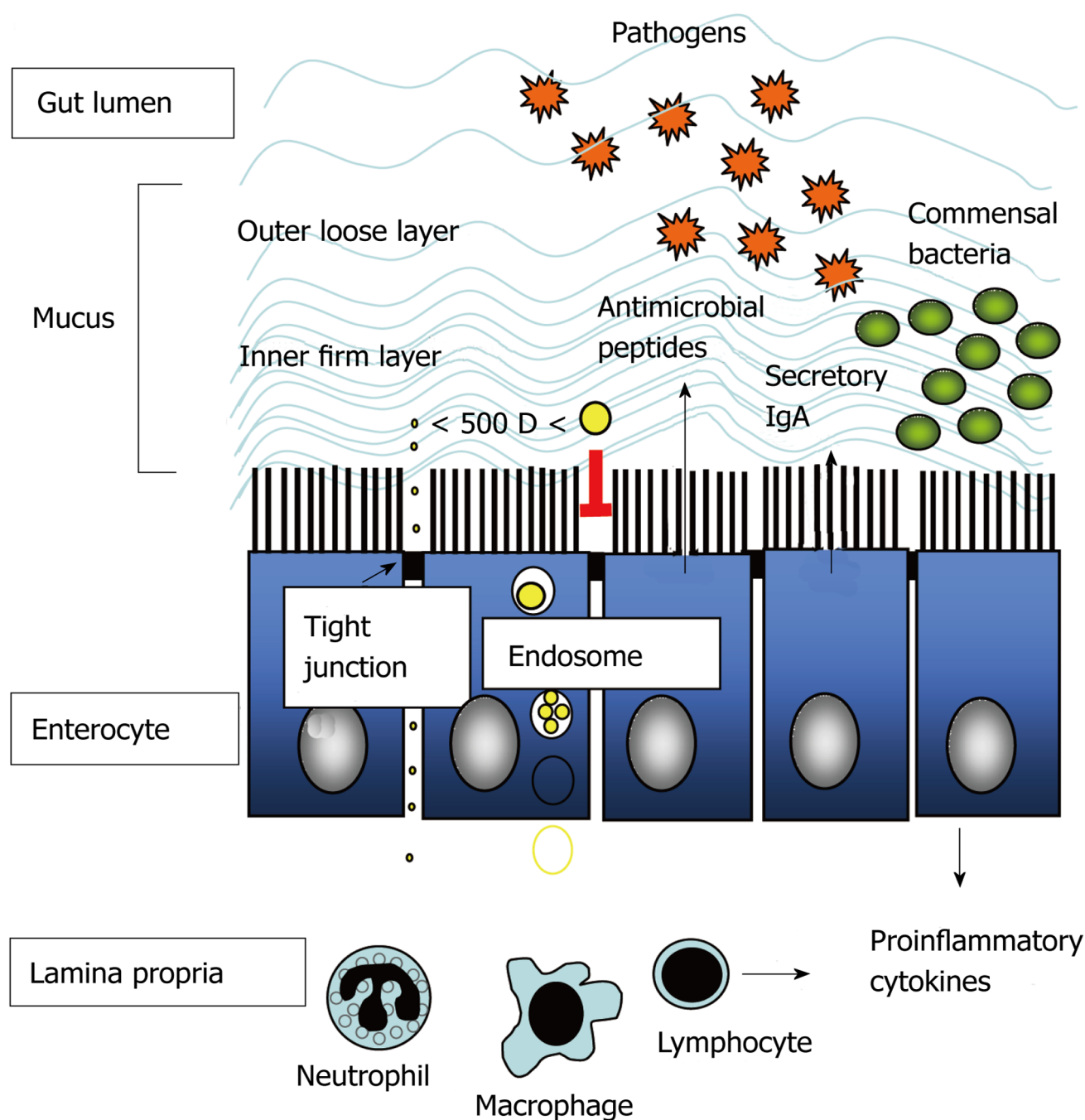


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Regulation of colon cancer recurrence and development of therapeutic strategies

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Abstract

Recurrence of colon cancer still remains a major issue which affects nearly 50% of patients treated by conventional therapeutics. Although the underlying causative factor(s) is not fully understood, development of drug-resistance has been associated with induction of cancer stem or stem-like cells (CSCs) which constitute a small sub-population of tumor cells known to be highly resistant to chemotherapy. In fact, the discovery of CSCs in a variety of tumors (including colon cancer) has changed the view of carcinogenesis and therapeutic strategies. Emerging reports have indicated that to improve patient outcomes, conventional anticancer therapies should be replaced with specific approaches targeting CSCs. Thus, therapeutic strategies that specifically target CSCs are being sought to reduce the risk of relapse and metastasis. In order to specifically

target colon CSCs (while sparing somatic intestinal stem cells), it is critical to identify unique deregulated pathways responsible for self-renewal of CSCs and colon cancer recurrence. Colon CSCs present a unique opportunity to better understand the biology of solid tumors. Thus, a better understanding of the clinical signs and symptoms of colon cancer patients (undergoing surgery or chemotherapy) during perioperative periods, along with the underlying regulatory events affecting the stem/progenitor cell self-renewal and differentiation of colon epithelial cells, is of immense importance. In this review we discuss the implication of clinical factors and the emerging role of CSCs during recurrence of colon cancer along with the development of new therapeutic strategies involving the use of natural agents.

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Key words: Chemo-resistance; 5-Fluorouracil; Oxaliplatin; β -catenin; Cancer stem cells

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INTRODUCTION

Colorectal cancer represents one of the most common cancers in the United States and worldwide. The estimated new cases and deaths from colon and rectal cancer (colorectal cancer) in the United States in 2010 are as follows: 102 900 colon cancer, 39 670 rectal cancer and 51 370 deaths from colon and rectal cancer combined^[1,2].

The lifetime risk of colorectal cancer is 1 in 18 with advancing age contributing to the risk^[3].

Traditionally colorectal cancer is staged based on histology/tumor size/invasion either by tumor node metastasis (TNM) staging or Dukes method. The American Joint Committee on Cancer endorses TNM staging according to which, stage I consists of tumor with size T1-2, N0, M0; stage II with T3-4, N0, M0; stage III with T1-4, N1-2, M0 and stage IV with T any, N any, M1. Currently stages I, II, III without any invasion of serosa are treated by surgical resection with or without adjuvant chemotherapy and stage IV with metastasis is treated with chemotherapy alone^[3]. The main chemotherapeutic agent used is 5-fluorouracil (5FU)/leucovorin + oxaliplatin, also known as the FOLFOX regimen. Though individual rates of recurrence for each stage are not known, surgically resected cases of colorectal cancer (CRC) are known to have a 40%-60% recurrence rate in the first 3 years after surgery with the majority in the second year^[4]. Lymph node metastasis and/or adjacent organ involvement in stage II is said to have a recurrence of 20%-30% and stage III 50%-80% recurrence after surgery^[3].

Various factors have been proposed to play a crucial role in the recurrence of cancer including: number of nodes positive at surgery, pre operative and operative conditions, body mass index (BMI)/obesity, physical activity post surgery and chemotherapy, certain tumor markers and genetic factors, *etc.* Research findings have delineated various factors at the molecular level that can potentially cause recurrence, the latest being the discovery of the association of cancer stem/stem-like cells (CSCs) with drug-resistance phenomenon. Similarly, newer therapeutic approaches are now under consideration with the advent of new chemotherapeutic or preventive agents and regimens. The following is a review of the various factors, at clinical and molecular level, that are being implicated in recurrence of CRC and current knowledge on developing potential therapeutic strategies to combat the recurrence of CRC.

DEVELOPMENT OF COLON CARCINOGENESIS

CRC is a result of a sequential process of carcinogenesis. There are 2 models to explain the occurrence of CRC. One model states that the initial step begins with somatic mutations in adenomatous polyposis coli (APC) gene, which is considered as the initiating step of transforming the normal mucosa to an adenoma (class I) by hyper proliferation^[5]. The hyper proliferation is brought about by accumulation of β -catenin that in turn enters the nucleus to trigger cell cycle leading to hyper proliferation^[3]. The next step involved is the activation of *K-ras*, which is a proto-oncogene that results in the transformation of an early adenoma to an intermediate adenoma (class II adenoma)^[3,5]. The third step is the loss of function gene deleted in colorectal cancer gene on chromosome 18q resulting in the formation of a class III adenoma^[3].

The last step is the mutations in *p53* gene that finally transforms an adenoma into an invasive/early cancer^[5]. It is predicted that the above 4 steps take approximately 10 years and hence a 10 years interval was selected as the screening interval for colonoscopies in people with normal colonic mucosa at initial colonoscopy^[3]. The second CRC model is based on "microsatellite instability" that causes mutations in DNA mismatch repair genes leading to accumulation of uncorrected replication errors resulting in hyper proliferation and eventually carcinoma^[3].

Surgery was seen as curative and hence became the mainstay of therapy. However, recurrence was seen in as many as 50% of patients after curative surgery^[6]. Later, various therapeutic approaches that aimed at targeting one or many steps in the above models surfaced by using chemotherapeutic regimens like FOLFOX, consisting of 5FU + oxaliplatin + leucovorin also known as mainstay for CRC chemotherapy^[3], FOLFIRI, consisting of 5FU + leucovorin + irinotecan mainly for patients with liver metastasis, neoadjuvant chemotherapy with Bevacizumab, a recombinant monoclonal antibody that targets vascular endothelial growth factor and hence antiangiogenic and Cetuximab, an antibody that inhibits epidermal growth factor receptor^[5]. In spite of these combinations of multiple chemotherapeutic agents, recurrence is seen frequently in CRC, especially in patients with metastasis.

CLINICAL SIGNS AND SYMPTOMS THAT PREDICT POOR PROGNOSIS AND RECURRENCE

Certain clinical signs and symptoms like bowel perforation, obstruction and change in bowel habits at presentation were hypothesized to predict poor prognosis as they are signs of advanced disease. Similarly, these signs were also said to be predictors of recurrence as per the prospective study of Aghili *et al.*^[4]. As per this study, 130 colorectal cancer patients with recurrence diagnosed during 1999-2005 were followed for a period of 20 mo with physical examination, serum carcinoembryonic antigen levels, chest X-ray and abdominopelvic sonography done every 2 mo in the first year, every 3 mo the second year, every 4-6 mo for the next 2 years and annually thereafter until the completion of the study. Patients were divided into "early recurrence group", those exhibiting recurrence within 2 years after surgery and "late recurrence group", patients with recurrence after 2 years. Bowel obstruction and change in bowel habits were seen more frequently in patients with early recurrence (10% and 20%, respectively) than in patients with late recurrence (1% and 4%, respectively). A plausible explanation for this could be that patients with early recurrence might have had more advanced disease at initial presentation; thus causing them to develop tumor related complications like luminal obstruction leading to change in bowel habits, perforation of bowel, earlier in the course of disease. On the other hand, patients with late recurrence

might not have had advanced disease at initial presentation, such as spread to serosa, adjacent organ involvement and lymph node involvement, that can potentially cause complications like bowel obstruction, perforation later on.

AGE, BMI AND PHYSICAL ACTIVITY

The effect of age was also studied by Aghili *et al.*^[4] and in their sample, they found that patients with early recurrence were younger, 48 ± 16 years than those with late recurrence, 54 ± 13 years. The reason was felt to be that younger patients at diagnosis had a more advanced cancer stage than the patients diagnosed with CRC at an older age. In addition, Agili *et al.*^[4] conducted their study in the Middle East and observed that in that geographic area, CRC is seen at a higher frequency in younger subjects when compared to the rest of the world and that most of these younger patients tend to have familial disease rather than sporadic CRC^[4]. This could explain the earlier recurrence in younger patients; patients with hereditary forms of CRC such as familial adenomatous polyposis, hereditary nonpolyposis CRC, are known to have disease incidence and progression early on in their lives, e.g., in their twenties, compared to sporadic CRC that tends to occur later on. Hence, screening also begins early for people with hereditary types of CRC in their family history. In the case of sporadic CRC, studies from our lab showed that over-expression of epidermal growth factor receptors (EGFR) plays a role in causing colon cancer as age progresses^[7-10]. Over-expression of EGFR was also seen to increase in CRC stem cells resistant to FOLFOX therapy^[10-12]. These findings suggest that advanced age certainly influences not only the occurrence but also the recurrence of CRC.

Obesity is a well known risk factor for CRC. Meyerhardt *et al.*^[13] conducted a prospective observational study of 1053 stage III CRC patients enrolled in a trial of adjuvant chemotherapy (National Cancer Institute sponsored The Cancer and Leukemia Group B Trial). Height and weight reports were taken during 4 mo after surgery and 6 mo after chemotherapy. They found that patients with class I, II and III obesity did not show any significant increase in CRC recurrence after chemotherapy when compared to normal weight patients with P trend = 0.54. The multivariate hazard ratio for cancer recurrence or death termed as “disease free survival” was found to be 1.00 and 1.24 for patients with class I, II and III obesity, respectively after factors like age, sex, physical activity, tumor-related prognostic factors, tobacco history and performance status were adjusted. Similarly, both weight loss and gain after adjuvant chemotherapy to 6 mo post chemotherapy did not show any effect on recurrence^[13] after the data were controlled for BMI and after exclusion of recurrence and/or death within 90 d of reporting weight at 6 mo post chemotherapy. When the exclusion of recurrence/death within 90 d was taken into account, patient weight loss of ≥ 5 kg during and

6 mo post chemotherapy was associated with a poorer outcome than patients who lost < 2 kg. The mechanism is thought to be due to increased levels of C-peptide in obese patients that inhibits the insulin receptor responsible for decreased production of inflammatory cytokines like interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α), C-reactive protein (CRP), *etc.*, that decrease P53 action, thereby leading to tumorigenesis^[13].

Many researchers^[14] have studied physical activity in association to CRC. Numerous case control and cohort studies showed that physical activity on a regular basis is associated with decreased incidence of CRC. Similarly, Harriss *et al.*^[14] reviewed studies to identify the existence of any relationship between physical activity and CRC recurrence. They found that in a prospective cohort study done by Haydon *et al.*^[15] on 526 CRC patients over a period of 5.3 years, an age adjusted disease specific survival of 12% and 39% in stage II, III respectively, in patients reported to be performing some sort of physical activity daily when compared to patients not involved in physical activity. Prospective studies of Allgayer *et al.*^[16] in CRC patients following primary therapy for CRC, showed that there is decreased urinary excretion of 8 oxo-dG indicative of DNA damage and thereby tumor formation/recurrence in patients ($n = 19$) undertaking moderate intensity exercise for 30-40 min for 2 wk. However, in the same study, similar findings were not seen in patients undertaking high intensity exercise defined as 50%-60% of maximal capacity for the same time period. The above results may be due to the existence of some unknown dose-response-like mechanism that plateaus after a certain dose of physical activity, giving the same or better results at moderate intensity rather than at high intensity. Another group of researchers, Meyerhardt *et al.*^[17] did a prospective study of recreational physical activity on 816 stage III CRC patients 6 mo post adjuvant chemotherapy. They found a significant difference of hazard ratio = 0.51 in patients who reported putting in 18-27 h per week of physical activity over 2 mo compared to patients who were inactive during the same time period. Various mechanisms like cyclooxygenase-2 expression reduction, a marker of anti-inflammatory response, physical activity induced decrease in IL-6, TNF- α , CRP, stimulation of toll-like receptor 4 that causes production of inflammatory cytokines and innate immunity activation were thought to be the mechanisms responsible^[14].

HISTOPATHOLOGICAL FEATURES

Numerous investigators have studied the role of histopathological features of tumors such as: number of lymph nodes, stage and grade of the primary tumor, adjacent organ involvement, the presence of inflammatory cells in the tumor, pathological type and gross pathological view of the tumor, in terms of causing recurrence. Among these Aghili *et al.*^[4] found that patients with Dukes C stage and adjacent organ involvement at initial

diagnosis had more early recurrence. In the same study, ulcerative tumors and non-mucinous adenocarcinomas seemed to recur more often within 2 years of surgery (48% and 62% respectively). On the other hand, number of lymph nodes involved and the site of primary tumor did not predict early or late recurrence. This finding is interesting as one would expect the number of lymph nodes involved to predict the rate of recurrence or prognosis. In fact, the number of lymph nodes involved by the primary or node positive primary at initial presentation was found to be an independent predictor of poor survival; and hence is part of a preoperative scoring system known as “Basingstoke predictive index” in patients who were considered to have surgery for liver metastasis^[18]. However, the prognostic potential of number of lymph nodes involved at presentation might vary with the stage of CRC at initial presentation, as the Basingstoke Predictive Index is applied for patients with liver metastasis, leaving the possibility that the number of lymph nodes involved at initial presentation might not have a similar prognostic value for patients with stage I, II and III of the disease.

PERIOPERATIVE PERIOD

authors felt that co-administration of opioids and COX inhibitors for management of pain during the perioperative period might contribute to decreased tumor recurrence. Similarly, certain alpha and beta blockers were also found to decrease the rate of recurrence through increased apoptosis, decreased tumor cell proliferation and by inhibiting Signal transducer and activator of transcription 3 activity^[19]. Addition of regional anesthesia was also thought to decrease the stress response and thereby reduce recurrence. However, volatile anesthetics like Thiopental and Halothane were known to reduce the natural killer cell activity by decreasing their density in circulation thereby increasing the chances of recurrence and metastasis in animal models^[20]. Other factors like anemia and perioperative hypothermia were found to be associated with a poorer outcome; whereas statins showed promising effects in reducing recurrence in animal models. These statin effects occurred *via*: antiangiogenic effect, anti-inflammatory and immunomodulatory effects through 3 hydroxy-3 methylglutaryl coenzyme A reductase dependant or independent pathways that alter the function of Lymphocyte function associated antigen, T helper 1, 2 cytokines and CRP, in turn inducing apoptosis and effecting signaling pathways of tumor progression^[19]. The role of statins seems to be a promising area of investigation since CRC patients with co-morbid cardiovascular disease conditions can benefit from statin therapy for both their CRC and cardiovascular disease states. Figure 1 summarizes the role of various factors that are implicated in the recurrence of colon cancer and the various drugs currently under clinical investigation for the treatment of recurrent colon cancer are presented in Table 1.

MOLECULAR BASIS OF CHEMO-RESISTANCE: STEM CELL REGULATION GONE AWRY

Researchers who studied extensively to find a cause/s for recurrence came up with the concept of cancer stem cell theory and colonel evolution model to explain recurrence. There is an emerging body of evidence suggesting that tumor cells resistant to chemotherapy represent a subpopulation of cells from the original tumor. These chemo-resistant cells are molecularly and phenotypically distinct and are also referred to as tumor-initiating, tumor-promoting or more commonly, cancer stem cells or cancer stem-like cells^[21]. Cancer stem cell theory states that the process of carcinogenesis is brought about by mutations in a normal stem cell/progenitor cell that leads to hyper proliferation and malignant transformation resulting in the formation of a host of cancer cells that have the same genome^[5,21-23]. These cancer stem cells, through their capacity to self-renew, are said to promote tumor growth and hence are difficult to kill by chemotherapy as current chemotherapeutic drugs are able to kill other cancer cells; the cancer stem cells.

Table 1 Drugs currently under clinical investigation for the treatment of recurrent colon cancer¹

Trail	Drug(s)/agent(s)	Target	Mechanism	Prior experiments
Phase II	R935788/fostamatinib disodium	Kinase inhibitor	Interference with cell communication thereby inhibiting tumor growth	On NCI-60 cell lines
Phase II	Anti ESO-TCR engineered lymphocytes + IL-2 + ALVAC ESO-1 vaccine		Tumor regression secondary to secretion of IFN-gamma by the anti-ESO-TCR induced cells + development of immunity from ALVAC ESO-1 vaccine	On HLA-A2 and ESO double positive tumors
Phase II	Bay 43-9006 + cetuximab	Kinase inhibitor	Inhibition of Raf pathway and VEGFR-2 by Bay 43-9006 + Ras and EGFR inhibition by cetuximab	With cetuximab
Phase II	MSLS + CRS + HIPEC		CRC + HIPEC for overall improvement in survival and MSLS to detect peritoneal carcinomatosis early on	Study results with increased survival of 48-63 mo from CRC + HIPEC when compared to 5-16 mo from chemotherapy alone in metastatic CRC patients
Phase II	Autologous CD8+ enriched young TIL + IL-2		TIL mediated regression of tumor bulk	Evidence of response rates in Metastatic melanoma upon infusion of young CD8+ enriched TIL + IL-2 after a lymphocyte depleting chemo preparative therapy.
Phase II	Echinomycin	Intercalating agent	Anti tumor properties from inhibition of stem cell RNA and DNA synthesis by intercalating into both strands of DNA thereby preventing synthesis	Evidence from trials of southwest oncology group for compounds with ability to modify fluopyrimidine activity
Phase III	Optimized chemotherapy + avastin strategy + tarceva			
Phase II	Bortezomib			

¹From www.cancer.gov. MSLS: Mandatory second look surgery; IL: Interleukin; CRS: Cytoreductive surgery; HIPEC: Hyperthermic intraperitoneal chemotherapy; TIL: Tumor infiltrating lymphocytes; CRC: Colorectal cancer.

whose survival leads to recurrence from self renewal, thus cause tumor formation^[5,21-23]. According to the clonal evolution model or classical theory, any normal cell can be affected by mutations, transforming it into a malignant cell that undergoes unrestricted cell divisions leading to the formation of a mass of cancer cells with genetic variability, resulting in tumor formation and progression^[5,21-23]. According to this theory, all the daughter cells originate from one parent cell and hence are homogenous. However, the trait of “homogeneity” cannot explain the concept of recurrence as current chemotherapeutic drugs can eliminate a specific cell type and, going by this model, should be able to eliminate all the cancer cells due to homogeneity. Todaro *et al*^[5] came up with a combination of both theories to explain recurrence, stating that CRC starts as a cancer stem cell disease with production and maintenance of cancer stem cells but progresses by clonal evolution thereby forming clones of cancer stem cells^[5] that are currently not, or minimally, targeted by existing chemotherapeutic drugs.

Recent findings clearly indicate crypt stem cells as the cells-of-origin of intestinal cancer^[24] as cancer stem cells have the capacity for unlimited self-renewal as well as the ability to initiate and drive tumor progression in an animal model^[23,25]. Thus, they would seem the most probable candidate responsible for tumor chemo-resistance and recurrence. Moreover, gene expression studies have revealed a higher expression of multidrug resistance genes and DNA mismatch repair genes as well as genes that inhibit apoptosis in the cells displaying higher expression of cancer stem cells surface antigens^[26].

Cancer stem cells can be responsible for recurrence due to the following reasons. One, the concept of existence of cancer stem cells and their clonal proliferation can explain the failure of current regimens that target the rest of the tumor cells, leading to recurrence. Secondly, there is evidence of cancer stem cells being accumulated; the presence of certain markers of cancer stem cells being found in chemotherapy resistant/recurrent tumors. Thus, the theory of colon cancer stem cells has opened up a new area of research to find chemotherapeutic or other drugs that can target these cells to prevent their recurrence.

INAPPROPRIATE EXPRESSION OF STEM CELL PATHWAYS

The human adult colonic epithelium undergoes perpetual regeneration fueled by intestinal epithelial stem and progenitor cells located at the colon crypt base. Perturbation of the pathways regulating stem cell renewal contributes significantly to neoplastic transformation. The link between genes important for normal stem cell development and colon cancer has been established^[23]. Since both normal stem cells and cancer stem cells share basic characteristics of self renewal, it seems reasonable to propose that newly arising cancer cells possess the machinery for self-renewal which is normally expressed in stem cells. Evidence shows that many pathways that are classically associated with oncogenesis may also regulate normal stem cell development that includes: Notch,

Sonic hedgehog and Wnt signaling pathways^[11,27,28].

One particularly interesting pathway that has also been shown to regulate both self-renewal and oncogenesis in different organs is the Wnt signaling pathway^[29-31]. Further, among these pathways, canonical Wnt signaling plays a major role in maintaining the fate of intestinal stem cells and progenitor cell proliferation^[21]. Cell fate decisions in the intestine have been shown to involve Notch signaling, which specifically directs cells toward a secretory lineage in the gut^[32]. All of the evidence suggests there is a close interaction of several key pathways in directing intestinal epithelial stem cell renewal and differentiation. Yet how these different pathways coordinate in the specific anatomical compartment of the intestine remains mostly unknown. Since colon cancer is one of the most common cancer types, understanding the proliferation program governing the stem/progenitor cell compartment and the differentiation program of colon epithelial cells is of particular importance.

TARGETING WNT/BETA-CATENIN SIGNALING

Recent studies have reported the pivotal role of Wnt/beta-catenin signaling pathway in the regulation of colonic epithelial stem cell self renewal in addition to its vital role in cellular proliferation, cellular movement and establishment of cell polarity^[33-35]. In contrast, deregulation of Wnt/beta-catenin signaling has been implicated in colon carcinogenesis^[36,37]. Wnt signaling has been defined as occurring either through the canonical or non-canonical pathways. Canonical Wnt signaling is characterized by the stabilization and cytoplasmic accumulation of beta-catenin, which then translocates to the nucleus to facilitate the activation of a variety of Wnt target genes.

In human cancers, including colon cancer, the frequent occurrences of mutations within this highly conserved signaling pathway have been reported^[38]. Because Wnt signaling plays such a critical role in the regulation of stem cell proliferation, targeting this pathway may yield important clinical benefits^[39]. Studies suggest that Wnt signaling is essential for maintaining colonic crypts and for the regulation of cellular differentiation^[40]. However, the regulatory role of Wnt/beta-catenin signaling in the maintenance and growth of CSCs still remains elusive. In a recently reported study the formation of epithelial tumors in mice has been linked with the activation of Wnt/beta-catenin signaling in epidermal stem cells^[41]. In agreement with these results, we have shown that the Wnt/beta-catenin pathway is majorly responsible for the regulation of growth and maintenance of CSCs enriched colonies derived from various human colon cancer cells termed as colonospheres^[28]. It is worth mentioning that colonospheres contain multi-potent cancer stem cells that are hypothesized to be causing recurrence. They are generated in vitro under serum free conditions and in the presence of special stem cell growth factors and are considered to be surrogate tumors. The colonospheres were

found to express: LGR5, CD44, CD166, Musashi-1 and EpCAM, that are markers of colon cancer stem cells^[11,28]. Apart from this, the colonospheres were also found to have elevated levels of total beta-catenin leading to transcriptional activation of the *TCF/LEF* gene responsible for the progression of cancer stem cells^[28]. In 2009, Fang *et al*^[6] did a prospective study on 620 CRC patients in various stages of the disease, post curative surgery (resection), for a median duration of 52 mo, using high density tissue microarray technology and immunohistochemical analysis, to determine the molecular markers that can predict recurrence. They found that certain target genes of the Wnt/Beta Catenin pathway like *Survivin*, *Cyclin D1*, *TCF 4*, (important components of Wnt pathway) and a cancer stem cell surface receptor TROP 2 were elevated in patients with disease recurrence^[6].

Recent studies^[22,42] have revealed that several multidrug resistance genes, including *MDR-1*, *ABCG2*, *ABCA3*, and *BRCP1* are also intrinsically expressed in stem and/or progenitor cells and may contribute to the side population phenotype of malignant cells. Wnt/β-catenin signaling seems to play an important role in *ABCB1/MDR-1* transcription^[43,44]. Putative TCF binding elements were also identified in the *ABCB1* promoter (-1813 to -275 bp)^[44]. Canonical Wnt signaling is believed to play an important role in the maintenance of hematopoietic progenitors and also in the lineage commitment of progenitors during hematopoiesis. Interestingly, many of the cell surface markers (including LGR5/GPR49, CD44, CD24 and EpCAM) that have been used to identify and isolate putative tumor stem cell populations in a variety of tissues including colon are direct Wnt targets. Since colon cancer stem cells are believed to be dependent on beta-catenin signaling to maintain their properties, targeting wnt/beta-catenin pathway may yield promising clinical benefits by inhibiting the proliferative capacity of CSCs or possibly force terminal differentiation.

PREVENTING RECURRENCE OF COLON CANCER WITH NATURAL COMPOUNDS

All cancers are thought to be preventable^[45-47]. The two most important ways to reduce cancer risk are the avoidance of cancer-causing biologic, chemical, and physical agents and the habitual consumption of diets high in foods that protect against cancer. It has been estimated that approximately, 30% to 40% of cancer incidents are preventable by consuming a healthy diet, regular physical activity, maintenance of optimum body weight and consumption of fruits and vegetables^[48]. Because of their safety, low toxicity, antioxidant properties, and general acceptance as dietary supplements, fruits, vegetables, and other dietary elements (phytochemicals and minerals) are being investigated for the prevention of cancer. Numerous recently published reports have indicated the usefulness of natural agents in reversing the recurrence of various cancers including colon cancer.

A thorough online search on the website: clinicaltri-

Table 2 Major natural compounds targeting Wnt/ β -catenin pathway undergoing clinical trials for the treatment of colon cancer

Category	Compound	Target	Mechanism	Clinical trials identification No.
Vitamins	Retinoids/ vitamin-A	Beta-catenin	Compete with TCFs, production of inhibitory protein-disabled-2	NCT00270647, NCT00712647
	Vitamin-D	Beta-catenin	Production of inhibitory proteins-dickkopf-1 and 4	NCT00208793, NCT00870961, NCT01198548, NCT00399607, NCT01150877, NCT00585637
Polyphenols	Vitamin-E	Unknown	Unknown	NCT00706121, NCT00270647
	Pyridoxine	Unknown	Unknown	NCT00559858
	Quercetin	Wnt/beta-catenin	Unknown	NCT00003365
	Epigallocatechin-3-gallate from green tea	Wnt/beta-catenin	Plietropic	NCT01239095, NCT00718094
	Curcumin	Wnt/beta-catenin	Plietropic	NCT00365209, NCT00295035, NCT00027495, NCT00973869, NCT00745134, NCT00118989, NCT00927485, NCT00641147
	Resveratrol	Wnt/beta-catenin	Unknown	NCT00256334, NCT00578396, NCT00433576, NCT00920803
	Dictyostelium discoideum	Wnt/beta-catenin	Activation of GSK-3 β by differentiation inducing factors-1,3	No current trails
Lipids	Omega 3 fatty acids and n-3 polyunsaturated fatty acids	Mitochondrial membrane of tumor cells	Enhanced reactive oxygen species generation and decreased cell anti-oxidant capacity leading to apoptosis in tumor cells	NCT01127867, NCT00145015, NCT01218841, NCT00432913, NCT00488904, NCT00168987, NCT00510692, NCT01070355, NCT01048463

ID numbers source: clinicaltrials.gov.

als.gov, will yield about 100 such trials that are ongoing with most promising natural compounds being investigated against various types of cancers^[46]. These include, but are not limited to, the use of (-)-epigallocatechin gallate (EGCG) (green tea), curcumin, resveratrol, genistein, pomegranate, lycopene, n-3 poly unsaturated fatty acids, folic acid, ellagic acid, lupeol and betulinic acid for targeting many cancers including solid tumors and gastrointestinal (GI) cancers (Table 2)^[46]. Almost all these natural compounds have also been found to synergistically increase the efficacy of other drugs in cell culture and animal models^[3,46,49-52]. Interestingly, these agents have also been shown to prevent or delay the progression of cancer, which could in part be due to their ability to attack CSCs by attenuating the Wnt and Hedgehog signaling pathways. There is strong evidence that natural compounds such as EGCG, curcumin, resveratrol and n-3 poly unsaturated fatty acids can be used to resensitize chemotherapy-resistant colon cancer cells. Among these natural compounds, EGCG, curcumin and resveratrol have been extensively studied for their efficacies against colon cancer.

