colorectal irritation during postnatal development. Neonatal male Sprague-Dawley rats (5 d old) were obtained from the Experiment Animal Center, Shanghai University of Traditional Chinese Medicine. The animals were maintained in a plastic cage containing corn chip bedding at a controlled temperature (21℃) in a light-dark cycle (12 h:12 h). The maximum number of rats per cage was five. All rats were used strictly in accordance with the National Institutions of Health Guide for the Care and Use of Laboratory Animals.

Neonatal rats were subjected to daily mechanical colorectal distention (CRD) from the age of 8 d to the age of 21 d. Neonatal rats received CRD twice daily, at 30-min intervals using a procedure modified from previous studies[6,13]. A balloon constructed from a condom (length 20.0 mm and diameter 3.0 mm) was inserted rectally into the descending colon. The balloon was distended with 0.5 mL air for 1 min. It was then deflated and withdrawn. The rats were reared until they reached adulthood (at least 6 wk old), and behavioral responses to visceral pain induced by acute CRD were then examined. SM was administered to CVH rats (n = 8) for 7 d. CVH rats without SM (n = 8) and normal rats (n = 8) were used as controls. After seven treatments, the abdominal withdrawal reflex (AWR) was monitored over a period of 30-90 min. The animals were then sacrificed by intraperitoneal anesthesia using sodium pentobarbital (80 mg/kg), and the hypothalamus was isolated immediately and frozen in liquid nitrogen.

SM treatment

In the CVH + SM group, one ignited moxa stick was suspended perpendicularly 2 cm above Tianshu (ST25)[11] and Shangjuxu (ST37) (5 mm lateral to the anterior tubercle of the tibia and 20 mm below the knee joint). ST25 and ST37 were the two key acupoints chosen in this study based on our clinical treatment of patients with IBS since the 1980s. The two pairs of acupoints (ST25 and ST37) were simultaneously treated with SM. Each treatment consisted of 30 min of moxibustion (15 min for each pair of acupoints). SM was administered once daily to CVH rats for 7 d. The animals were not anesthetized before SM, but were held in the supine position in one gloved hand. Rats from both the normal group and CVH group were also held in one gloved hand in the supine position, but not treated with SM[11], these were used as controls.

Colon stimulation and testing of the AWR

Behavioral responses to CRD in young adult rats were assessed by recording AWR scores, using a procedure modified from previous studies[5,13]. After anesthesia with diethyl ether, a balloon (3 cm in length, made using one finger of a latex glove) was inserted into the descending colon. The rats were then housed in small lucite cubicles (20 cm × 8 cm × 8 cm) on a platform and allowed to wake up and adapt for 20 min. CRD was induced by rapidly inflating the balloon at pressures of 20, 40, 60 and 80 mmHg for a duration of 10 s. AWR scores were observed by two blinded observers using the scale developed by Al-Chaer *et al*[13].

Real-time quantitative reverse transcription-polymerase chain reaction

Total RNA was isolated from the hypothalamus using TRIZOL Reagent (TAKARA Biotechnology Co., Ltd., China), and quantified using a UV-3000 spectrophotometer (UNICO, USA). First-strand cDNA was synthesized using oligo(dT)15 primer, Moloney murine leukemia virus reverse transcriptase (TAKARA Biotechnology Co., Ltd., China), and 4 μL RNA. CRH and GAPDH (housekeeping gene) primers were designed in different exons to amplify cDNA using ABI Prism 7500 SDS Software (Applied Biosystems Co., Ltd., USA). CRH primers were: sense 5'-TGGCCTGCAGTGCAATGC-3' and antisense 5'-CCTGGCACTCAGAATAATTCACAC-3'. Real-time quantitative polymerase chain reaction (qPCR) was performed with 5 μL of first-strand cDNA reaction in the presence of 0.5 μL dNTP, 10 μL specific buffer, 1 μL *Taq* polymerase, SYBR green fluorescent dye and the appropriate sense and antisense primers (0.5 μL) in a final volume of 50 μL (qPCR™ Core Kit, Shanghai DaWei' K Biology Technology Co., Ltd., China). PCR was carried out using the 7500 Sequence Detection System (Applied Biosystems). The reaction conditions were as follow: initial denaturation for 5 min at 95℃ followed by 40 cycles with denaturation at 95℃ for 15 s and annealing and extension at 60℃ for 45 s. Three SYBR cycle threshold values were

Table 1 Abdominal withdrawal reflex scores in response to graded colorectal distention at 20, 40, 60 and 80 mmHg

Group	*n*	AWR score			
		20 mmHg	40 mmHg	60 mmHg	80 mmHg
Normal	8	0.13 ± 0.13	0.38 ± 0.18	0.75 ± 0.25	1.50 ± 0.33
CVH	8	1.38 ± 0.18[b,d]	1.80 ± 0.25[b,d]	2.75 ± 0.25[b,d]	3.50 ± 0.27[b,d]
CVH + SM	8	0.25 ± 0.16	0.50 ± 0.19	1.13 ± 0.30	1.63 ± 0.18

AWR: Abdominal withdrawal reflex; SM: Suspended moxibustion; CVH: Chronic visceral hypersensitivity. [b]$P < 0.01$ *vs* normal group; [d]$P < 0.01$ *vs* CVH + SM group.

averaged for each sample, and the RNA input for the target gene was calculated from the standard curve.

Statistical analysis

All values are expressed as mean ± SE. Statistical analyses were performed using one-way ANOVA followed by Fisher's PLSD procedure using SPSS 10.0 (SPSS Inc., USA). Dunnett's T3 test was used if variances were unequal. $P < 0.05$ was considered to be significant.

RESULTS

AWR scores in response to graded CRD at 20, 40, 60 and 80 mmHg

As shown in Table 1, the AWR scores in response to graded CRD (20, 40, 60, and 80 mmHg) in the normal control group were lower than in the CVH group ($P < 0.01$). SM treatment significantly reduced AWR scores in the CVH rats in response to CRD (20, 40, 60 and 80 mmHg).

Relative hypothalamic CRH mRNA expression

The relative CRH mRNA expression level was significantly higher in the CVH group than in the normal control group ($P < 0.01$). However, the relative CRH mRNA expression level was markedly lower in the CVH + SM group than in the normal control group ($P < 0.01$) (Table 2).

DISCUSSION

In our previous study, we found that SM depressed AWR scores following CRD stimulation at 20 mmHg. However, in this study SM depressed AWR scores following CRD stimulation at 20, 40, 60 and 80 mmHg, which may be due to the increased number of treatments (1 treatment *vs* 7 treatments), and acupoints (1 pair of acupoints *vs* 2 pairs of acupoints). Overall, the results of this experiment demonstrated the efficacy of SM in decreasing CVH in a rat IBS model induced by mechanical colorectal irritation in the postnatal period.

We previously reported that EA can decrease the hypothalamic CRH levels in a rat IBS model[7]. Moreover, the rats were relaxed or asleep during SM, indicating that the procedure was not stressful to the animals[11]. Stress is known to lead to central CRH release, and we have confirmed that hypothalamic CRH levels are elevated in IBS rats. In the present study, we focused on whether SM could decrease hypothalamic CRH expression level in rats. Our results showed that the relative hypothalamic CRH mRNA expression level also decreased in IBS rats, suggesting that the modulation of hypothalamic CRH may mediate the decreased visceral sensitivity arising from SM.

Table 2 Relative hypothalamic corticotropin-releasing hormone mRNA expression

Group	*n*	CRH mRNA (relative expression)
Normal	8	0.29 ± 0.03
CVH	8	3.62 ± 0.23[b,d]
CVH + SM	8	0.47 ± 0.06

CRH: Corticotropin-releasing hormone; SM: Suspended moxibustion; CVH: Chronic visceral hypersensitivity. [b]$P < 0.01$ *vs* normal group; [d]$P < 0.01$ *vs* CVH + SM group.

The effects of CRH on different tissues are mediated *via* CRH receptors on the cell membrane[14]. CRH receptors are expressed in different brain regions[15,16] and in several peripheral organs[17]. Both central CRH receptor 1 (CRH-R1) and peripheral CRH-R1 are believed to be responsible for colorectal distension-induced sensitization[18]. Moreover, the activation of CRH-R2 reduces visceral sensitivity induced by colorectal distension in conscious rats.

We hope that this study will pave the way for further studies on the relationship between CRH, CRH receptors, and SM. It will be necessary to determine whether SM can regulate the expression or activity of CRH receptors in IBS rats. Further experiments with CRH receptor antagonists would shed light on the functional relationship between SM and changes in CRH levels.

In conclusion, SM increased pain thresholds in a rat model of IBS and decreased relative hypothalamic CRH mRNA expression level. We suggest that reduced hypothalamic CRH levels may mediate the beneficial effects of SM in a rat IBS model induced by mechanical colorectal irritation in the postnatal period.

COMMENTS

Background

The authors previously reported that electro-acupuncture can decrease hypothalamic corticotropin-releasing hormone (CRH) levels in a rat model of irritable bowel syndrome (IBS). However, it is still not known whether suspended moxibustion (SM) can decrease hypothalamic CRH levels. Previously, the authors reported that both SM and herb-partition moxibustion can decrease chronic visceral hypersensitivity (CVH) in a rat IBS model induced by mechanical colorectal irritation in the postnatal period. Moreover, the authors found that rats were relaxed or asleep during SM, indicating that the procedure was not stressful to the animals.

Research frontiers

The effects of CRH on different tissues are mediated *via* CRH receptors on the cell membrane. CRH receptors are expressed in different brain regions and in several peripheral organs. Both central CRH receptor 1 (CRH-R1) and peripheral CRH-R1 are believed to be responsible for colorectal distension-induced sensitization. Moreover, the activation of CRH-R2 reduces visceral sensitivity induced by colorectal distension in conscious rats.

Innovations and breakthroughs

This study is the first to report on the effects of SM on hypothalamic CRH levels in CVH rats.

Terminology

Moxibustion is an alternative or complementary therapy and is also used to treat IBS. Methods of moxibustion include SM (also named warming moxibustion), scarring moxibustion and herb-partition moxibustion. In the SM treatment, one ignited moxa-stick was suspended perpendicularly above the acupoints.

Peer review

The paper is very interesting. But there are some questions to be addressed before publication.

REFERENCES

1 **Drossman DA**, Camilleri M, Mayer EA, Whitehead WE. AGA technical review on irritable bowel syndrome. *Gastroenterology* 2002; **123**: 2108-2131

2 **Wang XM**, Liu HR, Ding GH, Chen YF, Wu HA, Li N, Zhou EH, Qin XD, Yuan LS. Effects of electroacupuncture on c-Fos expression in the spinal cord and brain of rats with chronic visceral hypersensitivity. *Neural Regen Res* 2009; **4**: 339-343

3 **Liu HR**, Wang XM, Zhou EH, Shi Y, Li N, Yuan LS, Wu HG. Acupuncture at both ST25 and ST37 improves the pain threshold of chronic visceral hypersensitivity rats. *Neurochem Res* 2009; **34**: 1914-1918

4 **Chu D**, Cheng P, Xiong H, Zhang J, Liu S, Hou X. Electroacupuncture at ST-36 relieves visceral hypersensitivity and decreases 5-HT(3) receptor level in the colon in chronic visceral hypersensitivity rats. *Int J Colorectal Dis* 2010; Epub ahead of print

5 **Tian XY**, Bian ZX, Hu XG, Zhang XJ, Liu L, Zhang H. Electroacupuncture attenuates stress-induced defecation in rats with chronic visceral hypersensitivity via serotonergic pathway. *Brain Res* 2006; **1088**: 101-108

6 **Cui KM**, Li WM, Gao X, Chung K, Chung JM, Wu GC. Electro-acupuncture relieves chronic visceral hyperalgesia in rats. *Neurosci Lett* 2005; **376**: 20-23

7 **Wu HG**, Liu HR, Zhang ZA, Zhou EH, Wang XM, Jiang B, Shi Z, Zhou CL, Qi L, Ma XP. Electro-acupuncture relieves visceral sensitivity and decreases hypothalamic corticotropin-releasing hormone levels in a rat model of irritable bowel syndrome. *Neurosci Lett* 2009; **465**: 235-237

8 **Vale W**, Spiess J, Rivier C, Rivier J. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 1981; **213**: 1394-1397

9 **Whitehead WE**, Crowell MD, Robinson JC, Heller BR, Schuster MM. Effects of stressful life events on bowel symptoms: subjects with irritable bowel syndrome compared with subjects without bowel dysfunction. *Gut* 1992; **33**: 825-830

10 **Ferrier L**. Significance of increased human colonic permeability in response to corticotrophin-releasing hormone (CRH). *Gut* 2008; **57**: 7-9

11 **Zhou EH**, Liu HR, Wu HG, Shi Y, Wang XM, Tan LY, Yao LQ, Zhong YS, Jiang Y, Zhang LL. Suspended moxibustion relieves chronic visceral hyperalgesia via serotonin pathway in the colon. *Neurosci Lett* 2009; **451**: 144-147

12 **Zhou EH**, Liu HR, Wu HG, Shi Y, Wang XM, Yao LQ, Zhong YS, Yang Y. Herb-partition moxibustion relieves chronic visceral hyperalgesia and 5-HT concentration in colon mucosa of rats. *Neurol Res* 2009; **31**: 734-737

13 **Al-Chaer ED**, Kawasaki M, Pasricha PJ. A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. *Gastroenterology* 2000; **119**: 1276-1285

14 **Chang CP**, Pearse RV 2nd, O'Connell S, Rosenfeld MG. Identification of a seven transmembrane helix receptor for corticotropin-releasing factor and sauvagine in mammalian brain. *Neuron* 1993; **11**: 1187-1195

15 **Primus RJ**, Yevich E, Baltazar C, Gallager DW. Autoradiographic localization of CRF1 and CRF2 binding sites in adult rat brain. *Neuropsychopharmacology* 1997; **17**: 308-316

16 **Lovenberg TW**, Liaw CW, Grigoriadis DE, Clevenger W, Chalmers DT, De Souza EB, Oltersdorf T. Cloning and characterization of a functionally distinct corticotropin-releasing factor receptor subtype from rat brain. *Proc Natl Acad Sci USA* 1995; **92**: 836-840

17 **Baigent SM**, Lowry PJ. mRNA expression profiles for corticotrophin-releasing factor (CRF), urocortin, CRF receptors and CRF-binding protein in peripheral rat tissues. *J Mol Endocrinol* 2000; **25**: 43-52

18 **Fukudo S**, Saito K, Sagami Y, Kanazawa M. Can modulating corticotropin releasing hormone receptors alter visceral sensitivity? *Gut* 2006; **55**: 146-148

S- Editor Shi ZF **L- Editor** Webster JR **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.666

World J Gastroenterol 2011 February 7; 17(5): 666-670
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Narrow-band imaging endoscopy with and without magnification in diagnosis of colorectal neoplasia

Qing-Jie Zhou, Jian-Min Yang, Bao-Ying Fei, Qi-Shun Xu, Wei-Quan Wu, Hong-Jun Ruan

Qing-Jie Zhou, Jian-Min Yang, Bao-Ying Fei, Qi-Shun Xu, Wei-Quan Wu, Hong-Jun Ruan, Department of Gastroenterology, Zhejiang Provincial People's Hospital, Hangzhou 310014, Zhejiang Province, China

Author contributions: Zhou QJ and Yang JM contributed equally to this work; Yang JM designed the research; Yang JM, Zhou QJ, Fei BY, Xu QS, Wu WQ and Ruan HJ performed the research; Zhou QJ and Yang JM analyzed data and wrote the paper.

