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AIM AND SCOPE

World Journal of Clinical Infectious Diseases (*World J Clin Infect Dis*, *WJCID*, online ISSN 2220-3176, DOI: 10.5495) is a peer-reviewed open access (OA) academic journal that aims to guide clinical practice and improve diagnostic and therapeutic skills of clinicians.

WJCID will focus on a broad spectrum of topics on infectious diseases that will cover epidemiology, immune-pathogenesis, genetic factors, host susceptibility to infection, vector control, novel approaches of treatment, molecular diagnostic and vaccines. It will provide a common stage to share the visions, new approaches, most advanced techniques, and to discuss research problems that will help everyone working in the field of various infections to exchange their views and to improve public health. *WJCID* will also focus on broad range of infections like opportunistic infections, zoonotic infections, tropical and neglected tropical diseases, emerging infections, *etc.* and following topics related to these issues: (1) Causative agents discussing various pathogens; (2) Vectors and Mode of transmission; (3) Host-pathogen interaction and immune-pathogenesis of the disease; (4) Epidemiology of the infection and vector control strategies; (5) Genetic factors covering both host and pathogen; (6) Molecular diagnostic techniques vaccines; and (7) Recent advances in cell tissue culture, lab techniques, *etc.* Various other related fields like medical microbiology, pharmacology of herbs, bioinformatics, *etc.* will be included.

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REVIEW

Acinetobacter baumannii: An emerging pathogenic threat to public health

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Abstract

Over the last three decades, *Acinetobacter* has gained importance as a leading nosocomial pathogen, partly due to its impressive genetic capabilities to acquire resistance and partly due to high selective pressure, especially in critical care units. This low-virulence organism has turned into a multidrug resistant pathogen and now alarming healthcare providers worldwide. *Acinetobacter baumannii* (*A. baumannii*) is a major species, contributing about 80% of all *Acinetobacter* hospital-acquired infections. It disseminates antibiotic resistance by virtue of its extraordinary ability to accept or donate resistance plasmids. The procedures for breaking the route of transmission are still proper hand washing and personal hygiene (both the patient and the healthcare professional), reducing patient's biofilm burden from skin, and judicious use of antimicrobial agents. The increasing incidence of extended-spectrum beta-lactamases and carbapenemases in *A. baumannii* leaves almost no cure for these "bad bugs". To control hospital outbreaks of multidrug resistant-*Acinetobacter* infection, we need to contain their dissemination or require new drugs or a rational combination therapy. The optimal treatment for multidrug-resistant *A. baumannii* infection has not been clearly established, and empirical therapy continues to require knowledge of susceptibility patterns of isolates from one's own institution. This review mainly focused on general features and introduction to *A. baumannii* and its epidemiological status, potential sources of infection, risk factors, and strategies to control infection to minimize spread.

Key words: *Acinetobacter*; *Acinetobacter baumannii*; Biofilm; Combination therapy; Hospital-acquired infection; Intensive care unit; Multidrug resistance; Nosocomial Pathogen; Risk factor

Core tip: *Acinetobacter*, is Gram-negative cocco-bacilli, originally regarded as low virulence bacteria, adopted now with increasing incidences, and recognized as a significant healthcare-associated multidrug-resistant classical pathogen. *Acinetobacter baumannii* (*A. baumannii*) accounts for nearly 80% of reported *Acinetobacter* infections. *A. baumannii* resist desiccation, and survive for several months on animate and inanimate surfaces. It has excellent colonizing potential, and contact transmission is a big challenge intermittent as well as endemic outbreaks. Strong biofilm formation is a part of virulence pathogenesis strategies of this organisms, and elimination of the identified source often require multiple interventions. This review mainly discusses on relevant epidemiological features of *A. baumannii*.

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IMPORTANCE

Once documented as a pathogen with low virulence, *Acinetobacter* is currently an important etiological agent of nosocomial infections, including hospital-acquired pneumonia and ventilator-associated pneumonia in patients admitted to intensive care units (ICUs), wound infections from war, and natural disasters such as a tsunami[1-3]. The National Nosocomial Infections Surveillance System reported a significant increase in the proportion of *Acinetobacter* among all Gram-negative aerobes during the 17 years of the study period (1986 through 2003)[2]. *Acinetobacter* was the only pathogen showing consistently increasing incidence in nosocomial pneumonias, and *Acinetobacter baumannii* (*A. baumannii*) was a major species among reported causes of nosocomial pneumonia[3].

Taxonomical aspect

The Gram-negative, non-fermentative aerobic bacteria which now recognized as belonging to the genus *Acinetobacter* have in the past been classified under various generic names. The genus *Acinetobacter* is now classified in the family *Moraxillaceae*, which includes Moraxella, Acinetobacter, Psychrobacter, and related organisms[4]. The genus *Acinetobacter* includes *Gram-negative coccobacilli* that have a G + C content of 39-47 mol% and that are strictly aerobic, non-motile, catalase-positive, and oxidase-negative. The negative oxidase test is important for rapid presumptive identification to differentiate the genus *Acinetobacter* from other similar non-fermentative organisms. But the transformation assay of Juni is the only test considered to be an unambiguous identification test for the genus *Acinetobacter*[5]. Most *Acinetobacter* species are non-fastidious and can be easily grown on simple microbiological media. Although variants appear, typical colonies are smooth, domed shaped pale yellow to grayish, about 2 mm with entire edge. Most species grow at ambient temperature, and pathogenic species such as *A. baumannii* grow well at 37 ℃. Enrichment medium such as Leeds selective medium as occasionally use, and are helpful in recovery of isolates from complex samples[6].

The genus *Acinetobacter* encompasses at least 25 DNA groups (genospecies) identified by DNA-DNA hybridization, 23 of which have been officially validated[7-10]. A recently submitted species of *Acinetobacter nosocomialis* (*A. nosocomialis*) and *A. pittii* are included in taxonomic nomenclature. *Acinetobacter* uses a wide variety of organic compounds as a carbon sources. This property has been used in developing the identification system for this organism. It is often difficult in clinical laboratories to differentiate the isolates of *Acinetobacter* at the species level according to their phenotypic characteristics[8], and can be inadequate for species confirmation, and should be used with caution. Automated systems available for distinguishing Gram-negative pathogens can identify *Acinetobacter* species but have limitations. *A. baumannii*, *Acinetobacter calcoaceticus* (*A. calcoaceticus*), genomic species 3, and 13TU are closely related and formally grouped as *A. baumannii-A. calcoaceticus* (Abc) complex (recently species 3 and 13TU are referred as *A. pittii* and *A. nosocomialis*, respectively). Molecular characterization, particularly 16S rRNA gene sequence analysis, can be of great help to resolve matters of dispute. Looking at the global dissemination of international clones, and their involvement in outbreaks, the rapid and discriminating genotyping methods are required for delineation of such clonal lineages[11]. Among the most common methods that are currently used involves pulse-field gel electrophoresis, amplified fragment length polymorphism, single locus genotyping, trilocus sequence-based typing, multi-locus sequence typing such as PubMLST, Pasteur's MLST, multi-locus variable-number tandem-repeats , resistance island typing, PCR with electrospray ionization mass spectrometry, next-generation whole genome sequencing, and PCR-based replicon of plasmid DNA. Most of these genotyping methods are not routinely used in hospitals and not cost effective, but extremely useful for to establish clonal relationships of the isolates and their taxonomical classification[11-15].

Habitat and colonization

Although *Acinetobacter* has emerged as an important pathogen, little is known about its natural reservoirs and habitat. Pathogenic members of the genus *Acinetobacter* contribute to the normal flora of human skin, upper respiratory tract, and gastrointestinal tract. The clinical consequences of *Acinetobacter* infections range from minimal to moderate to severe. *A. baumannii,* along with two other genetically closely related species (genomic species 3 and 13TU), is almost exclusively associated with human infection and is phenotypically difficult to differentiate routinely in clinical laboratories. Hence, the group is known as *A. baumannii-A. calcoaceticus*-complex (*Abc*-complex), and is often regarded *A. baumannii* in clinical practice as[16,17]. Although many consider *A. baumannii* to be ubiquitous, not everyone agrees. It is considered to be commensal with humans, and colonization is well documented. Therefore, the switch from colonization to infection is more favorable than it would be from more distant environmental sources[10]. Other species that are occasionally isolated from clinical samples are *A. calcoaceticus, A. hemolyticus, Acinetobacter johnsonii* (*A. johnsonii*), *Acinetobacter lowffi*, and *Acinetobacter ursingii*.