It has been known that in many malignancies, EGCG has concentration dependent inhibitory effects on Wnt signaling^[53]. In colon cancer cells, treatment of EGCG led to a potent inhibition of GSK3- α and GSK-3 β activity, the important regulatory molecules involved in Wnt signaling^[54]. It was observed that the amount of phosphorylated β -catenin was diminished and the overall amount of β -catenin and the TCF/LEF-mediated luciferase expressions were decreased by EGCG treatment^[54]. Further, it has also been reported that the canonical Wnt signaling could be inhibited at the level of TCF/LEF by EGCG in antler progenitor cells^[55]. These studies strongly indicate that EGCG can be a promising agent

in the management of colon cancer recurrence.

Similar to EGCG, curcumin and resveratrol have also been known for their plietropic effects against many cancers with most promising effects exhibiting against colon and other GI cancers. Our own study^[51] showed that the combination therapy of curcumin and resveratrol is highly effective in inhibiting the growth of colon cancer cells *in vitro* and *in vivo*. The combination of 5FU and oxaliplatin (FOLFOX) forms the backbone of colorectal cancer chemotherapeutics. Our studies *in vitro* demonstrated that curcumin, either alone or in combination with FOLFOX (5FU + oxaliplatin), caused a greater growth inhibition of FOLFOX-resistant colon cancer cells than either agent alone^[12]. Furthermore, in response to curcumin treatment caspase-3-mediated cleavage of β -catenin, E-cadherin, APC and decreased transactivation of β -catenin/TCF/LEF, DNA-binding activity of the β -catenin/TCF/LEF complex and the levels of c-Myc protein were observed^[56]. These results suggested that curcumin could impair both Wnt signaling and cell-cell adhesion pathways, leading to the G2/M phase arrest and apoptosis of colon cancer cells suggesting a promising potential against recurrence of colon cancer. Indeed, our recent findings suggested that curcumin can inhibit the growth of chemo-surviving colon cancer cells that are highly enriched in CSCs^[57]. Failure to eliminate these cells is thought to be one of the underlying causes of cancer recurrence. Our studies have shown that curcumin can not only synergize with FOLFOX but also with dasatinib to inhibit the growth of colon cancer cells^[12,57,58]. On the basis of these recent observations and the fact that curcumin and/or other natural derivatives has plietropic effects in down-regulating the survival signals seen in chemo-surviving cells, we have hypothesized that combination of non-toxic natural

agents like curcumin and FOLFOX can be a potential therapeutic strategy for colon cancer recurrence.

CONCLUSION

Drugs or natural compounds that target aberrant activation of the Wnt signaling cascade have enormous potential as novel cancer therapeutics. The constitutive activation of Wnt signaling primarily occurs during embryogenesis and tissue repair in the adult. Significant levels of toxicities are not expected with the use of anti-tumor agents that target Wnt signaling pathway. Various efforts are already underway to develop Wnt and/or beta-catenin antagonist that may specifically drive stem cells toward differentiation. As described above, there are a number of drugs and natural compounds that have already been identified to have therapeutic value against cancers associated with aberrant Wnt signaling. Because of the complex communication between cell signaling networks, cancer cells always show alterations in multiple cellular signaling pathways. Therefore, regulation of multiple cell signaling pathways controlling the behavior of cancer cells, such as inhibition of cell growth or induction of apoptosis, requires agents that could target multiple pathways. It is believed that many of natural products can perform these tasks. Identification of the target molecules and determination of the precise mechanism(s) by which different natural and/or synthetic agents exert their action is essential for the development of therapeutic strategies against various malignancies.

REFERENCES

- 1 Yang VW, Lewis J, Wang TC, Rustgi AK. Colon cancer: an update and future directions. *Gastroenterology* 2010; **138**: 2027-2028
- 2 NCI. National Cancer Institute, Colon and rectal cancer. 2010
- 3 Markle B, May EJ, Majumdar AP. Do nutraceuticals play a role in the prevention and treatment of colorectal cancer? *Cancer Metastasis Rev* 2010; **29**: 395-404
- 4 Aghili M, Izadi S, Madani H, Mortazavi H. Clinical and pathological evaluation of patients with early and late recurrence of colorectal cancer. *Asia Pac J Clin Oncol* 2010; **6**: 35-41
- 5 Todaro M, Francipane MG, Medema JP, Stassi G. Colon cancer stem cells: promise of targeted therapy. *Gastroenterology* 2010; **138**: 2151-2162
- 6 Fang YJ, Lu ZH, Wang GQ, Pan ZZ, Zhou ZW, Yun JP, Zhang MF, Wan DS. Elevated expressions of MMP7, TROP2, and survivin are associated with survival, disease recurrence, and liver metastasis of colon cancer. *Int J Colorectal Dis* 2009; **24**: 875-884
- 7 Nautiyal J, Rishi AK, Majumdar AP. Emerging therapies in gastrointestinal cancers. *World J Gastroenterol* 2006; **12**: 7440-7450
- 8 Majumdar APN, Jaszewski R. Aging of the esophagus, and stomach. New York: Krager, 2003
- 9 Majumdar APN, Basson MD. Effect of Aging on the Gastrointestinal Tract. New York: Academic Press, 2006
- 10 Levi E, Misra S, Du J, Patel BB, Majumdar AP. Combination of aging and dimethylhydrazine treatment causes an increase in cancer-stem cell population of rat colonic crypts. *Biochem Biophys Res Commun* 2009; **385**: 430-433
- 11 Kanwar SS, Yu Y, Nautiyal J, Patel BB, Padhye S, Sarkar FH, Majumdar AP. Difluorinated-curcumin (CDF): a novel curcumin analog is a potent inhibitor of colon cancer stem-like cells. *Pharm Res* 2011; **28**: 827-838
- 12 Yu Y, Kanwar SS, Patel BB, Nautiyal J, Sarkar FH, Majumdar AP. Elimination of Colon Cancer Stem-Like Cells by the Combination of Curcumin and FOLFOX. *Transl Oncol* 2009; **2**: 321-328
- 13 Meyerhardt JA, Niedzwiecki D, Hollis D, Saltz LB, Mayer RJ, Nelson H, Whittom R, Hantel A, Thomas J, Fuchs CS. Impact of body mass index and weight change after treatment on cancer recurrence and survival in patients with stage III colon cancer: findings from Cancer and Leukemia Group B 89803. *J Clin Oncol* 2008; **26**: 4109-4115
- 14 Harriss DJ, Cable NT, George K, Reilly T, Renehan AG, Haboubi N. Physical activity before and after diagnosis of colorectal cancer: disease risk, clinical outcomes, response pathways and biomarkers. *Sports Med* 2007; **37**: 947-960
- 15 Haydon AM, Macinnis RJ, English DR, Giles GG. Effect of physical activity and body size on survival after diagnosis with colorectal cancer. *Gut* 2006; **55**: 62-67
- 16 Allgayer H, Owen RW, Nair J, Spiegelhalder B, Streit J, Reichel C, Bartsch H. Short-term moderate exercise programs reduce oxidative DNA damage as determined by high-performance liquid chromatography-electrospray ionization-mass spectrometry in patients with colorectal carcinoma following primary treatment. *Scand J Gastroenterol* 2008; **43**: 971-978
- 17 Meyerhardt JA, Giovannucci EL, Holmes MD, Chan AT, Chan JA, Colditz GA, Fuchs CS. Physical activity and survival after colorectal cancer diagnosis. *J Clin Oncol* 2006; **24**: 3527-3534
- 18 Rees M, Tekkis PP, Welsh FK, O'Rourke T, John TG. Evaluation of long-term survival after hepatic resection for metastatic colorectal cancer: a multifactorial model of 929 patients. *Ann Surg* 2008; **247**: 125-135
- 19 Gottschalk A, Sharma S, Ford J, Durieux ME, Tiourine M. Review article: the role of the perioperative period in recurrence after cancer surgery. *Anesth Analg* 2010; **110**: 1636-1643
- 20 Melamed R, Bar-Yosef S, Shakhar G, Shakhar K, Ben-Eliyahu S. Suppression of natural killer cell activity and promotion of tumor metastasis by ketamine, thiopental, and halothane, but not by propofol: mediating mechanisms and prophylactic measures. *Anesth Analg* 2003; **97**: 1331-1339
- 21 Clevers H. Wnt/beta-catenin signaling in development and disease. *Cell* 2006; **127**: 469-480
- 22 O'Brien CA, Kreso A, Jamieson CH. Cancer stem cells and self-renewal. *Clin Cancer Res* 2010; **16**: 3113-3120
- 23 Ricci-Vitiani L, Lombardi DG, Pilozzi E, Biffoni M, Todaro M, Peschle C, De Maria R. Identification and expansion of human colon-cancer-initiating cells. *Nature* 2007; **445**: 111-115
- 24 Barker N, Ridgway RA, van Es JH, van de Wetering M, Begthel H, van den Born M, Danenberg E, Clarke AR, Sansom OJ, Clevers H. Crypt stem cells as the cells-of-origin of intestinal cancer. *Nature* 2009; **457**: 608-611
- 25 O'Brien CA, Pollett A, Gallinger S, Dick JE. A human colon cancer cell capable of initiating tumour growth in immunodeficient mice. *Nature* 2007; **445**: 106-110
- 26 Liu G, Yuan X, Zeng Z, Tunici P, Ng H, Abdulkadir IR, Lu L, Irvin D, Black KL, Yu JS. Analysis of gene expression and chemoresistance of CD133+ cancer stem cells in glioblastoma. *Mol Cancer* 2006; **5**: 67
- 27 Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. *Nature* 2001; **414**: 105-111
- 28 Kanwar SS, Yu Y, Nautiyal J, Patel BB, Majumdar AP. The Wnt/beta-catenin pathway regulates growth and maintenance of colonospheres. *Mol Cancer* 2010; **9**: 212

- 29 **Liu S**, Dontu G, Mantle ID, Patel S, Ahn NS, Jackson KW, Suri P, Wicha MS. Hedgehog signaling and Bmi-1 regulate self-renewal of normal and malignant human mammary stem cells. *Cancer Res* 2006; **66**: 6063-6071
- 30 **Dontu G**, Jackson KW, McNicholas E, Kawamura MJ, Abdallah WM, Wicha MS. Role of Notch signaling in cell-fate determination of human mammary stem/progenitor cells. *Breast Cancer Res* 2004; **6**: R605-R615
- 31 **Lowry WE**, Blanpain C, Nowak JA, Guasch G, Lewis L, Fuchs E. Defining the impact of beta-catenin/Tcf transactivation on epithelial stem cells. *Genes Dev* 2005; **19**: 1596-1611
- 32 **van Es JH**, Clevers H. Notch and Wnt inhibitors as potential new drugs for intestinal neoplastic disease. *Trends Mol Med* 2005; **11**: 496-502
- 33 **Takebe N**, Ivy SP. Controversies in cancer stem cells: targeting embryonic signaling pathways. *Clin Cancer Res* 2010; **16**: 3106-3112
- 34 **Korkaya H**, Paulson A, Charafe-Jauffret E, Ginestier C, Brown M, Dutcher J, Clouthier SG, Wicha MS. Regulation of mammary stem/progenitor cells by PTEN/Akt/beta-catenin signaling. *PLoS Biol* 2009; **7**: e1000121
- 35 **Brabletz S**, Schmalhofer O, Brabletz T. Gastrointestinal stem cells in development and cancer. *J Pathol* 2009; **217**: 307-317
- 36 **Koligs FT**, Bommer G, Göke B. Wnt/beta-catenin/tcf signaling: a critical pathway in gastrointestinal tumorigenesis. *Digestion* 2002; **66**: 131-144
- 37 **Morin PJ**, Sparks AB, Korinek V, Barker N, Clevers H, Vogelstein B, Kinzler KW. Activation of beta-catenin-Tcf signaling in colon cancer by mutations in beta-catenin or APC. *Science* 1997; **275**: 1787-1790
- 38 **Parsons DW**, Wang TL, Samuels Y, Bardelli A, Cummins JM, DeLong L, Silliman N, Ptak J, Szabo S, Willson JK, Markowitz S, Kinzler KW, Vogelstein B, Lengauer C, Velculescu VE. Colorectal cancer: mutations in a signalling pathway. *Nature* 2005; **436**: 792
- 39 **Kosinski C**, Li VS, Chan AS, Zhang J, Ho C, Tsui WY, Chan TL, Mifflin RC, Powell DW, Yuen ST, Leung SY, Chen X. Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors. *Proc Natl Acad Sci USA* 2007; **104**: 15418-15423
- 40 **Brittan M**, Wright NA. Stem cell in gastrointestinal structure and neoplastic development. *Gut* 2004; **53**: 899-910
- 41 **Honeycutt KA**, Waikel RL, Koster MI, Wang XJ, Roop DR. The effect of c-myc on stem cell fate influences skin tumor phenotype. *Mol Carcinog* 2010; **49**: 315-319
- 42 **Haraguchi N**, Utsunomiya T, Inoue H, Tanaka F, Mimori K, Barnard GF, Mori M. Characterization of a side population of cancer cells from human gastrointestinal system. *Stem Cells* 2006; **24**: 506-513
- 43 **Yamada T**, Takaoka AS, Naishiro Y, Hayashi R, Maruyama K, Maesawa C, Ochiai A, Hirohashi S. Transactivation of the multidrug resistance 1 gene by T-cell factor 4/beta-catenin complex in early colorectal carcinogenesis. *Cancer Res* 2000; **60**: 4761-4766
- 44 **Yamada T**, Mori Y, Hayashi R, Takada M, Ino Y, Naishiro Y, Kondo T, Hirohashi S. Suppression of intestinal polyposis in Mdr1-deficient ApcMin/+ mice. *Cancer Res* 2003; **63**: 895-901
- 45 **American Cancer Society**. Cancer facts & figures 2009. Proceedings of the American Cancer Society; 2009; Atlanta, US. Atlanta: American Cancer Society Inc., 2009
- 46 **Amin AR**, Kucuk O, Khuri FR, Shin DM. Perspectives for cancer prevention with natural compounds. *J Clin Oncol* 2009; **27**: 2712-2725
- 47 **Fairbanks M**. Cancer: Conquering a Deadly Disease. *School Library Journal* 2006; **52**: 247-248
- 48 **Glade MJ**. Food, nutrition, and the prevention of cancer: a global perspective. American Institute for Cancer Research/World Cancer Research Fund, American Institute for Cancer Research, 1997. *Nutrition* 1999; **15**: 523-526
- 49 **Sengupta A**, Ghosh S, Das RK, Bhattacharjee S, Bhattacharya S. Chemopreventive potential of diallylsulfide, lycopene and theaflavin during chemically induced colon carcinogenesis in rat colon through modulation of cyclooxygenase-2 and inducible nitric oxide synthase pathways. *Eur J Cancer Prev* 2006; **15**: 301-305
- 50 **Lim do Y**, Jeong Y, Tyner AL, Park JH. Induction of cell cycle arrest and apoptosis in HT-29 human colon cancer cells by the dietary compound luteolin. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G66-G75
- 51 **Majumdar AP**, Banerjee S, Nautiyal J, Patel BB, Patel V, Du J, Yu Y, Elliott AA, Levi E, Sarkar FH. Curcumin synergizes with resveratrol to inhibit colon cancer. *Nutr Cancer* 2009; **61**: 544-553
- 52 **Sarkar FH**, Li Y, Wang Z, Kong D. Cellular signaling perturbation by natural products. *Cell Signal* 2009; **21**: 1541-1547
- 53 **Dashwood WM**, Orner GA, Dashwood RH. Inhibition of beta-catenin/Tcf activity by white tea, green tea, and epigallocatechin-3-gallate (EGCG): minor contribution of H(2)O(2) at physiologically relevant EGCG concentrations. *Biochem Biophys Res Commun* 2002; **296**: 584-588
- 54 **Pahlke G**, Ngiewih Y, Kern M, Jakobs S, Marko D, Eisenbrand G. Impact of quercetin and EGCG on key elements of the Wnt pathway in human colon carcinoma cells. *J Agric Food Chem* 2006; **54**: 7075-7082
- 55 **Mount JG**, Muzylak M, Allen S, Althnaian T, McGonnell IM, Price JS. Evidence that the canonical Wnt signalling pathway regulates deer antler regeneration. *Dev Dyn* 2006; **235**: 1390-1399
- 56 **Jaiswal AS**, Marlow BP, Gupta N, Narayan S. Beta-catenin-mediated transactivation and cell-cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells. *Oncogene* 2002; **21**: 8414-8427
- 57 **Patel BB**, Gupta D, Elliott AA, Sengupta V, Yu Y, Majumdar AP. Curcumin targets FOLFOX-surviving colon cancer cells via inhibition of EGFRs and IGF-1R. *Anticancer Res* 2010; **30**: 319-325
- 58 **Nautiyal J**, Banerjee S, Kanwar SS, Yu Y, Patel BB, Sarkar FH, Majumdar AP. Curcumin enhances dasatinib-induced inhibition of growth and transformation of colon cancer cells. *Int J Cancer* 2011; **128**: 951-961

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Electrical bioimpedance and other techniques for gastric emptying and motility evaluation

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tric emptying studies and was essentially abandoned in favor of techniques such as electrogastrography and the gold standard, scintigraphy. The current research evaluating the utility of gastric EBI either combines this technique with other frequently used techniques or uses new methods for gastric EBI signal analysis. In this context, we discuss our results and those of other researchers who have worked with gastric EBI. In this review article, we present the following topics: (1) a description of the oldest methods and procedures for evaluating GME; (2) an explanation of the methods currently used to evaluate gastric activity; and (3) a perspective on the newest trends and techniques in clinical and research GME methods. We conclude that gastric EBI is a highly effective non-invasive, easy to use and inexpensive technique for assessing GME.

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Key words: Gastrointestinal motility; Gastric emptying; Bioimpedance technique; Diagnostic techniques; Digestive system

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Abstract

The aim of this article is to identify non-invasive, inexpensive, highly sensitive and accurate techniques for evaluating and diagnosing gastric diseases. In the case of the stomach, there are highly sensitive and specific methods for assessing gastric motility and emptying (GME). However, these methods are invasive, expensive and/or not technically feasible for all clinicians and patients. We present a summary of the most relevant international information on non-invasive methods and techniques for clinically evaluating GME. We particularly emphasize the potential of gastric electrical bioimpedance (EBI). EBI was initially used mainly in gas-

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A HISTORICAL PERSPECTIVE ON ASSESSING GASTRIC FUNCTION

The study of gastric anatomy, physiology and pathol-

ogy has been a subject of scientific interest since ancient times^[1-3]. Early physicians based their clinical diagnoses solely on patient symptoms. One of the first attempts to look inside a living human body occurred at the beginning of the nineteenth century, when a rudimentary endoscope was invented to assess the interior of the urinary tract^[4]. This instrument was also used to study other body cavities, such as the rectum and the pharynx^[5,6], and it marked the beginning of modern endoscopy^[7]. Endoscopy was further developed in the middle of the nineteenth century with the invention of the gastroscope, a specific apparatus for visually inspecting the stomach. The esophagoscope was invented in 1871 and esophageal manometry was introduced in 1885^[7]. After these inventions, the first modern instruments for assessing the interior of the gastric cavity were developed^[8]. Rudolph Schindler^[9] described many important diseases of the human digestive system and, together with Georg Wolf, developed a semiflexible gastroscope^[10,11]. Thereafter, the number of developed and improved techniques and apparatuses greatly increased, enabling additional applications, and there has been great scientific activity on this topic ever since.

GASTROINTESTINAL FUNCTION

To appreciate the existing techniques for evaluating gastrointestinal (GIT) function, it is important to have a basic understanding of the physiology and pathology of GIT motility and emptying. Like almost every other organ system, the number of basic physiological processes that occur in the GIT is immense; the major functions of the GIT include swallowing, motility, emptying (of every section), assimilation and elimination. When a piece of food is eaten, the person controls the food as it passes from the mouth to the esophagus, but involuntary functions control the digestive process beyond that point. The GIT cavity includes the mouth, pharynx, esophagus, stomach, small intestine, large intestine, sigmoid, rectum and anus. Among the functions listed above, motility is one of the cornerstones of GIT function. Motility enables swallowing, transit, emptying and elimination and is essential for proper assimilation^[12]. Beginning with swallowing and ending with elimination, motility in different parts of the GIT tract is required for GIT function^[13,14]. Two variables related to GIT motility are particularly important: (1) peristalsis, which is a function of the frequency and magnitude of the gastric contractions that are generated by the pacemaker area; and (2) gastric emptying, which is a measure of the average time that the stomach takes to empty half of its luminal content. The autonomic nervous system regulates GIT motility, controlling peristaltic activity through the myenteric system^[15,16]. Motility defects in any segment of the GIT tract can lead to pathologies that are then manifested as clinical symptoms or complications, such as type 1 and 2 diabetes^[17,18], or as sequelae to GIT surgery^[19,21] or anesthesia^[22,23]. In fact, alterations in GIT motility are

frequently viewed as signs of neuropathy of the myenteric plexus or other pathologies of neuropathic origin. One of the most common GIT motility alterations is reduced transit time (emptying time)^[24,25]. Abnormal gastric emptying is considered to be a clinical marker for a gastric or intestinal motility disorder^[26,27]. Therefore, GIT motility and emptying parameters are important for diagnosis and clinical evaluation. Clinically, this problem can be summarized as the need to better understand the relationship between clinical symptoms and gastric emptying disorders^[28,29]. In a review published in 2004, Quigley^[30] described the difficulties of correlating delayed gastric emptying with pathogenesis. The author found a relationship between delayed gastric emptying and female sex, low body weight, postprandial fullness, vomiting and stress^[31,32]. We and other authors have also found variations associated with the menstrual cycle^[33-36] and even with the anatomical position of the stomach^[37]. All of these studies are limited by the high sensitivity of gastric emptying (in humans) to external factors (the “confusion factor”). In our experience, accurately evaluating gastric function requires standardizing all of the factors that may affect gastric motility and emptying, such as: (1) the duration of the fasting state^[38,39]; (2) control of the patient’s stress and anxiety^[40,41]; (3) the venipuncture^[42,43]; (4) abstinence from smoking and the use of stimulants, such as coffee and certain drugs^[44,45]; (5) the phase of the menstrual cycle in females^[46]; (6) carbohydrate content of the meal^[47]; and (7) body posture^[47,48]. The gastric emptying rate provides only a rough representation of a complex phenomenon that integrates gastric and intestinal function, and its measured values are affected by the methodology employed, in addition to the external factors mentioned above^[48,49]. Functional dyspepsia, gastroparesis, irritable bowel syndrome and chronic constipation are some of the major GIT problems discussed in the literature that are related to gastric motility alterations. However, the relevance of delayed gastric emptying in diseases such as dyspepsia remains unclear^[50,51]. Gastric motility also seems to be a good clinical indication of gastro-esophageal reflux disease because fundoplication tends to accelerate gastric emptying^[52]. In fact, a delay in gastric emptying is common in almost all functional GIT disorders^[53].

TECHNIQUES AND PROCEDURES FOR MONITORING GASTRIC MOTILITY AND EMPTYING

When we consider gastric emptying solely as a consequence of motility, the gold standard technique for evaluating and diagnosing GIT motility defects is scintigraphy^[28,54]. This technique is essentially used to measure gastric emptying. However, scintigraphy provides information in a visual manner and requires the use of ionizing radiation^[55], with all of the associated disadvantages. Besides scintigraphy, many techniques, methods and

procedures for evaluating gastric motility and emptying have been developed in the last century^[28]. To date, none of these techniques has been recognized to be as precise and sensitive as scintigraphy, but some less invasive or less expensive techniques are reasonable alternatives. Invasive techniques for the clinical evaluation of gastric emptying include: (1) monitoring the intubation and absorption kinetics of orally-administered solutes; (2) radiological techniques; (3) scintigraphy; (4) manometry; (5) biomagnetic techniques; (6) breath tests; and (7) endoscopy. Non-invasive techniques include: (1) ultrasonography; (2) magnetic resonance imaging; (3) stethoscope method; (4) electrogastrography (EGG); and (5) electrical bioimpedance and applied potential tomography. All of these techniques have significant literature discussing their application to and use in evaluating disease and comparing different techniques (mainly to scintigraphy). Below, we divide the techniques into invasive and non-invasive and present a brief description of how each is used to assess gastric motility and emptying.

INVASIVE TECHNIQUES FOR EVALUATING GASTRIC MOTILITY AND EMPTYING

Intubation technique

In this technique, the remaining gastric volume is evaluated by liquid aspiration. This technique is not widely used because it is invasive and because it can only measure liquid. However, this technique is frequently used as a complement to absorption kinetic measurements for orally-administered solutes^[56], typically acetaminophen, paracetamol, ethanol, glucose or other substances. This technique estimates gastric emptying by measuring the solute concentrations in the patient's blood, at various times to determine the peak concentration, time to reach maximum concentration, and area under the solute curve^[57,58].

Radiological techniques

This technique consists of radiologically detecting radio-opaque material in the GIT cavity and observing the emptying patterns^[59,60]. Alternatively, in scintigraphy, a radionuclide-labeled meal is ingested and the emitted radiation is detected for image processing^[60,61]. Scintigraphy is strongly preferred to using radio-opaque markers^[62].

Scintigraphy

Scintigraphy is the gold standard for gastric emptying in clinical research and in clinical practice. It shows both the gastric emptying of liquids or solids over time and the intra-gastric distribution of the meal components. As mentioned above, this technique requires the patient to consume a radionuclide-labeled meal. Currently, this technique uses gamma-emitting liquid markers in a non-absorbable chelated form, such as ^[99Tc]-, ^[113In]- or ^[111In]-DTPA (P-Diethylene Triamine Pentaacetic Acid).

Digestible solid markers with a high labeling efficiency are also available (e.g., ^{99mTc})^[63]. To date, scintigraphy remains the most reliable method for measuring gastric emptying. In fact, this is the technique that is specified for use by formal procedural guidelines for measuring gastric emptying^[64].

Manometry

Manometry is another common technique for measuring gastric motility in many GIT regions (esophagus, small intestine and anus-rectum). In esophageal manometry, a thin, pressure-sensitive tube is passed by swallowing through the subject's mouth or nose and into the stomach. The pressure of the muscle contractions is then measured along several sections of the tube^[65]. A similar technique, antro-duodenal manometry, is used to measure the contractile activity of the distal stomach and duodenum. The changes in the intra-luminal pressure of the stomach and duodenum are measured by solid-state transducers incorporated into a catheter that is positioned under fluoroscopic guidance. Recordings may last for several hours and can be conducted while the patient is ambulatory (24 h)^[66].

Biomagnetism

Biomagnetic techniques can be used to evaluate gastric motility^[67,68], either by monitoring the misalignment of magnetic traces previously aligned in a strongly pulsed magnetic field or by a single small magnetic marker travelling through the GIT tract^[69]. In both cases, the magnetic field fluctuations are monitored on the surface of the skin and provide information about gastric motility^[70,71].

The breath test

This test uses ^[14C] octanoic acid as a marker for measuring solid gastric emptying. This isotope is stable and is used to avoid patient radiation exposure^[72,73]. The premise of the breath test is that ^{13/14C}-labeled octanoic acid is retained in the solid phase of a test meal during mixing and grinding in the stomach, rapidly absorbed from the chyme upon entering the duodenum and quickly and completely oxidized in the liver to labeled CO₂, which is rapidly exhaled in the breath^[74].

Endoscopy

Endoscopy is considered to be the preferred technique for internally evaluating gastric anatomy and physiology. It has also been proposed as a technique for assessing gastric emptying. However, its utility for evaluating gastric emptying and quantifying ingested food has not yet been validated^[75].

NON-INVASIVE TECHNIQUES FOR EVALUATING GASTRIC MOTILITY AND EMPTYING

Ultrasonography

Using ultrasonography, it is possible to assess antropyloro-

roduodenal motility and the flow of stomach contents. However, only the gastric antrum can be visualized using ultrasonic techniques; it is not possible to visualize the fundus and corpus of the stomach. The greatest disadvantage of this non-invasive technique is that it is time-consuming and requires an experienced and skilled operator^[76,77].

Magnetic resonance technique

This technique provides detailed visual images of the GIT tract; therefore, its advantage is the possibility of simultaneously measuring gastric emptying and the total volume of the gastric contents. Its main disadvantage is its high cost^[78].

Stethoscope gastric assessment

Acoustically assessing gastric activity through a stethoscope is used clinically but is rarely employed in research to evaluate gastric dysfunction in general^[79,80] or motility in particular^[81].

EKG and electrical bioimpedance

Some of the techniques described above are sensitive to gastric motility *per se*. Two techniques for evaluating gastric motility (EKG and electrical bioimpedance) record the electrical activity resulting from the smooth muscle innervation of the stomach. The first technique, EKG, appeared in 1921^[82]; it measures the electrical activity associated with gastric activity (and possibly activity elsewhere in the GIT) and correlates it with real-time gastric motility^[83].

Electrical bioimpedance

The electrical bio-impedance (EBI) technique for measuring gastric motility and emptying has been investigated only rarely over the past decade. In 1985, McClelland *et al.*^[84] suggested that the EBI technique should be used for evaluating gastric emptying only when using low electrical-conductivity liquids to increase epigastric impedance. These investigators monitored the effects of changing the electrical conductivity of the subject's meals on stomach motility. They also tested the effects of the drug metoclopramide. In this first attempt at gastric impedance evaluation, the subjects were intubated prior to the study and their gastric contents were aspirated after a washout period. The results were encouraging; motility, gastric emptying and the metoclopramide test showed significant results in the expected patterns. Furthermore, the impedance trace showed the correct activity frequency (in the 2-4 cycle/min range). These results and results from other researchers indicated that gastric EBI should be used with one of the following electrical configurations: a one-channel configuration (3 or 4 electrodes) or a multichannel configuration for potential tomography^[85]. For the sake of simplicity, we will only discuss the results of one-channel gastric EBI in this review. In general, the gastric electrical profiles

obtained through EBI are noisier than those obtained through other techniques. In 1992, Kothapalli studied the origin of the gastric electrical bioimpedance changes using a 3D model^[86]. The author demonstrated that the meal resistivity from the epigastric impedance signal is nonlinear, that the impedance signal varies linearly with the circular smooth muscles contractions of the stomach, and that the peristaltic wave changes do not modify the resistivity of the contents. The order of magnitude of the epigastric impedance signal is also a function of the electrode configuration.