Correspondence to: Dr. Jian-Min Yang, Department of Gastroenterology, Zhejiang Provincial People's Hospital, Hangzhou 310014, Zhejiang Province, China. jianminyang@hotmail.com

Telephone: +86-571-85893430 Fax: +86-571-85131448

Received: August 16, 2010 Revised: October 22, 2010
Accepted: October 29, 2010
Published online: February 7, 2011

Abstract

AIM: To evaluate the diagnostic efficacies of narrow-band imaging (NBI) endoscopy with and without high magnification in distinguishing neoplasia from non-neoplasia colorectal lesions.

METHODS: A total of 118 patients with 123 colorectal lesions examined by NBI endoscopy in the Zhejiang Provincial People's Hospital from September 2008 to April 2010 were enrolled in this study. These lesions were classified by pit pattern and capillary pattern, and then assessed by histopathology.

RESULTS: Ten lesions not meeting the diagnostic criteria were excuded, the overall diagnostic accuracy of NBI endoscopy in distinguishing neoplasia from non-neoplasia colorectal lesions was 91.2% (103/113), and that of NBI endoscopy with and without high magnification was 93.0% (40/43) and 90.0% (63/70), respectively. Both were significantly higher than that of conventional colonoscopy reported in the literature ($P < 0.05$), but there was no significant difference between the two groups ($P > 0.05$).

CONCLUSION: Besides NBI magnifying endoscopy, NBI endoscopy without magnification may also be used to distinguish neoplasia from non-neoplasia colorectal lesions.

Key words: Narrow-band imaging; Colorectal neoplasia; Magnifying endoscopy; Non-magnifying endoscopy; Diagnosis

Peer reviewers: Dr. Benjamin Perakath, Professor, Department of Surgery Unit 5, Christian Medical College, Vellore 632004, Tamil Nadu, India; Chakshu Gupta, MD, FCAP, Pathology and Laboratory Medicine, Heartland Regional Medical Center, 5325 Faraon Street, St. Joseph, MO 64506, United States; Dr. John B Schofield, MB, BS, MRCP, FRCP, Department of Cellular Pathology, Preston Hall, Maidstone, Kent, ME20 7NH, United Kingdom

Zhou QJ, Yang JM, Fei BY, Xu QS, Wu WQ, Ruan HJ. Narrow-band imaging endoscopy with and without magnification in diagnosis of colorectal neoplasia. *World J Gastroenterol* 2011; 17(5): 666-670 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/666.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.666

INTRODUCTION

Colorectal cancer is a common gastrointestinal malignancy with a slow process in occurrence and development involving multi-steps, multi-stages and multiple genes, and most of them arise from preexisting adenomas and have an adenoma-carcinoma sequence[1,2]. Early detection and removal of colorectal adenomas may greatly reduce both the incidence of colorectal cancer and cancer-related death[3].

Electronic colonoscopy is considered to be an effective examination for the detection of colorectal neoplastic lesions[4]. However, it is difficult to assess pre-malignant and early neoplastic lesions precisely using conventional white light endoscopy. In chromoendoscopy, a biocompatible dye, such as indigo carmine, can strengthen the surface structure of epithelial lesions[5], but the operation

is relatively cumbersome, time-consuming and costly, not conducive to observe the vascular structure, and may damage the DNA of epithelial cells.

Narrow-band imaging (NBI) is a novel technology that emerged in endoscopic diagnosis of early cancer, and it has better targeting for biopsy and higher diagnostic accuracy than conventional videoendoscopy by enhancing the visualization of surface mucosal and vascular patterns on the polyp surface[6]. Our study aimed to verify the diagnostic accuracy of NBI endoscopy in distinguishing neoplasia from non-neoplasia colorectal lesions, and evaluate the diagnostic efficacies of NBI endoscopy with and without high magnification.

MATERIALS AND METHODS

Patients

The patients who have poor bowel preparation, familial adenomatous polyposis, infectious bowel disease, inflammatory bowel disease and colorectal cancer were excluded. A total of 118 patients with 123 colorectal lesions examined by NBI endoscopy in the Zhejiang Provincial People's Hospital from September 2008 to April 2010 were enrolled in this study. Forty-six and 77 lesions were examined by NBI endoscopy with and without high magnification.

NBI and colonoscopy

A standard videoendoscopy system with two light sources was used for examination. One light source was for the standard optical filter (broadband) and the other was for the NBI system. The control knob on the grip of the endoscope allows single touch exchange of the standard filter for the NBI filter. Olympus CV-260SL, CLV-260SL, CF240I, H260AZI and SONY LMD-2140MD were used respectively for the endoscopic host, source, conventional endoscopy, magnifying endoscopy and monitor.

Methods

Researching methods, observation tables and patient's informed consent were obtained before the study. Polyethylene glycol lavage solution and diprivan propofol were used for bowel preparation and intravenous anesthesia, respectively, and the whole examination process was managed by an experienced endoscopist (the second author). The scope was entered to the ileocecal part using conventional observation mode, and back with white light and NBI. The lesions were classified by pit pattern and capillary pattern with NBI immediately when they were detected, and then biopsied or resected for pathological diagnosis by an experienced pathologist. The endoscopist and pathologist were unaware of each other's diagnosis, and finally the NBI endoscopic diagnosis was assessed based on the pathological diagnosis.

The NBI endoscopic diagnostic criteria followed Kudo's[7] classification of mucosal pit pattern and Sano's[8] classification of capillary pattern. We developed the comprehensive diagnostic criteria: Pit Ⅱ + CP1 as hyperplastic polyp, Pit ⅢL + CP2 or Pit Ⅳ + CP2 as adenoma, and Pit Ⅴ + CP3 as adenocarcinoma.

Table 1 Comparison of narrow-band imaging endoscopy and pathological diagnosis

	Narrow-band imaging diagnosis	Pathological diagnosis				Total
		Hyperplastic polyp	Tubular adenoma	Villous adenoma	Adenocarcinoma	
Pit pattern	Ⅱ	38	9	0	0	47
	Ⅲs	0	0	0	1	1
	ⅢL	4	51	1	0	56
	Ⅳ	0	0	6	0	6
	Ⅴ	0	0	0	13	13
Capillary pattern	CP1	35	4	0	0	39
	CP2	7	56	7	0	70
	CP3	0	0	0	14	14

Statistical analysis

Statistical differences of diagnostic accuracies were analyzed by the Mann-Whitney U test and χ^2 test. $P < 0.05$ was considered significantly different.

RESULTS

Among the 118 patients, 73 were males and 45 were females, with a mean age of 57.54 ± 14.01 years (range, 19-86 years). The location of the lesions was as follows: 53 in rectum, 22 in sigmoid colon, 14 in descending colon, 21 transverse colon and 13 in ascending colon, with a mean size of 10.13 ± 7.79 mm (range, 3-40 mm).

Pathologically, lesions were divided into non-neoplastic lesions, including hyperplastic and inflammatory lesions (42) and neoplastic lesions, including tubular adenoma (60), villous adenoma (7) and adenocarcinoma (14).

Based on the Kudo's classification of mucosal pit pattern and Sano's classification of capillary pattern, the lesions were classified with NBI and assessed by histopathology. The diagnostic accuracy, sensitivity and specificity to distinguish between non-neoplastic and neoplastic colorectal lesions were 94.7% (72/76), 88.9% (72/81) and 90.5% (38/42) for pit pattern delineation; and 91.7% (77/84), 95.1% (77/81) and 83.3% (35/42) for capillary pattern delineation, respectively (Table 1). According to the comprehensive diagnostic criteria, Pit Ⅱ + CP1 (Figure 1A and B) was defined as hyperplastic polyp, Pit ⅢL + CP2 (Figure 1C and D) or Pit Ⅳ + CP2 (Figure 1E and F) as adenoma, and Pit Ⅴ + CP3 (Figure 1G and H) as adenocarcinoma. Ten lesions failed to meet the diagnostic criteria. The overall diagnostic accuracy of NBI endoscopy in distinguishing neoplastic from non-neoplastic colorectal lesions was 91.2% (103/113) and that of NBI endoscopy with and without high magnification was 93.0% (40/43) and 90.0% (63/70), respectively (Table 2). Both were significantly higher than that of conventional colonoscopy reported in the literature[9] ($P < 0.05$), but there was no significant difference between the two groups ($P > 0.05$).

DISCUSSION

NBI is a novel imaging technology that uses special nar-

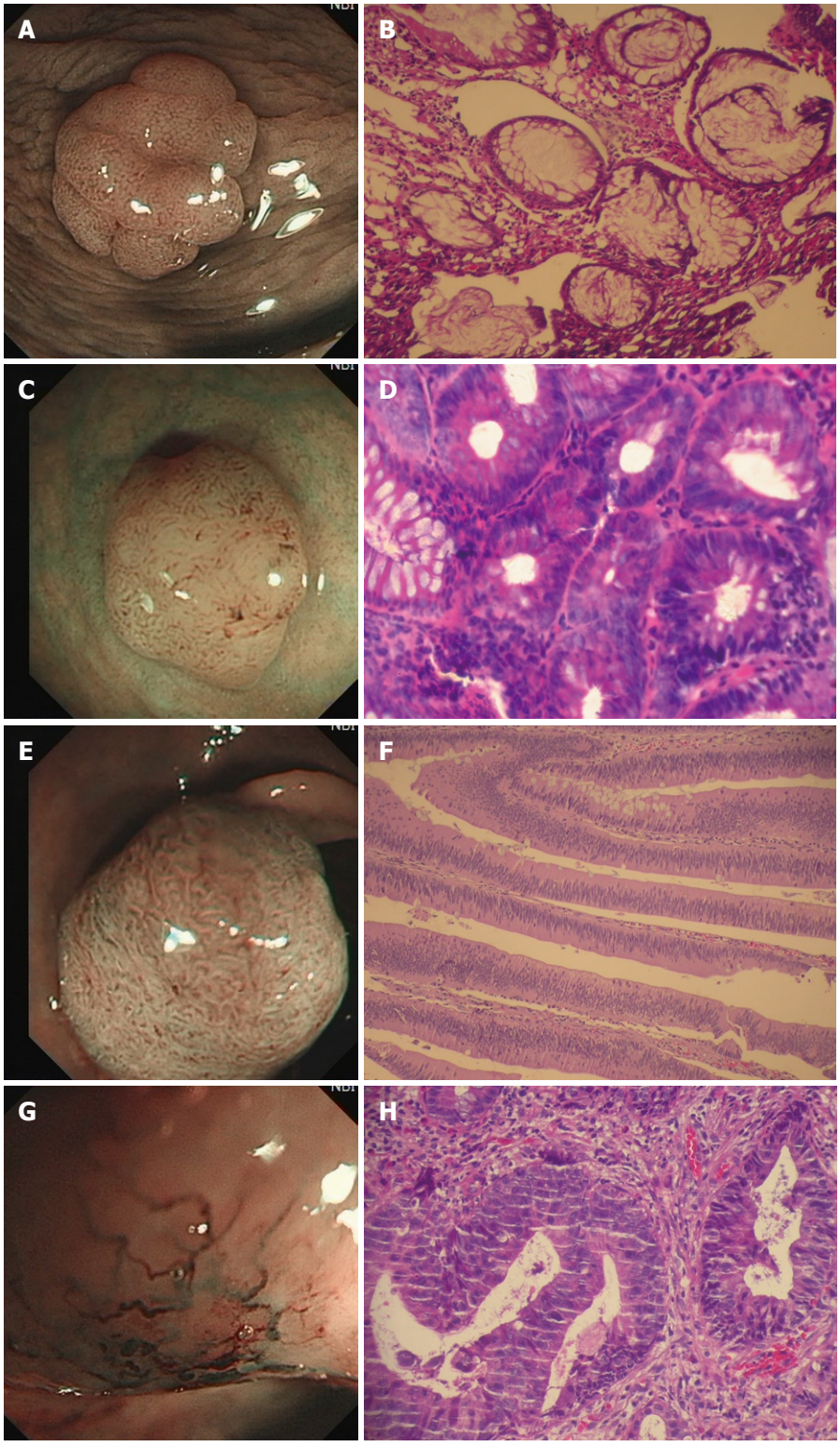


Figure 1 Narrow-band imaging endoscopic evaluation and the corresponding pathological images. A, B: A hyperplastic polyp with narrow-band imaging (NBI) magnifying endoscopy: the pits were asteroid, slightly larger than normal, with spacious space, and there was no significant microvascular structure. A: Pit Ⅱ + CP1 (H260AZI); B: Hyperplastic polyp, 100 ×; C, D: A tubular adenoma with NBI conventional endoscopy: the pits were tubular, larger than normal, showing oval microvascular structure. C: Pit ⅢL + CP2 (CF240I); D: Tubular adenoma, 400 ×; E, F: A villous adenoma with NBI conventional endoscopy: The pits were branching or gyrus-like, showing oval capillary. E: Pit Ⅳ + CP2 (CF240I); F: Villous adenoma, 100 ×; G, H: An adenocarcinoma with NBI magnifying endoscopy: the pits in the cancerous part completely disappeared, showing irregular angiogenesis network. G: Pit Ⅴ + CP3 (H260AZI); H: Adenocarcinoma, 200 ×.