EPIDEMIOLOGICAL ASPECT

Acinetobacter species account for a substantial proportion of epidemic and endemic nosocomial infections and occasional sporadic outbreaks[14,16,18,19]. Geographically distant outbreaks are being studied for their ancestral genetic pool and clonal lineage. Multilocus sequence typing analysis recognized Ⅰ to Ⅲ international clones, corresponds to their clonal complexes, and many of the isolates causing outbreaks are suspected phylogenetically to be closely related with these clonal groups[20]. It can cause a wide array of infections such as respiratory tract infections, bloodstream infections, urinary tract infec-

tions, meningitis, endocarditis, and wound infections. In a recent report, 6 out of 7 patients with *Acinetobacter* bloodstream infections found *A. baumannii* colonizing their gastrointestinal tract[21]. *A. baumannii* is a prevalent species that causes epidemic outbreaks of nosocomial *Acinetobacter* infections[17,22-24]. Although there are mixed opinions, *A. baumannii* is usually reported to have a known natural habitat around patient population and in healthcare facilities and is occasionally isolated from environmental samples such as soil and water. *A. baumannii* is an excellent colonizer and is known to form biofilms. Furthermore, the reports demonstrate a positive correlation between biofilm formation capabilities and the multidrug resistance (MDR) status of *A. baumannii*. Such phenotypes have the ability to mediate outbreaks[25]. The multifactorial nature of the pathogenicity of *A. baumannii* has been documented recently and various models are proposed, and the involvement the presence and expression of exoproteases and exopolysaccharides (mediating biofilms), iron acquisition resistance to serum, resistance to desiccation, adherence and colonization, epithelial cell invasion and extraordinary ability to acquire foreign genetic material through lateral transfer for own survival, are elaborated as virulence attributes[26-31]. *A. baumannii* survives for a relatively long time in environments such as dry animate and inanimate surfaces and, when conditions are favorable, leads to outbreaks. The exact natural habitat of many of the *Acinetobacter* species is yet to be fully understood and may require intense efforts to identify.

Towner describes that depending on the site of isolation and the population of species or strains involved, *Acinetobacter* can be broadly categorized into three groups[10]: (1) MDR isolates capable of colonizing and infecting hospitalized patients, usually mediating hospital outbreaks. Generally these are *A. baumannii*. The isolates usually belong to a single clone or limited clones. Intensive care units are the depots for such outbreaks (occasionally other units mediate their spread as well); and (2) Relatively less resistant, less virulent strains that occasionally cause outbreaks. These isolates can be a part of normal skin flora of humans or animals or are associated with food spoilage[10,32]. Examples of such isolates are *A. johnsonii*, *A. lwoffii*, and *A. radioresistens*[33]. Environmental sources of isolate that are sensitive to many routine antibiotics and rarely cause outbreaks. *A. calcoaceticus* is a classic example. Infection control practices are therefore reserved mainly for the resistant isolates, which are usually *A. baumannii*-complex members. The patterns of spread of these members are also peculiar and can be correlated to strains causing outbreaks. In many European and Asian hospitals, the clonal spread (single clone) of *A. baumannii* has been reported either in a single hospital or in multiple hospitals and the strain was susceptible only to colistin and tigecycline[34,35]. Epidemiological typing methods are often helpful in delineating their dissemination and the strains involved in an outbreak and can differentiate epidemic outbreaks from sporadic strains. Thus, overall diversity of habitat, predilection to accumulate antimicrobial resistance, resistance to desiccation, ability to form biofilm, and propensity to cause hospital infection outbreaks make *Acinetobacter* an remarkable microorganism.

A. baumannii strains are generally more resistant than other species of this genus and often express a MDR phenotype, as discussed previously. Therefore, treatment of nosocomial infections caused by *A. baumannii* has become complicated because of the widespread antimicrobial resistance among these organisms[36]. The rising trend of resistance in *A. baumannii* strains, particularly to newer antimicrobial agents, is a health care concern. The organism expresses multiple mechanisms of antibiotic resistance that likely leads to the development of multiply resistant or even "pan-resistant" strains. This situation is particularly a quandary in terms of therapeutic choices for epidemic outbreaks mediated by these phenotypes.

POTENTIAL SOURCES OF INFECTION AND CONTAMINATION

The source of *A. baumannii* infections can be endogenous or exogenous. Most frequently, the infection is exogenous in origin because of the ability of the organisms to survive longer in the environment and on dry surfaces and because they are resistant to desiccation. *A. baumannii* multiply not only on human and animal skin, but also in soil and water and thus have a diversity of reservoirs. Locations in the hospital environment where *A. baumannii* have been found include ventilator tubing, suction catheters, humidifiers, containers of distilled water, urine collection jugs, intravenous nutrition, multidose vials of medication, potable water, moist bedding articles, pillows, and inadequately sterilized reusable arterial pressure transducers[37-39]. *A. baumannii* have been found in or on water taps, sinks, and computer keyboards and on all other inanimate surfaces that can act as a reservoir[40,41]. Hospital food can also be a potential source of *Acinetobacter* infection[8]. A study of two hospital outbreaks in Leiden, the Netherlands, reported the isolation of the outbreak strains from the dust inside the respiratory ventilator, the apparatus used to cool or warm a patient[40].

The gloves, gowns, and unwashed hands of hospital staff including doctors and nurses are frequently contaminated and may act as a potential source of *Acinetobacter* infection[14,42]. Hospital staffs with damaged skin are at increased risk of being colonized with *Acinetobacter* and are more likely to contaminate medical equipment and devices and patients by direct contact, thereby causing outbreaks of infection[43]. Specific types of medical procedures are also reportedly associated with high rates of infection with *Acinetobacter*, such as wound irrigation and treatment, catheterization, and tracheostomy[44]. Thus, the mode of infection can be environmental contamination or cross-contamination[45]. Community-acquired *A. baumannii* pneumonia is one of the severe forms of infection found around Indian Ocean, with very high co-morbidities and

reportedly associated in part with casualties from natural disasters such as earthquake and tsunamis, and wound contamination occurring among soldiers following war-related injuries[46].

NOSOCOMIAL ACQUISITION AND RISK FACTORS

Several factors reported by different groups increase the risk of nosocomial infection with *A. baumannii*. Most vulnerable among them are mechanical ventilation (source of ventilator-associated pneumonia), intensive care and other critical care units, wound and burn units, prolonged hospital stay, prior antibiotic therapy, increased exposures to infected patients, colonized neighboring patients, and health care personnel. Other risk factors are a weakened immune system, chronic and debilitating disease, and diabetes. Infection secondary to an invasive procedure is widely reported and involves ventilator-associated pneumonia, secondary meningitis and bloodstream infection, urinary tract infection, surgical site infection, and catheter-related bloodstream infection. In most cases it is point source contamination. Postoperative complications from infection with *A. baumannii* have been reported; the major risk factors are skin and soft tissue, bone, central nervous system trauma or injuries, and combat wounds and injuries[47-49]. Post-disaster infections caused by *A. baumannii* have also been reported[50,51]. *A. baumannii* is intrinsically resistant to many antimicrobial agents and has a propensity to acquire resistance to other, newer antimicrobial agents as well[52]. Consequently, it has become more prevalent because of selective pressure from antimicrobial agents in ICUs. Analysis of the epidemiological profile of antibiotic-resistant *Acinetobacter* spp showed an increased risk of infection in patients in ICUs who probably spread large numbers of *A. baumannii* cells into their surroundings by shedding *A. baumannii*-infected or colonized cells, making the area more likely to be a source of infection for others[37,53]. Although airborne transmission has been documented, direct contact, including patient-to-patient and health care-provider-to-patient transmission, is more relevant.