Gastric EBI and a liquid meal with low electrical conductivity were used to measure the gastric emptying time^[27]. These investigators recorded the mean stomach impedance continuously for more than two hours. One practical limitation of this method is the need to immobilize the patient for a long period. Therefore, it appears that the change in the mean epigastric EBI magnitude depends strongly on the conductivity of the meal compared to the stomach and surrounding tissues. Typically, a conductive meal has a conductivity of $> 7 \text{ mScm}^{-1}$, a non-conductive meal has a conductivity of $< 2 \text{ mScm}^{-1}$, and a neutral meal has an average conductivity of 4.5 mScm^{-1} . However, Giouvanoudi *et al.*^[87] claimed that the author's half-gastric emptying times (T50s) were shorter than those expected based on scintigraphy. Thus, one of the conclusions from this report was that gastric EBI measurements are mainly influenced by gastric secretion; in other words, the gastric EBI of a neutral meal would be more influenced by gastric acid secretions than by the volume of the meal. It is known that the presence of a meal in the stomach stimulates gastric acid secretion, with a rate that initially increases and subsequently decreases. Gastric function studies using EBI measurements may therefore reflect gastric ionic concentration rather than the volume of the stomach contents. This consideration led to a proposed non-invasive method for continuously recording gastric acid secretions. In 2008, the same authors used gastric EBI to quantitatively determine gastric acidity.

The variations in the gastric EBI signals detected by these researchers, other researchers and our own group can be attributed to several factors: (1) the meal resistivity (lipids, proteins or carbohydrates) in long gastric EBI recordings (emptying studies)^[88,89]; (2) the meal volume; and (3) the contractions of the circular smooth gastric muscles in short gastric EBI recordings (motility studies).

One of the major problems in interpreting gastric EBI patterns (which is also a challenge in EGG) is overlapping signals, movements or impedance changes from other GIT regions. Gastric evaluation generally focuses on the stomach, but the esophagus and much of the large and small intestines are close enough to the gastric region to participate in the signal generation. The effect of this overlap on EGG has been discussed by Amaris *et al.*^[24], who investigated the possibility of overlapping dominant frequencies in cutaneous electrical activity

recorded from the stomach and colon. They concluded that the electrical activity arising from the colon can substantially affect EGG recordings.

It has been reported that small intestine peristalsis has frequencies above 7 cpm and that the large intestine has frequencies from 8 cpm to 12 cpm. In fact, peristalsis over the gross structure of the small intestine (duodenum, jejunum and ileum) ranges from 11-12 cpm in the duodenum (the highest frequency) to 8 cpm in the terminal ileum (the lowest frequency)^[90]. Because gastric movements are in the range of 2 cpm to 4 cpm, they are relatively easy to discriminate when measured by EBI. For short-term evaluations, EBI is more sensitive to gastric movements than to meal resistivity or gastric acid concentrations.

In recent years, EBI has been used in combination with other complementary techniques; it has also been used to search for new methodologies for analyzing and interpreting gastric signals. Combined synchronous EGG and gastric EBI has been studied by Zhangyong *et al.*^[91-93]. These researchers collected both surface signals (gastric EBI and EGG). They decomposed the signals by multiresolution analysis and by energy- and frequency-spectrum analysis. The signals were classified according to the dominant power and dominant frequency, and several variables were calculated: (1) the EGG and gastric EBI rhythms; (2) the signal power spectrum and dynamic spectrum; (3) the normal EGG rhythm and power rates; and (4) the gastric IBE. In 2007, they estimated either the gastric emptying time or gastric motility over long periods (30 to 60 min recordings) in healthy adults. Their results demonstrated that the gastric EBI signal and the synchronous EGG exhibit similar features in both the time and frequency domains. However, the gastric motility did not correlate with the synchronous EGG measurements, especially in cases of gastric disease; therefore, the EGG signal could not be directly correlated to the EBI signal. Nevertheless, these authors suggested that the gastric EBI and synchronous EGG signals should be analyzed together^[92]. These authors also evaluated volunteers with functional dyspepsia and a control group using the same signal analysis protocol. They observed significant differences in the time and frequency domains of the gastric signal between the two groups. They considered the main power frequency (position and dispersion), percentage of normal frequency, frequency instability coefficient, percentage of normal power and power instability coefficient. The authors found that patients with functional dyspepsia had abnormal motility, as measured by EBI and EGG. After one week of treatment, the patients showed normal EGG signals but abnormal gastric EBI. After three weeks of treatment, however, both the EBI and EGG signals were normal. One conclusion suggested by these results is that the EGG and gastric EBI signals are not directly correlated. The EGG technique measures gastric electrical activity, while EBI measures the electrical impedance

of the gastric region that is sensitive to internal gastric movements in short-term recordings. Normal gastric electrical activity does not imply normal gastric motility, which is directly detected by gastric EBI^[94]. Similar comparisons of EEG and antroduodenal manometry were discussed by Abid and Lindberg in 2007^[95].

Another approach is to analyze the frequency domain of the gastric EBI signal and consider alternative parameters with their corresponding interpretations. Moreover, it has been suggested that short evaluations are more useful for outpatient techniques that can be used in clinics or medical offices. In 2007, we suggested that electrical impedance could be used to assess gastric motility^[94]. Our principal aim was to use the global characteristics of the gastric EBI Fourier spectrum; we evaluated healthy subjects in the fasting state and after ingesting a meal. For the statistical analysis, we used the median of the area under the frequency spectrum in the region from 1 cpm to 6 cpm. The area from 2 cpm to 4 cpm corresponds to the gastric region rather than the complete region, which includes the intestines (assuming respiration is unaffected). Therefore, an analysis of the 1-6 cpm region should provide some insight into the relative activity changes of the stomach.

In 2009, we published new results for gastric motility using EBI techniques and short-term recordings. In that study, we proposed using EBI to evaluate gastric motility through considering the global features of the fast Fourier transform (FFT) spectra, and we mainly considered the median of the area under the FFT spectra. The study was performed in 11 healthy subjects who were evaluated under both fasting and postprandial conditions. The results indicated that the median of the area under the FFT spectrum is informationally equivalent to the main peak of the spectrum for purposes of determining the changes in gastric motility from the fasting to the postprandial state. This finding demonstrates that short EBI recordings are valid for evaluating gastric motility^[88]. In the same year, we published another study in which metoclopramide was used to generate physical stress in the gastric tract^[89]. We evaluated the differences in the short-term EBI signal for the gastric region in the fasting state, 60 min after the administration of metoclopramide (a drug that promotes gastric motility and gastric stress) and after food ingestion. We recorded the real component of the EBI signal from the gastric region for 1000 s (approximately 15 min). In that study, we compared the median of the area under the curve, the relative area under the curve at 2-4 cpm and 4-8 cpm and the main peak activity to the usual analysis. The frequency range was divided into four regions: R1 spanned 1-2 cpm, R2 spanned 2-4 cpm, R3 spanned 4-8 cpm, and R4 spanned 8-12 cpm. We found that the median of the area under the curve in the 2-8 cycles per minute (cpm) frequency range decreased from 4.7 cpm in the fasting condition to 4.0 cpm in the medicated state ($P = 0.004$). This result was consistent with the observed change in the relative

area under the FFT curve between 4 cpm and 8 cpm, which decreased from 38.3% to 26.6% ($P = 0.012$). In that study, we also demonstrated that the main peak position in the region from 2 cpm to 8 cpm decreased. The main peak activity was 4.72 cpm in the fasting state and declined to 3.45 cpm in the medicated state ($P = 0.025$). There was also a decrease from the fasting state to the postprandial state at 3.02 cpm ($P = 0.0013$). These results demonstrated that global changes in the GIT tract can be measured using short-term EBI, giving useful information on gastric motility. Therefore, we concluded that short-term EBI can be used to assess gastric motility changes in individuals experiencing gastric stress by analyzing the median of the areas under the FFT curve.

More recently, our group has used the short-term EBI technique to evaluate the physiological changes in gastric activity due to psychological stress using the Stroop and Raven test. Our analysis of the changes in the gastric EBI signal used the methodology described above and indicated a significant decrease in gastric motility during the stress test. These results demonstrated that short-term records of gastric EBI may be useful for evaluating the sympathetic nervous system response to acute psychological stress (data not yet published).

CONCLUSION

This review of a broad spectrum of the literature on assessing gastric function shows that the techniques for evaluating gastric motility and emptying have evolved from invasive to non-invasive and have become more sensitive, inexpensive and easier for general practitioners to use in small clinics and physician's offices. Although scintigraphy has been the gold standard for gastric evaluation, it is an invasive technique. This review has emphasized that electrical bioimpedance is a non-invasive alternative technique for evaluating gastric motility and emptying time. This technique can be implemented using small and inexpensive devices once the frequency and amplitude of the stimulation are known. The similarities between gastric EBI and EGG, both of which record information from cutaneous electrodes, enable simultaneous recordings for complementary signal analysis; one detects gastric electrical activity, while the other is sensitive to internal gastric movements. The relatively simple signal analysis required for gastric EBI could make this technique a good candidate for basic clinical evaluation and even an ambulatory method for assessing gastric motility and emptying. However, several important topics remain to be addressed by researchers: (1) To promote a general protocol for clinically assessing gastric motility and emptying in both healthy subjects and patients with the most common gastric diseases, there should be a summary of the major confounding factors for each technique. Implementing a standard protocol would enable systematic comparisons between groups of researchers, and improvements to specific methodologies could be directed in a common direction.

The limitations and scope of each technique would then be clearer, which would encourage using more than one complementary technique; (2) The need for a standard signal processing protocol is similar, but the number of possible signal analysis approaches is several times larger; (3) Improvements to existing methodologies and efforts to overcome the handicaps of existing techniques must be the main motivation of signal analysis research; and (4) Finally, with the goal of offering a technique for outpatient clinics, medical offices and (possibly) remote recordings, a simple, cheap, reasonably sensitive and compact instrument must be developed; gastric electrical bioimpedance is a good candidate technique for this type of instrument.

In summary, electrical bioimpedance is a non-invasive technique that shares some limitations with EGG but that is sensitive to gastric movement. It uses inexpensive, compact devices, making it a good candidate for potential ambulatory and home monitoring. Signal analysis has recently improved, enabling the detection of gastric changes due to food ingestion, medication and stress, among other factors. Further research should address alternative signal analysis approaches, validation, movement discrimination, limitations, variability factors, the normal range of significant parameters and assessment protocols. For these reasons, this technique has great potential to become a user-friendly methodology for assessing gastric motility and emptying.

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REFERENCES

- 1 **el-Assal GS.** Ancient Egyptian medicine. *Lancet* 1972; **2**: 272-274
- 2 **Cunha F.** More of the Ebers papyrus. *Am J Surg* 1949; **77**: 540-542
- 3 **Sródka A.** The short history of gastroenterology. *J Physiol Pharmacol* 2003; **54** Suppl 3: 9-21
- 4 **Verger-Kuhnke AB, Reuter MA, Beccaria ML.** [Biography of Phillip Bozzini (1773-1809) an idealist of the endoscopy]. *Actas Urol Esp* 2007; **31**: 437-444
- 5 **Verger-Kuhnke AB, Beccaria ML.** [The biography of Maximilian Nitze (1848-1906) and his contribution to the urology]. *Actas Urol Esp* 2007; **31**: 697-704
- 6 **Schultheiss D, Machters SA, Jonas U.** Air cystoscopy: the history of an endoscopic technique from the late 19th century. *BJU Int* 1999; **83**: 571-577
- 7 **Ségala M.** Description of an instrument for inspecting the urethra and bladder. *Lancet* 1827; **7**: 603
- 8 **Spaner SJ, Warnock GL.** A brief history of endoscopy, laparoscopy, and laparoscopic surgery. *J Laparoendosc Adv Surg Tech A* 1997; **7**: 369-373
- 9 **Schäfer PK, Sauerbruch T.** [Rudolf Schindler (1888--1968)--"father" of gastroscopy]. *Z Gastroenterol* 2004; **42**: 550-556
- 10 **Gilger MA, Boyle JT, Sondheimer JM, Colletti RB.** A medical position statement of the North American Society for

- Pediatric Gastroenterology and Nutrition. Indications for pediatric esophageal manometry. *J Pediatr Gastroenterol Nutr* 1997; **24**: 616-618
- 11 **Gilger MA**. Gastroenterologic endoscopy in children: past, present, and future. *Curr Opin Pediatr* 2001; **13**: 429-434
- 12 **Wenger MA**, Engel BT, Clemens TL, Cullen TD. Stomach motility in man as recorded by the magnetometer method. *Gastroenterology* 1961; **41**: 479-485
- 13 **Doglietto F**, Prevedello DM, Jane JA, Han J, Laws ER. Brief history of endoscopic transsphenoidal surgery—from Philipp Bozzini to the First World Congress of Endoscopic Skull Base Surgery. *Neurosurg Focus* 2005; **19**: E3
- 14 **Janssen P**, Vanden Berghe P, Verschueren S, Lehmann A, Depoortere I, Tack J. Review article: the role of gastric motility in the control of food intake. *Aliment Pharmacol Ther* 2011; **33**: 880-894
- 15 **Sobreira LF**, Zucoloto S, Garcia SB, Troncon LE. Effects of myenteric denervation on gastric epithelial cells and gastric emptying. *Dig Dis Sci* 2002; **47**: 2493-2499
- 16 **Quintana E**, Hernández C, Alvarez-Barrientos A, Esplugues JV, Barrachina MD. Synthesis of nitric oxide in postganglionic myenteric neurons during endotoxemia: implications for gastric motor function in rats. *FASEB J* 2004; **18**: 531-533
- 17 **Evers DJ**, Smeenk RM, Bottenberg PD, van Werkhoven ED, Boot H, Verwaal VJ. Effect of preservation of the right gastro-epiploic artery on delayed gastric emptying after cytoreductive surgery and HIPEC: a randomized clinical trial. *Eur J Surg Oncol* 2011; **37**: 162-167
- 18 **Ogawa E**, Hosokawa M, Harada N, Yamane S, Hamasaki A, Toyoda K, Fujimoto S, Fujita Y, Fukuda K, Tsukiyama K, Yamada Y, Seino Y, Inagaki N. The effect of gastric inhibitory polypeptide on intestinal glucose absorption and intestinal motility in mice. *Biochem Biophys Res Commun* 2011; **404**: 115-120
- 19 **Park JS**, Hwang HK, Kim JK, Cho SI, Yoon DS, Lee WJ, Chi HS. Clinical validation and risk factors for delayed gastric emptying based on the International Study Group of Pancreatic Surgery (ISGPS) Classification. *Surgery* 2009; **146**: 882-887
- 20 **Parks RW**, Parks TG. Pathogenesis, clinical features and management of hidradenitis suppurativa. *Ann R Coll Surg Engl* 1997; **79**: 83-89
- 21 **Skipworth RJ**, Parks RW, Stephens NA, Graham C, Brewster DH, Garden OJ, Paterson-Brown S. The relationship between hospital volume and post-operative mortality rates for upper gastrointestinal cancer resections: Scotland 1982-2003. *Eur J Surg Oncol* 2010; **36**: 141-147
- 22 **Qualls-Creekmore E**, Tong M, Holmes GM. Gastric emptying of enterally administered liquid meal in conscious rats and during sustained anaesthesia. *Neurogastroenterol Motil* 2010; **22**: 181-185
- 23 **Schurizek BA**. The effects of general anaesthesia on antroduodenal motility, gastric pH and gastric emptying in man. *Dan Med Bull* 1991; **38**: 347-365
- 24 **Amaris MA**, Sanmiguel CP, Sadowski DC, Bowes KL, Mintchev MP. Electrical activity from colon overlaps with normal gastric electrical activity in cutaneous recordings. *Dig Dis Sci* 2002; **47**: 2480-2485
- 25 **Daghasanli NA**, Braga FJ, Oliveira RB, Baffa O. Oesophageal transit time evaluated by a biomagnetic method. *Physiol Meas* 1998; **19**: 413-420
- 26 **Quigley EM**. Gastric motor and sensory function and motor disorders of the stomach. In: Feldman M, Friedman LS, Sleisenger MH. *Gastrointestinal and liver disease*. 7th ed. Philadelphia, PA: W.B. Saunders, 2002: 691-713
- 27 **Giouvanoudi A**, Amaee WB, Sutton JA, Horton P, Morton R, Hall W, Morgan L, Freedman MR, Spyrou NM. Physiological interpretation of electrical impedance epigastrogaphy measurements. *Physiol Meas* 2003; **24**: 45-55
- 28 **Smout A**, Horowitz M, Armstrong D. Methods to study gastric emptying. *Frontiers in gastric emptying. Dig Dis Sci* 1994; **39**: 130S-132S
- 29 **Cox-Reijven PL**, van Kreel B, Soeters PB. Bioelectrical impedance measurements in patients with gastrointestinal disease: validation of the spectrum approach and a comparison of different methods for screening for nutritional depletion. *Am J Clin Nutr* 2003; **78**: 1111-1119
- 30 **Quigley EM**. Review article: gastric emptying in functional gastrointestinal disorders. *Aliment Pharmacol Ther* 2004; **20** Suppl 7: 56-60
- 31 **Stanghellini V**, Malagelada JR, Zinsmeister AR, Go VL, Kao PC. Stress-induced gastroduodenal motor disturbances in humans: possible humoral mechanisms. *Gastroenterology* 1983; **85**: 83-91
- 32 **Stanghellini V**, Tosetti C, Paternico A, Barbara G, Morselli-Labate AM, Monetti N, Marengo M, Corinaldesi R. Risk indicators of delayed gastric emptying of solids in patients with functional dyspepsia. *Gastroenterology* 1996; **110**: 1036-1042
- 33 **Wallace M**, Hashim YZ, Wingfield M, Culliton M, McAuliffe F, Gibney MJ, Brennan L. Effects of menstrual cycle phase on metabolomic profiles in premenopausal women. *Hum Reprod* 2010; **25**: 949-956
- 34 **Brennan IM**, Feltrin KL, Nair NS, Hausken T, Little TJ, Gentilecore D, Wishart JM, Jones KL, Horowitz M, Feinle-Bisset C. Effects of the phases of the menstrual cycle on gastric emptying, glycemia, plasma GLP-1 and insulin, and energy intake in healthy lean women. *Am J Physiol Gastrointest Liver Physiol* 2009; **297**: G602-G610
- 35 **Gill RC**, Murphy PD, Hooper HR, Bowes KL, Kingma YJ. Effect of the menstrual cycle on gastric emptying. *Digestion* 1987; **36**: 168-174
- 36 **Cordova-Fraga T**, Bernal-Alvarado JJ, Gutierrez-Juarez G, Sosa M, Vargas-Luna M. Gastric activity studies using a magnetic tracer. *Physiol Meas* 2004; **25**: 1261-1270
- 37 **Cordova-Fraga T**, Sosa M, Wiechers C, De la Roca-Chiapas JM, Maldonado Moreles A, Bernal-Alvarado J, Huerta-Franco R. Effects of anatomical position on esophageal transit time: a biomagnetic diagnostic technique. *World J Gastroenterol* 2008; **14**: 5707-5711
- 38 **Cassilly D**, Kantor S, Knight LC, Maurer AH, Fisher RS, Semler J, Parkman HP. Gastric emptying of a non-digestible solid: assessment with simultaneous SmartPill pH and pressure capsule, antroduodenal manometry, gastric emptying scintigraphy. *Neurogastroenterol Motil* 2008; **20**: 311-319
- 39 **Benmair Y**, Fischel B, Frei EH, Gilat T. Evaluation of a magnetic method for the measurement of small intestinal transit time. *Am J Gastroenterol* 1977; **68**: 470-475
- 40 **De la Roca-Chiapas JM**, Solís-Ortiz S, Fajardo-Araujo M, Sosa M, Córdoba-Fraga T, Rosa-Zarate A. Stress profile, coping style, anxiety, depression, and gastric emptying as predictors of functional dyspepsia: a case-control study. *J Psychosom Res* 2010; **68**: 73-81
- 41 **Nygren J**, Thorell A, Jacobsson H, Larsson S, Schnell PO, Hylén L, Ljungqvist O. Preoperative gastric emptying. Effects of anxiety and oral carbohydrate administration. *Ann Surg* 1995; **222**: 728-734
- 42 **Kuo P**, Bellon M, Wishart J, Smout AJ, Holloway RH, Fraser RJ, Horowitz M, Jones KL, Rayner CK. Effects of metoclopramide on duodenal motility and flow events, glucose absorption, and incretin hormone release in response to intraduodenal glucose infusion. *Am J Physiol Gastrointest Liver Physiol* 2010; **299**: G1326-G1333
- 43 **Tan PC**, Khine PP, Vallikkannu N, Omar SZ. Promethazine compared with metoclopramide for hyperemesis gravidarum: a randomized controlled trial. *Obstet Gynecol* 2010; **115**: 975-981
- 44 **Okamura K**, Sasaki N, Yamada M, Yamada H, Inokuma H.

- Effects of mosapride citrate, metoclopramide hydrochloride, lidocaine hydrochloride, and cisapride citrate on equine gastric emptying, small intestinal and caecal motility. *Res Vet Sci* 2009; **86**: 302-308
- 45 **Kuo B**, McCallum RW, Koch KL, Sitrin MD, Wo JM, Chey WD, Hasler WL, Lackner JM, Katz LA, Semler JR, Wilding GE, Parkman HP. Comparison of gastric emptying of a non-digestible capsule to a radio-labelled meal in healthy and gastroparetic subjects. *Aliment Pharmacol Ther* 2008; **27**: 186-196
 - 46 **Caballero-Plasencia AM**, Valenzuela-Barranco M, Martín-Ruiz JL, Herrerías-Gutiérrez JM, Esteban-Carretero JM. Are there changes in gastric emptying during the menstrual cycle? *Scand J Gastroenterol* 1999; **34**: 772-776
 - 47 **Ferdinandis TG**, Dissanayake AS, De Silva HJ. Effects of carbohydrate meals of varying consistency on gastric myoelectrical activity. *Singapore Med J* 2002; **43**: 579-582
 - 48 **Jonderko K**, Kasicka-Jonderko A, Błońska-Fajfrowska B. Does body posture affect the parameters of a cutaneous electrogastrogram? *J Smooth Muscle Res* 2005; **41**: 133-140
 - 49 **Smout AJ**, Jebbink HJ, Akkermans LM, Bruijs PP. Role of electrogastrography and gastric impedance measurements in evaluation of gastric emptying and motility. *Dig Dis Sci* 1994; **39**: 110S-113S
 - 50 **Quigley EM**. Symptoms and gastric function in dyspepsia—goodbye to gastroparesis? *Neurogastroenterol Motil* 1996; **8**: 273-275
 - 51 **Bais JE**, Samsom M, Boudesteijn EA, van Rijk PP, Akkermans LM, Gooszen HG. Impact of delayed gastric emptying on the outcome of antireflux surgery. *Ann Surg* 2001; **234**: 139-146
 - 52 **Seok JW**. How to interpret gastric emptying scintigraphy. *J Neurogastroenterol Motil* 2011; **17**: 189-191
 - 53 **Tack J**, Coulie B, Verbeke K, Janssens J. Influence of delaying gastric emptying on meal-related symptoms in healthy subjects. *Aliment Pharmacol Ther* 2006; **24**: 1045-1050
 - 54 **Akkermans LM**, van Isselt JW. Gastric motility and emptying studies with radionuclides in research and clinical settings. *Dig Dis Sci* 1994; **39**: 95S-96S
 - 55 **Soulsby CT**, Khela M, Yazaki E, Evans DF, Hennessy E, Powell-Tuck J. Measurements of gastric emptying during continuous nasogastric infusion of liquid feed: electric impedance tomography versus gamma scintigraphy. *Clin Nutr* 2006; **25**: 671-680
 - 56 **Vidon N**, Pfeiffer A, Godbillon J, Rongier M, Gauron S, Hirtz J, Bernier JJ, Dubois JP. Evaluation of the gastric absorption and emptying of drugs under various pH conditions using a simple intubation method: application to diclofenac. *Br J Clin Pharmacol* 1989; **28**: 121-124
 - 57 **Heading RC**, Nimmo J, Prescott LF, Tothill P. The dependence of paracetamol absorption on the rate of gastric emptying. *Br J Pharmacol* 1973; **47**: 415-421
 - 58 **Clements JA**, Heading RC, Nimmo WS, Prescott LF. Kinetics of acetaminophen absorption and gastric emptying in man. *Clin Pharmacol Ther* 1978; **24**: 420-431
 - 59 **Bell FR**, Holbrook SE, Titchen DA. A radiological study of gastric (abomasal) emptying in calves before and after vagotomy. *J Physiol* 1977; **272**: 481-493
 - 60 **Kikuchi K**, Kusano M, Kawamura O, Mori M, Sekiguchi T. Measurement and evaluation of gastric emptying using radiopaque barium markers. *Dig Dis Sci* 2000; **45**: 242-247
 - 61 **Kattapuram SV**, Khurana JS, Scott JA, el-Khoury GY. Negative scintigraphy with positive magnetic resonance imaging in bone metastases. *Skeletal Radiol* 1990; **19**: 113-116
 - 62 **Scott SM**, Knowles CH, Williams NS, Lunniss PJ. Simple radio-opaque marker studies versus scintigraphy in slow colonic transit — a comparison. *Gastroenterology* 2000; **118**: A1196
 - 63 **Donohoe KJ**, Frey KA, Gerbaudo VH, Mariani G, Nagel JS, Shulkin B. Procedure guideline for brain death scintigraphy. *J Nucl Med* 2003; **44**: 846-851
 - 64 **Donohoe KJ**, Maurer AH, Ziessman HA, Urbain JL, Royal HD, Martin-Comin J. Procedure guideline for adult solid-meal gastric-emptying study 3.0. *J Nucl Med Technol* 2009; **37**: 196-200
 - 65 **Bogte A**, Bredenoord AJ, Oors J, Siersema PD, Smout AJ. Reproducibility of esophageal high-resolution manometry. *Neurogastroenterol Motil* 2011; **23**: e271-e276
 - 66 **Byrne KG**, Quigley EM. Antroduodenal manometry: an evaluation of an emerging methodology. *Dig Dis* 1997; **15** Suppl 1: 53-63
 - 67 **Carneiro AA**, Baffa O, Oliveira RB. Study of stomach motility using the relaxation of magnetic tracers. *Phys Med Biol* 1999; **44**: 1691-1697
 - 68 **iranda JR**, Corá LA, Américo MF, Romeiro FG. AC biosusceptometry technique to evaluate the gastrointestinal transit of pellets under influence of prandial state. *J Pharm Sci* 2010; **99**: 317-324
 - 69 **Córdova-Fraga T**, Carneiro AA, de Araujo DB, Oliveira RB, Sosa M, Baffa O. Spatiotemporal evaluation of human colon motility using three-axis fluxgates and magnetic markers. *Med Biol Eng Comput* 2005; **43**: 712-715
 - 70 **Benmair Y**, Dreyfuss F, Fischel B, Frei EH, Gilat T. Study of gastric emptying using a ferromagnetic tracer. *Gastroenterology* 1977; **73**: 1041-1045
 - 71 **Frei EH**, Benmair Y, Yerashlmi S, Dreyfuss F. Measurements of the emptying of the stomach with a magnetic tracer. *IEEE Trans Mag* 1970; **6**: 348-349
 - 72 **Choi MG**, Camilleri M, Burton DD, Zinsmeister AR, Forstrom LA, Nair KS. [13C]octanoic acid breath test for gastric emptying of solids: accuracy, reproducibility, and comparison with scintigraphy. *Gastroenterology* 1997; **112**: 1155-1162
 - 73 **Ghoos YF**, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. *Gastroenterology* 1993; **104**: 1640-1647
 - 74 **Perri F**, Pastore MR, Annese V. 13C-octanoic acid breath test for measuring gastric emptying of solids. *Eur Rev Med Pharmacol Sci* 2005; **9**: 3-8
 - 75 **Jung IS**, Kim JH, Youn Lee H, Park H, In Lee S. Endoscopic evaluation of gastric emptying and effect of mosapride citrate on gastric emptying. *Yonsei Med J* 2010; **51**: 33-38
 - 76 **Holt S**, McDicken WN, Anderson T, Stewart IC, Heading RC. Dynamic imaging of the stomach by real-time ultrasound—a method for the study of gastric motility. *Gut* 1980; **21**: 597-601
 - 77 **Georgescu DD**, Muntean MM, Georgescu CC, Simu MM, Georgescu LA. Ultrasound assessment of gastric motility patterns in patients with migraine and dyspepsia. *Ultrasound Med Biol* 2009; **35**: S163
 - 78 **Ajaj W**, Lauenstein T, Papanikolaou N, Holtmann G, Goehde SC, Ruehm SG, Debatin JF. Real-time high-resolution MRI for the assessment of gastric motility: pre- and post-pharmacological stimuli. *J Magn Reson Imaging* 2004; **19**: 453-458
 - 79 **Sugrue M**, Redfern M. Computerized phonoenterography: the clinical investigation of a new system. *J Clin Gastroenterol* 1994; **18**: 139-144
 - 80 **Dalle D**, Devroede G, Thibault R, Perrault J. Computer analysis of bowel sounds. *Comput Biol Med* 1975; **4**: 247-256
 - 81 **Mansy HA**, Sandler RH, inventors. Biomedical Acoustic Research Corp., Assignee. Acoustic detection of gastric motility dysfunction. United States Patent 6840913. 2005 Jan 11
 - 82 **Alvarez WC**. The electrogastrogram and what it shows. *JAMA* 1922; **78**: 1116-1118
 - 83 **Koyama S**, Hosoda S. [Evaluation of gastric motility using vector analysis of electrogastrography]. *J Smooth Muscle Res* 1994; **30**: 21-34
 - 84 **McClelland GR**, Sutton JA. Epigastric impedance: a non-

- invasive method for the assessment of gastric emptying and motility. *Gut* 1985; **26**: 607-614
- 85 **Podczeck F**, Mitchell CL, Newton JM, Evans D, Short MB. The gastric emptying of food as measured by gamma-scintigraphy and electrical impedance tomography (EIT) and its influence on the gastric emptying of tablets of different dimensions. *J Pharm Pharmacol* 2007; **59**: 1527-1536
- 86 **Kothapalli B**. Origin of changes in the epigastric impedance signal as determined by a three-dimensional model. *IEEE Trans Biomed Eng* 1992; **39**: 1005-1010
- 87 **Giouvanoudi AC**, Spyrou NM. Epigastric electrical impedance for the quantitative determination of the gastric acidity. *Physiol Meas* 2008; **29**: 1305-1317
- 88 **Huerta-Franco R**, Vargas-Luna M, Hernandez E, Capaccione K, Cordova T. Use of short-term bio-impedance for gastric motility assessment. *Med Eng Phys* 2009; **31**: 770-774
- 89 **Huerta-Franco MR**, Vargas-Luna M, Capaccione KM, Yañez-Roldán E, Hernández-Ledezma U, Morales-Mata I, Córdoba-Fraga T. Effects of metoclopramide on gastric motility measured by short-term bio-impedance. *World J Gastroenterol* 2009; **15**: 4763-4769
- 90 **Huizinga JD**, McKay CM, White EJ. The many facets of intestinal peristalsis. *Am J Physiol Gastrointest Liver Physiol* 2006; **290**: G1347-G1349; author reply G1347-G1349
- 91 **Zhangyong L**, Hong S, Yan W, Shu Z, Wei W, Chaoshi R. A new approach of gastric motility measurement and evaluation by bioimpedance. Proceedings of the 13th International Conference on Electrical Bioimpedance and the 8th Conference on Electrical Impedance Tomography. *IFMBE Proc* 2007; **17**: 691-694
- 92 **Li Z**, Ren C. Gastric motility measurement and evaluation of functional dyspepsia by a bio-impedance method. *Physiol Meas* 2008; **29**: S373-S382
- 93 **Li ZY**, Ren CS, Zhao S, Sha H, Deng J. Gastric motility functional study based on electrical bioimpedance measurements and simultaneous electrogastrography. *J Zhejiang Univ Sci B*. 2011; **12**: 983-989
- 94 **Huerta MR**, Vargas M, Vallejo JM, Hernández E, Córdoba T. Utility of short time bioelectrical impedance for the gastric motility assessment: preliminary results. Proceedings of 13th International Conference on Electrical Bioimpedance and the 8th Conference on Electrical Impedance Tomography. *IFMBE Proc* 2007; **17**: 725-728
- 95 **Abid S**, Lindberg G. Electrogastrography: poor correlation with antro-duodenal manometry and doubtful clinical usefulness in adults. *World J Gastroenterol* 2007; **13**: 5101-5107

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Encapsulated islets transplantation: Past, present and future

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Abstract

Islet transplantation could become an ideal treatment for severe diabetes to prevent hypoglycemia shock and irreversible diabetic complications, once some of the major and unresolved obstacles are overcome, including limited donor supplies and side effects caused by permanent immunosuppressant use. Approximately 30 years ago, some groups succeeded in improving the blood glucose of diabetic animals by transplanting encapsulated islets with semi-permeable membranes consisting of polymer. A semi-permeable membrane

protects both the inner islets from mechanical stress and the recipient's immune system (both cellular and humoral immunities), while allowing bidirectional diffusion of nutrients, oxygen, glucose, hormones and wastes, i.e., immune-isolation. This device, which enables immune-isolation, is called encapsulated islets or bio-artificial pancreas. Encapsulation with a semi-permeable membrane can provide some advantages: (1) this device protects transplanted cells from the recipient's immunity even if the xenogeneic islets (from large animals such as pig) or insulin-producing cells are derived from cells that have the potential for differentiation (some kinds of stem cells). In other words, the encapsulation technique can resolve the problem of limited donor supplies; and (2) encapsulation can reduce or prevent chronic administration of immunosuppressants and, therefore, important side effects otherwise induced by immunosuppressants. And now, many novel encapsulated islet systems have been developed and are being prepared for testing in a clinical setting.