row-band filters in the endoscopic system, which allow for a more detailed visualization of the mucosal architecture and vascular pattern. Current NBI technology limits the mucosal surface light penetration, thereby enhancing the visualization of the fine capillary vessel structure on the surface layer[10]. According to a previous pilot study by

Table 2 Diagnostic efficacies of narrow-band imaging endoscopy with and without magnification

Pathological diagnosis	NBI without magnification		NBI with magnification	
	Consistent	Inconsistent	Consistent	Inconsistent
Hyperplastic polyp	21	3	12	1
Adenoma	37	4	20	2
Adenocarcinoma	5	0	8	0
Total	63	7	40	3

NBI: Narrow-band imaging.

Machida *et al*[9], NBI with magnifying endoscopy achieved better visualization of the mucosal vascular network pattern than conventional white light imaging, and the diagnostic accuracy was higher than that of conventional colonoscopy and equivalent to chromoendoscopy. Furthermore, compared with chromoendoscopy, the NBI observation has the advantage of convenient application without the necessity of dye spraying, thus the procedure can be shortened in time and an overlooked lesion with accumulation of dark-blue dye at the dependent portion of colon can also be avoided[11].

According to previous pathological studies, most of the colorectal cancers arise from preexisting adenomas and such an adenoma-carcinoma sequence, and the adenoma shares many architectural features with the carcinoma in terms of vascular architecture including vessel diameter and spatial distribution which is considerably different from that in the non-neoplastic portion of colonic mucosa[12]. Therefore, NBI endoscopy has a significant advantage in the diagnosis of colorectal dysplasia accompanied with microvascular changes. In recent years, a number of researches[9,13-15] have shown that the diagnostic accuracy of NBI endoscopy in distinguishing neoplastic from non-neoplastic colorectal lesions was higher than that of conventional colonoscopy and equivalent to chromoendoscopy. In our study, we used Kudo's classification of mucosal pit pattern and Sano's classification of capillary pattern, and the diagnostic accuracy, sensitivity and specificity to distinguish between non-neoplastic and neoplastic colorectal lesions were 94.7% (72/76), 88.9% (72/81) and 90.5% (38/42) for pit pattern delineation, which is similar to the data reported in the literature (92.7%, 95.7% and 87.5%), significantly higher than that of conventional endoscopy (82.9%, 80.0% and 81.8%)[14]; and 91.7% (77/84), 95.1% (77/81), 83.3% (35/42) for capillary pattern delineation, which is slightly lower than the data reported in the literature (96.6%, 97.1% and 91.8%)[16], but there was no significant difference. It may be related to the relatively small number of the cases in this study. In addition, it is possible that endoscopists who are familiar with NBI endoscopy may also improve the diagnostic accuracy, but this requires further investigation.

In recent years, as NBI combined with magnifying endoscopy could enhance the contrast detailed morphological changes in the mucosal surface and clearly visualize the microvascular structures, most studies described the use of NBI endoscopy with magnification[13,17-22], but few data about diagnostic accuracy of NBI endoscopy without magnification were reported. However, magnifying endoscopy is not clinically used as standard endoscopic equipment in most institutions, not only in China but also in Japan and some Western countries, because magnifying endoscopy is much more expensive. This greatly restricted the wide application of NBI magnifying endoscopy. In order to evaluate the diagnostic efficacies of NBI conventional endoscopy in distinguishing neoplastic from non-neoplastic colorectal lesions, we compared the diagnostic accuracy of NBI with and without high magnification. The results showed that the overall diagnostic accuracy of NBI endoscopy in distinguishing neoplastic from non-neoplastic colorectal lesions was 91.2% (103/113), and that of NBI endoscopy with and without high magnification was 93.0% (40/43) and 90.0% (63/70), respectively. Both were significantly higher than that of conventional colonoscopy reported in the literature, but there was no significant difference between the two groups. Therefore, we believe that NBI endoscopy without high magnification could also greatly improve the diagnostic accuracy and may also be used to distinguish neoplastic from non-neoplastic colorectal lesions instead of NBI magnifying endoscopy. However, as the sample was relatively small in our study, it was not clear whether NBI without high magnification could improve the detection rate in the mass population screening; this requires further study and investigation.

As a new non-invasive endoscopic method, the diagnostic efficacies of NBI combined with magnifying endoscopy in distinguishing neoplastic from non-neoplastic colorectal lesions has been confirmed by extensive literatures[13,14,23-25]. We found in our study that the endoscopic system installed with the NBI system enables the clinicians to significantly improve the diagnostic accuracy. Therefore, even the institutions without the expensive magnifying endoscopy equipment can also use conventional NBI endoscopy to get an accurate diagnosis. Although these findings need to be confirmed in large prospective trials, this initial experience with conventional NBI endoscopy is encouraging and holds promise for future application in prospective studies. In addition, endoscopists, through training in NBI endoscopic practice, may also improve their own diagnostic accuracy and raise the detection rate of colorectal neoplasia[26].

COMMENTS

Background

Narrow-band imaging (NBI) is a novel technology developed for endoscopic diagnosis of early cancer, and it has a higher diagnostic accuracy than conventional endoscopy by enhancing the visualization of surface mucosal and vascular patterns on the lesion surface.

Research frontiers

In recent years, as NBI combined with magnifying endoscopy could enhance the contrast detailed morphological changes in the mucosal surface and clearly visualize the microvascular structures, most studies described the use of NBI endoscopy with magnification, but few data about diagnostic accuracy of NBI endoscopy without magnification were reported.

Innovations and breakthroughs

Compared with NBI magnifying endoscopy, NBI endoscopy without magnification may be used in distinguishing neoplasia from non-neoplasia colorectal lesions.

Applications

NBI endoscopy enables clinicians to significantly improve their diagnostic accuracy. Even those institutions equipped without magnifying endoscope can also use NBI conventional endoscopy to get an accurate diagnosis.

Terminology

NBI is a novel imaging technology that uses special narrow-band filters in the endoscopic system, which allow for a more detailed visualization of the mucosal architecture and vascular pattern.

Peer review

This paper compares narrow band imaging with and without high magnification in 100 patient with colorectal lesions over an 18 mo period. The authors show that both of these techniques are more accurate than conventional colonoscopy in distinguishing between neoplastic and non-neoplastic lesions with similar sensitivity. A larger study would have had more value.

REFERENCES

1 **Allen JI**. Molecular biology of colon polyps and colon cancer. *Semin Surg Oncol* 1995; **11**: 399-405
2 **Carethers JM**. The cellular and molecular pathogenesis of colorectal cancer. *Gastroenterol Clin North Am* 1996; **25**: 737-754
3 **Niv Y**, Dickman R, Figer A, Abuksis G, Fraser G. Case-control study of screening colonoscopy in relatives of patients with colorectal cancer. *Am J Gastroenterol* 2003; **98**: 486-489
4 **Rockey DC**, Paulson E, Niedzwiecki D, Davis W, Bosworth HB, Sanders L, Yee J, Henderson J, Hatten P, Burdick S, Sanyal A, Rubin DT, Sterling M, Akerkar G, Bhutani MS, Binmoeller K, Garvie J, Bini EJ, McQuaid K, Foster WL, Thompson WM, Dachman A, Halvorsen R. Analysis of air contrast barium enema, computed tomographic colonography, and colonoscopy: prospective comparison. *Lancet* 2005; **365**: 305-311
5 **Hurlstone DP**, Fujii T. Practical uses of chromoendoscopy and magnification at colonoscopy. *Gastrointest Endosc Clin N Am* 2005; **15**: 687-702
6 **Gono K**, Obi T, Yamaguchi M, Ohyama N, Machida H, Sano Y, Yoshida S, Hamamoto Y, Endo T. Appearance of enhanced tissue features in narrow-band endoscopic imaging. *J Biomed Opt* 2004; **9**: 568-577
7 **Kudo S**, Tamura S, Nakajima T, Yamano H, Kusaka H, Watanabe H. Diagnosis of colorectal tumorous lesions by magnifying endoscopy. *Gastrointest Endosc* 1996; **44**: 8-14
8 **Ikematsu H**, Matsuda T, Emura F, Saito Y, Uraoka T, Fu KI, Kaneko K, Ochiai A, Fujimori T, Sano Y. Efficacy of capillary pattern type IIIA/IIIB by magnifying narrow band imaging for estimating depth of invasion of early colorectal neoplasms. *BMC Gastroenterol* 2010; **10**: 33
9 **Machida H**, Sano Y, Hamamoto Y, Muto M, Kozu T, Tajiri H, Yoshida S. Narrow-band imaging in the diagnosis of colorectal mucosal lesions: a pilot study. *Endoscopy* 2004; **36**: 1094-1098
10 **Song LM**, Adler DG, Conway JD, Diehl DL, Farraye FA, Kantsevoy SV, Kwon R, Mamula P, Rodriguez B, Shah RJ, Tierney WM. Narrow band imaging and multiband imaging. *Gastrointest Endosc* 2008; **67**: 581-589
11 **Emura F**, Saito Y, Ikematsu H. Narrow-band imaging optical chromocolonoscopy: advantages and limitations. *World J Gastroenterol* 2008; **14**: 4867-4872
12 **Konerding MA**, Fait E, Gaumann A. 3D microvascular architecture of pre-cancerous lesions and invasive carcinomas of the colon. *Br J Cancer* 2001; **84**: 1354-1362
13 **Hirata M**, Tanaka S, Oka S, Kaneko I, Yoshida S, Yoshihara M, Chayama K. Magnifying endoscopy with narrow band imaging for diagnosis of colorectal tumors. *Gastrointest Endosc* 2007; **65**: 988-995
14 **Su MY**, Hsu CM, Ho YP, Chen PC, Lin CJ, Chiu CT. Comparative study of conventional colonoscopy, chromoendoscopy, and narrow-band imaging systems in differential diagnosis of neoplastic and nonneoplastic colonic polyps. *Am J Gastroenterol* 2006; **101**: 2711-2716
15 **Chang CC**, Hsieh CR, Lou HY, Fang CL, Tiong C, Wang JJ, Wei IV, Wu SC, Chen JN, Wang YH. Comparative study of conventional colonoscopy, magnifying chromoendoscopy, and magnifying narrow-band imaging systems in the differential diagnosis of small colonic polyps between trainee and experienced endoscopist. *Int J Colorectal Dis* 2009; **24**: 1413-1419
16 **Sano Y**, Ikematsu H, Fu KI, Emura F, Katagiri A, Horimatsu T, Kaneko K, Soetikno R, Yoshida S. Meshed capillary vessels by use of narrow-band imaging for differential diagnosis of small colorectal polyps. *Gastrointest Endosc* 2009; **69**: 278-283
17 **Horimatsu T**, Sano Y, Kaneko K, Ikematsu H, Katagiri A, Yano T, Fu KI, Muto M, Fujii S, Ochiai A, Yoshida S. Relationship between MVD and meshed-capillaries using magnifying NBI colonoscopy in colorectal precursor lesions. *Hepatogastroenterology* 2009; **56**: 372-377
18 **Kanao H**, Tanaka S, Oka S, Hirata M, Yoshida S, Chayama K. Narrow-band imaging magnification predicts the histology and invasion depth of colorectal tumors. *Gastrointest Endosc* 2009; **69**: 631-636
19 **Katagiri A**, Fu KI, Sano Y, Ikematsu H, Horimatsu T, Kaneko K, Muto M, Yoshida S. Narrow band imaging with magnifying colonoscopy as diagnostic tool for predicting histology of early colorectal neoplasia. *Aliment Pharmacol Ther* 2008; **27**: 1269-1274
20 **Hirata M**, Tanaka S, Oka S, Kaneko I, Yoshida S, Yoshihara M, Chayama K. Evaluation of microvessels in colorectal tumors by narrow band imaging magnification. *Gastrointest Endosc* 2007; **66**: 945-952
21 **Muto M**, Horimatsu T, Ezoe Y, Morita S, Miyamoto S. Improving visualization techniques by narrow band imaging and magnification endoscopy. *J Gastroenterol Hepatol* 2009; **24**: 1333-1346
22 **Uraoka T**, Saito Y, Matsuda T, Sano Y, Ikehara H, Mashimo Y, Kikuchi T, Saito D, Saito H. Detectability of colorectal neoplastic lesions using a narrow-band imaging system: a pilot study. *J Gastroenterol Hepatol* 2008; **23**: 1810-1815
23 **Fukuzawa M**, Saito Y, Matsuda T, Uraoka T, Itoi T, Moriyasu F. Effectiveness of narrow-band imaging magnification for invasion depth in early colorectal cancer. *World J Gastroenterol* 2010; **16**: 1727-1734
24 **Rastogi A**, Pondugula K, Bansal A, Wani S, Keighley J, Sugar J, Callahan P, Sharma P. Recognition of surface mucosal and vascular patterns of colon polyps by using narrow-band imaging: interobserver and intraobserver agreement and prediction of polyp histology. *Gastrointest Endosc* 2009; **69**: 716-722
25 **Chiu HM**, Chang CY, Chen CC, Lee YC, Wu MS, Lin JT, Shun CT, Wang HP. A prospective comparative study of narrow-band imaging, chromoendoscopy, and conventional colonoscopy in the diagnosis of colorectal neoplasia. *Gut* 2007; **56**: 373-379
26 **Adler A**, Pohl H, Papanikolaou IS, Abou-Rebyeh H, Schachschal G, Veltzke-Schlieker W, Khalifa AC, Setka E, Koch M, Wiedenmann B, Rösch T. A prospective randomised study on narrow-band imaging versus conventional colonoscopy for adenoma detection: does narrow-band imaging induce a learning effect? *Gut* 2008; **57**: 59-64