Community acquisition of *Acinetobacter* infection, although rare, has been reported[54,55], and a community-acquired MDR *Acinetobacter* carrying IMP1 metallo-β-lactamase, responsible for hospital infection, is recovered[55]. Community-acquired *A. baumannii* pneumonia[56,57], community-acquired bacteremia[58], urinary tract infection[59], and meningitis[54] have been reported. On the basis of the rising incidence of community-acquired *A. baumannii* infection, a concurrent spread of multidrug resistance is the greatest risk. Among, *A. baumannii* wound infections, three hypotheses usually described are a combination of wound with environmental bacteria, a wound contamination from previous cutaneous or oropharyngeal endogenous reservoir, and hospital acquisition[46].

STRATEGIES TO CONTROL INFECTION

Outbreaks, particularly endemic or periodic epidemic outbreaks, caused by MDR *A. baumannii* are difficult to control. It is still possible to effectively control *A. baumannii,* although eradication is in question[14]. Decontamination of the patient by treating the gut and skin has been reported. Antibiotics can be used to inhibit gut colonization by *A. baumannii* that remains susceptible, but the benefits are limited because of the risk of developing resistant phenotypes. Additional research is needed to clarify the role of such techniques for selective decontamination of gut compared with surfaces such as skin[60]. The role of various sites of *A. baumannii* colonization and the risk of epidemiological outbreaks have been assessed; selective gut decontamination was found to be less effective as an additional measure[61]. Selective decontamination of skin with chlorhexidine reduced a significant load of *A. baumannii* and has been proposed as the infection control measure to lower the number of endemic outbreaks[62]. Because *A. baumannii* is widely present in the hospital environment, it can contaminate any surface or article with which it comes in contact, *e.g.*, resuscitation bags, blood pressure cuffs, parenteral fluids and nutritional solutions, lotion dispensers, hand creams, bed linen, and mattresses. Therefore strict hand hygiene and personal cleanliness are essential in breaking the route of transmission[63]. Periodic disinfection of wards, units, and surfaces and sterilization of medical devices using appropriate methods are highly recommended. A periodic hospital environmental sample survey for microbiological contamination is advisable[64,65]. The epidemiological studies help to identify the source or reservoir of the infection and thus eventually to understand how to control the outbreaks[8]. Control of the environmental reservoir is a major part of an effective control strategy[64,66]. The researchers who conducted the study in the Netherlands controlled an outbreak by removing dust from the mechanical ventilator and continuous venovenous hemofiltration machines and replacing dust filters[40]. A study conducted in the United States reported *A. baumannii* as a model in eradication of MDR infections[67]. The control measures for *A. baumannii* infection have been discussed by many investigators[8,18,68,69]. Some of the specific control measures for *A. baumannii* infection are shown in Table 1.

One of the most associated factors with reservoirs is biofilm formation capability of *A. baumannii* wherein it is responsible in part for the intermittent release of pathogens that leads to outbreaks. Biofilm formation by this organism also facilitates its persistence, and thus acts as a source of infection[25]. Recently, a dynamic exchange of gene cassettes between integrons (a mobile genetic element responsible for recruitment of multiple resistance genes, *e.g.,* class 1 integron) in natural biofilms has been demonstrated[25,79]. This association of biofilm is important in higher tolerance or resistance to strong

Table 1 Some of the major infection control measures marked for *Acinetobacter baumannii* infection outbreaks

Sr	Effective control measure	Ref
1	Early detection of a colonized patient or the source or reservoir of an infection	[14,70]
2	Eradication of the source or reservoir	[71]
3	Isolation of an infected or colonized patient into an isolation cubicle	[18]
4	Cohort nursing	[72]
5	Emphasis on hand washing (with alcoholic-based disinfectants) before and after patient handling	[73]
6	Use of disposable gloves and aprons	[42]
7	Prohibition of sale of antibiotics without prescription/judicious use of antibiotics	[69,74]
8	Improved surveillance system for antimicrobial resistance	[75]
9	Adherence to infection control best practices	[76]
10	Education of hospital staff and community for infection control/proper drug use and maintenance of hygiene/contact precaution	[77,78]

antimicrobial and biocidal agent[80]. Biofilm producing virulence is also found associated with aminoglycoside resistance genes. Rajamohan *et al*[25] demonstrated an increased biocide resistance and multidrug resistance in *A. baumannii* associated with the ability to form stronger biofilms. In part, the resistance may be increasing due to low penetration of antimicrobials into biofilms, in addition to acquisition of resistance genes through mobile genetic elements[81]. The continuous presence of high selection pressure of antimicrobials and disinfectants in intensive care units is also been correlated to increased multidrug resistance, strong biofilm abilities, and survival of these variant within such biofilms[16,82]. Thus control of such variants are a challenge, and difficult with routine antimicrobial and biocidal agents.

Microbiology laboratories can provide frontline surveillance for antibiotic resistance and are therefore useful in combating nosocomial infections[83]. Rapid, accurate analysis of antimicrobial susceptibility will be useful in determining the precise use of antimicrobial agents. Hence, clinical input from a microbiologist is necessary to keep one step ahead in controlling nosocomial infections. Periodic surveillance by molecular typing of isolates from patients is recommended for early detection of an epidemic strain, which consequently serves as an effective control measure[84]. Empiric antimicrobial therapy based on such observations is useful when laboratory findings are impeded for one reason or another[85,86]. Such therapy has been successful against pneumonias, ventilator-associated pneumonias, and bloodstream infections caused by *A. baumannii*, especially in critically ill patients[87-90], although some failures have also been reported, and caution is advised[91]. Empiric carbapenem therapy is a popular example of such a regime[14,92,93]. With the rise of carbapenem resistance in MDR phenotypes, this approach seemingly faces difficulties[14]. MDR is a common phenomenon associated with *A. baumannii* that is on the increase[10,94-96]. There are no clear guidelines to treat *A. baumannii* infections, and antipseudomonal broad-spectrum penicillins and cephalosporins and the members of other categories such as monobactams, aminoglycosides, fluoroquinolones, carbapenems, glycylcyclines, polymyxins, and β-lactamase inhibitors are used to control infections involving *A. baumannii*. Selection of the appropriate antimicrobial agent for empirical therapy is therefore challenging and has to be based on local institutional and hospital findings. Treatment decisions are usually made on a case-by-case basis by a health care provider. Empirical treatment therefore is likely to differ for a given geographic location[97]. Antibiotic susceptibility testing and other phenotypic tests for detecting double-disk synergy should be used as a guide, in addition to approved governing guidelines. Institutional data mining and retrospective analysis are often of great help in this regard and are advised by Towner[10].

Because of the limited choice of antimicrobial agents, *A. baumannii* infections are treated mainly with extended-spectrum β-lactams; β-lactams with β-lactamase inhibitors such as tazobactam or sulbactam; and carbapenems. Colistin and sulbactam are still relatively effective against infection caused by MDR *A. baumannii*, but an anticipatory fear of the development of resistance is increasing in ICUs. Peptides and other novel antibacterial agents are in the experimental phases. A combination therapy (dual or triple therapy) of a carbapenem with sulbactam, tobramycin, colistin, and aztreonam is being assessed in laboratory synergy studies, but clinical trials are required before one can adopt such combination regimens[98]. A study containing pharmacokinetic-pharmacodynamic profiling of four antimicrobial drugs against *A. baumannii* suggested that a combination involving carbapenem is required for effective therapy[99]. A glycopeptide (vancomycin or teicoplanin)-colistin combination was found to be highly active (synergism) against *A. baumannii* both *in vitro* and in a simple animal model[100]. A complicated case of persistent MDR *A. baumannii* central nervous system infection (ventriculitis) was resolved by a prolonged triple combination therapy involving intraventricular colistin and tobramycin plus intravenous colistin, rifampin, and vancomycin[101]. In murine pneumonia and rabbit meningitis models of *A. baumannii* infection, imipenem or sulbactam were found to be appropriate for combination therapy when used with rifampin[102]. A comparative *in vitro* study of synergistic activities also demonstrated that imipenem has better synergism with colistin than does amikacin or ampicillin/sulbactam against carbapenem-resistant *A. baumannii*[103]. In another study, tigecycline, a recently developed novel broad-spectrum antibacterial agent, was used (off-label indication) in combination therapy to treat MDR *A. baumannii* superinfection. However, the studies had several limitations such as retrospective design, small number of patients, and tigecycline as a part of the combination[56]. Despite its association with nephrotoxicity, colistin has been used by different modes of administration. Nebulized colistin was found to be more efficient in *A. baumannii* pulmonary infections when administered solely in nebulized