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Key words: Islet transplantation; Encapsulated islets; Bio-artificial pancreas

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INTRODUCTION

Islet transplantation is a cell replacement therapy that involves transplantation of isolated islets to recipients with severe diabetes mellitus (DM), especially type 1

diabetes mellitus (T1DM)^[1]. Although the therapeutic outcome had been poor for approximately 30 years in the late 20th century, islet transplantation has been done clinically as one of the reliable therapeutic options since the development of the islet transplant protocol at Alberta University and has improved dramatically; it is called the “Edmonton protocol”^[1]. Since the success of the Alberta team, islet transplantation has been performed widely for the past 10 years. Approximately 550 islet transplantations have been performed in more than 40 institutions^[2,3]. According to a recent report, approximately 70% of patients did not need daily insulin at 1 year after transplantation and the graft function was well maintained with 82% graft survival at 5 years^[4].

One of the major obstacles of islet transplantation is the limited human donor supply. Moreover, permanent immunosuppressant use is also problematic because immunosuppressants have some harmful effects on recipients. Firstly, immunosuppressants provide immune-tolerance, but they enable opportunistic infection; that is, an infection caused by a pathogen that usually does not cause disease in a healthy host^[5]. A previous clinical report revealed that some of the patients that received islet transplantation had some infectious complications related to opportunistic infection, e.g., pneumonia, herpes infection and abscess formation^[2]. Secondly, immunosuppressants also induce some side effects such as those found in patients with malignant disease treated with chemotherapy: mouth ulceration, anemia, leukopenia, diarrhea, headache, neutropenia, nausea, vomiting and fatigue^[2]. Thirdly, immunosuppressants have islet toxicity. For example, it is known that tacrolimus and sirolimus, which are major immunosuppressants for islet transplantation, impair the islet viability and graft function^[6,7]. At present, islet transplantation should be done for patients with uncontrollable blood glucose and severe renal complications, and should not be performed in DM patients with no renal complications. If the problems concerning donors and immunosuppressants can be overcome, islet transplantation could become an ideal therapy for severe DM to prevent hypoglycemic shock and irreversible diabetic complications. We believe immune-isolation technology could be a powerful tool to resolve these problems.

Immune-isolation can be achieved by covering islets with semi-permeable membranes consisting of high polymer^[8,9], which is referred to as encapsulated islets or bio-artificial pancreas. Semi-permeable membranes protect the inner islets from both mechanical stress and the recipient's immune system (both cellular and humoral immunities), while allowing the bidirectional diffusion of glucose, oxygen, nutrients, hormone and wastes^[9] (Figure 1). Encapsulated islets could enable successful xenotransplantation with large animals, such as pigs, with a reduction or even absence of chronic administration of immunosuppressants, thus preventing important side-effects induced by immunosuppressants. Since the first encapsulated islet study was reported in 1977^[10], encapsulated islets have

been developed for the clinical setting.

ISLET ISOLATION AND TRANSPLANTATION

Islets are obtained from the donor pancreas by islet isolation. The procedure of islet isolation consists of pancreas digestion and islet purification steps. Pancreata are obtained from heart or brain dead donors or from living donors (for islet autotransplantation) with minimal warm ischemic time. Donor pancreata are preserved in cold preservation solution until islet isolation is started. University of Wisconsin (UW) solution has been used for this purpose^[11,12] and, currently, UW solution is used with oxygenated perfluorochemical called the two-layer method, for better preservation of the pancreas^[13]. ET-Kyoto solution, which is also cold preservation solution and used for lung preservation, has been used instead of UW solution for islet isolation in many institutions^[14]. ET-Kyoto solution has components similar to extracellular fluid and contains trehalose for a cytoprotective effect and ulinastatin for inhibiting trypsin. Pancreas digestion is performed with enzyme solution. After removing additional organs (duodenum, spleen, lymph nodes and vessels), the blended solution of collagenase and neutral protease is injected into the pancreatic main duct until the pancreas is distended. The pancreas is cut into pieces (approximately 7 to 9 pieces) and put into a Ricordi chamber with some marbles. Pancreas digestion is done by recirculating warmed (at 37 °C) enzyme solution and mechanical shaking of the Ricordi chamber. Pancreas digestion is stopped by cooling until an adequate number of islets are obtained by monitoring samples taken from the recirculating system. Digested pancreas contains many exocrine and connective tissues that may cause portal vein hypertension and thrombosis; thus, removing these cellular components is necessary. Purification is performed using a COBE 2991 cell processor with Ficoll or iodixanol gradient solution^[15]. Obtaining many good islets from the donor pancreas is necessary to cure severe diabetes, but it is impossible to obtain all islets contained in the pancreas by current isolation techniques. In the digestion stage, islet loss cannot be prevented if collagenase solution is not fully injected, if the digestion is inadequate, or if the digestion time is longer and isolated islets are injured. In the purification stage, some islets are discarded with other cells such as acinar and ductal cells if the gradient is similar. Over 11 000 islet equivalents per kilogram of body weight are recommended to cure DM^[1,12], but it is difficult to acquire this number of islets from one donor pancreas using current techniques. This is the reason why multiple donors are required. Culturing isolated islets before transplantation is done to evaluate them and many institutions perform islet culture^[12]. However, while culturing can reduce contamination of acinar cells (improving purity), isolated islets deteriorate rapidly in culture, reducing the number. Fresh islet transplantation is recommended for a good outcome^[1] and, if immediate transplantation cannot be done, cold pres-

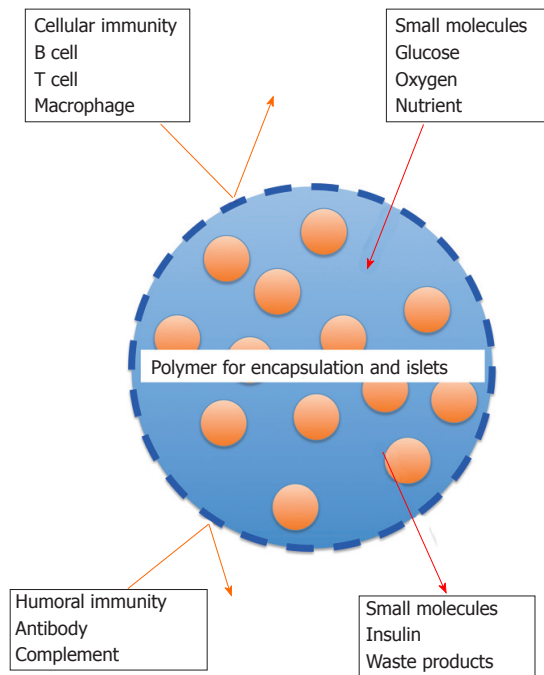


Figure 1 Scheme of the mechanism for encapsulated islets. Immune isolation can be achieved by covering islets with semi-permeable membranes consisting of high polymer. Semi-permeable membranes protect the inner islets from both mechanical stress and the recipient's immune system (both cellular and humoral immunities), while allowing bidirectional diffusion of glucose, oxygen, nutrients, insulin and waste.

ervation (4 °C in UW solution) before transplantation is recommended over 37 °C culture for preventing islet loss and preserving the size, shape and function of islets^[16].

While there are many transplantation sites (kidney^[17-19], muscle^[20], omentum^[21], testis^[22], bone marrow^[23] and some other organs) for islet transplantation in animal experiments, liver is the only the transplantation site used in a clinical setting. The islets are injected into the recipient liver *via* the portal vein. There are rare but severe complications due to this method: portal hypertension, portal thrombosis and bleeding^[12,24]. Portal thrombosis is an especially life-threatening complication. Recently, the Alberta group reported that portal thrombosis was detected in 3.7% of islet-transplanted patients, and a higher volume of islet graft (over 5.45 mL) and higher increase of the portal pressure at transplantation (over 4.5 mmHg) are risk factors for portal thrombosis^[25].

Islet transplantation is classified into three types by the cell sources: autotransplantation, allotransplantation and xenotransplantation. Autotransplantation means transplantation of self-islets when total pancreatectomy cannot be avoided in spite of benign diseases, i.e., chronic pancreatitis^[26,27], pancreatic arteriovenous malformation^[28] and trauma^[29]. The outcome of autotransplantation is excellent because transplanted islets are free from immunity and rejection and there is no need to use immunosuppressants. In allotransplantation, diabetic (especially T1DM) recipients are transplanted islets derived from different individual(s). Rejection cannot be prevented in allotransplantation and thus immunosuppressants are

Table 1 List of materials for encapsulated islets

Materials	Shapes of capsule	Donor source and recipient	Results
Alginate ^[36]	Microcapsule	Porcine and bovine to rat	Achieving normoglycemia for 9 mo
Polysulphone ^[41]	Macrocapsule	Porcine to rat	Normoglycemia over 1 mo
Polyvinyl alcohol ^[8]	Macrocapsule	Rat to mouse	Normoglycemia for 30 d
Low molecular weight dextran sulfate ^[47]	Microcapsule	<i>In vitro</i> assay	Inhibition of complement

necessary. In xenotransplantation, diabetic recipients are transplanted with islets derived from different animals. As in allotransplantation, rejection cannot be prevented but it is difficult to manage immunity by using any immunosuppressants. To utilize these cell sources, studies about encapsulated islets have been promoted.

MATERIALS FOR ENCAPSULATION

The materials for encapsulation must have two characteristics: Firstly, they must isolate the encapsulated islets from the immune system consisting of immune competent cells (T cells, B cells or macrophages), antibody and complement; and secondly, they must permit the diffusion of small molecules like glucose, oxygen and nutrients, and the diffusion of insulin and waste products (Figure 1 and Table 1). The function of encapsulated islets depends on the materials.

In 1980, Lim and Sun^[30] first developed microencapsulated islets using alginate and succeeded in achieving normoglycemia in diabetic rats for two-three weeks. Alginate is now the most famous material for encapsulation. Alginates are found in brown algae and in bacterial species^[31]. They consist of unbranched binary copolymers of 1-4 linked β -D-mannuronic acid (M) and α -L-guluronic acid (G), of widely varying composition and sequential structure (MMM-blocks, GGGblocks and MGM-blocks). The alkali-, ammonium- and magnesium-alginates are soluble in water. Gelation of alginates occurs when the carboxyl groups of the polymers are cross-linked with multi-valent cations (e.g., Ca^{2+} , Ba^{2+} , La^{3+} , Fe^{3+}) and poly-electrolytes^[32]. Alginate is a suitable material for encapsulated islets because alginate does not interfere with the islet function in releasing hormone and has good stability^[32]. Moreover, various materials such as poly (ethylene glycol) (PEG) and poly-L-lysine (PLL) have been used to improve the alginate capsule by reducing plasma absorption and making a semi-permeable membrane. The first report about an alginate/PEG capsule was published in 1999. Chandy *et al.*^[33] modified alginate encapsulated islets by including PEG and succeeded in improving the stability. Desai and colleagues clarified that islets encapsulated by alginate and PEG had good viability and insulin releasing function in an *in vitro* assay^[34]. The alginate/PLL capsule is the most

utilized combination: in 1985, Goosen *et al.*^[35] developed three layer capsules consisting of alginate/PLL/alginate layers and proved that the capsules had a good immune isolation effect by blocking the diffusion of serum immunoglobulin, albumin and hemoglobin. Lanza *et al.*^[36] transplanted encapsulated xenogeneic islets (porcine and bovine islets) into the peritoneal cavities of diabetic rats and found that there was no destruction of the islets for 9 mo and no fibrous adhesions around the capsule, while achieving normoglycemia. While the size of their capsule was approximately 800 μm , Strand *et al.*^[37] succeeded in developing thinner capsules (200 μm) with good immune isolation.

Similar to alginate, polysulphone (PSU) is suitable material for encapsulation. PSU has been used for renal dialysis as its hollow fibers remove tight, molecular-weight waste productions^[38]. PSU is focused on as a possible material for encapsulated islets because large amounts of insulin are absorbed by the PSU hollow fibers. Lambert *et al.*^[39,40] developed hydroxy-methylated PSU “macroencapsulated” islets which showed good insulin releasing function similar to naked islets while blocking endogenous retrovirus infection. Transplantation of their device also achieved normoglycemia in diabetic rats for over 1 mo^[41].

Polyvinyl alcohol (PVA) also has been used as a material for encapsulated islets. PVA is a water-soluble synthetic polymer that has been used as a material for many devices like contact lens solution or artificial tears for the treatment of dry eye. The first report of PVA encapsulated islets was published in 1992 from the Kyoto group. Inoue and colleagues developed a tube type of PVA macrocapsule with a mesh reinforcement. Two thousand rat islets were contained in the capsule and diabetic rats achieved the normoglycemia by allogeneic-transplantation of the device for 12 d^[42]. The device also had a good function of insulin release as indicated by the *in vitro* glucose concentration^[43]. After that, they developed various types of PVA capsules and evaluated the function. For example, transplantation of a bag type of PVA capsule encapsulating porcine islets achieved normoglycemia in diabetic rats for 2 wk^[44]. Sakurai *et al.*^[45] modified the device with an angiogenesis factor (fibroblast growth factor-2) and confirmed neovascularization around the capsule. Qi *et al.*^[8] evaluated the function of the sheet type of PVA capsule *in vitro* and *in vivo*. Rat islets in the PVA capsule had good function in insulin release and achieved normoglycemia in diabetic mice for 30 d. Sakata *et al.*^[46] also attempted to evaluate the therapeutic effect in renal function by transplantation of the device and confirmed an improvement of the hyperglycemia, serum blood urea nitrogen and creatinine and mesangial thickness.

Low molecular weight dextran sulfate (LMW-DS) was first introduced as a material for encapsulated islets in 2003^[47]. Ikada *et al.*^[47] developed a bio-artificial pancreas (encapsulated islets) using LMW-DS and succeeded in preventing complement attack. The Korsgren group revealed that LMW-DS is useful in preventing instant

blood-mediated inflammatory reaction (IBMIR: a rapid thrombotic reaction, in which binding of platelets to the islet surface, activation of the coagulation and complement systems, and leukocyte infiltration of the islets when the islets are exposed to blood occur)^[48-50].

Various materials have been studied and shown to have positive results, but encapsulated islets that can be utilized permanently have not been developed yet. Development of a material that can maintain good viability of the encapsulated islets and good immune-isolation for the long term is expected.

SHAPES OF ENCAPSULATED ISLETS

Encapsulated islets are classified into two types by the size: macrocapsules and microcapsules (Figure 2). Macrocapsules are also divided in two types, intravascular and extravascular types. An intravascular macrocapsule is a perfusion chamber that is directly connected to the host artery and vein^[51]. Blood flows into the hollow fibers and islets are placed near the fibers in this system. Islets could receive oxygen and nutrient supply from blood flow and were protected from immunity by the membrane. However, the intravascular macrocapsule had the severe problem of embolization in the hollow fibers caused by the formation of blood clots.

The extravascular macrocapsule is a diffusion chamber containing a large number of islets. Many shapes, including rod^[52], tube^[43,53] or sheet^[54] types, are used for the macroencapsulation. One of the merits of macrocapsules is the ease of implantation and removal with minimum risk when the device is infected. On the other hand, the permeability of the macrocapsule is less than that of the microcapsule because of the thicker membrane^[38].

Microencapsulated islets are microcapsules containing one or a few islets. Some different protocols for making the devices have been reported. For example, the alginate capsule is made by dropping it into ionic solution (Ca^{2+} , Ba^{2+} , *etc.*)^[55] and the agarose capsule is made by cooling with shaking^[56]. Microcapsules have some advantages in the transportation of oxygen and nutrients because of the smaller distance between the capsule surface and the islet^[38]. The response to glucose change is better than that by macrocapsules. The disadvantage of microcapsules is that they are difficult to remove completely when necessary.

The sizes of encapsulated islets should be selected according to the transplant sites and other characteristics.

CELL SOURCES

The purpose of encapsulation is to prevent loss of the transplanted islets due to immunity. The improvement of the immunosuppression protocol accounts for the present success of islet transplantation^[57]. Encapsulation can protect allogeneic islets from immunity without using immunosuppressants. Human islets are the ideal cell source, but clinical islet transplantation has been limited

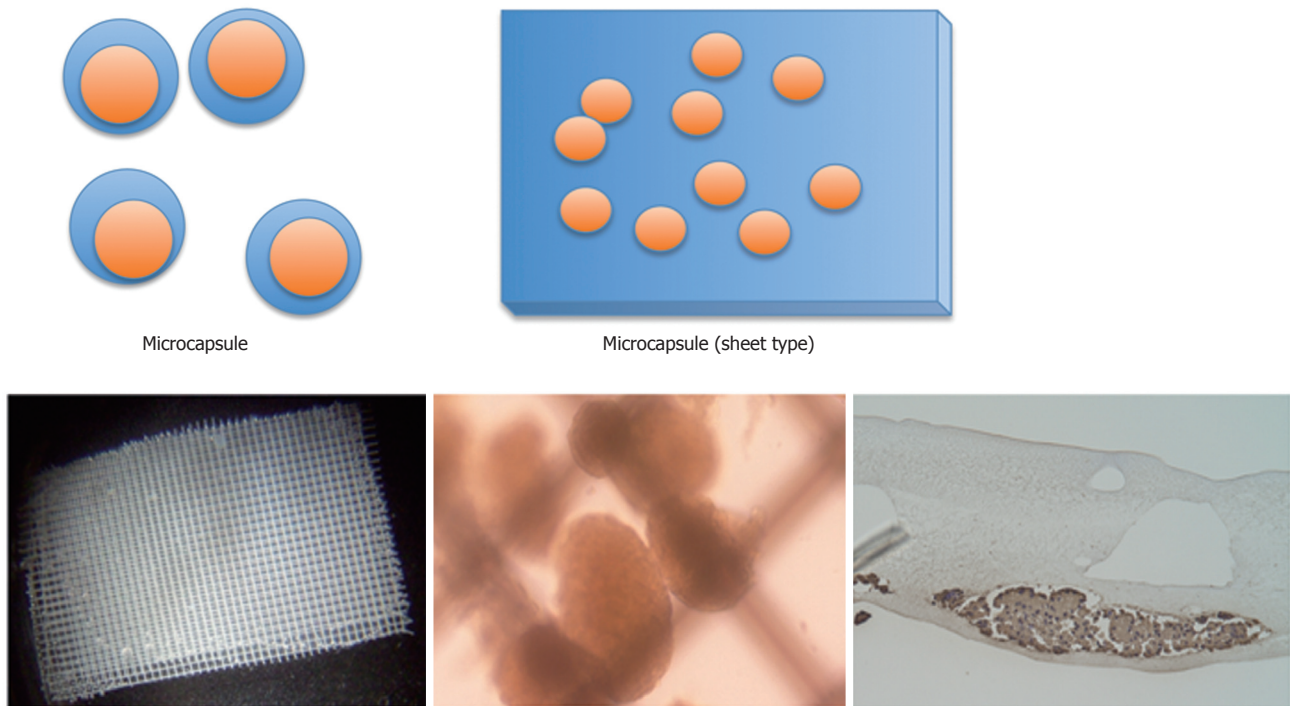


Figure 2 Scheme of size of the encapsulated islets. Encapsulated islets are classified into two types by the size: microcapsule (upper) and macrocapsule (lower). Microencapsulated islets are microcapsules containing a single or few islets. The microcapsule has some advantages in the transportation of oxygen and nutrients because of the smaller distance between the capsule surface and islet. The response to glucose change is better than that of a macrocapsule. The macrocapsule is a diffusion chamber containing a large amount of islets. Many shapes including rods, tubes or sheets (shown in the lower figure) are used for the macroencapsulation. One of the merits of a macrocapsule is the ease of implantation and removal with minimum risk when the device is infected. Left: Sheet type of polyvinyl alcohol macroencapsulated islets. Center: Inside of the macrocapsule. Intact islets are encapsulated in it. Right: Immunohistochemical staining of the macrocapsule for insulin. The islets in the macrocapsule are positive for insulin and viable.

by the donor supplies. To overcome this hurdle, cell sources in addition to allogeneic islets will be necessary.

One of the candidates is xenogeneic islets, especially those of pig^[58]. Historically, porcine insulin was mainly used clinically since insulin was discovered in the early 1920s^[59] before the development of recombinant insulin. Porcine insulin differs little from human insulin (only one difference in amino acid)^[60]. The pig is a large animal with a large pancreas that contains many islets. Therefore, porcine islets are considered an optimal donor source. There are many publications about encapsulated porcine islet xenotransplantation that could improve the blood glucose control in recipient animals. The first report about improved blood glucose by encapsulated porcine islets was published in 1991. Lanza *et al.*^[61] performed encapsulation of porcine islets by using alginate and transplanted the tube type of encapsulated islets into the peritoneal cavity of diabetic rats and confirmed the normalization of the blood glucose. Sun *et al.*^[62] succeeded in achieving normoglycemia in a diabetic monkey for over 150 d by transplanting microencapsulated porcine islets. In spite of the positive data for xenotransplantation, there are some obstacles for the clinical setting. The major obstacle is the risk of viral infection, especially porcine endogenous retrovirus (PERV). van der Laan *et al.*^[63] first described that PERV infection was detected in NOD/SCID mice that received porcine islet transplantation. Clemenceau *et al.*^[64] showed that PERV

DNA was transmitted into a mouse and human cell line. On the other hand, many publications described that there was no evidence of and no influence by HERV infection^[65-67]. Moreover, a recent study from a Minnesota group revealed that not many virus infections were detected in porcine organs, including islets, except porcine cytomegalovirus (PCMV). They concluded that pigs infected with PCMV should not be used as donors and pigs not older than 21 wk should be used to prevent viral infection^[68]. The other obstacle is a reluctance to transplant xenogeneic tissue based on religious beliefs and customs^[69]. In summary, while there are still some obstacles to promoting xenotransplantation, encapsulation technology can be a powerful tool for overcoming obstacles to xenotransplantation.

IDEAL TRANSPLANTATION SITE FOR ENCAPSULATED ISLETS

Intrahepatic transplantation is the current standard for islet transplantation and the liver is the only organ that has been successful as a transplantation site for clinical islet transplantation. However, several recent studies have clearly shown that most of the islets (approximately 60% islets) transplanted intraportally are immediately destroyed, mainly due to the IBMIR^[49,70]. Moreover, the infusion of islets with some other pancreatic tissues

(acinar cells, ductal cells or connective tissues) in the portal vein always has a risk of causing portal hypertension and portal vein embolization^[71]. Schneider *et al*^[72] transplanted microencapsulated rat islets (with alginate) into the livers of mice, but only a 1 wk normalization in the blood glucose level was achieved. We revealed^[70] that transplanted islets suffer from ischemia due to embolization by the islets themselves. The size of encapsulated islets is enlarged by encapsulation and thus encapsulated islets may tend to suffer from ischemia by the size and loss of permeability of the capsule. A thinner capsule (almost the same size as naked islets) is necessary for successful intraportal transplantation.

Furthermore, other transplant sites should be selected for encapsulated islet transplantation to avoid factors that injure islets by intraportal transplantation and the side effects of intraportal transplantation. In experimental studies, there are many descriptions of positive data on extrahepatic transplantation sites. The Kyoto and other groups^[73] succeeded in achieving normoglycemia in diabetic mice by transplantation of encapsulated islets in muscle, subcutaneous tissue^[58,74-76], renal subcapsule^[77] and omentum^[56]. These may be candidate transplantation sites in the near future because the loss of islets due to IBMIR, the risk of portal hypertension and portal vein thrombosis could be prevented. Especially, muscle and subcutaneous tissue are considered the best positions to transplant (easy to approach in comparison with intraperitoneal organs) and to remove when the graft fails in function or becomes an origin of infection. In our opinion, the ideal transplant site for encapsulated islets is a site that is managed easily and has good efficacy in transplantation.

CONCLUSION

Encapsulated islet transplantation was first performed in 1999. Encapsulated human islet transplantation was performed in a 38 year old man who had severe diabetes by a UCLA group^[78]. In 2000, Elliott *et al*^[79] transplanted encapsulated porcine islets into diabetic patients and confirmed no PERV infection in patients. Clinical trials have been few, but promising outcomes have been reported. Recently, Calafiore *et al*^[80] performed human islets microencapsulation using alginate and transplanted them into 10 T1DM patients in Italy. Their protocol is based on using clinical grade sodium alginate without pyrogen and endotoxin, using human islets which have high purity and viability (over 80%) and a minimally invasive transplant method by intraportal injection under ultrasound imaging with local anesthesia. This protocol is approved by the Italian Ministry of Health. An Australia group performed transplantation of barium-alginate encapsulated human islets to 4 T1DM patients without immunosuppression and followed them for 2.5 years as a phase 1 study^[81]. While no adverse events were detected, there was no improvement of the diabetic condition in the 4 patients. Laparoscopic biopsy revealed no cellular infiltration in

the capsule and islet necrosis. As compared with non-encapsulated islet transplantation with immunosuppression, the outcome of encapsulated islets is promising. Further improvements of the devices are necessary for the clinical setting, but, when successful, could cure severe DM without using immunosuppressants.

In conclusion, encapsulation technology can utilize allo- or xenogeneic cell sources to overcome limited donor supplies in islet transplantation.