S- Editor Tian L **L- Editor** Ma JY **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.671

World J Gastroenterol 2011 February 7; 17(5): 671-676
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

BRIEF ARTICLE

Fast-track rehabilitation program *vs* conventional care after colorectal resection: A randomized clinical trial

Gang Wang, Zhi-Wei Jiang, Jing Xu, Jian-Feng Gong, Yang Bao, Li-Fei Xie, Jie-Shou Li

Gang Wang, Zhi-Wei Jiang, Jian-Feng Gong, Yang Bao, Li-Fei Xie, Jie-Shou Li, Research Institute of General Surgery, Jinling Hospital, School of Medicine, Nanjing University, Nanjing 210002, Jiangsu Province, China
Jing Xu, Department of Medical Oncology, Jinling Hospital, School of Medicine, Nanjing University, Nanjing 210002, Jiangsu Province, China
Author contributions: Wang G, Jiang ZW and Li JS designed the research; Wang G, Jiang ZW, Xu J, Gong JF, Bao Y and Xie LF performed the research; Wang G analyzed the data; Wang G and Xu J wrote the paper.
Supported by Social Development Fund of Jiangsu Province, No. BS2007054
Correspondence to: Zhi-Wei Jiang, MD, Research Institute of General Surgery, Jinling Hospital, School of Medicine, Nanjing University, 305 Zhongshan East Road, Nanjing 210002, Jiangsu Province, China. surgery34@163.com
Telephone: +86-25-80860137 Fax: +86-25-84803956
Received: October 11, 2010 Revised: November 17, 2010
Accepted: November 24, 2010
Published online: February 7, 2011

Abstract

AIM: To compare the fast-track rehabilitation program and conventional care for patients after resection of colorectal cancer.

METHODS: One hundred and six consecutive patients who underwent fast-track rehabilitation program were encouraged to have early oral feeding and movement for early discharge, while 104 consecutive patients underwent conventional care after resection of colorectal cancer. Their gastrointestinal functions, postoperative complications and hospital stay time were recorded.

RESULTS: The restoration time of gastrointestinal functions in the patients was significantly faster after fast-track rehabilitation program than after conventional care (2.1 d *vs* 3.2 d, $P < 0.01$). The percentage of patients who developed complications was significantly lower 30 d after fast-track rehabilitation program than after conventional care (13.2% *vs* 26.9%, $P < 0.05$). Also, the percentage of patients who had general complications was significantly lower 30 d after fast-track rehabilitation program than after conventional care (6.6% *vs* 15.4%, $P < 0.05$). The postoperative hospital stay time of the patients was shorter after fast-track rehabilitation program than after conventional care (5 d *vs* 7 d, $P < 0.01$). No significant difference was observed in the readmission rate 30 d after fast-track rehabilitation program and conventional care (3.8% *vs* 8.7%).

CONCLUSION: The fast-track rehabilitation program can significantly decrease the complications and shorten the time of postoperative hospital stay of patients after resection colorectal cancer.

Key words: Perioperative care; Fast track; Rehabilitation; Colorectal cancer resection

Peer reviewer: Luca Stocchi, MD, Desk A 30, Department of Colorectal Surgery, Digestive Disease Institute, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, OH 44195, United States

Wang G, Jiang ZW, Xu J, Gong JF, Bao Y, Xie LF, Li JS. Fast-track rehabilitation program *vs* conventional care after colorectal resection: A randomized clinical trial. *World J Gastroenterol* 2011; 17(5): 671-676 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/671.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.671

INTRODUCTION

The concept of fast track rehabilitation program has been recently introduced with the intent to improve the management, stress, complications, shorten hospital stay time and reduce cost of patients after resection of colorectal cancer[1-7]. Fast track rehabilitation program is basically a

multidisciplinary perioperative care strategy for patients after resection of colorectal cancer, including preoperative education, effective anesthesia, postoperative analgesia techniques, early oral nutrition and ambulation[8-11]. However, the previous researches were mainly focused on the postoperative complications after conventional care rather than on the general complications after fast-track rehabilitation program. This study was to compare the complications, restoration of gastrointestinal functions, and hospital stay time of postoperative colorectal cancer patients after fast -track rehabilitation program and conventional care.

MATERIALS AND METHODS

Patients and procedures

Two hundred and thirty patients who underwent resection colorectal cancer in the Research Institute of General Surgery, Jinling Hospital (Nanjing, China) in July 2007 to August 2009 were enrolled in this study. Of the 230 patients, 115 who underwent resection of colorectal neoplastic disease served as a fast-track rehabilitation grogram group, and 115 who underwent resection of colorectal cancer served as a conventional care group. Nine patients with non-selective admission, preoperative distant metastasis, stoma, emergency situations, scheduled total colectomy or abdominoperineal resection, contraindications for epidural anesthesia or early ambulation were excluded from the fast-track rehabilitation grogram group, and 11 from the conventional care group. Finally, 106 patients in the fast-track rehabilitation program group and 104 patients in the conventional care group were analyzed in this study.

The contents of fast-track rehabilitation program include preoperative education of patients with no bowel preparation and fasting but with carbohydrate containing liquids 2 h before surgery, analgesia with routine oral non-steroidal anti-inflammatory medications and minimization of opioid pain management, avoidance of perioperative fluid overload, no routine use of nasogastric tubes, early removal of bladder catheters, early feeding and enforced ambulation on the day of surgery. In the fast track rehabilitation program group, minimal-access surgery or transverse curved incision used included right-sided hemicolectomy through a right horizontal incision above the umbilicus, sigmoid resection through a curved incision in the left iliac fossa and low anterior rectal resection through a mini-laparotomy in the subumbilicus which was extended toward the curvature if necessary. Principles of the perioperative care are shown in Table 1.

Discharge criteria for patients in both groups were the same, including tolerance to fluids and solid diet, adequate oral analgesia, passage of flatus or stool, and no surgical complication, basic self-care ability, and acceptance of discharge.

Clinical outcome

The intestinal function was defined as passage of flatus, morbidity requiring treatment during the first 30 postoperative days, postoperative hospital stay time, and readmission rate. No patient was lost during the follow-up. General complications were defined as those occurred in the cardiovascular, pulmonary, thromboembolic, urinary systems, while surgical complications were defined as wound complication, anastomotic leak, and bowel obstruction requiring reoperation as previously described[12].

Statistical analysis

Statistical analysis, based on an intention-to-treat analysis, was performed with the SPSS version 16.0 (Chicago, IL, USA). Mann-Whitney test was used to compare the continuous variables. χ^2 test and Fisher's exact test were used to compare the discrete variables. P <0.05 was considered statistically significant.

RESULTS

Of the 230 enrolled patients (115 in the fast track rehabilitation grogram group and 115 in the conventional care group), 210 were analyzed (106 in the fast track rehabilitation program group and 104 in the conventional care group). The relevant characteristics of patients and the types of surgery are shown in Table 2. No significant difference was observed in age, ASA status, types of surgery and tumor stages between the two groups.

The intestinal function of patients in the fast track rehabilitation program group and conventional care group became normal 2 d (range, 1-6 d) and 3 d (range, 1-8 d), respectively, after resection of colorectal cancer ($P < 0.01$). The median postoperative hospital stay time was 5 d (range, 2-41 d) and 7 d (range, 3-55 d), respectively, for the patients in the fast track rehabilitation program group and conventional care group ($P < 0.01$). The postoperative rehabilitation was also faster in patients of the fast track rehabilitation program group than in those of conventional care group. On the day of surgery, 11 patients (35%) in the fast track rehabilitation program group and no patient in the conventional care group were able to walk. On postoperative day 1, 56 patients (53%) in the fast track rehabilitation program group and 24 patients (23%) in the conventional care program group were able to walk. On postoperative day 2, 90 patients (85%) in the fast track rehabilitation program group and 61 patients (59%) in the conventional care group were able to walk ($P < 0.01$) (Table 3).

The urethral catheter in 81 patients (81%) of the fast track rehabilitation program group and in 21 patients (20%) of the conventional care group was removed on day 1 after resection of colorectal cancer ($P < 0.05$), and in 97 patients (92%) of the fast track rehabilitation program group and in 47 patients (45%) of the conventional care group on day 2 after resection of colorectal cancer ($P < 0.05$). Urinary retention occurred in 5 patients (5%) of the fast track rehabilitation program group and in 16 patients (15%) of the conventional care group. Urethral catheter was inserted again in 4 patients of the fast track rehabilitation program group and in 12 patients of the conventional care group.

Table 1 Principles of fast track rehabilitation program and conventional care

	Fast track rehabilitation program	Conventional care
Preoperative	Patients and their relatives were informed about the surgical procedure and postoperative course	Patient were educated in the standard manner
Day before surgery		
Bowel preparation	No bowel preparation was performed	Two oral sachets of fleet® bowel preparation
Carbohydrate load	4 units (preOp®)	No
Diet	Last meal 6 h before operation	Last meal at midnight
Day of surgery		
Pre-operative fasting	No, 2 units (preOp®) 2 h before surgery	Yes
Nasogastric tubes	No unless nausea and vomit	Routine placement
Pre-anesthetic medication	No	Oral diazepam 10 mg
Anesthesia	General anesthesia Remifentanil 1 μg/kg per minute Propofol 2-4 mg/kg per hour Cisatracium 0.15 mg/kg Ondansetron 4 mg Bupivacaine 0.25% 20 mL (incision) Epidural catheter T10-T12 Test: 3 mL 2% lidocaine with epinephrine Bupivacaine 0.5% (6 + 6) mL	General anesthesia Remifentanil 1 μg/kg per minute Propofol 2-4 mg/kg per hour Cisatracium 0.15 mg/kg Ondansetron 4 mg
Surgical management	Minimal invasive incision Infiltration of surgical wounds with Bupivacaine	Median laparotomy approach No infiltration of surgical wounds with local anesthetic drugs
Surgical drains	No, unless required in circumstances and discarded in early time (usually on postoperative day 1)	Routine placement usually discarded the day before discharge
Early post-operative care	Use of epidural catheter (0.125% Bupivacaine with 2.5 μg/mL Fentanyl) First oral drink 2 h after surgery IV infusion of Ringers lactate 1.5 L/d Mobilization in the evening (> 2 h out of bed)	Analgesia by bolus administration of diclofenac or morphine No oral application scheme IV infusion of Ringers lactate 2.5 L/d No mobilization scheme
Postoperative care		
Day 1 after surgery	Oral intake > 2 L (including 4 units CHL liquids) Semi-solid food intake Stop IV fluid administration Remove urine catheter Expand mobilization (> 6 h out of bed)	Diet increased on daily basis IV fluid administration (2.5 L/d) till adequate oral fluid intake Mobilization according to attending surgeon
Day 2 after surgery	Remove epidural add Diclofenac 3 × 50 mg/d Normal diet Expand mobilization (> 8 h) Plan discharge	Continue as on day 1 till discharge criteria fulfilled
Day 3 after surgery	Continue as on day 2 till discharge criteria fulfilled	Continue as on day 2 till discharge criteria fulfilled

Table 2 Characteristics of patients and their diagnosis

	Fast track rehabilitation group (*n* = 106)	Conventional care group (*n* = 104)	*P* value
Median age (range, yr)	57 (38-69)	55 (40-67)	0.462
Male/female	65/41	60/44	0.393
Colon/rectum	73/33	63/41	0.110
ASA score			0.384
Ⅰ	27	32	-
Ⅱ	60	56	-
Ⅲ	19	16	-
Operation			0.721
Right hemicolectomy	30	24	
Left hemicolectomy	18	26	
Sigmoid colectomy	28	32	
Anterior resection	30	22	
TNM stage			0.741
Ⅰ	19	17	
Ⅱ	56	61	
Ⅲ	31	26	

Table 3 Postoperative rehabilitation and hospital stay time of two groups *n* (%)

	Fast track rehabilitation group (*n* = 106)	Conventional care group (*n* = 104)	*P* value
Walk on surgery day	11 (35)	0 (0)	0.001
Walk on D 1	56 (53)	24 (23)	0.000
Walk on D 2	90 (85)	61 (59)	0.001
Days until flatus			0.001
mean ± SD	2.1 ± 2.0	3.2 ± 2.5	-
Median (range)	2 (1-6)	3 (1-8)	-
Hospital stay time (d)			0.001
mean ± SD	5.1 ± 3.1	7.6 ± 4.8	-
Median (range)	5 (2-41)	7 (3-55)	-

The nasogastric tube was maintained for 1-4 d in 3 patients (3%) of the fast track rehabilitation program group and for 1-11 d in 84 patients (81%) of the conventional

Table 4 General and surgical complications of two groups

	Fast track rehabilitation group (n = 106)	Conventional care group (n = 104)	P value
Overall complications	20	39	0.015
Patients with complications	14	28	0.016
General complications	10	23	0.042
Patients with general complications	7	16	0.048
Cardiac	2	5	-
Pulmonary	3	8	-
Thromboembolic	1	3	-
Urinary tract	2	5	-
Other	2	2	-
Overall surgical complications	10	16	0.221
Patients with surgical complications	7	12	0.230
Wound infection	4	7	-
Anastomotic leakage	4	2	-
Bowel obstruction	2	5	-
Death	2	1	0.572

care group ($P < 0.01$). The nasogastric tube was reinserted in 4 patients (4%) of the fast track rehabilitation program group and in 12 patients (11%) of the conventional care group due to nausea and vomit ($P < 0.05$).