Table 2 Some of the commonly reported mechanisms of resistance in *Acinetobacter baumannii* from different geographic locations

Category of mechanism	Gene involved	Geo-location	Ref
ESBL	*PER-1* type	Hungary, India, Turkey, Korea, France, Belgium, Romania	[114,127-132]
ESBL	*VEB-1* type	Belgium, France	[131,133]
ESBL	*KPC* type		[134]
ESBL	*CTX-M-2* type	Japan	[135]
Carbapenemase	*OXA* type	United Kingdom, transcontinental	[120,136]
Carbapenemase	*OXA* type	United States,	[119,122,123,137]
Carbapenemase	*OXA-51* type	United Kingdom, France, Iraq, United States	[123,138-140]
Carbapenemase	*OXA-23* type	United Kingdom, China, United States	[141,142]
Carbapenemase	*OXA-40* type	Spain, United States	[123,143]
Carbapenemase	*OXA-58* type	Greece, Italy, Bolivia	[144,145]
Carbapenemase (multiple)	*OXA, IMP, VIM*	Korea	[146]
Carbapenemase, MBL	*NDM*	Israel, Germany	[147,148]
Carbapenemase, MBL	*VIM*	Poland	[149]
Carbapenemase, MBL	*IMP*	Japan, Brazil	[150,151]
Carbapenemase, MBL	*SIM*	China	[152]

ESBL: Extended-spectrum β-lactamase; MBL: Metallo-β-lactamase

form or in combination with intravenous colistin against intravenous colistin alone[104]. Colistin is still considered a good choice against MDR *A. baumannii* compared with ampicillin/sulbactam[105-107] or rifampin+imipenem[107]. The nephrotoxicity associated with colistin is reported to be reversible and less frequent than once thought. Neurotoxicity is rare, although more posological research is needed[33]. At present, no new drugs that could be available in 5 years are currently in the pipeline; therefore, combination regimens of antibiotics are the only resources to combat this infection.

ANTIMICROBIAL RESISTANCE IN *A. BAUMANNII*

The three major forces that drive antimicrobial drug resistance are failure to maintain hospital hygiene, selective pressure due to irrational use of antibiotics, and mobile genetic elements encoding the bacterial resistance mechanism[96]. The resistance among *A. baumannii* strains to β-lactam agents is of great concern among clinicians. The β-lactams are broadly accepted for treatment because of the availability of a wide range of drugs, their broad spectrum of activity, minimum side effects, and most importantly, their relatively low cost in developing countries of Africa, Asia and Latin America. The restriction on the use of these agents because of the emergence of resistance is a loss to the community and a great blow to the health care system. The mechanism of resistance to β-lactam in *A. baumannii* can be attributed to an intrinsic property or an acquired phenomenon. This organism is a known reservoir of multiple plasmids carrying antibiotic resistance markers[16,95]. The later mobile genetic element is of concern because the acquisition of resistance genes can radically change the scenario of drug resistance. *Acinetobacter* spp are also known to donate resistance-plasmids and are therefore likely to rapidly disseminate resistance among other commensals or pathogens.

Acinetobacter harbors multiple mechanisms of drug resistance. The mechanism of resistance to β-lactam agents in *A. baumannii* involves production of a variety of chromosomal or plasmid-mediated β-lactamases, especially extended-spectrum β-lactamase (ESBL), alteration of drug-binding proteins, permeability changes in the cell membrane, loss of porins, and efflux pump, of which the presence of an array of β-lactamases is the predominant weapon[108-111]. *Acinetobacter* produce a variety of β-lactamases. The main mechanisms of resistance to extended-spectrum cephalosporins in *A. baumannii* are the over-expression of chromosomal cephalosporinases and plasmid-encoded Ambler class A, B, and D β-lactamases[112]. ESBL-producing *A. baumannii* strains are now reported from various geographic areas of the world. These include the TEM type, SHV type, CTX-M type, PER-1, and VEB-1 β-lactamases. The prevalence of ESBLs is much higher in the isolates from ICUs than in isolates from other hospital sites[113,114]. *A. baumannii* produces a variety of extended-spectrum β-lactamases, depending on its geographical location. The PER-1 ESBLs were from Turkey, Korea, Russia, Romania, Belgium, France, and India; VEB-1, from France and Belgium; TEM-116 and TEM-92, from China and Italy, respectively; SHV-12 from the Netherlands; CTX-M-2 and CTX-M-43 from Korea and Bolivia (Italy), respectively[114-118]. Table 2 demonstrates in brief the representative mechanisms reported from different geographic locations. It was believed that ESBL-producing *A. baumannii* strains remain susceptible to carbapenems. However, OXA-type ESBL-producing *A. baumannii* isolates resistant to carbapenems have been widely reported, including from the United States[119-122], that carry insertion sequence, IS*Aba1* upstream to OXA-like genes[123]. Although resistance in *A. baumannii* to polymyxins such as colistin is rare, recent reports suggest that an underlying mechanism of moderate resistance to colistin involves point mutation in pmrB, upregulation of pm-

rAB, and expression of pmrC, which lead to phosphoethanolamine modification of lipid A[33,124]. This finding means that we will not be able to use many more β-lactam drugs, which will further limit our options. Among carbapenem-resistant MDR *A. baumannii*, colistin is often the last resort. Recent findings suggest a slow rise of colistin-resistant isolates lead to Pan-drug resistant organisms[125]. With the help of rapid and powerful tools such as high throughput sequencing technologies *e.g.*, whole-genome sequencing, one can elucidate the origin of large outbreaks of such resistant pathogens, and the exact genetics behind resistance mechanisms[125,126].

FUTURE PROBLEMS

A contentment of multidrug resistance and their dissemination in *Acinetobacter baummannii* is not an easy task. While multiple drug resistance is increasing in this pathogen, and carbapenem resistance is rapidly spreading cross-continentally, there is a sharp decline in development of new antimicrobial agents that can control MDR *A. baumannii*. There is no new drug in pharmaceutical pipeline or none of the FDA-approved antimicrobial compounds tested had appreciable effect in control of MDR *A. baumannii*. The existing antimicrobials also failed to control the resistance development and effective elimination of MDR variants. A rational synergistic approach of some of the combination therapies although working, needs more in-depth understanding, and systematic studies are required in order to control probably outbreaks. Creation of pan-drug resistant variants will have to be avoided, and efforts on new anti-acinetobacter drug development would be invested.

CONCLUSION

Microbiological surveillance facilitates the ability to monitor changes in the trends of dominant microorganisms and their antimicrobial susceptibilities in hospitals. It helps to detect recent resistance mechanisms in these pathogens and to formulate antimicrobial usage policies for the hospital and adds to the epidemiological information about these organisms in particular regions of the country.

The MDR *Acinetobacter* clinical isolates, especially in the ICUs of hospitals, are a serious public health concern worldwide, and responsible for high mortality. The geographic variation in resistance patterns emphasizes the importance of local surveillance in determining the most suitable therapeutic option to treat *Acinetobacter* infections. The lack of therapeutic options for treating MDR organisms calls for systematic pharmacokinetic and pharmacodynamic studies of rational combination therapies until new, powerful drug appear in clinical practice for this purpose.