REFERENCES

- 1 **Shapiro AM**, Lakey JR, Ryan EA, Korbitt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343**: 230-238
- 2 **Shapiro AM**, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbitt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 2006; **355**: 1318-1330
- 3 **Shapiro AM**, Lakey JR, Paty BW, Senior PA, Bigam DL, Ryan EA. Strategic opportunities in clinical islet transplantation. *Transplantation* 2005; **79**: 1304-1307
- 4 **Ryan EA**, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005; **54**: 2060-2069
- 5 **Huurman VA**, Kalpoe JS, van de Linde P, Vaessen N, Ringers J, Kroes AC, Roep BO, De Fijter JW. Choice of antibody immunotherapy influences cytomegalovirus viremia in simultaneous pancreas-kidney transplant recipients. *Diabetes Care* 2006; **29**: 842-847
- 6 **Drachenberg CB**, Klassen DK, Weir MR, Wiland A, Fink JC, Bartlett ST, Cangro CB, Blahut S, Papadimitriou JC. Islet cell damage associated with tacrolimus and cyclosporine: morphological features in pancreas allograft biopsies and clinical correlation. *Transplantation* 1999; **68**: 396-402
- 7 **Tanemura M**, Saga A, Kawamoto K, Machida T, Deguchi T, Nishida T, Sawa Y, Doki Y, Mori M, Ito T. Rapamycin induces autophagy in islets: relevance in islet transplantation. *Transplant Proc* 2009; **41**: 334-338
- 8 **Qi M**, Gu Y, Sakata N, Kim D, Shirouzu Y, Yamamoto C, Hiura A, Sumi S, Inoue K. PVA hydrogel sheet macroencapsulation for the bioartificial pancreas. *Biomaterials* 2004; **25**: 5885-5892
- 9 **Murua A**, Portero A, Orive G, Hernández RM, de Castro M, Pedraz JL. Cell microencapsulation technology: towards clinical application. *J Control Release* 2008; **132**: 76-83
- 10 **Gates RJ**, Lazarus NR. Reversal of streptozotocin-induced diabetes in rats by intraperitoneal implantation of encapsulated neonatal rabbit pancreatic tissue. *Lancet* 1977; **2**: 1257-1259
- 11 **Munn SR**, Kaufman DB, Field MJ, Viste AB, Sutherland DE. Cold-storage preservation of the canine and rat pancreas prior to islet isolation. *Transplantation* 1989; **47**: 28-31
- 12 **Noguchi H**. Pancreatic islet transplantation. *World J Gastrointest Surg* 2009; **1**: 16-20
- 13 **Kuroda Y**, Kawamura T, Suzuki Y, Fujiwara H, Yamamoto K, Saitoh Y. A new, simple method for cold storage of the pancreas using perfluorochemical. *Transplantation* 1988; **46**: 457-460
- 14 **Noguchi H**, Ueda M, Hayashi S, Kobayashi N, Okitsu T, Iwanaga Y, Nagata H, Nakai Y, Matsumoto S. Ductal injec-

- tion of preservation solution increases islet yields in islet isolation and improves islet graft function. *Cell Transplant* 2008; **17**: 69-81
- 15 **Noguchi H**, Ikemoto T, Naziruddin B, Jackson A, Shimoda M, Fujita Y, Chujo D, Takita M, Kobayashi N, Onaca N, Levy MF, Matsumoto S. Iodixanol-controlled density gradient during islet purification improves recovery rate in human islet isolation. *Transplantation* 2009; **87**: 1629-1635
 - 16 **Noguchi H**, Naziruddin B, Jackson A, Shimoda M, Ikemoto T, Fujita Y, Chujo D, Takita M, Kobayashi N, Onaca N, Levy MF, Matsumoto S. Low-temperature preservation of isolated islets is superior to conventional islet culture before islet transplantation. *Transplantation* 2010; **89**: 47-54
 - 17 **Sakata N**, Kodama T, Chen R, Yoshimatsu G, Goto M, Egawa S, Unno M. Monitoring transplanted islets by high-frequency ultrasound. *Islets* 2011; **3**: 259-266
 - 18 **Sakata N**, Chan NK, Chrisler J, Obenaus A, Hathout E. Bone marrow cell cotransplantation with islets improves their vascularization and function. *Transplantation* 2010; **89**: 686-693
 - 19 **Sakata N**, Tan A, Chan N, Obenaus A, Mace J, Peverini R, Sowers L, Chinnock R, Hathout E. Efficacy comparison between intraportal and subcapsular islet transplants in a murine diabetic model. *Transplant Proc* 2009; **41**: 346-349
 - 20 **Christofferson G**, Carlsson PO, Phillipson M. Intramuscular islet transplantation promotes restored islet vascularity. *Islets* 2011; **3**: 69-71
 - 21 **McQuilling JP**, Arenas-Herrera J, Childers C, Pareta RA, Khanna O, Jiang B, Brey EM, Farney AC, Opara EC. New alginate microcapsule system for angiogenic protein delivery and immunoisolation of islets for transplantation in the rat omentum pouch. *Transplant Proc* 2011; **43**: 3262-3264
 - 22 **Gores PF**, Hayes DH, Copeland MJ, Korbitt GS, Halberstadt C, Kirkpatrick SA, Rajotte RV. Long-term survival of intratesticular porcine islets in nonimmunosuppressed beagles. *Transplantation* 2003; **75**: 613-618
 - 23 **Kover K**, Tong PY, Pacica D, Clements M, Bodker AM, Eidson C, Sheldon M, Southard A, Zaidi A, Moore WV. Bone marrow cavity: a supportive environment for islet engraftment. *Islets* 2011; **3**: 93-101
 - 24 **Brennan DC**, Shannon MB, Koch MJ, Polonsky KS, Desai N, Shapiro J. Portal vein thrombosis complicating islet transplantation in a recipient with the Factor V Leiden mutation. *Transplantation* 2004; **78**: 172-173
 - 25 **Kawahara T**, Kin T, Kashkoush S, Gala-Lopez B, Bigam DL, Kneteman NM, Koh A, Senior PA, Shapiro AM. Portal vein thrombosis is a potentially preventable complication in clinical islet transplantation. *Am J Transplant* 2011; **11**: 2700-2707
 - 26 **Najarian JS**, Sutherland DE, Baumgartner D, Burke B, Rynasiewicz JJ, Matas AJ, Goetz FC. Total or near total pancreatectomy and islet autotransplantation for treatment of chronic pancreatitis. *Ann Surg* 1980; **192**: 526-542
 - 27 **Farney AC**, Najarian JS, Nakhleh RE, Lloveras G, Field MJ, Gores PF, Sutherland DE. Autotransplantation of dispersed pancreatic islet tissue combined with total or near-total pancreatectomy for treatment of chronic pancreatitis. *Surgery* 1991; **110**: 427-437; discussion 437-439
 - 28 **Sakata N**, Egawa S, Motoi F, Mikami Y, Ishida M, Aoki T, Ottomo S, Fukuyama S, Rikiyama T, Katayose Y, Goto M, Unno M. Institutional indications for islet transplantation after total pancreatectomy. *J Hepatobiliary Pancreat Surg* 2008; **15**: 488-492
 - 29 **Jindal RM**, Ricordi C, Shriver CD. Autologous pancreatic islet transplantation for severe trauma. *N Engl J Med* 2010; **362**: 1550
 - 30 **Lim F**, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 1980; **210**: 908-910
 - 31 **Leung YF**, O'Shea GM, Goosen MF, Sun AM. Microencapsulation of crystalline insulin or islets of Langerhans: an insulin diffusion study. *Artif Organs* 1983; **7**: 208-212
 - 32 **de Vos P**, Hamel AF, Tatarkiewicz K. Considerations for successful transplantation of encapsulated pancreatic islets. *Diabetologia* 2002; **45**: 159-173
 - 33 **Chandy T**, Mooradian DL, Rao GH. Evaluation of modified alginate-chitosan-polyethylene glycol microcapsules for cell encapsulation. *Artif Organs* 1999; **23**: 894-903
 - 34 **Desai NP**, Sojomihardjo A, Yao Z, Ron N, Soon-Shiong P. Interpenetrating polymer networks of alginate and polyethylene glycol for encapsulation of islets of Langerhans. *J Microencapsul* 2000; **17**: 677-690
 - 35 **Goosen MF**, O'Shea GM, Gharapetian HM, Chou S, Sun AM. Optimization of microencapsulation parameters: Semi-permeable microcapsules as a bioartificial pancreas. *Biotechnol Bioeng* 1985; **27**: 146-150
 - 36 **Lanza RP**, Jackson R, Sullivan A, Ringeling J, McGrath C, Kührtreiber W, Chick WL. Xenotransplantation of cells using biodegradable microcapsules. *Transplantation* 1999; **67**: 1105-1111
 - 37 **Strand BL**, Gåserød O, Kulseng B, Espevik T, Skjåk-Baek G. Alginate-polylysine-alginate microcapsules: effect of size reduction on capsule properties. *J Microencapsul* 2002; **19**: 615-630
 - 38 **Beck J**, Angus R, Madsen B, Britt D, Vernon B, Nguyen KT. Islet encapsulation: strategies to enhance islet cell functions. *Tissue Eng* 2007; **13**: 589-599
 - 39 **Petersen P**, Lambert N, Zschocke P, Stenglein S, Planck H, Ammon HP, Becker HD. Hydroxymethylated polysulphone for islet macroencapsulation allows rapid diffusion of insulin but retains PERV. *Transplant Proc* 2002; **34**: 194-195
 - 40 **Lambert N**, Wesche J, Petersen P, Zschocke P, Enderle A, Planck H, Ammon HP. Macroencapsulation of rat islets without alteration of insulin secretion kinetics. *Exp Clin Endocrinol Diabetes* 2001; **109**: 116-119
 - 41 **Lambert N**, Wesche J, Petersen P, Doser M, Zschocke P, Becker HD, Ammon HP. Encapsulation of islets in rough surface, hydroxymethylated polysulfone capillaries stimulates VEGF release and promotes vascularization after transplantation. *Cell Transplant* 2005; **14**: 97-108
 - 42 **Inoue K**, Fujisato T, Gu YJ, Burczak K, Sumi S, Kogire M, Tobe T, Uchida K, Nakai I, Maetani S. Experimental hybrid islet transplantation: application of polyvinyl alcohol membrane for entrapment of islets. *Pancreas* 1992; **7**: 562-568
 - 43 **Aung T**, Kogire M, Inoue K, Fujisato T, Gu Y, Burczak K, Shinohara S, Mitsuo M, Maetani S, Ikada Y. Insulin release from a bioartificial pancreas using a mesh reinforced polyvinyl alcohol hydrogel tube. An in vitro study. *ASAIO J* 1993; **39**: 93-96
 - 44 **Miyamoto M**, Inoue K, Gu Y, Tun T, Cui W, Fujiwara I, Ohyanagi H, Hayashi H, Yamazaki T, Setoyama H, Kawakami Y, Ida J, Kogire M, Imamura M, Iwata H, Ikada Y. Improved large-scale isolation of breeder porcine islets: possibility of harvesting from nonheart-beating donor. *Cell Transplant* 1998; **7**: 397-402
 - 45 **Sakurai T**, Satake A, Sumi S, Inoue K, Nagata N, Tabata Y, Miyakoshi J. The efficient prevascularization induced by fibroblast growth factor 2 with a collagen-coated device improves the cell survival of a bioartificial pancreas. *Pancreas* 2004; **28**: e70-e79
 - 46 **Sakata N**, Gu Y, Qi M, Yamamoto C, Hiura A, Sumi S, Sunamura M, Matsuno S, Inoue K. Effect of rat-to-mouse bioartificial pancreas xenotransplantation on diabetic renal damage and survival. *Pancreas* 2006; **32**: 249-257
 - 47 **Murakami Y**, Iwata H, Kitano E, Kitamura H, Ikada Y. Dextran sulfate as a material for the preparation of a membrane for immunoisolation. *J Biomater Sci Polym Ed* 2003; **14**: 875-885
 - 48 **Goto M**, Johansson H, Maeda A, Elgue G, Korsgren O, Nilsson B. Low molecular weight dextran sulfate prevents the instant blood-mediated inflammatory reaction induced by adult porcine islets. *Transplantation* 2004; **77**: 741-747

- 49 **Johansson H**, Goto M, Dufrane D, Siegbahn A, Elgue G, Gianello P, Korsgren O, Nilsson B. Low molecular weight dextran sulfate: a strong candidate drug to block IBMIR in clinical islet transplantation. *Am J Transplant* 2006; **6**: 305-312
- 50 **Goto M**, Tjernberg J, Dufrane D, Elgue G, Brandhorst D, Ekdahl KN, Brandhorst H, Wennberg L, Kurokawa Y, Satomi S, Lambris JD, Gianello P, Korsgren O, Nilsson B. Dissecting the instant blood-mediated inflammatory reaction in islet xenotransplantation. *Xenotransplantation* 2008; **15**: 225-234
- 51 **Monaco AP**, Maki T, Ozato H, Carretta M, Sullivan SJ, Borland KM, Mahoney MD, Chick WL, Muller TE, Wolfrum J. Transplantation of islet allografts and xenografts in totally pancreatectomized diabetic dogs using the hybrid artificial pancreas. *Ann Surg* 1991; **214**: 339-360; discussion 361-362
- 52 **Jones KS**, Sefton MV, Gorczynski RM. In vivo recognition by the host adaptive immune system of microencapsulated xenogeneic cells. *Transplantation* 2004; **78**: 1454-1462
- 53 **Hayashi H**, Inoue K, Aung T, Tun T, Yuanjun G, Wenjing W, Shinohara S, Kaji H, Doi R, Setoyama H, Kato M, Imamura M, Maetani S, Morikawa N, Iwata H, Ikada Y, Miyazaki J. Application of a novel B cell line MIN6 to a mesh-reinforced polyvinyl alcohol hydrogel tube and three-layer agarose microcapsules: an in vitro study. *Cell Transplant* 1996; **5**: S65-S69
- 54 **Lee JI**, Nishimura R, Sakai H, Sasaki N, Kenmochi T. A newly developed immunoisolated bioartificial pancreas with cell sheet engineering. *Cell Transplant* 2008; **17**: 51-59
- 55 **Johnson AS**, O'Sullivan E, D'Aoust LN, Omer A, Bonner-Weir S, Fisher RJ, Weir GC, Colton CK. Quantitative assessment of islets of Langerhans encapsulated in alginate. *Tissue Eng Part C Methods* 2011; **17**: 435-449
- 56 **Kobayashi T**, Aomatsu Y, Iwata H, Kin T, Kanehiro H, Hisanga M, Ko S, Nagao M, Harb G, Nakajima Y. Survival of microencapsulated islets at 400 days posttransplantation in the omental pouch of NOD mice. *Cell Transplant* 2006; **15**: 359-365
- 57 **Laugharne M**, Cross S, Richards S, Dawson C, Ilchyshyn L, Saleem M, Mathieson P, Smith R. Sirolimus toxicity and vascular endothelial growth factor release from islet and renal cell lines. *Transplantation* 2007; **83**: 1635-1638
- 58 **Dufrane D**, Goebbels RM, Gianello P. Alginate macroencapsulation of pig islets allows correction of streptozotocin-induced diabetes in primates up to 6 months without immunosuppression. *Transplantation* 2010; **90**: 1054-1062
- 59 **Rosenfeld L**. Insulin: discovery and controversy. *Clin Chem* 2002; **48**: 2270-2288
- 60 **Brogden RN**, Heel RC. Human insulin. A review of its biological activity, pharmacokinetics and therapeutic use. *Drugs* 1987; **34**: 350-371
- 61 **Lanza RP**, Butler DH, Borland KM, Staruk JE, Faustman DL, Solomon BA, Muller TE, Rupp RG, Maki T, Monaco AP. Xenotransplantation of canine, bovine, and porcine islets in diabetic rats without immunosuppression. *Proc Natl Acad Sci USA* 1991; **88**: 11100-11104
- 62 **Sun AM**, Vacek I, Sun YL, Ma X, Zhou D. In vitro and in vivo evaluation of microencapsulated porcine islets. *ASAIO J* 1992; **38**: 125-127
- 63 **van der Laan LJ**, Lockey C, Griffeth BC, Frasier FS, Wilson CA, Onions DE, Hering BJ, Long Z, Otto E, Torbett BE, Salomon DR. Infection by porcine endogenous retrovirus after islet xenotransplantation in SCID mice. *Nature* 2000; **407**: 90-94
- 64 **Clémenceau B**, Jégou D, Martignat L, Sai P. Microchimerism and transmission of porcine endogenous retrovirus from a pig cell line or specific pathogen-free pig islets to mouse tissues and human cells during xenografts in nude mice. *Diabetologia* 2002; **45**: 914-923
- 65 **Denner J**, Specke V, Karlas A, Chodnevskaja I, Meyer T, Moskalenko V, Kurth R, Ulrichs K. No transmission of porcine endogenous retroviruses (PERVs) in a long-term pig to rat xenotransplantation model and no infection of immunosuppressed rats. *Ann Transplant* 2008; **13**: 20-31
- 66 **Garkavenko O**, Dieckhoff B, Wynyard S, Denner J, Elliott RB, Tan PL, Croxson MC. Absence of transmission of potentially xenotic viruses in a prospective pig to primate islet xenotransplantation study. *J Med Virol* 2008; **80**: 2046-2052
- 67 **Irgang M**, Laue C, Velten F, Kurth R, Schrezenmeier J, Denner J. No evidence for PERV release by islet cells from German landrace pigs. *Ann Transplant* 2008; **13**: 59-66
- 68 **Abrahante JE**, Martins K, Papas KK, Hering BJ, Schuurman HJ, Murtaugh MP. Microbiological safety of porcine islets: comparison with source pig. *Xenotransplantation* 2011; **18**: 88-93
- 69 **Sattar SP**, Ahmed MS, Madison J, Olsen DR, Bhatia SC, El-lahi S, Majeed F, Ramaswamy S, Petty F, Wilson DR. Patient and physician attitudes to using medications with religiously forbidden ingredients. *Ann Pharmacother* 2004; **38**: 1830-1835
- 70 **Sakata N**, Obenaus A, Chan N, Mace J, Chinnock R, Hathout E. Factors affecting islet graft embolization in the liver of diabetic mice. *Islets* 2009; **1**: 26-33
- 71 **Walsh TJ**, Eggleston JC, Cameron JL. Portal hypertension, hepatic infarction, and liver failure complicating pancreatic islet autotransplantation. *Surgery* 1982; **91**: 485-487
- 72 **Schneider S**, von Mach MA, Kraus O, Kann P, Feilen PJ. Intraportal transplantation of allogenic pancreatic islets encapsulated in barium alginate beads in diabetic rats. *Artif Organs* 2003; **27**: 1053-1056
- 73 **Balamurugan AN**, Gu Y, Tabata Y, Miyamoto M, Cui W, Hori H, Satake A, Nagata N, Wang W, Inoue K. Bioartificial pancreas transplantation at prevascularized intermuscular space: effect of angiogenesis induction on islet survival. *Pancreas* 2003; **26**: 279-285
- 74 **Kawakami Y**, Iwata H, Gu YJ, Miyamoto M, Murakami Y, Balamurugan AN, Imamura M, Inoue K. Successful subcutaneous pancreatic islet transplantation using an angiogenic growth factor-releasing device. *Pancreas* 2001; **23**: 375-381
- 75 **Wang W**, Gu Y, Tabata Y, Miyamoto M, Hori H, Nagata N, Touma M, Balamurugan AN, Kawakami Y, Nozawa M, Inoue K. Reversal of diabetes in mice by xenotransplantation of a bioartificial pancreas in a prevascularized subcutaneous site. *Transplantation* 2002; **73**: 122-129
- 76 **Wang W**, Gu Y, Hori H, Sakurai T, Hiura A, Sumi S, Tabata Y, Inoue K. Subcutaneous transplantation of macroencapsulated porcine pancreatic endocrine cells normalizes hyperglycemia in diabetic mice. *Transplantation* 2003; **76**: 290-296
- 77 **Dufrane D**, Goebbels RM, Saliez A, Guiot Y, Gianello P. Six-month survival of microencapsulated pig islets and alginate biocompatibility in primates: proof of concept. *Transplantation* 2006; **81**: 1345-1353
- 78 **Soon-Shiong P**. Treatment of type I diabetes using encapsulated islets. *Adv Drug Deliv Rev* 1999; **35**: 259-270
- 79 **Elliott RB**, Escobar L, Garkavenko O, Croxson MC, Schroeder BA, McGregor M, Ferguson G, Beckman N, Ferguson S. No evidence of infection with porcine endogenous retrovirus in recipients of encapsulated porcine islet xenografts. *Cell Transplant* 2000; **9**: 895-901
- 80 **Calafiore R**, Basta G, Luca G, Lemmi A, Racanicchi L, Mancuso F, Montanucci MP, Brunetti P. Standard technical procedures for microencapsulation of human islets for graft into nonimmunosuppressed patients with type 1 diabetes mellitus. *Transplant Proc* 2006; **38**: 1156-1157
- 81 **Tuch BE**, Keogh GW, Williams LJ, Wu W, Foster JL, Vaithilingam V, Philips R. Safety and viability of microencapsulated human islets transplanted into diabetic humans. *Diabetes Care* 2009; **32**: 1887-1889

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Host-microbial interactions and regulation of intestinal epithelial barrier function: From physiology to pathology

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restitution and reorganization of tight junctions, all of which are pivotal for fortifying barrier function. Recent studies indicate that aberrant bacterial lipopolysaccharide-mediated signaling in gut mucosa may be involved in the pathogenesis of chronic inflammation and carcinogenesis. Our perception of enteric commensals has now changed from one of opportunistic pathogens to active participants in maintaining intestinal homeostasis. This review attempts to explain the dynamic interaction between the intestinal epithelium and commensal bacteria in disease and health status.

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Abstract

The gastrointestinal tract is the largest reservoir of commensal bacteria in the human body, providing nutrients and space for the survival of microbes while concurrently operating mucosal barriers to confine the microbial population. The epithelial cells linked by tight junctions not only physically separate the microbiota from the lamina propria, but also secrete proinflammatory cytokines and reactive oxygen species in response to pathogen invasion and metabolic stress and serve as a sentinel to the underlying immune cells. Accumulating evidence indicates that commensal bacteria are involved in various physiological functions in the gut and microbial imbalances (dysbiosis) may cause pathology. Commensal bacteria are involved in the regulation of intestinal epithelial cell turnover, promotion of epithelial

INTRODUCTION

The gastrointestinal tract is the largest reservoir of commensal bacteria in the human body. Food intake through the oral route serves as a port to the outside environment and allows for entry of exogenous organisms, and nutrients in the gastrointestinal tract support growth and survival of both the host and commensals. With this unique feature, the healthy gut is required to perform

digestive and absorptive functions while it concurrently maintains a barrier against luminal microbes. Accumulating evidence indicates that the taxonomically complex intestinal microbes constitute a dynamic community (microbiota) that is now known to have a strong impact on human physiology.

Humans are born germ-free, yet, rapidly after birth, bacteria populates the digestive tract and establishes a microbial ecosystem in the gut^[1]. The bacterial density gradually increases along the proximal to distal segments of the gastrointestinal tract and rises to an estimated 10^{11} to 10^{12} bacteria per gram of colonic content. The enteric bacterial population consists of up to 100 trillion (10^{14}) cells, which is ten times the number of cells of the human body^[2,3]. The gut microbiota is highly diverse and displays an individual-specific composition determined by host genotype and environmental factors. It had been estimated that more than 500 bacterial species inhabit the human gut, based mainly on culturing techniques^[4,5]. With the advancement of metagenomic technology, our knowledge of the diversity of bacterial species has expanded rapidly beyond the list obtained from traditional microbiological methods, by which many gut bacteria are not culturable. Around 15 000 to 36 000 species of bacteria have now been identified in the human gastrointestinal tract using culture-independent rRNA sequence analysis^[6,7]. A recent paper from the Metagenomics of the Human Intestinal Tract project revealed a total of 3.3 million non-redundant microbial genes in human fecal specimens^[8]. Much to our surprise, this number is approximately 150 times larger than the protein-encoding gene set in human cells (approximately 20 000 genes according to data of Human Genome Project)^[9,10]. Commonly identified enteric commensal bacteria include the phyla of Firmicutes (species such as *Lactobacillus*, *Clostridium*, *Enterococcus*), Bacteroidetes (species such as *Bacteroides*), Proteobacteria (species such as *Escherichia coli*) and Actinobacteria (species such as *Bifidobacteria*)^[6,11].

Commensal bacteria were traditionally considered simply as co-living organisms residing in the gut lumen without much interaction with the host, and their quiet presence in the intestines did not draw interest from the gastroenterological field for several decades. Paradoxically, cardiologists and researchers in critical care medicine have paid much more attention to these bacteria in situations of gut barrier damage. In the event of their invasion to the systemic circulation and/or extraintestinal sterile organs, gut-derived bugs may pose a serious risk to the individual by inadvertently triggering septic shock, systemic inflammatory response syndrome and subsequent multiple organ failure^[12,13]. Abnormal enteric bacterial translocation and gut-derived sepsis have been documented clinically and observed in animal models of intestinal ischemia/reperfusion^[14-16], bowel obstruction^[17,18] and hemorrhagic and traumatic shock^[19,20].

The beneficial effects of our co-evolved microorganisms have begun to be seen recently^[3,21]. It is now generally believed that commensal bacteria are involved

in various physiological functions in the gut, whereas dysbiosis (a term that describes the condition of having microbial imbalances within the body) may cause pathology^[6,22]. This review will discuss the classical view and the recent knowledge of host-microbe interaction in the gastrointestinal tract. Early studies investigated the maintenance of a passive intestinal barrier to confine the luminal bacteria and to fend off invasions of opportunistic microbes; current research is focused on the beneficial effects of commensal bacteria on the hosts as well as the influence from an active intestinal barrier on the microfloral population in order to maintain gut homeostasis and, in a broader aspect, to promote the health of the host.

INTESTINAL BARRIERS FOR LUMINAL CONFINEMENT OF COMMENSAL BACTERIA

There is no doubt that tight control of the location, number and population of enteric bacteria by the hosts is prerequisite for health-promoting effects. Luminal confinement of commensal microflora is a main task of the gut mucosa. To prevent microbial dissemination or invasion of sterile extraintestinal viscera, physical barriers composed of epithelial cells and mucus layer, chemical barriers with antimicrobial peptides, and immune barriers including secretory IgA, act as front lines of defense. If these foremost barriers fail and bacteria translocation occurs, activation of immune cells in the lamina propria including phagocytes and lymphocytes are next in line to carry out antimicrobial actions (Figure 1).

Epithelial barrier limits the space for bacterial growth

The luminal surface of the gastrointestinal tract from the stomach to the rectum is covered by a single layer of epithelial cells. These epithelial cells with their well-ordered brush borders constitute a large surface area that is multiplied both by the macroscopic features of valvulae conniventes and the microscopic structures of finger-like villi. The vast interior surface area of the gut lining allows for efficient nutrient uptake for the individual. On the other hand, this large surface area also has to tolerate noxious luminal contents and form a competent barrier and/or defense mechanism in face of a massive load of antigenic substances and microbes. It is worth noting that this amazing balancing act between uptake and exclusion is managed by intestinal epithelial cells with a dynamic turnover pace.

Crypt-villus axis and enterocytic turnover rates: The turnover rates of intestinal epithelial cells (enterocytes) are governed by the pace of crypt cell proliferation and villus/surface cell shedding. The newly proliferated stem cells in the crypt regions differentiate into epithelial cells with high expression of brush border enzymes and transporters, and concurrently migrate upward to the apex of the villi where cell apoptosis and detachment occurs at the so-called "extrusion zone" (Figure 1)^[23].

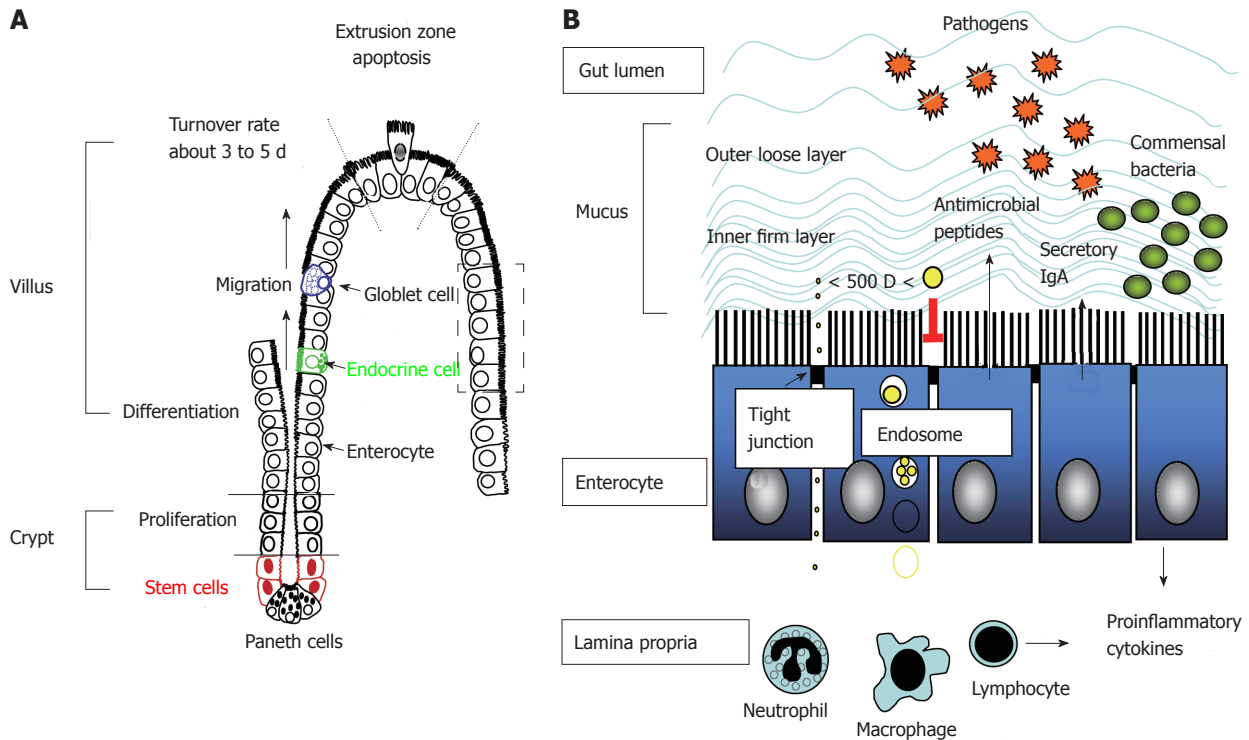


Figure 1 Intestinal crypt-villus axis and formation of intestinal barriers for luminal confinement of commensal bacteria. A: Stem cells in the crypt regions undergo proliferation and differentiation into columnar epithelial cells (enterocytes) with high expression of brush border enzymes and transporters, and concurrently migrate upward to the apex of the villi where cell apoptosis and shedding occurs at the so-called "extrusion zone". The stem cells also differentiate into Paneth cells that migrate downward to the crypt bottom, as well as into mucin-secreting goblet cells and enteroendocrine cells that migrate upwards to the villous tips. During the differentiation and migration process, tight junctional proteins are formed at the cell-cell contact sites to seal off gaps between enterocytes; B: Enteric microbes are restricted in the gut lumen by physical barriers composed of epithelium and mucus, chemical barriers with antimicrobial peptides, and immune barriers such as secretory immunoglobulin A (IgA). The tight junctional complexes between plasma membranes of two cells exclude the influx of bacteria and molecules larger than 500 dalton through paracellular routes, whereas endosomal degradation limits transcellular transport of particles and proteins. If the epithelial barrier is breached and invasion of bacteria occurs, the underlying immune cells in the lamina propria such as phagocytes (macrophages and neutrophils) and lymphocytes are responsible for antimicrobial and inflammatory responses.

These stem cells also differentiate into Paneth cells that migrate downward to the bottom of the crypt, as well as into goblet cells and enteroendocrine cells in the epithelial layer that migrate upwards to the villous tips. The cell migration process along the crypt-villus axis is dependent on dynamic turnover of focal cell-matrix adhesions. Although the order of apoptosis and sloughing of cells on villous tips is still in debate, accumulating evidence indicates that the apoptotic signaling cascade proceeds along with the purse-string action of cell extrusion^[24,25].

During the differentiation and migration process, epithelial tight junctional proteins are formed at the cell-cell contact sites to seal off gaps between cells. The physical barrier constituted by these closely linked epithelial cells is the rate-limiting factor that determines intestinal permeability. Physiological epithelial apoptosis and extrusion at the villous tips does not compromise barrier function^[26,27]. Abundant studies have indicated that tight junctional proteins are present at the base of basolateral membranes between two neighboring enterocytes flanking the extruding cells, and thus barrier functions are sustained at the villous tips^[26,27]. Nevertheless, excessive epithelial cell death caused by pathogenic microbes^[28-32], metabolic stress^[15,16], and nonsteroidal anti-inflammatory

drugs, acidic or enzymatic agents^[33,34], may lead to villous surface denudation and gut leakiness if crypt proliferation and enterocytic migration were not sufficient to cover the wounded area. Conversely, high rates of cell proliferation and resistance to cell apoptosis are known to be two equally important determining factors during the early stages of colorectal carcinogenesis^[35,36]. The balance between these two events, i.e. cell death and proliferation of epithelial cells, is now recognized as a single key determinant for gut homeostasis.