No significant difference was observed in re-admission rate between the two groups within 30 d after resection of colorectal cancer. Four patients (4%) in the fast track rehabilitation program group were readmitted due to wound infection, and 9 patients (9%) in the conventional care group were readmitted due to bowel obstruction, vomit and wound infection.

The incidence of complications was 19% in patients of the fast track rehabilitation program group and 38% in those of the conventional care group ($P < 0.05$) during the first 30 postoperative days. One or more complications occurred in 14 patients (13%) of the fast track rehabilitation program group and in 28 patients (27%) of the conventional care group ($P < 0.05$). The overall incidence of general complications was lower in patients of the fast track rehabilitation program group than in those of the conventional care group ($P < 0.05$). The incidence of complications in the cardiac and pulmonary system was also significantly lower in patients of the fast track rehabilitation program group than in those of the conventional care group ($P < 0.05$). A significant difference was observed in surgical complications between the two groups. Two patients died in the fast track rehabilitation program group due to cardiovascular failure and multiple organ failure, respectively. One patient died in the conventional care group due to cardiovascular failure. The types of complications are listed in Table 4.

DISCUSSION

The results of the present study indicate that fast-track rehabilitation program can significantly accelerate the restoration of gastrointestinal function and reduce the postoperative complications as well as hospital stay time of patients after resection of colorectal cancer. The results of this study show that preoperative education of patients, epidural anesthesia or regional anesthesia[13], early ambulation and early postoperative oral nutrition are the important predictors for the rehabilitation of patients after resection of colorectal cancer.

Preoperative education of patients is regarded as one of the crucial factors for fast-track rehabilitation. It is necessary to explain the detailed treatment plan, different stages of fast-track rehabilitation program and relevant measures for recovery for the patients in order to make them better understand the importance of fast-track rehabilitation program. Better cooperation of patients can bring better outcomes of fast track rehabilitation program. Generally, since the gastric emptying time of solid meal and fluid is 6 and 2 h, respectively[14], the patients should be encouraged to have liquid meal 2 h before operation instead of fasting. It has been shown that preoperative oral carbohydrate is safe and can efficiently reduce complications[15-17].

The role of epidural anesthesia or regional anesthesia in fast-track rehabilitation program should be stressed. Postoperative epidural analgesia can avoid stress-induced neurological, endocrinological and homeostatic changes or the blocking of sympathetic nerve-related surgical stress response, reduce complications such nausea, vomiting and enteroparalysis after operation, early ambulation, improve the intestinal function and shorten the hospital stay time of patients after resection of colorectal cancer[18-24]. In this study, epidural analgesia significantly shortened the bedridden time and potentially reduced the cardiopulmonary and thromboembolic complications. The rate of cardiopulmonary and thromboembolic complications was much lower in patients of the fast track rehabilitation program group than in those of the conventional care group ($P < 0.05$).

Early postoperative oral nutrition also plays an essential part in fast-track rehabilitation program. Food intake can stimulate gastrointestinal peristalsis, and early feeding during the first 24 h after surgery promotes the recovery of ileus. It has been illustrated that early postoperative oral nutrition attenuates catabolism and potentially decreases infectious complications[25,26]. Consistent with this, early postoperative oral nutrition has been suggested as a routine procedure of abdominal surgery[26]. Enforced postoperative mobilization of patients can reduce protein loss due to long-term bedridden, pulmonary infection and venous thrombosis. In this study, complete analgesia, control of nausea and vomiting, early postoperative oral nutrition and early ambulation efficiently reduced the postoperative complication of ileus and improved the recovery of intestinal function.

In this study, the early removal of gastric tube and urethral catheter decreased not only the infectious complications in cardiopulmonary and urinary systems but also the symptoms of patients. The shortened fasting

time, preoperative carbohydrate load and intraoperative fluid restriction effectively protected against homeostasis in patients after resection of colorectal cancer. The outcome of fast-track rehabilitation program was better than that of conventional care.

Fast track rehabilitation program can improve the symptoms of patients after resection of colorectal cancer better than conventional care, thus benefiting their surgery, anesthesia, pain management, physical therapy and social work. The primary work of fast track rehabilitation program is the preoperative education of patients to make them understand the whole plan and the aim of each stage. Therefore, it is necessary to get the cooperation from nurses, because they need to work professionally and nicely. Although there must be lots of difficulties in fast track rehabilitation program, it is an inevitable stage to test a new set of rules and guidelines.

Recently, laparoscopic surgery, applied in treatment of colorectal and early gastric cancer, can significantly reduce trauma and speed up the rehabilitation of patients after surgery. It was reported that the hospital stay time is shorter and the morbidity and readmission rate are lower after laparoscopic surgery[27,28]. However, these studies only compared open surgery with laparoscopic surgery rather than laparoscopic surgery with fast-track rehabilitation program[27,28]. Therefore, further studies are needed to focus on the potential influence of laparoscopy-assisted surgery with or without fast-track rehabilitation program on the recovery of patients after resection of colorectal cancer. Laparoscopic surgery and fast-track rehabilitation program can effectively promote the recovery of patients after resection of colorectal cancer. We believe that laparoscopic surgery in combination with fast track rehabilitation program is significantly advantageous over other procedures for patients after resection of colorectal cancer.

In conclusion, fast track rehabilitation program plays an important role in the recovery of patients after resection of colorectal cancer, which can accelerate the restoration of their gastrointestinal function, decrease their postoperative complications, and shorten their hospital stay time.

COMMENTS

Background

Fast-track rehabilitation program, first reported by Kehlet *et al*, can reduce the postoperative complications and hospital stay time of patients after resection of colorectal cancer without compromising the surgical outcome. The concept of fast track rehabilitation program has been recently introduced in colorectal surgery. It is basically a multidisciplinary perioperative care strategy for patients after resection of colorectal cancer.

Research frontiers

The previous studies seemed to compare the postoperative complications rather than the general complications of fast tract rehabilitation program and conventional care.

Innovations and breakthroughs

The gastrointestinal function, postoperative complications, and hospital stay time of patients after resection of colorectal cancer were studied during their fast track rehabilitation program. The accelerated restoration of gastrointestinal function and decreased postoperative complications may shorten the hospital stay time of patients after resection of colorectal cancer.

Applications

Surgical care has changed dramatically over the past half century and will continue to improve with the time. Extensive studies on the optimized care will allow us to develop more appropriate perioperative surgical care programs for patients after resection of colorectal cancer.

Terminology

Fast track rehabilitation program, basically a multidisciplinary strategy for patients after resection of colorectal cancer, is to optimize the preoperative, perioperative and postoperative factors for reducing their physiological and psychological stress surgery.

Peer review

This manuscript describes a prospective randomized trial comparing fast track rehabilitation program and conventional care for patients after resection of colorectal cancer. The data are sound support the hypothesis of the authors.

REFERENCES

1 **Basse L**, Thorbøl JE, Løssl K, Kehlet H. Colonic surgery with accelerated rehabilitation or conventional care. *Dis Colon Rectum* 2004; **47**: 271-277; discussion 277-278
2 **Bradshaw BG**, Liu SS, Thirlby RC. Standardized perioperative care protocols and reduced length of stay after colon surgery. *J Am Coll Surg* 1998; **186**: 501-506
3 **Delaney CP**, Zutshi M, Senagore AJ, Remzi FH, Hammel J, Fazio VW. Prospective, randomized, controlled trial between a pathway of controlled rehabilitation with early ambulation and diet and traditional postoperative care after laparotomy and intestinal resection. *Dis Colon Rectum* 2003; **46**: 851-859
4 **DiFronzo LA**, Yamin N, Patel K, O'Connell TX. Benefits of early feeding and early hospital discharge in elderly patients undergoing open colon resection. *J Am Coll Surg* 2003; **197**: 747-752
5 **Anderson AD**, McNaught CE, MacFie J, Tring I, Barker P, Mitchell CJ. Randomized clinical trial of multimodal optimization and standard perioperative surgical care. *Br J Surg* 2003; **90**: 1497-1504
6 **Gatt M**, Anderson AD, Reddy BS, Hayward-Sampson P, Tring IC, MacFie J. Randomized clinical trial of multimodal optimization of surgical care in patients undergoing major colonic resection. *Br J Surg* 2005; **92**: 1354-1362
7 **Khoo CK**, Vickery CJ, Forsyth N, Vinall NS, Eyre-Brook IA. A prospective randomized controlled trial of multimodal perioperative management protocol in patients undergoing elective colorectal resection for cancer. *Ann Surg* 2007; **245**: 867-872
8 **Kehlet H**, Wilmore DW. Multimodal strategies to improve surgical outcome. *Am J Surg* 2002; **183**: 630-641
9 **Wind J**, Polle SW, Fung Kon Jin PH, Dejong CH, von Meyenfeldt MF, Ubbink DT, Gouma DJ, Bemelman WA. Systematic review of enhanced recovery programmes in colonic surgery. *Br J Surg* 2006; **93**: 800-809
10 **Fearon KC**, Ljungqvist O, Von Meyenfeldt M, Revhaug A, Dejong CH, Lassen K, Nygren J, Hausel J, Soop M, Andersen J, Kehlet H. Enhanced recovery after surgery: a consensus review of clinical care for patients undergoing colonic resection. *Clin Nutr* 2005; **24**: 466-477
11 **Kehlet H**. Fast-track colonic surgery: status and perspectives. *Recent Results Cancer Res* 2005; **165**: 8-13
12 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213
13 **Wilmore DW**, Kehlet H. Management of patients in fast track surgery. *BMJ* 2001; **322**: 473-476
14 **Ljungqvist O**, Søreide E. Preoperative fasting. *Br J Surg* 2003; **90**: 400-406
15 **Reissman P**, Teoh TA, Cohen SM, Weiss EG, Nogueras JJ,

Wexner SD. Is early oral feeding safe after elective colorectal surgery? A prospective randomized trial. *Ann Surg* 1995; **222**: 73-77
16 **Lewis SJ**, Egger M, Sylvester PA, Thomas S. Early enteral feeding versus "nil by mouth" after gastrointestinal surgery: systematic review and meta-analysis of controlled trials. *BMJ* 2001; **323**: 773-776
17 **Feo CV**, Romanini B, Sortini D, Ragazzi R, Zamboni P, Pansini GC, Liboni A. Early oral feeding after colorectal resection: a randomized controlled study. *ANZ J Surg* 2004; **74**: 298-301
18 **Wu CL**, Cohen SR, Richman JM, Rowlingson AJ, Courpas GE, Cheung K, Lin EE, Liu SS. Efficacy of postoperative patient-controlled and continuous infusion epidural analgesia versus intravenous patient-controlled analgesia with opioids: a meta-analysis. *Anesthesiology* 2005; **103**: 1079-1088; quiz 1109-1110
19 **Carli F**, Mayo N, Klubien K, Schricker T, Trudel J, Belliveau P. Epidural analgesia enhances functional exercise capacity and health-related quality of life after colonic surgery: results of a randomized trial. *Anesthesiology* 2002; **97**: 540-549
20 **Clemente A**, Carli F. The physiological effects of thoracic epidural anesthesia and analgesia on the cardiovascular, respiratory and gastrointestinal systems. *Minerva Anestesiol* 2008; **74**: 549-563
21 **Steinberg RB**, Liu SS, Wu CL, Mackey DC, Grass JA, Ahlén K, Jeppsson L. Comparison of ropivacaine-fentanyl patient-controlled epidural analgesia with morphine intravenous patient-controlled analgesia for perioperative analgesia and recovery after open colon surgery. *J Clin Anesth* 2002; **14**: 571-577
22 **Taqi A**, Hong X, Mistraletti G, Stein B, Charlebois P, Carli F. Thoracic epidural analgesia facilitates the restoration of bowel function and dietary intake in patients undergoing laparoscopic colon resection using a traditional, nonaccelerated, perioperative care program. *Surg Endosc* 2007; **21**: 247-252
23 **Marret E**, Remy C, Bonnet F. Meta-analysis of epidural analgesia versus parenteral opioid analgesia after colorectal surgery. *Br J Surg* 2007; **94**: 665-673
24 **Augestad KM**, Delaney CP. Postoperative ileus: impact of pharmacological treatment, laparoscopic surgery and enhanced recovery pathways. *World J Gastroenterol* 2010; **16**: 2067-2074
25 **Andersen HK**, Lewis SJ, Thomas S. Early enteral nutrition within 24h of colorectal surgery versus later commencement of feeding for postoperative complications. *Cochrane Database Syst Rev* 2006; CD004080
26 **Gatt M**, MacFie J. Randomized clinical trial of the impact of early enteral feeding on postoperative ileus and recovery (Br J Surg 2007; 94: 555-561). *Br J Surg* 2007; **94**: 1044-1045
27 **Kim HJ**, Lee IK, Lee YS, Kang WK, Park JK, Oh ST, Kim JG, Kim YH. A comparative study on the short-term clinicopathologic outcomes of laparoscopic surgery versus conventional open surgery for transverse colon cancer. *Surg Endosc* 2009; **23**: 1812-1817
28 **Steele SR**, Brown TA, Rush RM, Martin MJ. Laparoscopic vs open colectomy for colon cancer: results from a large nationwide population-based analysis. *J Gastrointest Surg* 2008; **12**: 583-591