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REVIEW

Physiological functions and clinical implications of fibrinogen-like 2: A review

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Abstract

Fibrinogen-like 2 (FGL2) encompasses a transmembrane (mFGL2) and a soluble (sFGL2) form with differential tertiary structure and biological activities. Typically, mFGL2 functions as prothrombinase that is capable of initiating coagulation in tissue without activation of the blood clotting cascade, whereas sFGL2 largely acts as an immunosuppressor that can repress proliferation of alloreactive T lymphocytes and maturation of bone marrow dendritic cells. Protein sequences of FGL2 exhibit evolutionary conservation across wide variety of species, especially at the carboxyl terminus that contains fibrinogen related domain (FRED). The FRED of FGL2 confers specificity and complexity in the action of FGL2, including receptor recognition, calcium affiliation, and substrate binding. Constitutive expression of FGL2 during embryogenesis and in mature tissues suggests FGL2 might be physiologically important. However, excessive induction of FGL2 under certain medical conditions (*e.g.*, pathogen invasion) could trigger complement activation, inflammatory response, cellular apoptosis, and immune dysfunctions. On the other hand, complete absence of FGL2 is also detrimental as lack of FGL2 can cause autoimmune glomerulonephritis and acute cellular rejection of xenografts. All these roles involve mFGL2, sFGL2, or their combination. Although it is not clear how mFGL2 is cleaved off its host cells and secreted into the blood, circulating sFGL2 has been found correlated with disease severity and viral loading among patients with human hepatitis B virus or hepatitis C virus infection. Further studies are warranted to understand how FGL2 expression is regulated under physiological and pathological conditions. Even more interesting is to determine whether mFGL2 can fulfill an immunoregulatory role through its FRED at carboxyl end of the molecule and, and vice versa, whether sFGL2 is procoagulant upon binding to a target cell. Knowledge in this area should shed light on development of sFGL2 as an alternative immunosuppressive agent for organ transplantation or as a biomarker for predicting disease progression, monitoring therapeutic effects, and targeting FGL2 for repression in ameliorating fulminant viral hepatitis.

Key words: Fibrinogen-like 2; Prothrombinase; Immunosuppressor; Infectious disease

Core tip: Fibrinogen-like 2 (FGL2) protein promotes coagulation as a prothrombinase, or acts as an immunosuppressor to repress function of T lymphocytes and dendritic cells and induce apoptosis of B lymphocytes. Ectopic expression of FGL2 has been proven relevant for the pathogenesis of viral infections. Induction of FGL2 in response to pathogen invasion causes focal prothrombin activation and fibrin deposition. This process may lead to inflammation, microvascular thrombosis, and subsequent organ failure. FGL2-mediated immunosuppression can facilitate pathogen proliferation and expansion. The understanding of FGL2-mediated

pathophysiology offers an insight into biomarker development and clinical intervention of FGL2-associated medical conditions such as viral hepatitis.

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INTRODUCTION

Fibrinogen-like 2 (FGL2), also known as fibroleukin, is a multifunctional protein. FGL2 has been found to be not only physiologically important[1-3], but also involved in pathogenesis of viral infections[4,5], pregnancy failure[6], autoimmune disorders[7,8], allograft rejections[9], and tumor growth[10]. Alternations in FGL2 expression or structure are tied to several highly virulent viral infections, including human immunodeficiency virus (HIV) infection, severe acute respiratory syndrome (SARS), and hepatitis B and C[4,5,11]. In this review, constitutive expression and physiological roles of FGL2 that have been identified to date will be illustrated to help understand pathological properties of FGL2 during pathogen invasion when ectopic expression of FGL2 occurs. While FGL2 might have a potential to be used as a biomarker or therapeutic target, some research gaps will be explored to expand possible clinical applications of FGL2. Although signal transduction pathways involved in regulation of *FGL2* transcription and post-transcriptional modifications are important, in-depth discussion of these molecular mechanisms is not in the scope of this review.

STRUCTURE AND FUNCTION

Several studies have suggested that FGL2 is highly conserved, sharing over 70% homology among human, mouse and rat[12-15]. A phylogenetic tree analysis suggests an even closer evolutional relationship between human *Fgl2* and pig *Fgl2*[16]. This extraordinary evolutionary conservation across different species suggests that FGL2 might be an indispensable protein with critical biological function(s).

Two distinct forms of the FGL2 protein have been identified, membrane-associated FGL2 (mFGL2) and soluble FGL2 (sFGL2). mFGL2 integrates with phospholipids of cellular membranes and is expressed as a type Ⅱ transmembrane protein[14], while sFGL2 can be secreted into the vasculature. Native sFGL2 exists as an oligomer consisting of four disulfide-linked FGL2 monomers[1,17]. The difference in the tertiary structure between mFGL2 and sFGL2 suggests that the two forms of FGL2 may function differently.

mFGL2 has been found to be expressed on endothelial cells, epithelial cells, macrophages, and dendritic cells[18-20]. The first 19 to 26 amino acids at the N-terminus are highly hydrophobic and predicted to serve as the transmembrane domain of mFGL2[14,21]. Three tentative serine protease active sites at positions 91, 142, and 423 are conserved between human and mouse FGL2[14]. The residue serine 91 of human FGL2, corresponding to the serine 89 of murine FGL2, has been revealed to be capable of cleaving prothrombin into thrombin[14,22]. FGL2 was thus speculated and has been demonstrated to function as a prothrombinase[15,22,23]. FGL2 activates prothrombin to generate thrombin that in turn converts fibrinogen into fibrin, a process equivalent to factor Ⅱ (F Ⅱ) activation[15,22,23]. However, unlike F Ⅱ, the proteolytic activity of FGL2 is independent of factor *X* and cannot be inhibited by antithrombin Ⅲ[22,24]. Instead, full proteolytic activity of FGL2 is contingent on its physical association with membrane phospholipids, factor Va, and calcium[22]. Therefore, prothrombinase activity appears to be intrinsic to mFGL2, but similar function of sFGL2, if there is any, has yet to be demonstrated.

sFGL2 is known to be secreted by cytotoxic and regulatory T lymphocytes upon activation, but not by helper T lymphocytes and B lymphocytes[8,12,17,19,21,25]. A stretch of hydrophobic amino acids at N-terminus of FGL2 may serve as signal peptide for sFGL2 secretion[14], but the mechanism whereby sFGL2 is cleaved and released outside of the host cell remains to be determined. Glycosylation of the amino acids at positions of 172, 228, 256, and 329 were found to be critical to maintain the solubility of sFGL2[16]. Previous studies indicate that sFGL2 lacks procoagulant activity of mFGL2[12,17], rather, it functions largely as an immunosuppressor or pro-apoptotic effector molecule[26]. sFGL2 has been shown to inhibit maturation of bone marrow-derived dendritic cells; maintain immunosuppressive activity of regulator T cells (Tregs); and suppress T cell proliferation in response to the stimulation by alloantigens, anti-CD3/Cd28 antibodies, or CoA[8,26,27]. A fibrinogen related domain (FRED) at the carboxyl terminus is believed to account for the immunosuppressive activity of sFGL2 as a monoclonal antibody against FRED abrogated the sFGL2-mediated suppression of T cell proliferation[26]. sFGL2 binds specifically to Fc gamma receptor (FcγR) Ⅱ B/CD32. This receptor is expressed on sinusoidal endothelial cells (SECs) within the liver[28]; glomerular mesangial cells within the kidney[29]; and immunoregulatory cells such as dendritic cells (DCs), B lymphocytes, macrophages, and activated T lymphocytes[16,30,31]. Binding of sFGL2 to SECs, glomerular mesangial cells, B lymphocytes, or macrophages caused apoptosis of the target cells[8,28,30]. Binding of sFGL2 to bone marrow DCs can inhibit lipase (LPS)-induced DC maturation[30] and thereby impairs the ability of DCs to stimulate alloreactive T cell proliferation[26]. In addition, sFGL2 can bind directly to T lymphocytes to inhibit their proliferation and polarize allogeneic immune response toward a Th2 cytokine profile by inducing interleukin 4 (IL-4)/IL-10 while inhibiting IL-2/interferon gamma (IFN-γ) produc-

tion[8,26]. Consistent with these observations, the levels of Th2 cytokines and the activity of DCs, B lymphocytes, and T lymphocytes have all been found to be increased in FGL2-deficient mice[8].

PHYSIOLOGICAL ROLES

Dissecting potential functions of a protein under normal physiological conditions sets a foundation for understanding of pathological properties of this molecule. Constitutive expression of FGL2 has been detected in the heart, lung, small bowel, spleen, ovary, uterus, liver, and kidney[13,32]. Expression of FGL2 is regulated tightly and associated with several physiological processes, including sperm maturation[1], embryo development[3,33], and smooth muscle contraction[2,34].