Paracellular epithelial permeability: The intestinal epithelial cells are joined at their apical side by tight junctions (TJs). The tight junctional complexes form the narrowest distance between plasma membranes of two cells, thus excluding the influx of bacteria through paracellular routes. The transmembranous junctional proteins, e.g., claudins, occludin or junction-associated molecule, are linked to intracellular zonula occludens (ZO) which are bridges to cytoskeletal actin and myosin filaments^[37,38].

The organization of TJ proteins and perijunctional actinomyosins are regulated by a complex network of signaling pathways. Contraction of the actinomyosin filaments that open up paracellular junctions is mediated

by the phosphorylation of myosin light chain (MLC) *via* activation of myosin light chain kinase (MLCK) or Rho-associated kinase (ROCK)^[17,39]. In addition to the physical opening of TJs, ROCK also mediates the endocytosis of TJ proteins into vacuolar apical compartments^[39]. Different isoforms of protein kinase C (PKC) are involved in the processes of TJ opening and assembly^[40]. The atypical PKC zeta is the sole isoform found located at intercellular contact sites^[41,42]. Recent evidence shows that PKC zeta directly interacts with and phosphorylates occludin, causing the redistribution of occludin away from intercellular junctions in cell culture monolayers^[43]. A large body of evidence showed that abnormal passage of bacteria across the epithelial layer may occur *via* the paracellular routes in disease states. Increased epithelial MLC-dependent paracellular permeability was associated with enhanced bacterial translocation to extraintestinal organ routes in experimental models of colitis and bowel obstruction^[17,18,44-46]. Increased paracellular permeability and tight junctional disruption were also documented in *in vitro* cultures of human intestinal epithelial Caco-2 cells challenged with Gram-negative bacterial lipopolysaccharide (LPS)^[31].

Transcellular epithelial permeability: Transcellular transport of particles and proteins are limited by endosomal degradation within enterocytes. Dietary proteins are mostly digested by gastric and pancreatic proteases, as well as integral brush border enzymes, and converted to small peptides and amino acids, which are then absorbed by enterocytes *via* electrogenic or sodium-dependent transporters. Although a small amount of intact protein may be endocytosed into epithelial cells in physiological conditions, most of it is sorted into lysosomal compartments for degradation and therefore, transcytosis of whole proteins is prevented^[47-49].

Most commensal bacteria are separated from the epithelial surface by the mucus layer and these bacteria do not internalize into epithelial cells. However, increased translocation of nonpathogenic bacteria *via* the transcellular routes has been documented in epithelial cells under inflammatory situations and metabolic stresses, such as low dose immunoreactive fibronectin-gamma (IFN γ)^[50], tumor necrosis factor-alpha (TNF α) during glutamine deprivation^[51], uncoupling of mitochondrial oxidative phosphorylation^[52,53], low dose nitric oxide^[54,55] and hypoxia^[56]. Other studies^[57-62] have documented the internalization of bacterial LPS and their binding to intracellular receptors in cell culture models and in mouse enterocytes. Recent reports also showed that commensal bacteria may be engulfed into intestinal epithelial cells in the presence of pathogenic invasive bacterial strains. Using a polarized human intestinal epithelial cell model system, it was demonstrated that *Campylobacter jejuni* (a common enteric pathogen identified in humans and chickens) not only penetrates into epithelial cells itself but also promotes the internalization and translocation of non-invasive, nonpathogenic *E. coli* *via* a lipid raft-

dependent mechanism^[63].

Antigen sampling and uptake of bacterial particles by follicle-associated epithelium [mainly by microfold (M) cells] on Peyer's patches (PP) is another route of transcellular transport for luminal substances. These PPs are specialized lymphoid follicles in the gut with a large number of dendritic cells in the dome region^[64]. This particular form of luminal antigen transport across follicle-associated epithelium has been implicated in induction of oral tolerance and was reviewed previously^[48,65]. Recent evidence shows that although most enteric bacteria resides in the mucus blanket, there are exceptions, i.e., segmented filamentous bacteria (SFB), a *Clostridium*-related species that anchors on the gut epithelial cells adjacent to M cells^[66,67].

Chemical and immune barriers shape the microbial population

The epithelial cells linked by tight junctions not only physically separate the microbiota from the lamina propria, but also secrete proinflammatory cytokines and reactive oxygen species in response to pathogen invasion and metabolic stress, and serves as a warning system to the underlying immune cells to combat microbes^[68-71]. The epithelial layer is now considered as an active participant in innate immunity. Other chemical and immune barriers to restrict and shape the enteric bacterial population include antimicrobial peptides, sIgA, phagocytes and lymphocytes.

Antimicrobial peptides: Antimicrobial peptides (AMPs) or host defense peptides are small cationic peptides that exhibit broad-spectrum antibiotic activity against Gram-positive and Gram-negative bacteria, fungi, yeasts and viruses^[72]. Defensins (cryptdins) are stored in the granules of Paneth cells situated besides the proliferative crypt stem cells, and are secreted into the luminal space in response to bacteria and microbial molecules, e.g., oligonucleotides and LPS^[73-75]. Triggers for the production of cathelicidin-related AMPs in epithelial cells and neutrophils include bacterial flagellin and LPS^[76,77].

Accumulating data indicate a crucial role of AMPs in shaping the commensal bacterial population. A developmental switch of gut AMP expression during the neonatal period is correlated with the establishment of commensal microflora. Previous studies showed that production of mouse cathelin-related antimicrobial peptides (mCRAMP) can be observed in the first two weeks after birth and gradually disappears with the onset of stem cell proliferation and establishment of the crypt-villus axis. The synthesis of mCRAMP was found to play a role in the inhibition of growth of *Listeria monocytogenes*, which is a commensal bacteria populated in the mother's vaginal canal but a potential pathogen in the neonatal gut^[78]. In addition, Paneth cells and defensin production appear after 2 wk of birth, which accompanies the development of intestinal crypts^[79,80].

Human Paneth cell defensins HD-5 and HD-6 are

stored in their inactive form and are activated by trypsin after secretion^[81], whereas mouse procrptidins (α -defensins) are activated by matrix metalloproteinase-7 (MMP-7)^[82]. An elegant study using mice overexpressing human α -defensin HD-5 and others lacking functional α -defensins by genetic deficiency of MMP-7 showed that there is no change in the total number of commensal bacteria, but only alterations in the ratio of the two major bacterial phyla *Firmicutes* and *Bacteroides*^[83]. Interestingly, overexpression of *HD-5* inhibited the adherence of SFB to epithelial cells close to M cells on PP in mice^[83]. The physiological significance of the attaching SFB has been discussed, including stimulation of sIgA production and regulation of T lymphocyte differentiation^[83-85]. Much exploration is needed to understand the interactions between AMP synthesis and the shaping of the commensal bacterial population.

Secretory IgA: The presence of sIgA in the luminal space of the gastrointestinal tract has long been associated with the prevention of infection and dissemination by pathogen neutralization^[86]. However, recent evidence shows that sIgA is also involved in homeostatic control of the commensal microbiota. Enteric commensal bacteria were found to be coated with highly specific anti-commensal sIgA^[87]. The intestinal IgA production is profoundly affected by the colonization of commensal microflora, as evidenced by the low level of IgA in germ-free animals, which is corrected after inoculation with luminal bacteria^[88,89]. Recent studies showed that luminal sIgA selectively adhered to M cells in the mouse and human intestinal PP *via* a novel IgA receptor and mediated translocation of bacteria and antigenic products to the underlying dendritic cells^[90,91]. The luminal bacterial uptake by the sIgA into PPs induces naïve B cells to differentiate into IgA-committed plasma cells^[92] and causes a decrease in proinflammatory cytokine expression that accompanies the neutralization of pathogenic bacteria^[93]. These IgA-committed B cells in PPs and the mesenteric lymph nodes subsequently drain into the thoracic duct and bloodstream, and finally return home to the intestinal mucosa^[94]. The sIgA produced by these lamina propria plasma cells is then transported across the epithelial cells *via* the polymeric immunoglobulin receptor into gut lumen^[95]. The roundtrip, bidirectional transport of sIgA and the bacterial coating mediated by sIgA have been implicated in the mechanism of antigen neutralization that curtails luminal bacterial overgrowth^[96].

Phagocytes and lymphocytes: Once the mucus and epithelial barrier are breached, phagocytes residing in and infiltrated into the lamina propria are next in line for mucosal defense. The phagocytic functions of macrophages and neutrophils are just one part of innate immunity, in which these cells also produce large amounts of reactive oxygen species (ROS, e.g., superoxide and hydrogen peroxide) *via* catalytic activities of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase and

myeloperoxidase. Aside from phagocytic sources, intestinal epithelial cells also contain isoforms of NADPH oxidase, e.g., NOX1, and generate superoxide upon stimulation with pro-inflammatory cytokines or with microbial molecules^[69-71,97]. These oxidative free radicals are efficient in killing bacteria through lipid peroxidation, protein nitrosylation, and DNA strand breakage, which eventually leads to death of the microbial targets^[98,99].

The adaptive arm of the gut immune system, termed gut-associated lymphoid tissues, include lymphocytes scattered in the lamina propria, intraepithelial lymphocytes and those aggregated into lymphoid nodules, such as PP and mesenteric lymph nodes. Depending on the cytokine production profile, the differentiated T helper lymphocytes are mainly subgrouped into Th1, Th2, Th3/Tr1 and Th17. The classical dichotomy of Th1/Th2 paradigm of CD4(+) T-cell subsets are associated with inflammation and allergy, respectively; whereas the Th3/Tr1 subgroups are involved in immunoregulatory and suppressive events. The identification of an additional subset, known as Th17 cells, has further illustrated the complexity and diversity of effector T cells with pro-inflammatory characteristics.

Studies using germ-free mice have shown that the frequency of Th17 cells in the lamina propria of the large intestine is significantly elevated in the absence of commensal bacteria^[100], suggesting that enteric microbes are involved in the reduction of the numbers of this pro-inflammatory T lymphocyte subset. The differentiation of Th17 cells is promoted by interleukin 6 (IL-6) and transforming growth factor-beta, whereas IL-23 is required for the subsequent expansion of committed Th17 cells and production of IL-17^[101]. An IL-25-IL-23-IL-17 axis was recently implicated in abnormal reactions towards the individual's own commensal bacteria that cause autoimmune chronic inflammation in the gut^[100]. Commensal-dependent expression of epithelial IL-25 restricted the expansion of Th17 cells by decreasing the expression of macrophage-derived IL-23^[100], suggesting that commensal bacteria may promote immune cell hyporesponsiveness through epithelial signaling. Conversely, other reports have demonstrated that specific microbes, i.e., SFB, induce the differentiation of Th17 cells in the intestine of gnotobiotic mice^[85,102]. Taken together, these findings indicated that eco-imbalance with particular strains of bacteria or dysbiosis may be a cause for inflammatory responses in the intestine.

COMMENSAL BACTERIA REGULATES INTESTINAL EPITHELIAL BARRIER FUNCTIONS

The traditional concept regarding commensal bacterial as a potential threat to the human body is now changed by evidence of the beneficial effects of gut microbiota in promoting epithelial barrier integrity (Figure 2). At this point, the various health-promoting effects of commensal

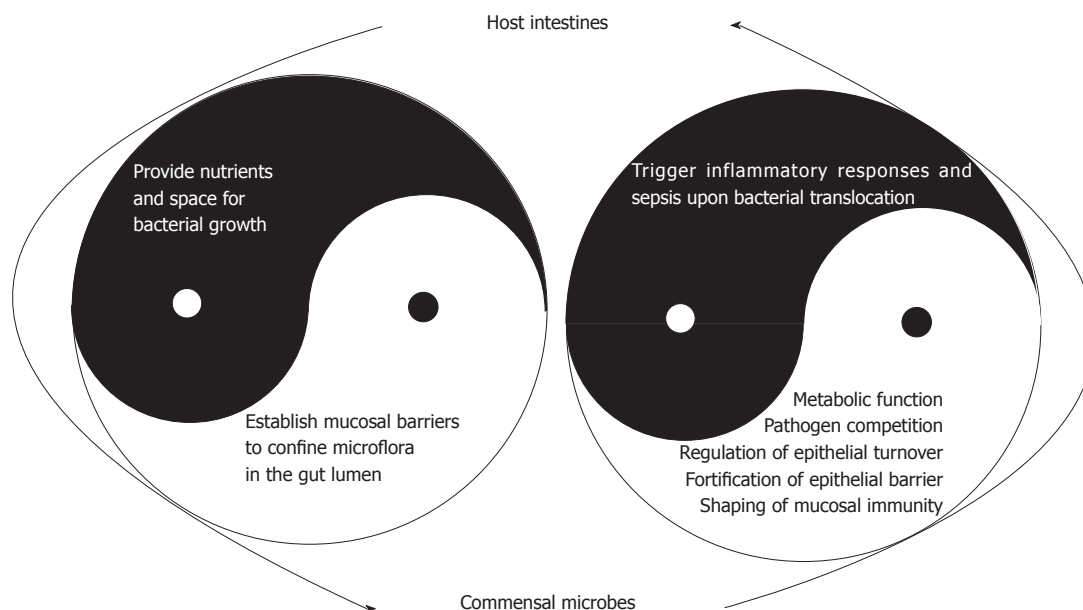


Figure 2 Dynamic interactions between host intestine and commensal microbes to achieve balance for maintenance of gut homeostasis. The survival and growth of enteric microbes relies on energy supply from food nutrients, and are dependent on the space and anchor provided by the host intestines. Conversely, luminal microbes are capable of fermenting non-digestible dietary substances, generating short chain fatty acids and essential vitamins, and providing caloric sources for the host. These symbiotic bacteria also play important roles in pathogen competition, regulation of the turnover rate of enterocytes and fortification of epithelial barrier functions, as well as shaping of the mucosal immunity. From the host's point of view, tight physical, chemical and immune barriers of intestines are pivotal in the keeping of the number and location of the microfloral population in check in order to maintain the health-promoting effects, and to prevent bacterial dissemination and the triggering of local and systemic inflammatory responses. The balance of Yin-Yang between the host intestine and commensal microbes is central to maintaining homeostasis.

sal bacteria have justified the use of the term “symbionts” for these microbes. These enteric bacteria are no longer regarded as an intruder of the human body that requires annihilation and expulsion, but their presence is recognized as part of the human physiology. This consensus has been long-awaited, since the theory that “certain types of bacteria especially those with lactic acid-producing ability in the digestive tract could prolong life” was established by Dr. Eli Metchnikoff, the 1908 Nobel Prize Laureate. Of course, there was no knowledge of the existence of commensal bacteria at the turn of the 1920s, let alone the understanding that *Lactobacillus spp.* was a constituent of the gut microbiota system. This early theory did lead to the much later recognition by the World Health Organization that particular types of microorganisms which, when administered in adequate amounts, confer a health benefit on the host and the coining of the term “probiotics”.

Enteric microbes are responsible for numerous protective and metabolic functions, and are involved in various structure- and immune-enhancing effects of the gut (Table 1). The presence of commensal bacteria protects against enteric pathogen colonization through competition for nutrients and receptors^[103,104], and by synthesis or induction of anti-microbial factors^[105,106]. The metabolic role of enteric bacteria involves degradation of non-digestible dietary substances, production of essential vitamins, and generation of short chain fatty acids (SC-FAs)^[107,108]. In addition, enteric microbes play an active role in the shaping of mucosal immunity, an aspect that has been discussed in detail in other review papers^[3,21].

Other important functions of commensal microbes have just begun to emerge, suggesting that luminal bacteria signal the interfacing epithelial layer and control the turnover rate of enterocytes^[3,109], and fortify the epithelial regenerative and barrier functions^[109-114].

Microbial effects in intestinal epithelial cell turnover rates

When evaluating the effect of commensal microbes on intestinal epithelial cell turnover rates and crypt-villus axis, it is important to consider the balance between cell proliferation and cell death. Increased epithelial cell apoptosis without sufficient proliferation or restitution results in barrier damage, whereas decreased cell death with hyperproliferation runs the risk of tumor formation. A number of reports comparing germ-free, gnotobiotic and conventionally-raised animals have indicated that luminal bacteria signals the epithelial layer to control cell apoptosis, proliferation and differentiation^[109-113]. Germ-free piglets display aberrant intestinal morphology with longer villi and shorter crypts than their conventional counterparts. Decreased epithelial apoptosis and crypt cell proliferation were observed in the intestine of germ-free animals compared to those raised conventionally^[110,111]. Oral inoculation of commensal bacteria obtained from feces or administration of non-pathogenic *E. coli* to these gnotobiotic pigs stimulates epithelial apoptosis, increases crypt depth for compensatory proliferation, and induces brush border enzyme activities compared to those raised in a germ-free environment^[109-112]. Previous studies also showed that commensals and non-pathogenic *E. coli* LPS mediate

Table 1 Functions of commensal bacteria in the gut

Protective functions
Pathogen displacement
Competition for nutrients
Competition for receptors
Production of anti-microbial factors
Metabolic functions
Fermentation of non-digestible dietary substances
Generation of short chain fatty acids
Salvage of energy source
Synthesis of essential vitamins (vitamin K and B12, niacin, biotin and folate)
Structural functions
Regulation of epithelial cell turnover
Promotion of epithelial cell differentiation
Fortification of epithelial barrier
Stabilization of tight junctions
Immune functions
Induction of secretory IgA
Induction of oral tolerance
Shaping of immune microenvironment

pro-apoptotic effects on epithelial cells in human colon explants depleted of IL-10, as well as human intestinal epithelial cell lines^[31,115,116].

Further evidence of a role for commensal bacteria in regulation of epithelial cell turnover and restitution was seen in colitis models with mucosal deformation by oral administration of dextran sodium sulfate (DSS, a sulfated polysaccharide that is directly toxic to colonic epithelial cells^[117]). Animals with commensal bacterial depletion are more susceptible to oral DSS-induced mucosal injury, with more extensive denudation of the surface epithelium resulting in ulceration or erosion of mucosa compared to conventionalized counterparts^[114]. Impaired epithelial proliferation and regenerative ability were seen in the intestines of germ-free mice upon DSS-induced injury^[113]. Moreover, worsened histopathological score, decreased enterocyte proliferation and delayed wound healing were documented in DSS-induced colitis in mice deficient of proinflammatory signal pathways in response to ligands of bacterial LPS or lipoteichoic acid (LTA)^[113,114]. Oral ingestion of bacterial products LPS or LTA prior to DSS challenge conferred protection in wild type mice with colons depleted of commensal microflora^[114], suggesting that luminally administered bacterial products are important for protection against DSS-induced epithelial injury. Contradictory data were seen in animals with colitis-prone genetic background, showing that IL-10^{-/-} mice fail to develop spontaneous colitis and intestinal histopathology if reared in germ-free conditions, suggesting that the presence of commensal bacteria may trigger chronic intestinal inflammation in the background of IL-10 deficiency^[118]. The discrepancy further emphasizes the critical role of commensal bacteria in the shift between immune suppression and inflammation in intestines, and they may stimulate differential responses in enterocytes and immune cells.

A recent study has indicated that commensal bacteria promote epithelial restitution and wound closure

through mechanisms that involve ROS^[119]. Epithelial restitution is dependent on cell migration, a process that requires phosphorylation of focal adhesion kinase (FAK) for the dynamic turnover of focal cell-matrix adhesions^[120]. It was demonstrated that commensal bacteria stimulate the production of epithelial-derived oxidative free radicals that induce oxidation and inactivation of FAK phosphatases, which in turn results in increased phosphorylation of FAK^[119]. Another report has shown that hydrogen peroxide promotes intestinal epithelial cell migration *via* induction of FAK phosphorylation by a phosphatidylinositol 3 kinase-dependent mechanism^[121]. In addition, NOX1 (a superoxide-generating oxidase which is highly expressed on colonic epithelial cells) plays a crucial role in regulation of epithelial proliferation and differentiation by modulating Wnt/Notch signaling^[122]. It seems plausible that bacterial contact on epithelial surface or microbial influx to the mucosa due to barrier dysfunction may serve as triggers for ROS production from enterocytes and phagocytes to promote cell renewal and wound healing.

The enteric microbiota thrives in a largely anaerobic luminal environment and generates a spectrum of SCFAs, including butyrate, succinate and propionate, as well as other terminal products such as lactate^[107]. SCFAs are important energy sources for the colonic epithelium and for the host, and also regulate colonic epithelial cell growth and differentiation^[108,123]. Butyrate was shown to increase alkaline phosphatase activity, a marker of colonocyte differentiation, in highly proliferative epithelial cells, correlated with cell cycle arrest^[124,125]. Besides its role in promoting cell differentiation, butyrate plays a role in prevention of colonic cancer by terminating cell cycle progression and promoting apoptosis of transformed colonocytes through mechanisms associated with inhibition of histone deacetylase activity and induction of p21WAF1/Cip1 proteins^[124,126,127].

Microbial effects in fortification of epithelial tight junctional structures

Strong evidence that commensal bacteria regulate epithelial permeability came from studies with probiotics in various disease models. Probiotics are defined as non-pathogenic microorganisms that confer health benefits for the host^[128] and several strains of commensal bacteria have been included in the category so far. Pretreatment with multispecies or single strain of probiotics (e.g., VSL3, nonpathogenic *Escherichia coli* Nissle 1917, or *Lactobacillus rhamnosus*) inhibited gut leakiness and prevented the colonic cell apoptosis in colitis mice models induced by DSS challenge^[129-131] and IL-10 gene deficiency^[132]. The maintenance of epithelial barrier was associated with restoration of tight junctional structures and increased expression of ZO-1 and MLCK^[130,131]. Oral administration of probiotics containing *Lactobacillus* sp., *Enterococcus faecalis* (previously *Streptococcus faecalis*) and *Bifidobacterium brevis* prevented the increase of transepithelial macromolecular flux in rat intestines caused by acute or chronic psycho-

logical stress^[133,134]. Studies *in vitro* have shown that probiotics, such as *E.coli* strain Nissle 1917 and *Lactobacillus plantarum*, reduced the epithelial hyperpermeability caused by enteropathogenic *Escherichia Coli* in human intestinal epithelial cells by silencing PKC ζ and reorganizing ZO-2^[135,136]. Beneficial effects of probiotics in maintaining colonic barrier function and reducing bacterial influx and plasma endotoxin levels were also seen in clinical studies and endotoxemic rat models^[137,138]. Several strains of *Lactobacillus* stabilize tight junctional structures after free radical-induced or cyclooxygenase-dependent epithelial barrier dysfunction^[139-141]. It is noteworthy that administration of these probiotics does not lead to changes in intestinal epithelial permeability in healthy control animals^[133], emphasizing that the presence of probiotics is critical for the prevention of intestinal barrier dysfunction only upon injury. In addition, bacterial fermentation products of SCFAs also directly increase the transepithelial resistance of intestinal epithelial monolayers *in vitro* by accelerating the assembly of tight junctions that is regulated by AMP-activated protein kinase and PI3K signaling pathways^[142,143]. A *Lactobacillus*-derived molecule, polyphosphate, was recently identified to suppress oxidant-induced intestinal permeability in mouse small intestine^[144]. The findings of specific molecules secreted by probiotics and/or commensal bacteria may benefit the development of natural product supplementations to enhance the intestinal barrier functions.

ABERRANT RECOGNITION OF MICROBIAL PRODUCTS RESULTS IN INTESTINAL PATHOLOGY

Chronic inflammation

Intestinal epithelial cells are constantly bombarded with pathogenic, cytotoxic, metabolic stresses which trigger apoptotic and necrotic cell death, leading to gut barrier damage, microbial influx and inflammatory responses^[15,16,31,32,145,146]. Evidence supporting the notion that gut permeability defects precedes the onset of mucosal inflammation was found in spontaneous enterocolitis models of IL-10^{-/-} and SAMP1/YitC mice^[147-149]. Moreover, mucosal inflammation was seen in areas adjacent to epithelium with TJ disruption (loss of endogenous E-cadherin) due to the expression of a dominant negative N-cadherin mutant lacking an extracellular domain in mice^[150]. Recent studies using epithelial-specific knockout models provide direct evidence of the cause-and-effect relationship between cell death-dependent epithelial barrier defects and intestinal inflammation. Mice with conditional deletion of caspase-8 or Fas-Associated protein with Death Domain on intestinal epithelial cells spontaneously developed epithelial cell necrosis and inflammatory lesions in the ileum and colon^[145,146]. On the other hand, a number of studies have demonstrated that pro-inflammatory cytokines (e.g., IFN γ and TNF α) and phagocytic mediators (e.g., free radicals and proteases)

cause tight junctional breakdown and intestinal permeability rise^[139,151,152], and thus argue in favor of inflammation as the cause for epithelial barrier disruption. Regardless of the starting point, a feed-forward vicious cycle between barrier dysfunction and inflammatory reaction is crucial for the perpetuation and aggravation of chronic inflammation in intestines.

Several lines of evidence suggest a critical role of dysbiosis in the pathogenesis of inflammatory bowel disease (IBD). In IBD patients, not only the quantity of commensal bacteria in the intestine is reduced (about ten-fold lower than control subjects), but also the diversity of the microbiota is altered^[6,153,154]. Reduction of major classes of commensals, *Firmicutes* and *Bacteroidetes*, and increase of mucosal adherent bacteria are documented in patients^[6,153-155]. Experimental models such as IL-2- or IL-10-deficient mice that spontaneously develop colitis do not develop disease when raised in a germ-free environment^[156,157]. In addition, monoassociation with *Bacteroides vulgatus* or *E. coli* is sufficient to induce colitis in human leukocyte antigen-B27 transgenic rats^[158]. Recent findings that transmission of colitogenic commensal bacteria is able to trigger colitis in the genetically intact recipient mice further strengthen this view. Mice with genetic deficiency in *RAG-1* and *T-bet* displayed dysbiosis and developed spontaneous colonic inflammation that resembles human ulcerative colitis^[159]. Interestingly, T-bet-competent wild type pups develop colitis after being crossfostered to female mutant mice, suggesting a communicable nature of this form of colitis by the gut microbiota^[160].

Aberrant bacterial signaling by microbe-associated molecular pattern receptors, e.g., nucleotide-binding oligomerisation domain 2 (NOD2) and toll-like receptors (TLRs), on mucosal cells is incriminated in the development of chronic intestinal inflammation. Mutations in the gene encoding *NOD2* were identified in patients with Crohn's disease^[161,162]. *NOD2* has been known as a cytosolic innate receptor able to sense peptidoglycan from Gram-positive and -negative bacteria inside enterocytes to trigger RIP2- and nuclear factor kappa B (NF- κ B)-mediated pro-inflammatory responses and to induce antimicrobial defensin synthesis^[163,164]. Recent studies demonstrated that *NOD2*-deficient mice display altered microbiota composition, and elevated bacterial load in the feces and terminal ileum compared to their wild-type counterparts^[165,166], supporting that *NOD2* dysfunctions and its subsequent dysbiosis may result in the breakdown of gut homeostasis and predispose to chronic inflammation.

Accumulating evidence points out that changes in the expression levels of receptors to Gram-negative bacterial LPS in the intestinal mucosa may be involved in the pathogenesis of IBD and colorectal cancer^[167-170]. The multi-unit receptor for LPS (CD14/TLR4/MD-2 complex) was originally detected on blood monocytes in the context of the pathogenesis of septic shock^[171,172]. It becomes clear now that intestinal epithelial cells and resident macrophages bear a distinct expression pattern of receptors unlike circulating monocytes and perito-

neal macrophages. Recent data show that in purified enterocytes isolated from normal human biopsy samples, CD14 protein is constitutively expressed, whereas TLR4 is barely detectable^[167-170,173]. Moreover, human intestinal macrophages isolated from normal jejunal specimens do not express innate immune receptors, such as receptors for LPS (CD14), Fc α (CD89), Fc γ (CD64, CD32, CD16), CR3 (CD11b/Cd18) and CR4 (CD11a/CD18)^[174]. Low TLR4 levels have also been reported in the lamina propria macrophages in comparison to blood monocytes in normal human subjects^[175]. It is noteworthy that these intestinal resident macrophages show downregulated LPS-induced production of proinflammatory cytokines, but retain potent phagocytic and bactericidal activities in physiological conditions^[174,176]. The distinct characteristics of LPS receptors on enterocytes and mucosal macrophages may reflect its tolerance to the presence of commensal bacteria, which is crucial for limiting unwanted inflammation and for maintaining gut homeostasis.

Polymorphism of *CD14* and *TLR4* genes was identified in subsets of IBD patients^[177-183], suggesting that abnormal bacterial LPS signaling may play a role in the pathogenesis. Since both intestinal epithelial cells and lamina propria macrophages express CD14 and TLR4 proteins at variable levels, their changes related to chronic colitis will be discussed in a cell type-specific fashion. Upregulated epithelial *TLR4* expression was observed in IBD patients compared to normal subjects^[167,168]. A similar increase in TLR4 was found in crypt epithelial cells in DSS-induced mouse colitis models^[184,185]. Moreover, *CD14* mRNA and protein levels in the intestinal epithelial cells of DSS-induced and spontaneous colitic mice were also higher than those in healthy animals^[184,186]. These findings suggest that at the interface with commensal microbes, altered expression of *LPS* receptor components (*CD14* and *TLR4*) on enterocytes may trigger epithelial-derived proinflammatory signals.

A wide array of differential expression patterns and subcellular location of *LPS* receptors was seen in different intestinal epithelial cell lines that correlated with their responsiveness to LPS for proinflammatory cytokine synthesis. For example, Caco-2 cells that express cell surface CD14 but have low levels of *TLR4* mRNA and proteins, similar to normal human enterocytes, neither activate their NF- κ B pathway nor produce IL-8 after LPS challenge^[57,68,116,187], showing one of the possible mechanisms for endotoxin tolerance by enterocytes. Transfection of TLR4/MD2 to Caco-2 cells restores the responsiveness to LPS and synergistic activation of NF- κ B and IL-8 reporter genes^[187]. Moreover, HT29 cells that express membrane-bound CD14 and cytoplasmic TLR4 are responsive to IFN γ for upregulation of intracellular TLR4 levels and the cells are sensitized for LPS-induced IL-8 production^[57,116]. Among human intestinal epithelial cell lines that express constitutively high cell surface levels of TLR4, such as SW480 and T84 cells, exposure to LPS stimulates the activation of NF- κ B and AP-1 signaling and the production of TNF α and IL-8^[57,8,187]. It is clear

from *in vitro* data that induction or heightened expression of individual *LPS* receptor components on intestinal epithelial cells may overrule their hyporesponsiveness to luminal bacterial LPS as a trigger for proinflammatory signals. Augmented expression of *LPS* receptors was also noted in lamina propria macrophages in inflamed tissues of IBD patients^[168,175,188,189]. Heightened *TLR4* expression was localized to intestinal macrophages in biopsy or surgical specimens obtained from both ulcerative colitis and Crohn's disease patients^[175]. In Crohn's disease patients, recent studies found increased subsets of CD14 macrophages in comparison to the typical resident macrophages (CD14 CD33⁺) in the intestinal lamina propria^[168,188,189]. The CD14 population of macrophages exhibit potent antigen-presenting ability to evoke differentiation of Th17 cells^[188] and produce large amounts of proinflammatory cytokines (e.g., TNF α and IL-23) that stimulate lamina propria mononuclear cells to synthesize IFN γ in a positive feedback loop^[189]. These abnormal CD14 macrophages may decrease the threshold to mount an inflammatory response upon exposure to low concentrations of LPS and to commensal bacteria, and may amplify the production of proinflammatory cytokines from different cell types through the positive feedback loop of IL-23/IFN γ ^[189,190].