S- Editor Tian L **L- Editor** Wang XL **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v17.i5.677

World J Gastroenterol 2011 February 7; 17(5): 677-680
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

CASE REPORT

In-vivo characterization of DALM in ulcerative colitis with high-resolution probe-based confocal laser endomicroscopy

Giovanni D De Palma, Stefania Staibano, Saverio Siciliano, Francesco Maione, Maria Siano, Dario Esposito, Giovanni Persico

Giovanni D De Palma, Saverio Siciliano, Francesco Maione, Dario Esposito, Giovanni Persico, Department of Surgery and Advanced Technologies, Center of Excellence for Technical Innovation in Surgery, Section of Diagnostic and Therapeutic Endoscopy, University of Naples Federico II, School of Medicine, 80131, Naples, Italy
Stefania Staibano, Maria Siano, Department of Biomorphological and Functional Sciences, Section of Pathology, University of Naples Federico II, School of Medicine, 80131, Naples, Italy
Author contributions: De Palma GD and Staibano S contributed to conception and design, analysis and interpretation of data, drafting the article, critical revision of the article, final approval of the article; Siciliano S, Siano M, Esposito D and Maione F contributed to data gathering, analysis and interpretation of the data, drafting the article, final approval of the article; Persico G contributed to critical revision of the article and final approval of the article.
Correspondence to: Giovanni D De Palma, Professor, Department of Surgery and Advanced Technologies, Center of Excellence for Technical Innovation in Surgery, Section of Diagnostic and Therapeutic Endoscopy, University of Naples Federico II, School of Medicine, Via Pansini, 5, 80131, Naples, Italy. giovanni.depalma@unina.it
Telephone: +39-81-7462773 Fax: +39-81-7462752
Received: July 20, 2010 Revised: September 9, 2010
Accepted: September 16, 2010
Published online: February 7, 2011

Abstract

Recently, the use of confocal laser endomicroscopy (CLE) in the diagnosis of chronic ulcerative colitis (CUC) was reported. In this brief report we aimed to assess the application of probe-based CLE to characterize colonic mucosa and dysplasia in CUC. The study involved a patient presenting long-standing CUC. Confocal imaging of both the inflamed mucosa, a circumscribed lesion (dysplasia-associated lesional mass), and adjacent colonic mucosa are demonstrated and the correlation between the CLE and histological images. Inflamed mucosa and dysplasia showed specific alteration of crypt architecture, cellular infiltration, and vessel architecture with an excellent correlation between CLE and standard histological examination.

Key words: Colonoscopy; Confocal laser endomicroscopy; Chronic ulcerative colitis; Dysplasia-associated lesional mass; Histology

Peer reviewer: Dr. Sanchoy Sarkar, Department of Gastroenterology, Royal Liverpool University Hospital, Prescot Street, Liverpool, L78XP, United Kingdom

De Palma GD, Staibano S, Siciliano S, Maione F, Siano M, Esposito D, Persico G. *In-vivo* characterization of DALM in ulcerative colitis with high-resolution probe-based confocal laser endomicroscopy. *World J Gastroenterol* 2011; 17(5): 677-680 Available from: URL: http://www.wjgnet.com/1007-9327/full/v17/i5/677.htm DOI: http://dx.doi.org/10.3748/wjg.v17.i5.677

INTRODUCTION

The term dysplasia-associated lesional mass (DALM) has been adopted to describe the group of endoscopically visible lesions, within the colitic colon (in the course of IBD), that refers to a heterogeneous population of lesions that demonstrate plaque-like, mass, stricture, sessile, or pedunculated morphology, that have an associated dysplasia in the surrounding mucosa[1-3].

With recent rapid advances in videoendoscopic instrument systems, improved endoscopic skills, and improved detection techniques such as pan-colonic dye spraying, the proportion of lesions that are discovered macroscopically is likely to increase[4].

Recently, the use of confocal laser endomicroscopy

(CLE) in the diagnosis of chronic ulcerative colitis (CUC) was reported[5,6].

This report describes the CLE findings in a case of DALM in CUC in correlation with histopathology diagnosis.

CASE REPORT

A 48-year-old woman with a 23 years history of left-sided ulcerative colitis underwent a surveillance colonoscopy.

A mild anemia (hemoglobin level, 110 g/L) with low serum iron levels (210 μg/L); normal value, 53-167 μg/L) and a high erythrocyte sedimentation rate (37 mm/L per hour; normal value, 1-10 mm/L per hour) were the only blood chemistry abnormalities identified.

Colonoscopy revealed a left-sided Mayo CU-1 CUC with a 2 cm plaque-like lesion at the sigmoid colon (Figure 1A and B). A morphological characterization of the lesion with CLE was undertaken.

Probe-based CLE procedure

The procedure was performed using the Cellvizio® Endomicroscopy System (Mauna Kea Technologies, Paris, France) by a Coloflex UHD-type probe (1 μm lateral resolution; 12 frames/s).

This system uses a 2.5-mm catheter probe (Coloflex UHD-type probe) that is inserted through the endoscope-working channel to obtain dynamic imaging of the mucosa. This probe has a field of view of 240 μm × 200 μm, with a lateral resolution of 1 μm. Probe-based CLE (pCLE) imaging data were collected at a scan rate of 12 frames/s with a scanning field of 30 000 pixels. Single video frames were reconstructed into 1 larger static image (4 mm × 2 mm) by a special computer software ("mosaicing" Mauna Kea Technologies).

Five mililiters of 10% sodium fluorescein were injected intravenously before CLE image acquisition as a contrast agent.

Confocal imaging of both the inflamed mucosa, the circumscribed lesion (DALM), and adjacent colorectal mucosa was performed by placing the tip of the probe in direct contact with the target tissue site.

Mucosal biopsy specimens were collected from the observation sites using biopsy forceps. Fixed samples were embedded in paraffin and sectioned transversely, and stained with hematoxylin-eosin to facilitate the comparison between confocal images and histology.

pCLE Images

pCLE imaging of inflamed mucosa showed dilation of crypt openings, more irregular arrangement of crypts, and enlarged spaces between crypt, crypt destruction, and/or crypt fusion and crypt abscess. Microvascular alterations with fluorescein leaks into the crypt lumen (therefore making the lumen brighter than the surrounding epithelium) were observed (Figure 2A and B).

DALM was characterized by "dark" cells, with mucin depletion and goblet cell/crypt density attenuation; the architectural pattern was irregular, as well as the epithelial thickness, with villiform structures and "dark" epithelial border. The blood vessels were dilatated and irregularly-branching, with poor orientation to adjunct tissue, and fluorescein extravasation (Figure 3A and B).

pCLE imaging of colorectal mucosa adjacent to lesion (1 to 2 cm around DALM) showed the switch from the inflamed mucosa, to the neoplastic mucosa as evidenced by DALM (Figure 4).

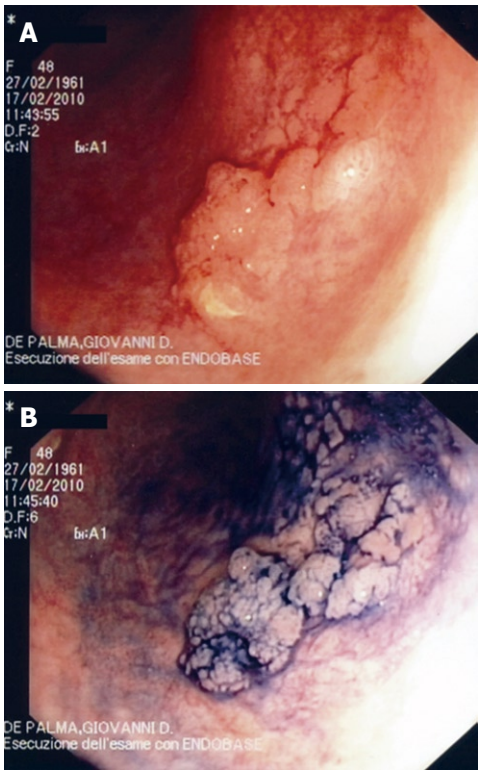


Figure 1 Conventional "white light" imaging of a plaque-like lesion of sigmoid colon in ulcerative colitis (A) and 0.5% indigo carmine chromoscopy of the lesion (B). The lesion is "unmasked" and clearly delineated.

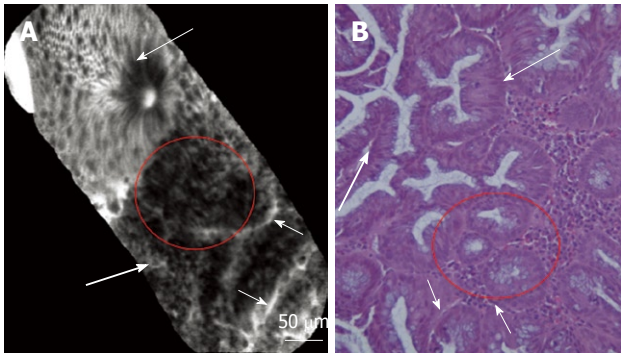


Figure 2 Confocal (A) and histological (B) images of colonic mucosa showing the switch from normal mucosa to inflamed mucosa. Normal crypt architecture is classically represented by ordered and regular crypt orifices covered by a homogeneous epithelial layer with visible "black-hole" goblet cells within the subcellular matrix (long thin arrows). Inflamed mucosa showing irregular arrangement of crypts, crypt fusion (red circles) and capillaries alterations (short arrows) and inflammatory cells (lymphocytes: long thick arrows). Magnification, × 200.

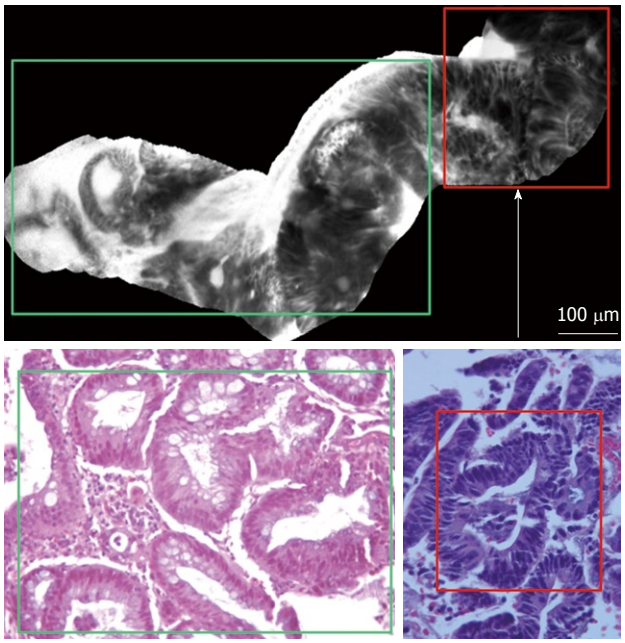


Figure 3 Confocal images of colonic mucosa evidencing the switch from the inflamed mucosa, to the neoplastic mucosa. Inflamed mucosa (green rectangle) is characterized by dilation of crypt openings, enlarged spaces between crypt, and microvascular alterations with fluorescein leaks into the crypt lumen (white arrow) therefore making the lumen brighter than the surrounding epithelium. Dysplastic mucosa (red rectangle) is characterized by "dark" cells, irregular architectural patterns with villiform structures and a "dark" epithelial border. Histology images show high-power hematoxylin and eosin stain of the tissue sampled, evidencing respectively inflamed area with features suggestive of chronic ulcerative colitis (green rectangle) and low grade dysplasia (red rectangle). Magnification, × 200.

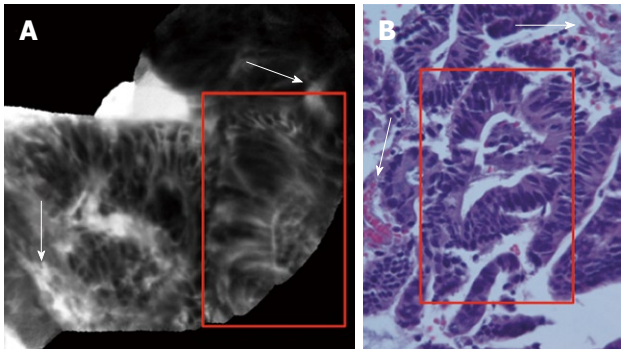


Figure 4 Confocal (A) and histological (B) images of dysplasia-associated lesional mass showing "dark" cells, with mucin depletion and goblet cell/crypt density attenuation; the architectural pattern is irregular, as well as the epithelial thickness, with villiform structures and "dark" epithelial border (red rectangles). There is gross distortion of the vascular architecture with tortuous and dilated vessels (white arrows). The hematoxylin and eosin stain histology shows a low grade dysplasia (red rectangle; hematoxylin and eosin staining; original magnification, × 200).

DISCUSSION

CLE is a new technology that has enabled endoscopists to collect real-time *in vivo* histological images or "virtual biopsies" of the gastrointestinal mucosa during endoscopy.