FGL2 might play a protective role during sperm maturation in epididymis[1]. The expression of *Fgl2* messenger RNA (mRNA) under normal physiological conditions has been identified in the tubule principal cells of hamster epididymis[1]. FGL2 was found to be secreted from the principal cells into the tubule lumen where sFGL2 binds specifically to the nonviable, but not the viable, spermatozoa[1]. This process forms sFGL2-protein complex that coats and envelops dying sperms to restrict release and spread of detrimental enzymes and immunogenic molecules from defective spermatozoa. Nevertheless, FGL2-deficient male mice were still fertile[35], suggesting that the potential protective role of FGL2 is limited, or becomes prominent only under certain medical conditions when increased apoptosis of spermatozoa occurs.

Expression of FGL2 has been shown to change dynamically during murine embryogenesis[3]. FGL2 was first detectable at the implantation site at E5.5 of gestation among CBA mice that exhibited the rate of pregnancy failure equivalent to that expected on the basis of embryonic chromosome abnormalities[3]. By E6.5, the embryo itself became positive for FGL2, but the level diminished at the maternal-fetal trophoblastic interface by E7.5 and was barely detectable in the developing embryo at E8.5 to E9.5[3]. By E13.5, however, FGL2 was detected in somites (future vertebra) and adjacent neural tube within the embryo[3]. This spatio-temporally coordinated expression of FGL2 , in forms of mFGL2 or sFGL2, during embryonic development suggests that FGL2 might play a physiological role in embryogenesis. Congruent with this speculation, the level of *FGL2* messenger RNA was higher in gravid myometrium tissues from pregnant women than in the hysterectomy samples from premenopausal non-pregnant women ($P < 0.001$)[36]. The physiological function of FGL2 has been further demonstrated in animal studies. Knocking-out *Fgl2* gene (*Fgl2*$^{-/-}$) in mice led to higher rate of pregnancy failure than wild type (*Fgl2*$^{+/+}$) mice[33]. Early miscarriage of mouse embryos between the time of implantation (E4.5) and formation of vascularized placenta (E9.5) were associated with absence of physiological expression of FGL2[3], suggesting that FGL2 might aid embryo implantation and placenta development.

Given that sFGL2 can suppress T cell activation[26], it is likely that pregnancy failures among *Fgl2*$^{-/-}$ mice might have been caused by loss of local suppression of classical T cells or natural killer T cells that otherwise might contribute to immune rejection of developing fetus half of whose antigens are encoded by paternal genes[37]. In addition, mFGL2 might function as prothombinase to improve coagulation and reduce hemorrhage at the implantation site that is often seen in FGL2-deficient embryos but not in wild type embryos[3,23,38].

FGL2 might be involved in modulation of vascular and nonvascular smooth muscle contraction. Expression of FGL2 was detected in mouse cardiomyocytes[2]. *Fgl2*$^{-/-}$ murine embryos had significantly lower heart rates than *Fgl2*$^{+/+}$ embryos[2]. About 33% of *Fgl2*$^{-/-}$ pups died within 3 d after birth due to acute congestive cardiac failure resulted from myocardial contractile dysfunction[2]. These data suggest that FGL2 is critical for normal myocardial function during prenatal and postnatal development in mice[2], but it is not clear how FGL2 deficiency is linked to abnormal myocardial contraction. O' Brien *et al*[34] investigated expression of FGL2 in biopsies of human uterine myometrium incised during cesarean delivery [pregnant not in labor (PNL)] or at intrapartum [pregnant in labor (PL)]. They noticed that both *FGL2* mRNA and FGL2 protein were expressed more prominently in PL samples than in PNL samples. Interestingly, up-regulation of thrombin receptors, F2R and F2RL3, were found to be correlated with FGL2 elevation in the myometrium in labor[34]. Increased level of FGL2 can lead to thrombin accumulation in myometrium. Thrombin in turn binds to these receptors and causes cytosolic enrichment of calcium[39]. This process may ultimately result in myometrial smooth muscle contraction[39,40]. Pretreatment with a thrombin-specific inhibitor hirudin prevented myometrial contraction[40]. Therefore, FGL2 appears to modulate vascular and nonvascular muscle contraction through generation of thrombin.

PATHOGENESIS

Disturbances in the tight control that balances the time and location of constitutive FGL2 expression have been implicated in the pathogenesis of pathogen invasion[4,5,11], miscarriage/pre-eclampsia[6,41,42], allograft rejections[9], autoimmune diseases[7,8], and tumor growth[10,43]. Pathogenesis of these disorders share some common features rooted from ectopic expression of mFGL2 or sFGL2. This section focuses on potential mechanisms behind the pathogenesis of the infectious diseases that involve abnormal activities of mFGL2, sFGL2, or both.

HIV-1 infection typically advances through acute phase to asymptomatic stages and finally to full-blown acquired immune deficiency syndrome (AIDS). Acute stage is characterized by elevated expression of genes involved in immune activation and defenses, resulting

in partial control of HIV infection and progression to asymptomatic stage. Expression of a host of immunosuppressive genes including *FGL2* is activated at the asymptomatic stage[5]. Up-regulation of sFGL2 may help to dampen the immunopathological consequences of sustained immune activation during acute phase of HIV infection, however, the host immune system is probably too naïve to assume that their mission is over and wind down immunosurveillance by turning on the expression of *FGL2* and other immunosuppressive genes. Elevation of FGL2 at the asymptomatic stage might have facilitated HIV to escape the host immune protection and thus leading to uncontrolled viral proliferation and AIDS[5].

The SARS coronavirus (SARS-CoV) is the etiologic agent responsible for the outbreak of SARS in Asia in 2003. The infection resulted in a mortality rate of 50% among patients over 60 years of age[44]. A homozygous mutation (dbSNP ID: rs2075761; JSNP ID: IMSJST003521) at the amino acid position 53 (FGL2 G53E) appears to be weakly associated with level of nasopharyngeal shedding of SARS-CoV (P = 0.041)[45]. Subsequent studies from the same group confirmed that FGL2 G53E was a dominant risk variant for SARS-CoV infection[46]. Individuals carrying this mutation had about 40% higher SARS-infection rate than those without this mutation (P < 0.0001)[46].

It is worth noting that the G53E point mutation is not located near known important functional motifs, including FRED, glycosylation sites, and serine prothrombinase sites. There is no report to date exploring whether and how the G53E mutation might affect the function of sFGL2. Nevertheless, SARS-CoV was shown to be capable of activating transcription of *FGL2* gene[47]. Transfection of plasmids expressing the nucleocapsid protein of SARS-CoV into human macrophage cell line or African green money kidney epithelial cells activated *FGL2* expression[47]. However, Siu *et al*[48] was not able to reproduce the results in the Vero cell line, nor in human embryonic kidney cells or cultured human airway epithelial cells. Therefore, it remains controversial as to whether SARS-CoV regulates *FGL2* transcription. Both SARS-CoV and murine hepatitis virus strain 1 (MHV1) are categorized as group 2 coronaviruses[49] and produced very similar pathological features. MHV1 infection of mice is thus proposed as an animal model for SARS research[50]. A study *in vivo* in A/J mice suggested that FGL2 might contribute to the pathogenesis of SARS-like severe pulmonary disease induced by MHV1[50]. However, there has been no direct evidence indicating that FGL2 might be involved in SARS infection.

FGL2 may contribute to pathogenesis of human hepatitis B virus (HBV) and hepatitis C virus (HCV) infections. Patients with HBV or HCV infection have higher levels of FGL2 than healthy controls[4,11,51]. Strong fibrin deposition and necrosis were co-localized with robust FGL2 expression in liver biopsies from 21 out of 23 patients with acute-on-chronic hepatitis B characterized by recurrent flares of hepatocellular injury, but not among all 13 patients with minimal chronic hepatitis that exhibited no major active liver pathology[23,51]. Similarly, the plasma levels of FGL2 were over 2-fold higher among patients with HCV infection (n = 80) than healthy controls (n = 30, P < 0.001)[4].