Other reports indicated that a decrease in IL-10-producing intestinal macrophage subsets (CD11b F4/80 CD11c⁺) also plays a role in the development of chronic intestinal inflammation^[191,192]. Studies in IL-10-deficient colitis mouse models have demonstrated that bone marrow-derived macrophages from IL-10^{-/-} mice produce large amounts of IL-12 and IL-23 upon stimulation with heat-killed bacterial antigens, whereas those from wild type mice produce high levels of IL-10 but neither IL-12 nor IL-23^[190], which is correlated to the phenomenon where IL-10^{-/-} mice fail to develop spontaneous colitis and intestinal histopathology if reared in germ-free conditions^[118]. These findings suggest that commensal microbes or bacterial LPS may stimulate different subsets of macrophages, leading to varied patterns of macrophage-derived cytokine production (IL-10 vs IL-12/IL-23) that determine the progress to immune hyporesponsiveness or development of colitis^[118,190]. It remains unknown whether the low baseline levels of Fc α and Fc γ on normal intestinal resident macrophages are also upregulated in IBD patients, which may increase opsonization and phagocytosis for more efficient antigen presenting capability to stimulate long-term immune memory and chronic reactions.

Dysregulation of enterocytic apoptosis, proliferation and tumorigenesis

The abnormal *TLR4* overexpression on enterocytes and intestinal macrophages in IBD patients suggests that bacterial LPS stimulation may initiate mucosal-derived proinflammatory signals in the pathogenesis of chronic colitis. Based on this theory, a number of laboratories investigated the possibility that targeted deficiency of

TLR4 signaling might decrease gut inflammation. Unexpectedly, mice with spontaneous mutation or targeted knock-out of TLR4 and MyD88 displayed poorer colitis scores and lower survival rates in DSS models^[114,193,194]. Besides the heightened mucosal inflammatory responses, the lack of TLR4 signaling also resulted in other abnormalities, such as elevated epithelial cell apoptosis, decreased crypt cell proliferation, and impaired epithelial restitution accompanied with more severe mucosal ulceration in the DSS-induced colitis model^[114,193,194]. The findings in these TLR4^{-/-} and MyD88^{-/-} mice were similar to those with commensal bacteria depletion in DSS-induced colonic injury, whereby more extensive denudation of the surface epithelium results in ulceration or erosion of mucosa accompanied by pronounced compensatory crypt proliferation^[114]. These novel observations point out that presence of commensal bacteria and LPS-mediated TLR4 signaling may also be involved in epithelial cell survival that is critical in maintaining epithelial barrier integrity in physiological conditions and recovery to gut homeostasis in diseased states.

Many studies have shown that a lack of NF- κ B signaling leads to increased epithelial apoptosis and impaired epithelial restitution after DSS challenge in colitis development^[114,193-196]. Mice with epithelial-specific deficiency of IKK γ /NEMO develop spontaneous chronic intestinal inflammation associated with increased epithelial apoptosis and bacterial translocation^[195]. Targeted ablation of IKK β in intestinal epithelial cells also resulted in severe cell apoptosis upon radiation^[197] or ischemic challenge^[198], further supporting a universal role of IKK β for cell survival against various types of stresses. Another study also showed that enterocyte-specific knockout of Raf-1 leads to NF- κ B inactivation that is responsible for increased epithelial apoptosis and impaired epithelial proliferation and regeneration after oral DSS challenge^[194]. Taken together, the aforementioned studies indicated that epithelial-derived TLR4/NF- κ B pathways are involved in anti-apoptotic events.

From a physiological point of view, LPS signaling in the normally tolerant gut epithelial cells may serve as a warning system to the underlying immune cells while trying to promote epithelial restitution and maintain epithelial barrier functions *via* multiple pathways for proinflammatory, anti-apoptotic and proliferative effects. Short-term epithelial TLR4/NF- κ B signaling is crucial for preventing pathogenic epithelial cell death and epithelial barrier disruption, which may help limit the exposure of the immune cells to bacterial antigens and toxins that could cause full-blown reactions. On the other hand, a chronic epithelial-derived LPS signaling may shift the normal cell cycle into tumorigenic phenotypes in the long run.

A strong link between inflammation and cancer formation was suggested by the higher incidence of gastric and colorectal cancer in patients with early onset of IBD^[199,200]. Accumulating evidence indicates that *TLR4* expression in intestinal epithelial cells is upregulated in patients with colorectal cancer^[169,170], suggesting that altered expression

pattern and malformed signals of epithelial LPS receptor components may also play crucial roles in tumorigenesis. Aberrant reactions to bacterial LPS by CD14/TLR4 may induce an imbalance of apoptosis and proliferation, resulting in cancer formation. Recent data showed that TLR4^{-/-} and MyD88^{-/-} mice failed to develop colitis-associated and carcinogen-induced colorectal tumors^[201-204]. TLR4 may be responsible for upregulated production of cyclooxygenases and activation of epidermal growth factor receptors which may contribute to cancer formation^[193,201]. A recent study pointed out that MyD88-dependent signaling controls the expression of several key modifier genes of intestinal tumorigenesis and has a critical role in both spontaneous and carcinogen-induced tumor development^[202].

Mice with epithelial-specific IKK β deficiency had a lower incidence of tumor formation, partly due to increased levels of epithelial apoptosis, compared to wild type animals after injection with azoxymethane (AOM) followed by treatment with DSS^[205]. It is noteworthy that deletion of IKK β in myeloid cells led to smaller tumor size, but no change of tumor incidence compared to wild type mice after AOM-DSS challenge^[205]. These findings suggest that IKK β in different cell types contributes to tumorigenesis *via* variable cellular functions, of which epithelial-specific IKK β promotes tumor formation by conferring resistance to cell apoptotic pathways, whereas IKK β signals in myeloid cells are involved in boosting epithelial cell cycle progression and cell division^[205]. Therefore, it is important to identify the different types of mucosal cells (enterocytes or macrophages) responding to LPS when explaining the pathogenesis of intestinal inflammation and colorectal cancer formation.

In summary, LPS/TLR4-mediated signals which are normally downregulated in the gut epithelium are now linked with various pathological phenomena and disease states such as chronic inflammation, anti-apoptosis, hyperproliferation and tumorigenesis in the gastrointestinal tract.

CONCLUSION

With such a variety of species and the large numbers of commensal bacteria which undergoes wax and wane processes throughout the host's life, homeostasis of the gut is maintained by dynamic cross-talks between luminal microbes and intestinal epithelium. This delicate balance is complicated by the need to maintain both oral tolerance and mucosal defense. It remains to be resolved whether the expression of pattern recognition receptors, such as *NOD2*, *CD14* and *TLR4*, on enterocytes along the crypt-villus axis is differentially regulated in order to respond to microbes for proliferative, differentiative and apoptotic signals at different stages. Aberrant recognition and abnormal signaling caused by luminal bacteria may result in epithelial barrier dysfunction and/or carcinogenesis. The understanding of the interaction between host epithelium and commensal bacteria will provide us with novel information for the development of prophylactic and therapeutic interventions for patients with chronic inflammation and colorectal cancer.

REFERENCES

- 1 **Palmer C**, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol* 2007; **5**: e177
- 2 **Ley RE**, Peterson DA, Gordon JI. Ecological and evolutionary forces shaping microbial diversity in the human intestine. *Cell* 2006; **124**: 837-848
- 3 **O'Hara AM**, Shanahan F. The gut flora as a forgotten organ. *EMBO Rep* 2006; **7**: 688-693
- 4 **Andoh A**, Benno Y, Kanauchi O, Fujiyama Y. Recent advances in molecular approaches to gut microbiota in inflammatory bowel disease. *Curr Pharm Des* 2009; **15**: 2066-2073
- 5 **Manson JM**, Rauch M, Gilmore MS. The commensal microbiology of the gastrointestinal tract. *Adv Exp Med Biol* 2008; **635**: 15-28
- 6 **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
- 7 **Frank DN**, Pace NR. Gastrointestinal microbiology enters the metagenomics era. *Curr Opin Gastroenterol* 2008; **24**: 4-10
- 8 **Qin J**, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C, Nielsen T, Pons N, Levenez F, Yamada T, Mende DR, Li J, Xu J, Li S, Li D, Cao J, Wang B, Liang H, Zheng H, Xie Y, Tap J, Lepage P, Bertalan M, Batto JM, Hansen T, Le Paslier D, Linneberg A, Nielsen HB, Pelletier E, Renault P, Sicheritz-Ponten T, Turner K, Zhu H, Yu C, Li S, Jian M, Zhou Y, Li Y, Zhang X, Li S, Qin N, Yang H, Wang J, Brunak S, Doré J, Guarner F, Kristiansen K, Pedersen O, Parkhill J, Weissenbach J, Bork P, Ehrlich SD, Wang J. A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 2010; **464**: 59-65
- 9 **Mount DW**, Pandey R. Using bioinformatics and genome analysis for new therapeutic interventions. *Mol Cancer Ther* 2005; **4**: 1636-1643
- 10 **Southan C**. Has the yo-yo stopped? An assessment of human protein-coding gene number. *Proteomics* 2004; **4**: 1712-1726
- 11 **Ley RE**, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; **444**: 1022-1023
- 12 **Balzan S**, de Almeida Quadros C, de Cleve R, Zilberstein B, Ceccanello I. Bacterial translocation: overview of mechanisms and clinical impact. *J Gastroenterol Hepatol* 2007; **22**: 464-471
- 13 **Leaphart CL**, Tepas JJ. The gut is a motor of organ system dysfunction. *Surgery* 2007; **141**: 563-569
- 14 **Yu LCH**. Protective Mechanism against Gut Barrier Dysfunction in Mesenteric Ischemia/Reperfusion. *Adapt Med* 2010; **2**: 11-22
- 15 **Hsiao JK**, Huang CY, Lu YZ, Yang CY, Yu LC. Magnetic resonance imaging detects intestinal barrier dysfunction in a rat model of acute mesenteric ischemia/reperfusion injury. *Invest Radiol* 2009; **44**: 329-335
- 16 **Huang CY**, Hsiao JK, Lu YZ, Lee TC, Yu LC. Anti-apoptotic PI3K/Akt signaling by sodium/glucose transporter 1 reduces epithelial barrier damage and bacterial translocation in intestinal ischemia. *Lab Invest* 2011; **91**: 294-309
- 17 **Wu CC**, Lu YZ, Wu LL, Yu LC. Role of myosin light chain kinase in intestinal epithelial barrier defects in a rat model of bowel obstruction. *BMC Gastroenterol* 2010; **10**: 39
- 18 **Wu LL**, Chiu HD, Peng WH, Lin BR, Lu KS, Lu YZ, Yu LC. Epithelial inducible nitric oxide synthase causes bacterial translocation by impairment of enterocytic tight junctions via intracellular signals of Rho-associated kinase and protein kinase C zeta. *Crit Care Med* 2011; **39**: 2087-2098
- 19 **Thuijls G**, de Haan JJ, Derikx JP, Daissormont I, Hadfoune M, Heineman E, Buurman WA. Intestinal cytoskeleton degradation precedes tight junction loss following hemorrhagic shock. *Shock* 2009; **31**: 164-169
- 20 **Luyer MD**, Buurman WA, Hadfoune M, Speelmans G, Knol J, Jacobs JA, Dejong CH, Vriesema AJ, Greve JW. Strain-specific effects of probiotics on gut barrier integrity following hemorrhagic shock. *Infect Immun* 2005; **73**: 3686-3692
- 21 **Kelly D**, Conway S. Bacterial modulation of mucosal innate immunity. *Mol Immunol* 2005; **42**: 895-901
- 22 **Kassinen A**, Krogius-Kurikka L, Mäkituokko H, Rinttilä T, Paulin L, Corander J, Malinen E, Apajalahti J, Palva A. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. *Gastroenterology* 2007; **133**: 24-33
- 23 **Yen TH**, Wright NA. The gastrointestinal tract stem cell niche. *Stem Cell Rev* 2006; **2**: 203-212
- 24 **Bullen TF**, Forrest S, Campbell F, Dodson AR, Hershman MJ, Pritchard DM, Turner JR, Montrose MH, Watson AJ. Characterization of epithelial cell shedding from human small intestine. *Lab Invest* 2006; **86**: 1052-1063
- 25 **Rosenblatt J**, Raff MC, Cramer LP. An epithelial cell destined for apoptosis signals its neighbors to extrude it by an actin- and myosin-dependent mechanism. *Curr Biol* 2001; **11**: 1847-1857
- 26 **Madara JL**. Maintenance of the macromolecular barrier at cell extrusion sites in intestinal epithelium: physiological rearrangement of tight junctions. *J Membr Biol* 1990; **116**: 177-184
- 27 **Watson AJ**, Chu S, Sieck L, Gerasimenko O, Bullen T, Campbell F, McKenna M, Rose T, Montrose MH. Epithelial barrier function in vivo is sustained despite gaps in epithelial layers. *Gastroenterology* 2005; **129**: 902-912
- 28 **Moss SF**, Sordillo EM, Abdalla AM, Makarov V, Hanzely Z, Perez-Perez GI, Blaser MJ, Holt PR. Increased gastric epithelial cell apoptosis associated with colonization with cagA + *Helicobacter pylori* strains. *Cancer Res* 2001; **61**: 1406-1411
- 29 **Paesold G**, Guiney DG, Eckmann L, Kagnoff MF. Genes in the Salmonella pathogenicity island 2 and the Salmonella virulence plasmid are essential for Salmonella-induced apoptosis in intestinal epithelial cells. *Cell Microbiol* 2002; **4**: 771-781
- 30 **Flynn AN**, Buret AG. Tight junctional disruption and apoptosis in an in vitro model of Citrobacter rodentium infection. *Microb Pathog* 2008; **45**: 98-104
- 31 **Yu LC**, Flynn AN, Turner JR, Buret AG. SGLT-1-mediated glucose uptake protects intestinal epithelial cells against LPS-induced apoptosis and barrier defects: a novel cellular rescue mechanism? *FASEB J* 2005; **19**: 1822-1835
- 32 **Yu LC**, Huang CY, Kuo WT, Sayer H, Turner JR, Buret AG. SGLT-1-mediated glucose uptake protects human intestinal epithelial cells against Giardia duodenalis-induced apoptosis. *Int J Parasitol* 2008; **38**: 923-934
- 33 **Omatsu T**, Naito Y, Handa O, Hayashi N, Mizushima K, Qin Y, Hirata I, Adachi S, Okayama T, Kishimoto E, Takagi T, Kokura S, Ichikawa H, Yoshikawa T. Involvement of reactive oxygen species in indomethacin-induced apoptosis of small intestinal epithelial cells. *J Gastroenterol* 2009; **44** Suppl 19: 30-34
- 34 **Redlak MJ**, Power JJ, Miller TA. Prevention of deoxycholate-induced gastric apoptosis by aspirin: roles of NF-kappaB and PKC signaling. *J Surg Res* 2008; **145**: 66-73
- 35 **Renahan AG**, O'Dwyer ST, Haboubi NJ, Potten CS. Early cellular events in colorectal carcinogenesis. *Colorectal Dis* 2002; **4**: 76-89
- 36 **Oumouna-Benachour K**, Oumouna M, Zerfaoui M, Hans C, Fallon K, Boulares AH. Intrinsic resistance to apoptosis of colon epithelial cells is a potential determining factor in the susceptibility of the A/J mouse strain to dimethylhydrazine-induced colon tumorigenesis. *Mol Carcinog* 2007; **46**: 993-1002
- 37 **Turner JR**. Molecular basis of epithelial barrier regulation:

- from basic mechanisms to clinical application. *Am J Pathol* 2006; **169**: 1901-1909
- 38 **Ivanov AI**, Parkos CA, Nusrat A. Cytoskeletal regulation of epithelial barrier function during inflammation. *Am J Pathol* 2010; **177**: 512-524
- 39 **Utech M**, Ivanov AI, Samarin SN, Bruewer M, Turner JR, Mrsny RJ, Parkos CA, Nusrat A. Mechanism of IFN- γ -induced endocytosis of tight junction proteins: myosin II-dependent vacuolarization of the apical plasma membrane. *Mol Biol Cell* 2005; **16**: 5040-5052
- 40 **González-Mariscal L**, Tapia R, Chamorro D. Crosstalk of tight junction components with signaling pathways. *Biochim Biophys Acta* 2008; **1778**: 729-756
- 41 **Dodane V**, Kachar B. Identification of isoforms of G proteins and PKC that colocalize with tight junctions. *J Membr Biol* 1996; **149**: 199-209
- 42 **Stuart RO**, Nigam SK. Regulated assembly of tight junctions by protein kinase C. *Proc Natl Acad Sci USA* 1995; **92**: 6072-6076
- 43 **Suzuki T**, Elias BC, Seth A, Shen L, Turner JR, Giorgianni F, Desiderio D, Guntaka R, Rao R. PKC ϵ regulates occludin phosphorylation and epithelial tight junction integrity. *Proc Natl Acad Sci USA* 2009; **106**: 61-66
- 44 **Moriez R**, Salvador-Cartier C, Theodorou V, Fioramonti J, Eutamene H, Bueno L. Myosin light chain kinase is involved in lipopolysaccharide-induced disruption of colonic epithelial barrier and bacterial translocation in rats. *Am J Pathol* 2005; **167**: 1071-1079
- 45 **Ferrier L**, Mazelin L, Cenac N, Desreumaux P, Janin A, Emilie D, Colombel JF, Garcia-Villar R, Fioramonti J, Bueno L. Stress-induced disruption of colonic epithelial barrier: role of interferon- γ and myosin light chain kinase in mice. *Gastroenterology* 2003; **125**: 795-804
- 46 **Clayburgh DR**, Barrett TA, Tang Y, Meddings JB, Van Eldik LJ, Watterson DM, Clarke LL, Mrsny RJ, Turner JR. Epithelial myosin light chain kinase-dependent barrier dysfunction mediates T cell activation-induced diarrhea in vivo. *J Clin Invest* 2005; **115**: 2702-2715
- 47 **Fujita M**, Reinhart F, Neutra M. Convergence of apical and basolateral endocytic pathways at apical late endosomes in absorptive cells of suckling rat ileum in vivo. *J Cell Sci* 1990; **97** (Pt 2): 385-394
- 48 **Yu LCH**. The epithelial gatekeeper against food allergy. *Pediatr Neonatol* 2009; **50**: 247-254
- 49 **Yu LC**. Intestinal epithelial barrier dysfunction in food hypersensitivity. *J Allergy (Cairo)* 2012; **2012**: 596081
- 50 **Clark E**, Hoare C, Tanianis-Hughes J, Carlson GL, Warhurst G. Interferon gamma induces translocation of commensal *Escherichia coli* across gut epithelial cells via a lipid raft-mediated process. *Gastroenterology* 2005; **128**: 1258-1267
- 51 **Clark EC**, Patel SD, Chadwick PR, Warhurst G, Curry A, Carlson GL. Glutamine deprivation facilitates tumour necrosis factor induced bacterial translocation in Caco-2 cells by depletion of enterocyte fuel substrate. *Gut* 2003; **52**: 224-230
- 52 **Nazli A**, Yang PC, Jury J, Howe K, Watson JL, Söderholm JD, Sherman PM, Perdue MH, McKay DM. Epithelia under metabolic stress perceive commensal bacteria as a threat. *Am J Pathol* 2004; **164**: 947-957
- 53 **Lewis K**, Lutgendorff F, Phan V, Söderholm JD, Sherman PM, McKay DM. Enhanced translocation of bacteria across metabolically stressed epithelia is reduced by butyrate. *Inflamm Bowel Dis* 2010; **16**: 1138-1148
- 54 **Inaba T**, Alexander JW, Ogle JD, Ogle CK. Nitric oxide promotes the internalization and passage of viable bacteria through cultured Caco-2 intestinal epithelial cells. *Shock* 1999; **11**: 276-282
- 55 **Wang G**, Moniri NH, Ozawa K, Stamler JS, Daaka Y. Nitric oxide regulates endocytosis by S-nitrosylation of dynamin. *Proc Natl Acad Sci USA* 2006; **103**: 1295-1300
- 56 **Wells CL**, VandeWesterlo EM, Jechorek RP, Erlandsen SL. Effect of hypoxia on enterocyte endocytosis of enteric bacteria. *Crit Care Med* 1996; **24**: 985-991
- 57 **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
- 58 **Cario E**, Brown D, McKee M, Lynch-Devaney K, Gerken G, Podolsky DK. Commensal-associated molecular patterns induce selective toll-like receptor-trafficking from apical membrane to cytoplasmic compartments in polarized intestinal epithelium. *Am J Pathol* 2002; **160**: 165-173
- 59 **Drewe J**, Beglinger C, Fricker G. Effect of ischemia on intestinal permeability of lipopolysaccharides. *Eur J Clin Invest* 2001; **31**: 138-144
- 60 **Ferreira RC**, Forsyth LE, Richman PI, Wells C, Spencer J, MacDonald TT. Changes in the rate of crypt epithelial cell proliferation and mucosal morphology induced by a T-cell-mediated response in human small intestine. *Gastroenterology* 1990; **98**: 1255-1263
- 61 **Hornef MW**, Frisan T, Vandewalle A, Normark S, Richter-Dahlfors A. Toll-like receptor 4 resides in the Golgi apparatus and colocalizes with internalized lipopolysaccharide in intestinal epithelial cells. *J Exp Med* 2002; **195**: 559-570
- 62 **Tomita M**, Ohkubo R, Hayashi M. Lipopolysaccharide transport system across colonic epithelial cells in normal and infective rat. *Drug Metab Pharmacokinet* 2004; **19**: 33-40
- 63 **Kalischuk LD**, Inglis GD, Buret AG. *Campylobacter jejuni* induces transcellular translocation of commensal bacteria via lipid rafts. *Gut Pathog* 2009; **1**: 2
- 64 **Keita AV**, Gullberg E, Ericson AC, Salim SY, Wallon C, Kald A, Artursson P, Söderholm JD. Characterization of antigen and bacterial transport in the follicle-associated epithelium of human ileum. *Lab Invest* 2006; **86**: 504-516
- 65 **van Wijk F**, Knippels L. Initiating mechanisms of food allergy: Oral tolerance versus allergic sensitization. *Biomed Pharmacother* 2007; **61**: 8-20
- 66 **Roze KR**, Cooper D, Lam K, Costerton JW. Microbial flora of the mouse ileum mucous layer and epithelial surface. *Appl Environ Microbiol* 1982; **43**: 1451-1463
- 67 **Sanford SE**. Light and electron microscopic observations of a segmented filamentous bacterium attached to the mucosa of the terminal ileum of pigs. *J Vet Diagn Invest* 1991; **3**: 328-333
- 68 **Eckmann L**, Jung HC, Schürer-Maly C, Panja A, Morzycka-Wroblewska E, Kagnoff MF. Differential cytokine expression by human intestinal epithelial cell lines: regulated expression of interleukin 8. *Gastroenterology* 1993; **105**: 1689-1697
- 69 **Kuwano Y**, Kawahara T, Yamamoto H, Teshima-Kondo S, Tominaga K, Masuda K, Kishi K, Morita K, Rokutan K. Interferon- γ activates transcription of NADPH oxidase 1 gene and upregulates production of superoxide anion by human large intestinal epithelial cells. *Am J Physiol Cell Physiol* 2006; **290**: C433-C443
- 70 **Kuwano Y**, Tominaga K, Kawahara T, Sasaki H, Takeo K, Nishida K, Masuda K, Kawai T, Teshima-Kondo S, Rokutan K. Tumor necrosis factor α activates transcription of the NADPH oxidase organizer 1 (NOXO1) gene and upregulates superoxide production in colon epithelial cells. *Free Radic Biol Med* 2008; **45**: 1642-1652
- 71 **Kawahara T**, Kuwano Y, Teshima-Kondo S, Takeya R, Sumimoto H, Kishi K, Tsunawaki S, Hirayama T, Rokutan K. Role of nicotinamide adenine dinucleotide phosphate oxidase 1 in oxidative burst response to Toll-like receptor 5 signaling in large intestinal epithelial cells. *J Immunol* 2004;

- 172: 3051-3058
- 72 **De Smet K**, Contreras R. Human antimicrobial peptides: defensins, cathelicidins and histatins. *Biotechnol Lett* 2005; **27**: 1337-1347
 - 73 **Vaishnava S**, Behrendt CL, Ismail AS, Eckmann L, Hooper LV. Paneth cells directly sense gut commensals and maintain homeostasis at the intestinal host-microbial interface. *Proc Natl Acad Sci USA* 2008; **105**: 20858-20863
 - 74 **Tanabe H**, Ayabe T, Bainbridge B, Guina T, Ernst RK, Darveau RP, Miller SI, Ouellette AJ. Mouse paneth cell secretory responses to cell surface glycolipids of virulent and attenuated pathogenic bacteria. *Infect Immun* 2005; **73**: 2312-2320
 - 75 **Rumio C**, Besusso D, Palazzo M, Selleri S, Sfondrini L, Dubini F, Ménard S, Balsari A. Degranulation of paneth cells via toll-like receptor 9. *Am J Pathol* 2004; **165**: 373-381
 - 76 **Yu FS**, Cornicelli MD, Kovach MA, Newstead MW, Zeng X, Kumar A, Gao N, Yoon SG, Gallo RL, Standiford TJ. Flagellin stimulates protective lung mucosal immunity: role of cathelicidin-related antimicrobial peptide. *J Immunol* 2010; **185**: 1142-1149
 - 77 **Ouhara K**, Komatsuzawa H, Shiba H, Uchida Y, Kawai T, Sayama K, Hashimoto K, Taubman MA, Kurihara H, Sugai M. Actinobacillus actinomycetemcomitans outer membrane protein 100 triggers innate immunity and production of beta-defensin and the 18-kilodalton cationic antimicrobial protein through the fibronectin-integrin pathway in human gingival epithelial cells. *Infect Immun* 2006; **74**: 5211-5220
 - 78 **Ménard S**, Förster V, Lotz M, Gütle D, Duerr CU, Gallo RL, Henriques-Normark B, Pütsep K, Andersson M, Glocker EO, Hornef MW. Developmental switch of intestinal antimicrobial peptide expression. *J Exp Med* 2008; **205**: 183-193
 - 79 **Bry L**, Falk P, Huttner K, Ouellette A, Midtvedt T, Gordon JI. Paneth cell differentiation in the developing intestine of normal and transgenic mice. *Proc Natl Acad Sci USA* 1994; **91**: 10335-10339
 - 80 **Darmoul D**, Brown D, Selsted ME, Ouellette AJ. Cryptdin gene expression in developing mouse small intestine. *Am J Physiol* 1997; **272**: G197-G206
 - 81 **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
 - 82 **Shirafuji Y**, Tanabe H, Satchell DP, Henschen-Edman A, Wilson CL, Ouellette AJ. Structural determinants of pro-cryptdin recognition and cleavage by matrix metalloproteinase-7. *J Biol Chem* 2003; **278**: 7910-7919
 - 83 **Salzman NH**, Hung K, Haribhai D, Chu H, Karlsson-Sjöberg J, Amir E, Tegatz P, Barman M, Hayward M, Eastwood D, Stoeckl 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
 - 84 **Snel J**, Bakker MH, Heidt PJ. Quantification of antigen-specific immunoglobulin A after oral booster immunization with ovalbumin in mice mono-associated with segmented filamentous bacteria or Clostridium innocuum. *Immunol Lett* 1997; **58**: 25-28
 - 85 **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
 - 86 **Corthésy B**. Roundtrip ticket for secretory IgA: role in mucosal homeostasis? *J Immunol* 2007; **178**: 27-32
 - 87 **van der Waaij LA**, Limburg PC, Mesander G, van der Waaij D. In vivo IgA coating of anaerobic bacteria in human faeces. *Gut* 1996; **38**: 348-354
 - 88 **Moreau MC**, Ducluzeau R, Guy-Grand D, Muller MC. Increase in the population of duodenal immunoglobulin A plasmacytes in axenic mice associated with different living or dead bacterial strains of intestinal origin. *Infect Immun* 1978; **21**: 532-539
 - 89 **Hapfelmeier S**, Lawson MA, Slack E, Kirundi JK, Stoeckl M, Heikenwalder M, Cahenzli J, Velykoredko Y, Balmer ML, Endt K, Geuking MB, Curtiss R, McCoy KD, Macpherson AJ. Reversible microbial colonization of germ-free mice reveals the dynamics of IgA immune responses. *Science* 2010; **328**: 1705-1709
 - 90 **Rey J**, Garin N, Spertini F, Corthésy B. Targeting of secretory IgA to Peyer's patch dendritic and T cells after transport by intestinal M cells. *J Immunol* 2004; **172**: 3026-3033
 - 91 **Kadaoui KA**, Corthésy B. Secretory IgA mediates bacterial translocation to dendritic cells in mouse Peyer's patches with restriction to mucosal compartment. *J Immunol* 2007; **179**: 7751-7757
 - 92 **Massacand JC**, Kaiser P, Ernst B, Tardivel A, Bürki K, Schneider P, Harris NL. Intestinal bacteria condition dendritic cells to promote IgA production. *PLoS One* 2008; **3**: e2588
 - 93 **Boullier S**, Tanguy M, Kadaoui KA, Caubet C, Sansonetti P, Corthésy B, Phalipon A. Secretory IgA-mediated neutralization of Shigella flexneri prevents intestinal tissue destruction by down-regulating inflammatory circuits. *J Immunol* 2009; **183**: 5879-5885
 - 94 **Mora JR**, Iwata M, Eksteen B, Song SY, Junt T, Senman B, Otipoby KL, Yokota A, Takeuchi H, Ricciardi-Castagnoli P, Rajewsky K, Adams DH, von Andrian UH. Generation of gut-homing IgA-secreting B cells by intestinal dendritic cells. *Science* 2006; **314**: 1157-1160
 - 95 **Johansen FE**, Pekna M, Norderhaug IN, Haneberg B, Hietala MA, Krajci P, Betsholtz C, Brandtzaeg P. Absence of epithelial immunoglobulin A transport, with increased mucosal leakiness, in polymeric immunoglobulin receptor/secretory component-deficient mice. *J Exp Med* 1999; **190**: 915-922
 - 96 **Macpherson AJ**, Slack E. The functional interactions of commensal bacteria with intestinal secretory IgA. *Curr Opin Gastroenterol* 2007; **23**: 673-678
 - 97 **Kim KA**, Kim JY, Lee YA, Song KJ, Min D, Shin MH. NOX1 participates in ROS-dependent cell death of colon epithelial Caco2 cells induced by Entamoeba histolytica. *Microbes Infect* 2011; **13**: 1052-1061
 - 98 **Stark G**. Functional consequences of oxidative membrane damage. *J Membr Biol* 2005; **205**: 1-16
 - 99 **Cuzzocrea S**. Role of nitric oxide and reactive oxygen species in arthritis. *Curr Pharm Des* 2006; **12**: 3551-3570
 - 100 **Zaph C**, Du Y, Saenz SA, Nair MG, Perrigoue JG, Taylor BC, Troy AE, Kobuley DE, Kastelein RA, Cua DJ, Yu Y, Artis D. Commensal-dependent expression of IL-25 regulates the IL-23-IL-17 axis in the intestine. *J Exp Med* 2008; **205**: 2191-2198
 - 101 **Weaver CT**, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol* 2007; **25**: 821-852
 - 102 **Ivanov II**, Frutos Rde L, Manel N, Yoshinaga K, Rifkin DB, Sartor RB, Finlay BB, Littman DR. Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* 2008; **4**: 337-349
 - 103 **Leatham MP**, Banerjee S, Autieri SM, Mercado-Lubo R, Conway T, Cohen PS. Precolonized human commensal Escherichia coli strains serve as a barrier to E. coli O157: H7 growth in the streptomycin-treated mouse intestine. *Infect Immun* 2009; **77**: 2876-2886
 - 104 **Jankowska A**, Laubitz D, Antushevich H, Zabielski R, Grzesiuk E. Competition of Lactobacillus paracasei with Salmonella enterica for adhesion to Caco-2 cells. *J Biomed Biotechnol* 2008; **2008**: 357964
 - 105 **Schlee M**, Harder J, Köten B, Stange EF, Wehkamp J, Feller-