CLE can be performed currently with 2 devices: one integrated into an endoscope (Pentax, Japan, herein termed eCLE) and one as a stand-alone probe (herein termed pCLE) capable of passage through the accessory channel of most endoscopes (Cellvizio, Mauna Kea Technologies, Paris, France)[7-9]. There are no data, at present, comparing pCLE with eCLE to demonstrate the superiority of any one system. pCLE has several advantages and disadvantages compared with eCLE. Advantages include the greater versatility of pCLE probes, which can be used in conjunction with virtually any endoscope (high-resolution endoscopes, NBI, cholangioscope, *etc.*), ad hoc usage (such as when a lesion is detected with a normal endoscope) and acquisition at video frame rate of 12 frames/s. allowing *in vivo* imaging of capillary flow. Disadvantages include a slightly lower resolution (approximately 1 μm compared with 0.7 μm for eCLE) and smaller field of view (240-600 μm).

Recently, the use of eCLE in the diagnosis of CUC was reported. Watanabe *et al*[5] and Li *et al*[6] reported on real-time inflammation activity assessment by CLE. The inflammation activity assessment includes crypt architecture, cellular infiltration, and vessel architecture. These studies evidenced that images taken with the CLE provided information that was equivalent to conventional histology, differentiating between active and non-active CUC patients during ongoing endoscopy.

Hurlstone *et al*[10] assessed the clinical applicability and predictive power of the CLE for the *in vivo* differentiation of ALM and DALM in CUC. The study evidenced that ALM and DALM can be differentiated with a high overall accuracy, enabling the safe selection of patients suitable for endoluminal resection *vs* immediate referral for surgery.

To the best of our knowledge, this is the first report that addressed the application of pCLE for the *in vivo* characterization of colonic mucosa and DALM in a patient with CUC during ongoing videocolonoscopy.

Our study showed that the pCLE system permits high-quality cellular, subsurface vascular and stromal imaging *in vivo*, with an excellent correlation between CLE and standard histopathologic examination for both inflammation and dysplasia in ulcerative colitis.

Post-acquisition specifically-developed software ("mosaicing", Mauna Kea Technologies) was used to reconstitute the dynamic high-resolution pictures into a larger static image. By the use of mosaicing, the image area could be increased 2- to 4-fold, and image definition could be further enhanced to allow finer detail visualization. As a result of the large static image comprising of many single pictures of a video sequence, evaluation of the examination is easier and more efficient. Thereby, these features lead to an excellent correlation between CLE and standard histopathologic examination (Figures 1-4).

The main aspect of inflamed mucosa consisted of dilation of crypt openings, enlarged spaces between crypt, and microvascular alterations with fluorescein leaks into the crypt lumen (therefore making the lumen brighter than the surrounding epithelium); DALM showed typical neoplastic features of Mainz CLE criteria for prediction of intraepithelial neoplasia[11] (Figure 4).

In conclusion, this is the first study to address the novel applicability of pCLE for the *in vivo* characterization of mucosal inflammation and dysplasia in CUC. With appropriate training and careful patient selection, CLE imaging may become a suitable imaging modality in patients with CUC. The ability to target biopsies to areas suggestive of dysplasia *in vivo* allows rapid, highly accurate diagnosis "on table", reducing inappropriate, non-significant histopathology.

REFERENCES

1 **Blackstone MO**, Riddell RH, Rogers BH, Levin B. Dysplasia-associated lesion or mass (DALM) detected by colonoscopy in long-standing ulcerative colitis: an indication for colectomy. *Gastroenterology* 1981; **80**: 366-374

2 **Leidenius M**, Kellokumpu I, Husa A, Riihelä M, Sipponen P. Dysplasia and carcinoma in longstanding ulcerative colitis: an endoscopic and histological surveillance programme. *Gut* 1991; **32**: 1521-1525

3 **Lennard-Jones JE**, Melville DM, Morson BC, Ritchie JK, Williams CB. Precancer and cancer in extensive ulcerative colitis: findings among 401 patients over 22 years. *Gut* 1990; **31**: 800-806

4 **Kiesslich R**, Goetz M, Lammersdorf K, Schneider C, Burg J, Stolte M, Vieth M, Nafe B, Galle PR, Neurath MF. Chromoscopy-guided endomicroscopy increases the diagnostic yield of intraepithelial neoplasia in ulcerative colitis. *Gastroenterology* 2007; **132**: 874-882

5 **Watanabe O**, Ando T, Maeda O, Hasegawa M, Ishikawa D, Ishiguro K, Ohmiya N, Niwa Y, Goto H. Confocal endomicroscopy in patients with ulcerative colitis. *J Gastroenterol Hepatol* 2008; **23** Suppl 2: S286-S290

6 **Li CQ**, Xie XJ, Yu T, Gu XM, Zuo XL, Zhou CJ, Huang WQ, Chen H, Li YQ. Classification of inflammation activity in ulcerative colitis by confocal laser endomicroscopy. *Am J Gastroenterol* 2010; **105**: 1391-1396

7 **De Palma GD**. Confocal laser endomicroscopy in the "in vivo" histological diagnosis of the gastrointestinal tract. *World J Gastroenterol* 2009; **15**: 5770-5775

8 **Kiesslich R**, Goetz M, Vieth M, Galle PR, Neurath MF. Confocal laser endomicroscopy. *Gastrointest Endosc Clin N Am* 2005; **15**: 715-731

9 **Hoffman A**, Goetz M, Vieth M, Galle PR, Neurath MF, Kiesslich R. Confocal laser endomicroscopy: technical status and current indications. *Endoscopy* 2006; **38**: 1275-1283

10 **Hurlstone DP**, Thomson M, Brown S, Tiffin N, Cross SS, Hunter MD. Confocal endomicroscopy in ulcerative colitis: differentiating dysplasia-associated lesional mass and adenoma-like mass. *Clin Gastroenterol Hepatol* 2007; **5**: 1235-1241

11 **Kiesslich R**, Burg J, Vieth M, Gnaendiger J, Enders M, Delaney P, Polglase A, McLaren W, Janell D, Thomas S, Nafe B, Galle PR, Neurath MF. Confocal laser endoscopy for diagnosing intraepithelial neoplasias and colorectal cancer in vivo. *Gastroenterology* 2004; **127**: 706-713

S- Editor Sun H **L- Editor** Rutherford A **E- Editor** Zheng XM

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2011 February 7; 17(5): I
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Acknowledgments to reviewers of *World Journal of Gastroenterology*

Many reviewers have contributed their expertise and time to the peer review, a critical process to ensure the quality of *World Journal of Gastroenterology*. The editors and authors of the articles submitted to the journal are grateful to the following reviewers for evaluating the articles (including those published in this issue and those rejected for this issue) during the last editing time period.

Rakesh Aggarwal, Additional Professor, Department of Gastroenterology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

Sk Md Fazle Akbar, Dr., Assistant Professor, Third Department of Internal Medicine, Ehime University School of Medicine, Shigenobu-Cho, Ehime 791-0295, Japan

Giedrius Barauskas, Professor, Department of Surgery, Kaunas University of Medicine, Eiveniu str. 2, Kaunas, LT-50009, Lithuania

Luigi Bonavina, Professor, Department of Surgery, Policlinico San Donato, University of Milano, via Morandi 30, Milano 20097, Italy

Xian-Ming Chen, MD, Associate Professor, Department of Medical Microbiology and Immunology, Creighton University, 2500 California Plaza, Omaha, NE 68178, United States

Sung-Gil Chi, Professor, School of Life Sciences and Biotechnology, Korea University, #301, Nok-Ji Building, Seoul 136-701, South Korea

T Choli-Papadopoulou, Dr., Associate Professor, Department of Biochmeistry, Aristotle University of Thessaloniki, School of Chemistry, Thessaloniki, 55124, Greece

Ashraf Dahaba, Dr., MD, PhD, MSc, Professor, Department of Anaesthesiology, Medical University of Graz Medical University, Auenbruggerplatz 29, A-8036 Graz, Austria

Robert JL Fraser, Associate Professor, Investigations and Procedures Unit, Repatriation General Hospital, Daw Park, Australia

Pankaj Garg, Dr., Consultant, Department of General Surgery, Fortis Super Speciality Hospital, Mohali, Punjab, Panchkula, 134112, India

Jon C Gould, MD, FACS, Associate Professor of Surgery, University of Wisconsin School of Medicine and Public Health, 600 Highland Avenue, H4/726, Madison, WI 53792, United States

Pietro Invernizzi, MD, PhD, Division of Internal Medicine and Hepatobiliary Immunopathology Unit, IRCCS Istituto Clinico Humanitas, via A. Manzoni 113, 20089 Rozzano, Milan, Italy

Bhupendra Kumar Jain, Dr., MS, Professor of Surgery and Head, Department of Surgery, GTB Hospital and University College of Medical Sciences, Delhi 110 095, India

Ingmar Königsrainer, MD, Department of General, Visceral and Transplant Surgery, Hoppe Seyler Str. 3, 72076 Tübingen, Germany

Rupert Leong, Associate Professor, Director of Endoscopy, Concord Hospital, ACE Unit, Level 1 West, Hospital Rd, Concord NSW 2139, Australia

Fabrizio Montecucco, MD, Assistant, Division of Cardiology, Department of Internal Medicine, University of Geneva, Avenue de la Roseraie 64, 1211 Geneva, Switzerland

Patrick O'Dwyer, MB, BCh, BAO, FRCS (1),MCh, FRCS (Glasg), University Department of Surgery,Western Infirmary, Glasgow, G11 6NT, United Kingdom

Josep M Pique, MD, Department of Gastroenterology, Hospital Clínic of Barcelona, Villarroel, 170, Barcelona 08036, Spain

Marco Romano, MD, Professor, Dipartimento di Internistica Clinica e Sperimentale-Gastroenterologia, II Policlinico, Edificio 3, II piano, Via Pansini 5, 80131 Napoli, Italy

Norbert Senninger, Professor, Department of General Surgery, University Clinics, Westphalian-Wilhelm's-University, Waldeyerstrasse 1, D-48149 Muenster, Germany

Paul E Sijens, PhD, Associate Professor, Radiology, UMCG, Hanzeplein 1, 9713GZ Groningen, The Netherlands

Hiroaki Takeuchi, MD, PhD, Kochi Medical School, Nankoku-City, Kochi 783-8505, Japan

Hans L Tillmann, Professor, Medizinische Klinik und Poliklinik II, University Leipzig, Philipp Rosenthal, Str. 27, Leipzig 04103, Germany

Frank I Tovey, OBE, ChM, FRCS, Honorary Research Felllow, Department of Surgery, University College London, London, United Kingdom

Li Yuyuan, Professor, Department of Gastroenterology, First Municipal People's Hospital of Guangzhou, 1 Panfu Road, Guangzhou 510180, Guangdong Province, China

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2011 February 7; 17(5): I
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Meetings

Events Calendar 2011

January 14-15, 2011
AGA Clinical Congress of Gastroenterology and Hepatology: Best Practices in 2011 Miami, FL 33101, United States

January 20-22, 2011
Gastrointestinal Cancers Symposium 2011, San Francisco, CA 94143, United States

January 27-28, 2011
Falk Workshop, Liver and Immunology, Medical University, Franz-Josef-Strauss-Allee 11, 93053 Regensburg, Germany

January 28-29, 2011
9. Gastro Forum München, Munich, Germany

February 4-5, 2011
13th Duesseldorf International Endoscopy Symposium, Duesseldorf, Germany

February 13-27, 2011
Gastroenterology: New Zealand CME Cruise Conference, Sydney, NSW, Australia

February 17-20, 2011
APASL 2011-The 21st Conference of the Asian Pacific Association for the Study of the Liver
Bangkok, Thailand

February 22, 2011-March 04, 2011
Canadian Digestive Diseases Week 2011, Vancouver, BC, Canada

February 24-26, 2011
Inflammatory Bowel Diseases 2011-6th Congress of the European Crohn's and Colitis Organisation, Dublin, Ireland

February 24-26, 2011
2nd International Congress on Abdominal Obesity, Buenos Aires, Brazil

February 24-26, 2011
International Colorectal Disease Symposium 2011, Hong Kong, China

February 26-March 1, 2011
Canadian Digestive Diseases Week, Westin Bayshore, Vancouver, British Columbia, Canada

February 28-March 1, 2011
Childhood & Adolescent Obesity: A whole-system strategic approach, Abu Dhabi, United Arab Emirates

March 3-5, 2011
42nd Annual Topics in Internal Medicine, Gainesville, FL 32614, United States

March 7-11, 2011
Infectious Diseases: Adult Issues in the Outpatient and Inpatient Settings, Sarasota, FL 34234, United States

March 14-17, 2011
British Society of Gastroenterology Annual Meeting 2011, Birmingham, England, United Kingdom

March 17-19, 2011
41. Kongress der Deutschen Gesellschaft für Endoskopie und Bildgebende Verfahren e.V., Munich, Germany

March 17-20, 2011
Mayo Clinic Gastroenterology & Hepatology 2011, Jacksonville, FL 34234, United States

March 18, 2011
UC Davis Health Informatics: Change Management and Health Informatics, The Keys to Health Reform, Sacramento, CA 94143, United States

March 25-27, 2011
MedicReS IC 2011 Good Medical Research, Istanbul, Turkey

March 26-27, 2011
26th Annual New Treatments in Chronic Liver Disease, San Diego, CA 94143, United States

April 6-7, 2011
IBS-A Global Perspective, Pfister Hotel, 424 East Wisconsin Avenue, Milwaukee, WI 53202, United States

April 7-9, 2011
International and Interdisciplinary Conference Excellence in Female Surgery, Florence, Italy