The expression of FGL2 seemed to correlate with the progression of viral hepatitis. Mean levels of plasma sFGL2 were nearly 3-fold higher among HCV-patients with cirrhosis (n = 60) than those without cirrhosis (n = 20, P = 0.001)[4]. Fibrosis stage is an established indication for disease severity and efficacy of anti-viral treatment. Plasma levels of sFGL2 among HCV-patients with advanced fibrosis (stage 3-4, n = 22) were twice that of patients with moderate fibrosis (stage 2, n = 19, P = 0.01) and over 3-fold higher than those with mild fibrosis (stage < 1, n = 35, P = 0.001)[4]. However, the levels of FGL2 among HCV-patients with inactive alcoholic cirrhosis were comparable to the controls[4], suggesting that it is the activity and progression of HCV infection, not the end stage cirrhosis,that accounts for the high levels of FGL2 among HCV-patients.

How does HBV-infection or HCV-infection lead to increased FGL2 expression, and why do high levels of FGL2 correlate with the disease progression? Han *et al*[11] found that HBV core protein or *X* protein were both capable of binding directly to the promoter of *FGL2* gene and activating its transcription in a hepatocellular carcinoma cell line. On the other hand, sFGL2 has been found to function as an immunosuppressor to inhibit maturation of dendritic cells, reduce production of IFNγ, and impair proliferation of alloreactive T lymphocytes among the patients with HBV or HCV[52-54]. In contrast, FGL2-deficiency was associated with the development of T cell leukemia/lymphoma[55]. Collectively, HBV/HCV might up-regulate sFGL2 expression. High levels of sFGL2 in turn might jeopardize the host immune integrity and thus may facilitate viral replication and expansion. In addition, FGL2-mediated accumulation of fibrin could restrain or block blood flow in the liver and cause hepatocyte necrosis or even liver failure[23,51].

Observations from animal studies have provided further insights into the pathogenesis of viral hepatitis. Mouse hepatitis virus 3 (MHV3)-a member of Coronaviridae-has served as a model for dissecting pathological determinants of diseases caused by coronaviruses. *Fgl2* mRNA was detected in Kupffer (macrophage) cells and reticuloendothelial cells in the liver of BALB/cJ mice within 8 h post MHV3-infection[56]. FGL2 protein was detected within 24 h following MHV3 infection in endothelium of intrahepatic veins and hepatic sinusoids where concomitant fibrin deposition and subsequent focal hepatocyte necrosis occurred[56]. Ning *et al*[57,58] demonstrated that the nucleocapsid protein of MHV3 was capable of activating transcription of mouse *Fgl2* gene *in vitro*. Serum level of sFGL2 was shown to correlate with liver cytopathology among the mice infected by MHV3[19]. More compelling evidence suggesting that

FGL2 might contribute to pathogenesis of viral infection comes from the elegant studies by Shalev *et al*[27] and by Marsden *et al*[23], who have demonstrated that MHV3 caused fibrin deposition and hepatocellular necrosis only in *Fgl2*$^{+/+}$ mice but not in *Fgl2*$^{-/-}$ mice. Further, they have showed that administration of anti-FGL2 monoclonal antibody (mAb) improved liver histology and survival rate[27]. Similarly, target deletion of *Fgl2* gene in C57BI/6 mice[23] or depletion of *Fgl2* mRNA by introducing dual short hairpin RNA into BALB/cJ mice before exposing to MHV3 can alleviate liver pathogenesis and improve survival rate[59].

Given FGL2-mAb directed to the FRED region that conveys immunosuppressive activity reduced MHV3 viral titers among infected mice[27], it is reasonable to postulate that FGL2-mediated-immunosuppression might play a role in viral hepatitis. However, several lines of evidence suggest that FGL2-mediated immunosuppression is not a major determinant in the pathogenesis of viral hepatitis. First, MHV-3 viral loads in the livers did not vary between *Fgl2*$^{+/+}$ and *Fgl2*$^{-/-}$ mice[23]. Second, although administration of FGL2 neutralizing antibodies abrogated hepatitis in mice infected by MHV3, a high viral load persisted[60]. Finally, in spite of similar MHV3 viral load between the spleen and the liver of MHV3-infected BALB/cJ mice, the pathogenesis was restricted in the liver with complete absence of disease in the spleen. Recent studies have demonstrated that the progression of fulminant viral hepatitis usually exhibits a similar pattern, viral-induced up-regulation of the *Fgl2* gene precedes focal deposits of fibrin in sinusoids, followed by accumulation of inflammatory cells and focal hepatocyte necrosis. The roles of FGL2 in the rapid development of confluent multicellular hepatic necrosis are probably fulfilled through several interrelated processes: (1) FGL2-mediated fibrin deposition may hamper or terminate sinusoidal blood flow and cause hepatocyte necrosis[61]; (2) FGL2 may bind directly to the (FcγR) IIB receptors on sinusoidal endothelial cells and trigger cellular apoptosis[28]; and (3) FGL2 may stimulate inflammation through generation of thrombin. Thrombin is known to be able to stimulate endothelial cells to produce IL-8[62,63]. Knocking-down *Fgl2* using *Fgl2* RNA interference (RNAi) caused a reduction of LPS-mediated IL-8 production[20]. IL-8 is a potent chemo-attractant for polymorphonuclear leukocytes that have been identified at the sites of FGL2-mediated inflammation.

It is puzzling why FGL2 induction and hepatocellular necrosis occurred only among BALB/cJ mice but not among A/J mice infected by MHV3[23,51]. These BALB/c-susceptible and A/J-resistant phenotypes might be attributable to several factors. First, MHV3 induced significantly greater apoptosis of macrophages from A/J mice than from BALB/cJ mice[64]. MHV3 is propagated in macrophages[19]. Apoptosis of macrophages might decrease the extent of viral replication and result in higher MHV3 viral load in BALB/cJ than in A/J mice. Additionally, macrophages are a source of FGL2 production[19,56,64]. *Fgl2*$^{+/+}$- macrophages exhibited a robust procoagulant response to MHV3; whereas procoagulant activity from *Fgl2*$^{-/-}$ macrophages exposed to MHV3 was comparable to the control levels[23]. Therefore, reduction in the number of macrophages due to apoptosis might translate into a diminished level of FGL2. In accordance with this hypothesis, apoptosis of macrophages mediated by target ablation of the gene encoding an inhibitory receptor B and T lymphocyte attenuator (*BTLA*$^{-/-}$) repressed FGL2 induction by MHV3 in the liver[19]. The reduction of FGL2 was associated with decreased tissue lesions and mortality among *BTLA*$^{-/-}$ mice infected with MHV3[19]. Adoptive transfer of macrophages into *BTLA*$^{-/-}$ mice increased their mortality rates close to those seen in *BTLA*$^{+/+}$ mice infected by MHV3[19]. This finding appears to be clinically relevant. Biopsies analysis indicates that the numbers of CD68^{+} macrophages were strikingly higher in the liver from patients with active HBV-infection than from normal controls and the patients with inactive chronic hepatitis[65]; Next, MHV3 induced over 100-fold lower level of *Fgl2* mRNA and significantly less amount of FGL2 protein in macrophages isolated from A/J mouse than from BALB/c mice (Fung *et al* 1991)[66]; Thirdly, baseline number and percentage of CD4^{+}Foxp3^{+}-Tregs in the spleen and thymus were 1-fold to 2-fold greater among BALB/cJ mice than among A/J mice[27]. These Tregs expressed FGL2 and depended on FGL2 for their immunosuppressive activity[8,27]; therefore, higher numbers of Tregs in BALB/cJ mice may confer faster viral replication and worse pathology. Adoptive transfer of *Fgl2*$^{+/+}$ Tregs or *Fgl2*$^{+/+}$ splenocytes that contain Tregs into *Fgl2*$^{-/-}$ mice 1 h before exposing to MHV3 recapitulated the susceptible phenotype seen in *Fgl2*$^{+/+}$ mice infected by MHV3[27]; Finally, sFGL2 binds to the inhibitory FcrRIIB receptor on DCs and B cells from BALB/cJ mice but not the DCs and B cells from A/J mice due to an allelic polymorphism of the FcγRIIB receptor in A/J mice[31]. As a result, FGL2-mediated immunosuppression might be less significant among A/J mice than among BALB/c mice, which may explain why A/J mouse is able to clear MHV3 shortly after the viral infection[67].

