

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

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Received: June 28, 2013 Revised: July 23, 2013

Accepted: August 16, 2013

Published online: August 25, 2013

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.

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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.

Yang G, Hooper WC. Physiological functions and clinical implications of fibrinogen-like 2: A review. *World J Clin Infect Dis* 2013; 3(3): 37-46 Available from: URL: <http://www.wjgnet.com/2220-3176/full/v3/i3/37.htm> DOI: <http://dx.doi.org/10.5495/wjcid.v3.i3.37>

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 evolutionary 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 prothrombinase 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: IMS-JST003521) 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 monkey 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 IFN γ , 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 C57Bl/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 pro-

duction^[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 FcγRIIB 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 seen 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.

ACKNOWLEDGEMENTS

We thank Drs. Michael Soucie and Christopher Bean for their critical comments on our manuscript.

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P-Reviewer Watanabe T S-Editor Song XX L-Editor A
E-Editor Lu YJ





Published by **Baishideng Publishing Group Co., Limited**
Flat C, 23/F., Lucky Plaza,
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