April 15-16, 2011
Falk Symposium 177, Endoscopy Live Berlin 2011 Intestinal Disease Meeting, Stauffenbergstr. 26, 10785 Berlin, Germany

April 18-22, 2011
Pediatric Emergency Medicine: Detection, Diagnosis and Developing Treatment Plans, Sarasota, FL 34234, United States

April 20-23, 2011
9th International Gastric Cancer Congress, COEX, World Trade Center, Samseong-dong, Gangnam-gu, Seoul 135-731, South Korea

April 25-27, 2011
The Second International Conference of the Saudi Society of Pediatric Gastroenterology, Hepatology & Nutrition, Riyadh, Saudi Arabia

April 25-29, 2011
Neurology Updates for Primary Care, Sarasota, FL 34230-6947, United States

April 28-30, 2011
4th Central European Congress of Surgery, Budapest, Hungary

May 7-10, 2011
Digestive Disease Week, Chicago, IL 60446, United States

May 12-13, 2011
2nd National Conference Clinical Advances in Cystic Fibrosis, London, England, United Kingdom

May 19-22, 2011
1st World Congress on Controversies in the Management of Viral Hepatitis (C-Hep), Palau de Congressos de Catalunya, Av. Diagonal, 661-671 Barcelona 08028, Spain

May 21-24, 2011
22nd European Society of Gastrointestinal and Abdominal Radiology Annual Meeting and Postgraduate Course, Venise, Italy

May 25-28, 2011
4th Congress of the Gastroenterology Association of Bosnia and Herzegovina with international participation, Hotel Holiday Inn, Sarajevo, Bosnia and Herzegovina

June 11-12, 2011
The International Digestive Disease Forum 2011, Hong Kong, China

June 13-16, 2011
Surgery and Disillusion XXIV SPIGC, II ESYS, Napoli, Italy

June 14-16, 2011
International Scientific Conference on Probiotics and Prebiotics-IPC2011, Kosice, Slovakia

June 22-25, 2011
ESMO Conference: 13th World Congress on Gastrointestinal Cancer, Barcelona, Spain

June 29-2, 2011
XI Congreso Interamericano de Pediatria "Monterrey 2011", Monterrey, Mexico

September 2-3, 2011 Falk Symposium 178, Diverticular Disease, A Fresh Approach to a Neglected Disease, Gürzenich Cologne, Martinstr. 29-37, 50667 Cologne, Germany

September 10-11, 2011
New Advances in Inflammatory Bowel Disease, La Jolla, CA 92093, United States

September 10-14, 2011
ICE 2011-International Congress of Endoscopy, Los Angeles Convention Center, 1201 South Figueroa Street Los Angeles, CA 90015, United States

September 30-October 1, 2011
Falk Symposium 179, Revisiting IBD Management: Dogmas to be Challenged, Sheraton Brussels Hotel, Place Rogier 3, 1210 Brussels, Belgium

October 19-29, 2011
Cardiology & Gastroenterology | Tahiti 10 night CME Cruise, Papeete, French Polynesia

October 22-26, 2011
19th United European Gastroenterology Week, Stockholm, Sweden

October 28-November 2, 2011
ACG Annual Scientific Meeting & Postgraduate Course, Washington, DC 20001, United States

November 11-12, 2011
Falk Symposium 180, IBD 2011: Progress and Future for Lifelong Management, ANA Interconti Hotel, 1-12-33 Akasaka, Minato-ku, Tokyo 107-0052, Japan

December 1-4, 2011
2011 Advances in Inflammatory Bowel Diseases/Crohn's & Colitis Foundation's Clinical & Research Conference, Hollywood, FL 34234, United States

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2011 February 7; 17(5): I-VI
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

Instructions to authors

GENERAL INFORMATION

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a weekly, open-access (OA), peer-reviewed journal supported by an editorial board of 1144 experts in gastroenterology and hepatology from 60 countries.

The biggest advantage of the OA model is that it provides free, full-text articles in PDF and other formats for experts and the public without registration, which eliminates the obstacle that traditional journals possess and usually delays the speed of the propagation and communication of scientific research results. The open access model has been proven to be a true approach that may achieve the ultimate goal of the journals, i.e. the maximization of the value to the readers, authors and society.

Maximization of personal benefits

The role of academic journals is to exhibit the scientific levels of a country, a university, a center, a department, and even a scientist, and build an important bridge for communication between scientists and the public. As we all know, the significance of the publication of scientific articles lies not only in disseminating and communicating innovative scientific achievements and academic views, as well as promoting the application of scientific achievements, but also in formally recognizing the "priority" and "copyright" of innovative achievements published, as well as evaluating research performance and academic levels. So, to realize these desired attributes of *WJG* and create a well-recognized journal, the following four types of personal benefits should be maximized. The maximization of personal benefits refers to the pursuit of the maximum personal benefits in a well-considered optimal manner without violation of the laws, ethical rules and the benefits of others. (1) Maximization of the benefits of editorial board members: The primary task of editorial board members is to give a peer review of an unpublished scientific article via online office system to evaluate its innovativeness, scientific and practical values and determine whether it should be published or not. During peer review, editorial board members can also obtain cutting-edge information in that field at first hand. As leaders in their field, they have priority to be invited to write articles and publish commentary articles. We will put peer reviewers' names and affiliations along with the article they reviewed in the journal to acknowledge their contribution; (2) Maximization of the benefits of authors: Since *WJG* is an open-access journal, readers around the world can immediately download and read, free of charge, high-quality, peer-reviewed articles from *WJG* official website, thereby realizing the goals and significance of the communication between authors and peers as well as public reading; (3) Maximization of the benefits of readers: Readers can read or use, free of charge, high-quality peer-reviewed articles without any limits, and cite the arguments, viewpoints, concepts, theories, methods, results, conclusion or facts and data of pertinent literature so as to validate the innovativeness, scientific and practical values of their own research achievements, thus ensuring that their articles have novel arguments or viewpoints, solid evidence and correct conclusion; and (4) Maximization of the benefits of employees: It is an iron law that a first-class journal is unable to exist without first-class editors, and only first-class editors can create a first-class academic journal. We insist on strengthening our team cultivation and construction so that every employee, in an open, fair and transparent environment, could contribute their wisdom to edit and publish high-quality articles, thereby realizing the maximization of the personal benefits of editorial board members, authors and readers, and yielding the greatest social and economic benefits.

Aims and scope

The major task of *WJG* is to report rapidly the most recent results in basic and clinical research on esophageal, gastrointestinal, liver, pancreas and biliary tract diseases, *Helicobacter pylori*, endoscopy and gastrointestinal surgery, including: gastroesophageal reflux disease, gastrointestinal bleeding, infection and tumors; gastric and duodenal disorders; intestinal inflammation, microflora and immunity; celiac disease, dyspepsia and nutrition; viral hepatitis, portal hypertension, liver fibrosis, liver cirrhosis, liver transplantation, and metabolic liver disease; molecular and cell biology; geriatric and pediatric gastroenterology; diagnosis and screening, imaging and advanced technology.

Columns

The columns in the issues of *WJG* will include: (1) Editorial: To introduce and comment on major advances and developments in the field; (2) Frontier: To review representative achievements, comment on the state of current research, and propose directions for future research; (3) Topic Highlight: This column consists of three formats, including (A) 10 invited review articles on a hot topic, (B) a commentary on common issues of this hot topic, and (C) a commentary on the 10 individual articles; (4) Observation: To update the development of old and new questions, highlight unsolved problems, and provide strategies on how to solve the questions; (5) Guidelines for Basic Research: To provide guidelines for basic research; (6) Guidelines for Clinical Practice: To provide guidelines for clinical diagnosis and treatment; (7) Review: To review systemically progress and unresolved problems in the field, comment on the state of current research, and make suggestions for future work; (8) Original Article: To report innovative and original findings in gastroenterology; (9) Brief Article: To briefly report the novel and innovative findings in gastroenterology and hepatology; (10) Case Report: To report a rare or typical case; (11) Letters to the Editor: To discuss and make reply to the contributions published in *WJG*, or to introduce and comment on a controversial issue of general interest; (12) Book Reviews: To introduce and comment on quality monographs of gastroenterology and hepatology; and (13) Guidelines: To introduce consensuses and guidelines reached by international and national academic authorities worldwide on basic research and clinical practice gastroenterology and hepatology.

Name of journal

World Journal of Gastroenterology

CSSN

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

Indexed and Abstracted in
Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, and Digital Object Identifer. ISI, Thomson Reuters, 2009 Impact Factor: 2.092 (33/65 Gastroenterology and Hepatology).

Published by
Baishideng Publishing Group Co., Limited

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statisital review is performed after peer review. We invite an expert in Biomedical Statistics from to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, Ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJG* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of informed consent
Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Statement of human and animal rights
When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions
Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/1007-9327office. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm) before attempting to submit on-

line. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjg@wjgnet.com, or by telephone: +86-10-5908-0039. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece.

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-59080039 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision for acceptance is made only when at least two experts recommend an article for publication. Reviewers for accepted manuscripts are acknowledged in each manuscript, and reviewers of articles which were not accepted will be acknowledged at the end of each issue. To ensure the quality of the articles published in *WJG*, reviewers of accepted manuscripts will be announced by publishing the name, title/position and institution of the reviewer in the footnote accompanying the printed article. For example, reviewers: Professor Jing-Yuan Fang, Shanghai Institute of Digestive Disease, Shanghai, Affiliated Renji Hospital, Medical Faculty, Shanghai Jiaotong University, Shanghai, China; Professor Xin-Wei Han, Department of Radiology, The First Affiliated Hospital, Zhengzhou University, Zhengzhou, Henan Province, China; and Professor Anren Kuang, Department of Nuclear Medicine, Huaxi Hospital, Sichuan University, Chengdu, Sichuan Province, China.

Abstract

There are unstructured abstracts (no more than 256 words) and structured abstracts (no more than 480). The specific requirements for structured abstracts are as follows:

An informative, structured abstracts of no more than 480 words should accompany each manuscript. Abstracts for original contributions should be structured into the following sections. AIM (no more than 20 words): Only the purpose should be included. Please write the aim as the form of "To investigate/study/…"; MATERIALS AND METHODS (no more than 140 words); RESULTS (no more than 294 words): You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$; CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both. The main text format of these sections, editorial, topic highlight, case report, letters to the editors, can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm.

Illustrations

Figures should be numbered as 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Figures should be either Photoshop or Illustrator files (in tiff, eps, jpeg formats) at high-resolution. Examples can be found at: http://www.wjgnet.com/1007-9327/13/4520.pdf; http://www.wjgnet.com/1007-9327/13/4554.pdf; http://www.wjgnet.com/1007-9327/13/4891.pdf; http://www.wjgnet.com/1007-9327/13/4986.pdf; http://www.wjgnet.com/1007-9327/13/4498.pdf. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use

uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A:...; B:...; C:...; D:...; E:...; F:...; G: …*etc.* It is our principle to publish high resolution-figures for the printed and E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc.*, and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of P values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of P values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc.*, in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that...".

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list, e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in boldfaced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in boldfaced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixudiarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315223018.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

Editorial: http://www.wjgnet.com/1007-9327/g_info_20100315220036.htm

Frontier: http://www.wjgnet.com/1007-9327/g_info_20100315220305.htm

Topic highlight: http://www.wjgnet.com/1007-9327/g_info_20100315220601.htm

Observation: http://www.wjgnet.com/1007-9327/g_info_20100312232427.htm

Guidelines for basic research: http://www.wjgnet.com/1007-9327/g_info_20100315220730.htm

Guidelines for clinical practice: http://www.wjgnet.com/1007-9327/g_info_20100315221301.htm

Review: http://www.wjgnet.com/1007-9327/g_info_20100315221554.htm

Original articles: http://www.wjgnet.com/1007-9327/g_info_20100315221814.htm

Brief articles: http://www.wjgnet.com/1007-9327/g_info_20100312231400.htm

Case report: http://www.wjgnet.com/1007-9327/g_info_20100315221946.htm

Letters to the editor: http://www.wjgnet.com/1007-9327/g_info_20100315222254.htm

Book reviews: http://www.wjgnet.com/1007-9327/g_info_20100312231947.htm

Guidelines: http://www.wjgnet.com/1007-9327/g_info_20100312232134.htm

RESUBMISSION OF THE REVISED MANUSCRIPTS

Please revise your article according to the revision policies of *WJG*. The revised version includes manuscript and high-resolution image figures. The author should re-submit the revised manuscript online, along with printed high-resolution color or black and white photos; Copyright transfer letter, and responses to the reviewers, and science news are sent to us *via* email.

Editorial Office

World Journal of Gastroenterology
Editorial Department: Room 903, Building D,
Ocean International Center,
No. 62 Dongsihuan Zhonglu,
Chaoyang District, Beijing 100025, China
E-mail: wjg@wjgnet.com
http://www.wjgnet.com
Telephone: +86-10-5908-0039
Fax: +86-10-85381893

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1007-9327/g_info_20100315222818.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315222607.htm.

Proof of financial support

For paper supported by a foundation, authors should provide a copy of the document and serial number of the foundation.

Links to documents related to the manuscript

WJG will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Science news releases

Authors of accepted manuscripts are suggested to write a science news item to promote their articles. The news will be released rapidly at EurekAlert/AAAS (http://www.eurekalert.org). The title for news items should be less than 90 characters; the summary should be less than 75 words; and main body less than 500 words. Science news items should be lawful, ethical, and strictly based on your original content with an attractive title and interesting pictures.

Publication fee

Authors of accepted articles must pay a publication fee.
EDITORIAL, TOPIC HIGHLIGHTS, BOOK REVIEWS and LETTERS TO THE EDITOR are published free of charge.