Interestingly, although A/J mice can clear MHV3 by 10 d to 14 d post infection, pretreatment of A/J mice with corticosteroids, methylprednisolone, abolished their resistance to MHV3 and the animals died within 10 d of infection[67]. It turned out that methylprednisolone stabilized *Fgl2* mRNA and hence increased accumulation of *Fgl2* mRNA, which in turn translates into more FGL2 protein[67,68]. Immunofluorescence analysis of the liver tissue from methylprednisolone-treated and MHV3-infected A/J mouse showed increased expression of FGL2 in areas of inflammation around hepatic sinusoids[67].

Taken together, the level of FGL2 correlates positively with the development and severity of typical MHV cytopathology[51,64]. Animal studies have suggested that elevation of FGL2 might be one of critical determinants of susceptibility to hepatitis virus infection[51].

POTENTIAL VALUE AS A BIOMARKER OR THERAPEUTIC TARGET

It would be highly desirable to measure a substance in readily available specimens such as blood or urine that would lead to disease diagnosis, reflect disease burden, correlate with therapeutic results, or simply be utilized as a surveillance marker to predict disease prognosis. FGL2 appears to be such a candidate. Variations in the plasma level of FGL2 among healthy human volunteers were minimal, regardless of race, gender, or age[4]. In contrast, plasma levels of FGL2 correlated positively with HCV titers and degree of inflammation in the liver[4]. The level of FGL2 dropped significantly following an effective anti-viral therapy among patients with biopsy-proven HCV hepatitis (n = 32, $P < 0.001$)[4]. Furthermore, as discussed previously, FGL2 expression has been found to be associated with progression and severity of disease. HCV-patients with cirrhosis had significantly higher levels of FGL2 (164.1 ± 121.8 ng/mL, n = 60) compared with patients without cirrhosis (57.7 ± 52.8 ng/mL, n = 20, P = 0.001) and patients with inactive end stage alcoholic cirrhosis (18.8 ± 17.4 ng/mL, n = 24, $P < 0.001$)[4]. Similarly, FGL2 was detected in peripheral blood mononuclear cells (PBMC) from 28 of 30 patients (93%) with severe hepatitis B, but only 1 of 10 (10%) patients with mild chronic hepatitis B[51]. FGL2-procoagulant activity was more than 10-fold higher on PBMCs from patients with acute-on-chronic hepatitis B than from healthy controls[51].

Plasma levels of FGL2 have been found to correlate with diseases other than viral hepatitis. For example, the levels of plasma FGL2 were significantly higher among patients with fatty liver disease than healthy controls[69]. Likewise, although the elevation of FGL2 was not associated with clinical features of systemic sclerosis, the mean serum level of FGL2 among patients with systemic sclerosis (28.7 ± 17 ng/mL) was significantly higher than that among healthy controls (11.4 ± 5.5 ng/mL, $P < 0.001$)[70].

Recent research has provided exciting insight into clinical application of FGL2 as a therapeutic target. Animal studies suggested that effective disease intervention could be achieved through modulation of FGL2 expression at DNA or protein level. For example, tail-vein injection of antisense plasmid complementary to the exon 1 of mouse *Fgl2* gene into BALB/cJ mice caused marked reduction of inflammatory cell infiltration, fibrin deposition, and hepatocyte necrosis[71]. All 18 mice receiving *Fgl2* antisense plasmid were alive on 3 d post MHV3-infection[71]. Six of 18 mice (33%) recovered from fulminant viral hepatitis[71]. In contrast, no mice in the control group (n = 18) survived beyond 3 d postinfection[71]. Similar effects have been observed by targeting FGL2 protein directly. Administration of FGL2-mAb resulted in a dose-dependent reduction of MHV3 viral titers among infected mice and improved liver histology and survival rate[27, 60].

Infections after organ transplantation remain a significant cause of mortality among the recipients[72,73]. For example, Sanders-Pinheiro *et al*[73] reported nearly 80% of kidney transplant recipients (n = 80) had infections and 53.8% of death resulted from infections. High rate of severe infections have also been seem among liver recipients with HIV/HCV-coinfection[74]. Current steroid or steroid-free immunosuppression scheme following an organ transplantation has been found to be associated with cardiovascular disease and infections[72,73,75]. Therefore, novel regimen is in great need to overcome or minimize adverse effects of immunosuppression[30]. Intravenous injection of recombinant sFGL2 into donor mice receiving skin transplantation prolonged the survival of skin allografts from 7.8 ± 1.99 d to 15 ± 2.56 d ($P < 0.001$)[30]. This finding might be clinically significant in that FGL2 could induce immune tolerance without relying on prolonged immunosuppression and thus help to reduce the risk of development of cardiovascular disease, infections, or cancer. Interestingly, monomeric FGL2 has been found to exhibit greater immunosuppressive activity than native oligomer sFGL2[16]. Monomeric FGL2 could be a better candidate in clinical usage than native sFGL2 in terms of its stronger potency, higher permeability and usually less antigenicity due to its lower molecular weight.

In summary, blood FGL2 might not be suitable for diagnosis as a disease-specific biomarker, but could emerge as an indicator to monitor disease progression and therapeutic effects for certain disorders such as HBV or HCV infection. While target-specific repression of mFGL2 expression has showed promising clinical implications for hepatitis therapy, sFGL2 may be used as novel immunosuppression agent for organ and tissue transplantations.

GAPS AND FUTURE DIRECTIONS

Although several reports have dealt with transcriptional regulation of *Fgl2* expression[11,57,76,77], it remains to be determined how the transcription and translation of *Fgl2* gene are regulated differentially in response to MHV3 infection across different cell types, tissues, and strains of animals. For example, Ding *et al*[56,61] reported that MHV3 induced *Fgl2* mRNA expression in the lung, liver, and spleen, but barely in the brain or kidneys of BALB/cJ mice despite of comparable viral titers in all tissues. At any time during the course of MHV-3 infection, FGL2 protein was only detectable in the liver but not any other tissues that were also positive for *Fgl2* mRNA[56]. In addition, MHV3 regulated FGL2 expression differentially even in the same type of cells from different strains of mice[78]. Exploring the mechanism responsible for cell- and tissue-specific expression of FGL2 may provide some insights into targeting FGL2 for more practical and effective clinical applications.

Further studies are needed to clarify unique and com-

mon functions between mFGL2 and sFGL2. Given that the FRED is conserved between mFGL2 and sFGL2, it is plausible to speculate that mFGL2 could also exhibit FRED-mediated immunosuppressive activity[26]. Although monomeric FGL2 has been found to be capable of suppressing allogeneic T cell proliferation[16], there is no report to date demonstrating that mFGL2 can act like sFGL2 to elicit immunologic response. Conversely, a valid question to ask is whether sFGL2 secreted by Tregs maintains prothrombinase activity, given FRED contains several prothrombinase-related functional motifs, including calcium ion loop and fibrinogen knob binding pocket[16]. FGL2 produced by the principle cells along epididymis epithelium can be secreted into the lumen where sFGL2 sequesters defective spermatozoa through forming polymerized protein matrix around dying cells[1]. However, it is not clear whether epididymal sFGL2 exhibits mFGL2-like prothrombinase activity and how the sFGL2 binds specifically to compromised spermatozoa to eliminate defective cells[1].

Sepsis is a life-threatening immune response to infection. Systemic coagulation and inflammation are hallmarks of this complication. Increased fibrin formation is also a characteristic clinical feature of sepsis[79,80]. Infection is often in concert with elevated production of inflammatory cytokines such as TNF-α and IFN-γ. TNF-α and IFN-γ have been found to induce *Fgl2* expression[77,81]. As discussed previously, FGL2 can activate coagulation and provoke inflammation through thrombin- and fibrin-generation, but the role of FGL2 in sepsis onset and progression needs further investigation.

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Acknowledgments

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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]

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10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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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

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Write as mean ± SD or mean ± SE.

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