- mann K. Probiotic lactobacilli and VSL#3 induce enterocyte beta-defensin 2. *Clin Exp Immunol* 2008; **151**: 528-535
- 106 **Lu R**, Fasano S, Madayiputhiya N, Morin NP, Nataro J, Fasano A. Isolation, identification, and characterization of small bioactive peptides from *Lactobacillus* GG conditional media that exert both anti-Gram-negative and Gram-positive bactericidal activity. *J Pediatr Gastroenterol Nutr* 2009; **49**: 23-30
- 107 **Resta SC**. Effects of probiotics and commensals on intestinal epithelial physiology: implications for nutrient handling. *J Physiol* 2009; **587**: 4169-4174
- 108 **O'Keefe SJ**. Nutrition and colonic health: the critical role of the microbiota. *Curr Opin Gastroenterol* 2008; **24**: 51-58
- 109 **Shirkey TW**, Siggers RH, Goldade BG, Marshall JK, Drew MD, Laarveld B, Van Kessel AG. Effects of commensal bacteria on intestinal morphology and expression of proinflammatory cytokines in the gnotobiotic pig. *Exp Biol Med* (Maywood) 2006; **231**: 1333-1345
- 110 **Willing BP**, Van Kessel AG. Enterocyte proliferation and apoptosis in the caudal small intestine is influenced by the composition of colonizing commensal bacteria in the neonatal gnotobiotic pig. *J Anim Sci* 2007; **85**: 3256-3266
- 111 **Danielsen M**, Hornshøj H, Siggers RH, Jensen BB, van Kessel AG, Bendixen E. Effects of bacterial colonization on the porcine intestinal proteome. *J Proteome Res* 2007; **6**: 2596-2604
- 112 **Kozakova H**, Kolinska J, Lojda Z, Rehakova Z, Sinkora J, Zakostelecka M, Splichal I, Tlaskalova-Hogenova H. Effect of bacterial monoassociation on brush-border enzyme activities in ex-germ-free piglets: comparison of commensal and pathogenic *Escherichia coli* strains. *Microbes Infect* 2006; **8**: 2629-2639
- 113 **Pull SL**, Doherty JM, Mills JC, Gordon JL, Stappenbeck TS. Activated macrophages are an adaptive element of the colonic epithelial progenitor niche necessary for regenerative responses to injury. *Proc Natl Acad Sci USA* 2005; **102**: 99-104
- 114 **Rakoff-Nahoum S**, Paglino J, Eslami-Varzaneh F, Edberg S, Medzhitov R. Recognition of commensal microflora by toll-like receptors is required for intestinal homeostasis. *Cell* 2004; **118**: 229-241
- 115 **Jarry A**, Bossard C, Bou-Hanna C, Masson D, Espaze E, Denis MG, Laboisse CL. Mucosal IL-10 and TGF-beta play crucial roles in preventing LPS-driven, IFN-gamma-mediated epithelial damage in human colon explants. *J Clin Invest* 2008; **118**: 1132-1142
- 116 **Yu LC**, Turner JR, Buret AG. LPS/CD14 activation triggers SGLT-1-mediated glucose uptake and cell rescue in intestinal epithelial cells via early apoptotic signals upstream of caspase-3. *Exp Cell Res* 2006; **312**: 3276-3286
- 117 **Kitajima S**, Takuma S, Morimoto M. Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. *Exp Anim* 1999; **48**: 137-143
- 118 **Rakoff-Nahoum S**, Hao L, Medzhitov R. Role of toll-like receptors in spontaneous commensal-dependent colitis. *Immunity* 2006; **25**: 319-329
- 119 **Swanson PA**, Kumar A, Samarin S, Vijay-Kumar M, Kundu K, Murthy N, Hansen J, Nusrat A, Neish AS. Enteric commensal bacteria potentiate epithelial restitution via reactive oxygen species-mediated inactivation of focal adhesion kinase phosphatases. *Proc Natl Acad Sci USA* 2011; **108**: 8803-8808
- 120 **Sanders MA**, Basson MD. Collagen IV regulates Caco-2 cell spreading and p130Cas phosphorylation by FAK-dependent and FAK-independent pathways. *Biol Chem* 2008; **389**: 47-55
- 121 **Basuroy S**, Dunagan M, Sheth P, Seth A, Rao RK. Hydrogen peroxide activates focal adhesion kinase and c-Src by a phosphatidylinositol 3 kinase-dependent mechanism and promotes cell migration in Caco-2 cell monolayers. *Am J Physiol Gastrointest Liver Physiol* 2010; **299**: G186-G195
- 122 **Coant N**, Ben Mkaddem S, Pedruzzi E, Guichard C, Tréton X, Ducroc R, Freund JN, Cazals-Hatem D, Bouhnik Y, Wörther PL, Skurnik D, Grodet A, Fay M, Biard D, Lesuffleur T, Deffert C, Moreau R, Groyer A, Krause KH, Daniel F, Ogier-Denis E. NADPH oxidase 1 modulates WNT and NOTCH1 signaling to control the fate of proliferative progenitor cells in the colon. *Mol Cell Biol* 2010; **30**: 2636-2650
- 123 **Wong JM**, de Souza R, Kendall CW, Emam A, Jenkins DJ. Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* 2006; **40**: 235-243
- 124 **Comalada M**, Bailón E, de Haro O, Lara-Villoslada F, Xaus J, Zarzuelo A, Gálvez J. The effects of short-chain fatty acids on colon epithelial proliferation and survival depend on the cellular phenotype. *J Cancer Res Clin Oncol* 2006; **132**: 487-497
- 125 **Orchel A**, Dzierzewicz Z, Parfiniewicz B, Weglarz L, Wilczok T. Butyrate-induced differentiation of colon cancer cells is PKC and JNK dependent. *Dig Dis Sci* 2005; **50**: 490-498
- 126 **Waldecker M**, Kautenburger T, Daumann H, Busch C, Schrenk D. Inhibition of histone-deacetylase activity by short-chain fatty acids and some polyphenol metabolites formed in the colon. *J Nutr Biochem* 2008; **19**: 587-593
- 127 **Crim KC**, Sanders LM, Hong MY, Taddeo SS, Turner ND, Chapkin RS, Lupton JR. Upregulation of p21Waf1/Cip1 expression in vivo by butyrate administration can be chemoprotective or chemopromotive depending on the lipid component of the diet. *Carcinogenesis* 2008; **29**: 1415-1420
- 128 **Ohland CL**, Macnaughton WK. Probiotic bacteria and intestinal epithelial barrier function. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G807-G819
- 129 **Mennigen R**, Nolte K, Rijcken E, Utech M, Loeffler B, Senninger N, Bruewer M. Probiotic mixture VSL#3 protects the epithelial barrier by maintaining tight junction protein expression and preventing apoptosis in a murine model of colitis. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1140-G1149
- 130 **Ukena SN**, Singh A, Dringenberg U, Engelhardt R, Seidler U, Hansen W, Bleich A, Bruder D, Franzke A, Rogler G, Suerbaum S, Buer J, Gunzer F, Westendorf AM. Probiotic *Escherichia coli* Nissle 1917 inhibits leaky gut by enhancing mucosal integrity. *PLoS One* 2007; **2**: e1308
- 131 **Miyachi E**, Morita H, Tanabe S. *Lactobacillus rhamnosus* alleviates intestinal barrier dysfunction in part by increasing expression of zonula occludens-1 and myosin light-chain kinase in vivo. *J Dairy Sci* 2009; **92**: 2400-2408
- 132 **Madsen KL**, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. *Lactobacillus* species prevents colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 1999; **116**: 1107-1114
- 133 **Zareie M**, Johnson-Henry K, Jury J, Yang PC, Ngan BY, McKay DM, Soderholm JD, Perdue MH, Sherman PM. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. *Gut* 2006; **55**: 1553-1560
- 134 **Laudanno OM**, Cesolari JA, Godoy A, Sutich E, Sarangone S, Catalano J, San Miguel P. Bioflora probiotic in immunomodulation and prophylaxis of intestinal bacterial translocation in rats. *Dig Dis Sci* 2008; **53**: 2667-2670
- 135 **Zyrek AA**, Cichon C, Helms S, Enders C, Sonnenborn U, Schmidt MA. Molecular mechanisms underlying the probiotic effects of *Escherichia coli* Nissle 1917 involve ZO-2 and PKCzeta redistribution resulting in tight junction and epithelial barrier repair. *Cell Microbiol* 2007; **9**: 804-816
- 136 **Anderson RC**, Cookson AL, McNabb WC, Kelly WJ, Roy NC. *Lactobacillus plantarum* DSM 2648 is a potential probiotic that enhances intestinal barrier function. *FEMS Microbiol Lett* 2010; **309**: 184-192
- 137 **Schiffrin EJ**, Parlesak A, Bode C, Bode JC, van't Hof MA, Grathwohl D, Guigoz Y. Probiotic yogurt in the elderly with intestinal bacterial overgrowth: endotoxaemia and innate immune functions. *Br J Nutr* 2009; **101**: 961-966

- 138 **Ewaschuk J**, Endersby R, Thiel D, Diaz H, Backer J, Ma M, Churchill T, Madsen K. Probiotic bacteria prevent hepatic damage and maintain colonic barrier function in a mouse model of sepsis. *Hepatology* 2007; **46**: 841-850
- 139 **Seth A**, Yan F, Polk DB, Rao RK. Probiotics ameliorate the hydrogen peroxide-induced epithelial barrier disruption by a PKC- and MAP kinase-dependent mechanism. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G1060-G1069
- 140 **Montalto M**, Maggiano N, Ricci R, Curigliano V, Santoro L, Di Nicuolo F, Vecchio FM, Gasbarrini A, Gasbarrini G. Lactobacillus acidophilus protects tight junctions from aspirin damage in HT-29 cells. *Digestion* 2004; **69**: 225-228
- 141 **Karczewski J**, Troost FJ, Konings I, Dekker J, Kleerebezem M, Brummer RJ, Wells JM. Regulation of human epithelial tight junction proteins by Lactobacillus plantarum in vivo and protective effects on the epithelial barrier. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G851-G859
- 142 **Suzuki T**, Yoshida S, Hara H. Physiological concentrations of short-chain fatty acids immediately suppress colonic epithelial permeability. *Br J Nutr* 2008; **100**: 297-305
- 143 **Peng L**, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J Nutr* 2009; **139**: 1619-1625
- 144 **Segawa S**, Fujiya M, Konishi H, Ueno N, Kobayashi N, Shigyo T, Kohgo Y. Probiotic-derived polyphosphate enhances the epithelial barrier function and maintains intestinal homeostasis through integrin-p38 MAPK pathway. *PLoS One* 2011; **6**: e23278
- 145 **Welz PS**, Wullaert A, Vlantis K, Kondylis V, Fernández-Majada V, Ermolaeva M, Kirsch P, Sterner-Kock A, van Loo G, Pasparakis M. FADD prevents RIP3-mediated epithelial cell necrosis and chronic intestinal inflammation. *Nature* 2011; **477**: 330-334
- 146 **Günther C**, Martini E, Wittkopf N, Amann K, Weigmann B, Neumann H, Waldner MJ, Hedrick SM, Tenzer S, Neurath MF, Becker C. Caspase-8 regulates TNF- α -induced epithelial necroptosis and terminal ileitis. *Nature* 2011; **477**: 335-339
- 147 **Madsen KL**, Malfair D, Gray D, Doyle JS, Jewell LD, Fedorak RN. Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflamm Bowel Dis* 1999; **5**: 262-270
- 148 **Olson TS**, Reuter BK, Scott KG, Morris MA, Wang XM, Hancock LN, Burcin TL, Cohn SM, Ernst PB, Cominelli F, Meddings JB, Ley K, Pizarro TT. The primary defect in experimental ileitis originates from a nonhematopoietic source. *J Exp Med* 2006; **203**: 541-552
- 149 **Reuter BK**, Pizarro TT. Mechanisms of tight junction dysregulation in the SAMP1/YitFc model of Crohn's disease-like ileitis. *Ann N Y Acad Sci* 2009; **1165**: 301-307
- 150 **Hermiston ML**, Gordon JI. Inflammatory bowel disease and adenomas in mice expressing a dominant negative N-cadherin. *Science* 1995; **270**: 1203-1207
- 151 **Bruewer M**, Luegering A, Kucharzik T, Parkos CA, Madara JL, Hopkins AM, Nusrat A. Proinflammatory cytokines disrupt epithelial barrier function by apoptosis-independent mechanisms. *J Immunol* 2003; **171**: 6164-6172
- 152 **Chin AC**, Lee WY, Nusrat A, Vergnolle N, Parkos CA. Neutrophil-mediated activation of epithelial protease-activated receptors-1 and -2 regulates barrier function and transepithelial migration. *J Immunol* 2008; **181**: 5702-5710
- 153 **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
- 154 **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
- 155 **Kleessen B**, Kroesen AJ, Buhr HJ, Blaut M. Mucosal and invading bacteria in patients with inflammatory bowel disease compared with controls. *Scand J Gastroenterol* 2002; **37**: 1034-1041
- 156 **Schultz M**, Tonkonogy SL, Sellon RK, Veltkamp C, Godfrey VL, Kwon J, Grenther WB, Balish E, Horak I, Sartor RB. IL-2-deficient mice raised under germfree conditions develop delayed mild focal intestinal inflammation. *Am J Physiol* 1999; **276**: G1461-G1472
- 157 **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
- 158 **Rath HC**, Herfarth HH, Ikeda JS, Grenther WB, Hamm TE, Balish E, Taurog JD, Hammer RE, Wilson KH, Sartor RB. Normal luminal bacteria, especially Bacteroides species, mediate chronic colitis, gastritis, and arthritis in HLA-B27/human beta2 microglobulin transgenic rats. *J Clin Invest* 1996; **98**: 945-953
- 159 **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
- 160 **Garrett WS**, Gallini CA, Yatsunenka T, Michaud M, DuBois A, Delaney ML, Punit S, Karlsson M, Bry L, Glickman JN, Gordon JI, Onderdonk AB, Glimcher LH. Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* 2010; **8**: 292-300
- 161 **Gutiérrez A**, Holler E, Zapater P, Sempere L, Jover R, Pérez-Mateo M, Schoelmerich J, Such J, Wiest R, Francés R. Antimicrobial peptide response to blood translocation of bacterial DNA in Crohn's disease is affected by NOD2/CARD15 genotype. *Inflamm Bowel Dis* 2011; **17**: 1641-1650
- 162 **Lacher M**, Helmbrecht J, Schroepf S, Koletzko S, Ballauff A, Classen M, Uhlig H, Hubertus J, Hartl D, Lohse P, von Schweinitz D, Kappler R. NOD2 mutations predict the risk for surgery in pediatric-onset Crohn's disease. *J Pediatr Surg* 2010; **45**: 1591-1597
- 163 **Lecat A**, Piette J, Legrand-Poels S. The protein Nod2: an innate receptor more complex than previously assumed. *Biochem Pharmacol* 2010; **80**: 2021-2031
- 164 **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
- 165 **Rehman A**, Sina C, Gavrilova O, Häslér R, Ott S, Baines JF, Schreiber S, Rosenstiel P. Nod2 is essential for temporal development of intestinal microbial communities. *Gut* 2011; **60**: 1354-1362
- 166 **Petnicki-Ocwieja T**, Hrnčir T, Liu YJ, Biswas A, Hudcovic T, Tlaskalova-Hogenova H, Kobayashi KS. Nod2 is required for the regulation of commensal microbiota in the intestine. *Proc Natl Acad Sci USA* 2009; **106**: 15813-15818
- 167 **Cario E**, Podolsky DK. Differential alteration in intestinal epithelial cell expression of toll-like receptor 3 (TLR3) and TLR4 in inflammatory bowel disease. *Infect Immun* 2000; **68**: 7010-7017
- 168 **Frolova L**, Drastich P, Rossmann P, Klimesova K, Tlaskalova-Hogenova H. Expression of Toll-like receptor 2 (TLR2), TLR4, and CD14 in biopsy samples of patients with inflammatory bowel diseases: upregulated expression of TLR2 in terminal ileum of patients with ulcerative colitis. *J Histochem Cytochem* 2008; **56**: 267-274
- 169 **Doan HQ**, Bowen KA, Jackson LA, Evers BM. Toll-like receptor 4 activation increases Akt phosphorylation in colon

- cancer cells. *Anticancer Res* 2009; **29**: 2473-2478
- 170 **Wang EL**, Qian ZR, Nakasono M, Tanahashi T, Yoshimoto K, Bando Y, Kudo E, Shimada M, Sano T. High expression of Toll-like receptor 4/myeloid differentiation factor 88 signals correlates with poor prognosis in colorectal cancer. *Br J Cancer* 2010; **102**: 908-915
 - 171 **da Silva Correia J**, Soldau K, Christen U, Tobias PS, Ulevitch RJ. Lipopolysaccharide is in close proximity to each of the proteins in its membrane receptor complex. transfer from CD14 to TLR4 and MD-2. *J Biol Chem* 2001; **276**: 21129-21135
 - 172 **Cuschieri J**, Billgren J, Maier RV. Phosphatidylcholine-specific phospholipase C (PC-PLC) is required for LPS-mediated macrophage activation through CD14. *J Leukoc Biol* 2006; **80**: 407-414
 - 173 **Martín-Villa JM**, Ferre-López S, López-Suárez JC, Corell A, Pérez-Blas M, Arnaiz-Villena A. Cell surface phenotype and ultramicroscopic analysis of purified human enterocytes: a possible antigen-presenting cell in the intestine. *Tissue Antigens* 1997; **50**: 586-592
 - 174 **Smythies LE**, Sellers M, Clements RH, Mosteller-Barnum M, Meng G, Benjamin WH, Orenstein JM, Smith PD. Human intestinal macrophages display profound inflammatory anergy despite avid phagocytic and bacteriocidal activity. *J Clin Invest* 2005; **115**: 66-75
 - 175 **Hausmann M**, Kiessling S, Mestermann S, Webb G, Spöttl T, Andus T, Schölmerich J, Herfarth H, Ray K, Falk W, Rogler G. Toll-like receptors 2 and 4 are up-regulated during intestinal inflammation. *Gastroenterology* 2002; **122**: 1987-2000
 - 176 **Smith PD**, Smythies LE, Mosteller-Barnum M, Sibley DA, Russell MW, Merger M, Sellers MT, Orenstein JM, Shimada T, Graham MF, Kubagawa H. Intestinal macrophages lack CD14 and CD89 and consequently are down-regulated for LPS- and IgA-mediated activities. *J Immunol* 2001; **167**: 2651-2656
 - 177 **Brand S**, Staudinger T, Schnitzler F, Pfennig S, Hofbauer K, Dambacher J, Seiderer J, Tillack C, Konrad A, Crispin A, Göke B, Lohse P, Ochsenkühn T. The role of Toll-like receptor 4 Asp299Gly and Thr399Ile polymorphisms and CARD15/NOD2 mutations in the susceptibility and phenotype of Crohn's disease. *Inflamm Bowel Dis* 2005; **11**: 645-652
 - 178 **Franchimont D**, Vermeire S, El Housni H, Pierik M, Van Steen K, Gustot T, Quertinmont E, Abramowicz M, Van Gossum A, Devière J, Rutgeerts P. Deficient host-bacteria interactions in inflammatory bowel disease? The toll-like receptor (TLR)-4 Asp299gly polymorphism is associated with Crohn's disease and ulcerative colitis. *Gut* 2004; **53**: 987-992
 - 179 **De Jager PL**, Franchimont D, Waliszewska A, Bitton A, Cohen A, Langelier D, Belaiche J, Vermeire S, Farwell L, Goris A, Libioulle C, Jani N, Dassopoulos T, Bromfield GP, Dubois B, Cho JH, Brant SR, Duerr RH, Yang H, Rotter JL, Silverberg MS, Steinhart AH, Daly MJ, Podolsky DK, Louis E, Hafler DA, Rioux JD. The role of the Toll receptor pathway in susceptibility to inflammatory bowel diseases. *Genes Immun* 2007; **8**: 387-397
 - 180 **Baumgart DC**, Buning C, Geerdts L, Schmidt HH, Genschel J, Fiedler T, Gentz E, Molnar T, Nagy F, Lonovics J, Lochs H, Wiedenmann B, Nickel R, Witt H, Dignass A. The c.1-260C>T promoter variant of CD14 but not the c.896A>G (p.D299G) variant of toll-like receptor 4 (TLR4) genes is associated with inflammatory bowel disease. *Digestion* 2007; **76**: 196-202
 - 181 **Browning BL**, Huebner C, Petermann I, Gearry RB, Barclay ML, Shelling AN, Ferguson LR. Has toll-like receptor 4 been prematurely dismissed as an inflammatory bowel disease gene? Association study combined with meta-analysis shows strong evidence for association. *Am J Gastroenterol* 2007; **102**: 2504-2512
 - 182 **Hsiao CH**, Wei SC, Wong JM, Lai HS, Chang MH, Ni YH. Pediatric Crohn disease: clinical and genetic characteristics in Taiwan. *J Pediatr Gastroenterol Nutr* 2007; **44**: 342-346
 - 183 **Guo QS**, Xia B, Jiang Y, Morré SA, Cheng L, Li J, Crusius JB, Peña AS. Polymorphisms of CD14 gene and TLR4 gene are not associated with ulcerative colitis in Chinese patients. *Postgrad Med J* 2005; **81**: 526-529
 - 184 **Ortega-Cava CF**, Ishihara S, Rumi MA, Kawashima K, Ishimura N, Kazumori H, Udagawa J, Kadowaki Y, Kinoshita Y. Strategic compartmentalization of Toll-like receptor 4 in the mouse gut. *J Immunol* 2003; **170**: 3977-3985
 - 185 **Ortega-Cava CF**, Ishihara S, Rumi MA, Aziz MM, Kazumori H, Yuki T, Mishima Y, Moriyama I, Kadota C, Oshima N, Amano Y, Kadowaki Y, Ishimura N, Kinoshita Y. Epithelial toll-like receptor 5 is constitutively localized in the mouse cecum and exhibits distinctive down-regulation during experimental colitis. *Clin Vaccine Immunol* 2006; **13**: 132-138
 - 186 **Meijssen MA**, Brandwein SL, Reinecker HC, Bhan AK, Podolsky DK. Alteration of gene expression by intestinal epithelial cells precedes colitis in interleukin-2-deficient mice. *Am J Physiol* 1998; **274**: G472-G479
 - 187 **Abreu MT**, Vora P, Faure E, Thomas LS, Arnold ET, Arditi M. Decreased expression of Toll-like receptor-4 and MD-2 correlates with intestinal epithelial cell protection against dysregulated proinflammatory gene expression in response to bacterial lipopolysaccharide. *J Immunol* 2001; **167**: 1609-1616
 - 188 **Kamada N**, Hisamatsu T, Honda H, Kobayashi T, Chinen H, Kitazume MT, Takayama T, Okamoto S, Koganei K, Sugita A, Kanai T, Hibi T. Human CD14+ macrophages in intestinal lamina propria exhibit potent antigen-presenting ability. *J Immunol* 2009; **183**: 1724-1731
 - 189 **Kamada N**, Hisamatsu T, Okamoto S, Chinen H, Kobayashi T, Sato T, Sakuraba A, Kitazume MT, Sugita A, Koganei K, Akagawa KS, Hibi T. Unique CD14 intestinal macrophages contribute to the pathogenesis of Crohn disease via IL-23/IFN-gamma axis. *J Clin Invest* 2008; **118**: 2269-2280
 - 190 **Kamada N**, Hisamatsu T, Okamoto S, Sato T, Matsuoka K, Arai K, Nakai T, Hasegawa A, Inoue N, Watanabe N, Akagawa KS, Hibi T. Abnormally differentiated subsets of intestinal macrophage play a key role in Th1-dominant chronic colitis through excess production of IL-12 and IL-23 in response to bacteria. *J Immunol* 2005; **175**: 6900-6908
 - 191 **Denning TL**, Wang YC, Patel SR, Williams IR, Pulendran B. Lamina propria macrophages and dendritic cells differentially induce regulatory and interleukin 17-producing T cell responses. *Nat Immunol* 2007; **8**: 1086-1094
 - 192 **Takada Y**, Hisamatsu T, Kamada N, Kitazume MT, Honda H, Oshima Y, Saito R, Takayama T, Kobayashi T, Chinen H, Mikami Y, Kanai T, Okamoto S, Hibi T. Monocyte chemoattractant protein-1 contributes to gut homeostasis and intestinal inflammation by composition of IL-10-producing regulatory macrophage subset. *J Immunol* 2010; **184**: 2671-2676
 - 193 **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
 - 194 **Edelblum KL**, Washington MK, Koyama T, Robine S, Baccharini M, Polk DB. Raf protects against colitis by promoting mouse colon epithelial cell survival through NF-kappaB. *Gastroenterology* 2008; **135**: 539-551
 - 195 **Nenci A**, Becker C, Wullaert A, Gareus R, van Loo G, Danese S, Huth M, Nikolaev A, Neufert C, Madison B, Gumucio D, Neurath MF, Pasparakis M. Epithelial NEMO links innate immunity to chronic intestinal inflammation. *Nature* 2007; **446**: 557-561
 - 196 **Matharu KS**, Mizoguchi E, Cotoner CA, Nguyen DD, Mingle B, Iweala OI, McBee ME, Stefka AT, Prioult G, Haigis KM, Bhan AK, Snapper SB, Murakami H, Schauer DB,

- Reinecker HC, Mizoguchi A, Nagler CR. Toll-like receptor 4-mediated regulation of spontaneous *Helicobacter*-dependent colitis in IL-10-deficient mice. *Gastroenterology* 2009; **137**: 1380-1390.e1-3
- 197 Egan LJ, Eckmann L, Greten FR, Chae S, Li ZW, Myhre GM, Robine S, Karin M, Kagnoff MF. IkappaB-kinasebeta-dependent NF-kappaB activation provides radioprotection to the intestinal epithelium. *Proc Natl Acad Sci USA* 2004; **101**: 2452-2457
- 198 Chen LW, Egan L, Li ZW, Greten FR, Kagnoff MF, Karin M. The two faces of IKK and NF-kappaB inhibition: prevention of systemic inflammation but increased local injury following intestinal ischemia-reperfusion. *Nat Med* 2003; **9**: 575-581
- 199 Bernstein CN, Blanchard JF, Kliever E, Wajda A. Cancer risk in patients with inflammatory bowel disease: a population-based study. *Cancer* 2001; **91**: 854-862
- 200 Itzkowitz SH, Harpaz N. Diagnosis and management of dysplasia in patients with inflammatory bowel diseases. *Gastroenterology* 2004; **126**: 1634-1648
- 201 Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, Hsu D, Xu R, Harpaz N, Dannenberg AJ, Subbaramaiah K, Cooper HS, Itzkowitz SH, Abreu MT. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. *Gastroenterology* 2007; **133**: 1869-1881
- 202 Rakoff-Nahoum S, Medzhitov R. Regulation of spontaneous intestinal tumorigenesis through the adaptor protein MyD88. *Science* 2007; **317**: 124-127
- 203 Li Q, Yu YY, Zhu ZG, Ji YB, Zhang Y, Liu BY, Chen XH, Lin YZ. Effect of NF-kappaB constitutive activation on proliferation and apoptosis of gastric cancer cell lines. *Eur Surg Res* 2005; **37**: 105-110
- 204 Morais C, Pat B, Gobe G, Johnson DW, Healy H. Pyrrolidine dithiocarbamate exerts anti-proliferative and pro-apoptotic effects in renal cell carcinoma cell lines. *Nephrol Dial Transplant* 2006; **21**: 3377-3388
- 205 Greten FR, Eckmann L, Greten TF, Park JM, Li ZW, Egan LJ, Kagnoff MF, Karin M. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell* 2004; **118**: 285-296

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Authorship: Authorship credit should be in accordance with the standard proposed by International Committee of Medical Journal Editors, 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.

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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.

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Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

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Data that are not statistically significant should not be noted.

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^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 ¹F, ²F, ³F; 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

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Format

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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 Pixu-diarrrhoea. *Shijie Huaren Xiaobua 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**. Hyper tension, 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. *HRS-A Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

- 10 **Sherlock S**, Dooley J. Diseases of the liver and biliary 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. Wicczorek 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 \pm SD or mean \pm 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 ν (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 $24.5 \mu\text{g/L}$; CO₂ volume fraction, 50 mL/L CO₂, not 5% CO₂; 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.

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Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.

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