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Mannose-binding lectin and the balance between immune protection and complication

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Abstract

The innate immune system is evolutionarily ancient and biologically primitive. Historically, it was first identified as an element of the immune system that provides the first-line response to pathogens, and increasingly it is recognized for its central housekeeping role and its essential functions in tissue homeostasis, including coagulation and inflammation, among others. A pivotal link between the innate immune system and other functions is mannose-binding lectin (MBL), a pattern recognition molecule. Multiple studies have demonstrated that MBL deficiency increases susceptibility to infection, and the mechanisms associated with this susceptibility to infection include reduced opsonophagocytic killing and reduced activation of the lectin complement pathway. Results from our laboratory have demonstrated that MBL and MBL-associated serine protease (MASP)-1/3 together mediate coagulation factor-like activities, including thrombin-like activity. MBL and/or MASP-1/3-deficient hosts demonstrate *in vivo* evidence that MBL and MASP-1/3 are involved with hemostasis following injury. *Staphylococcus aureus*-infected MBL null mice developed disseminated intravascular coagulation, which was associated with elevated blood IL-6 levels (but not TNF- α) and systemic inflammatory responses. Infected MBL null mice also develop liver injury. These findings suggest that MBL deficiency may manifest as disseminated intravascular coagulation and organ failure with infection. Beginning from these observations, this review focuses on the interaction of innate immunity and other homeostatic systems, the derangement of which may lead to complications in infection and other inflammatory states.

Keywords

coagulation; complement; deficiency; infection; inflammation; mannose-binding lectin; MASP; pattern recognition molecules

Innate immunity is the front-line of the host defense system, which protects from invading pathogens and abnormal self-derived components that are generated by a variety of causes, including infection. Successful innate immune protection requires an orchestrated network of cellular and humoral factors, including both recognition and effector mechanisms. The effectors include innate immune cells, such as epithelial cells and phagocytes; and innate immune soluble factors such as cytokines, chemokines, complement proteins, coagulation factors, and soluble pattern recognition molecules. Activities of these effector cells and

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molecules are initiated in part by pattern recognition molecules. Such molecules are found as membrane proteins on innate immune cells, as proteins embedded in extracellular matrices, or circulating as soluble proteins in the blood. One such protein circulating in blood is mannose-binding lectin (MBL) [1,2]. MBL, through its carbohydrate recognition domain (CRD), recognizes molecular patterns that are present on many pathogens or exposed neoepitopes on apoptotic and injured cells and tissues [1,2]. These molecular patterns include sugars such as α -mannose and N-acetyl- β -glucosamine (GlcNAc), both of which are recognized by MBL [3–5]. MBL functions as an opsonin and forms complexes with MBL-associated serine proteases (MASPs), which can activate complement and coagulation proteases [6–9]. MASPs are evolutionarily heterogeneous. While MASP-1 seems to be an ancient type of protease, MASP-2 is evolutionarily (and functionally) very close to C1r and C1s [10]. Other molecules also associate with the MBL–MASP complex, suggesting that activities of MBL–MASP are further regulated and directed. However, the regulatory mechanisms of these MBL-initiated activities are not well understood and this is the subject of continuing investigation [11,12].

MBL deficiency is a primary immunodeficiency that results from genetic defects that occur in 5–30% of the population, depending on ethnicity [13,14]. Human MBL gene defects can be caused by single nucleotide polymorphisms (SNPs) in the promoter region and exon 1 of the coding region [13,15]. Combinations of these SNPs result in reduced blood concentration and/or dysfunctional MBL proteins [16,17]. As a result, human MBL blood levels vary significantly, from undetectable to as high as 10 μ g/ml [13,15,17]. Compelling clinical and animal model studies have associated MBL deficiency with increased susceptibility to many pathogens, including influenza A virus, herpes simplex virus-2, *Pseudomonas aeruginosa* and *Staphylococcus aureus* [2,18–22]. The infection susceptibility phenotype was confirmed to be due to lack of MBL, and evidence from our laboratory shows that MBL-mediated anti-infection mechanisms include opsonic function, activation of the lectin complement pathway and regulation of inflammation [20,21]. Worsening infection, as may be seen in MBL deficiency, not only causes bacteremia but also triggers various systemic derangements, including chaotic systemic inflammation [20,21,23].

Clinical investigations have documented that mortality from infection is associated with complications such as organ failure and disseminated intravascular coagulation (DIC) [24–27]. DIC is a coagulation disorder in which thrombosis (clotting) and bleeding simultaneously progress throughout the body [25,28]. While DIC is often associated with organ failure it is also known as an independent predictor of mortality and adverse events in many settings, including infection and trauma [24,25]. Not every patient with similar clinical conditions and symptoms develops DIC, suggesting genetic differences are involved. However, no genetic test or biomarker has been identified to predict DIC. Recent findings from our laboratory suggest that MBL deficiency may be involved with causes of organ failure and DIC at an early phase of infection [6]. Recent studies, including our own, demonstrate that the MBL–MASP complex mediates coagulation and that MBL-deficient mice have impaired coagulation when tissue is injured even without infection [6,8,9,29–31]. These observations suggest that MBL deficiency may be a risk factor for developing complications from infection, and investigation of the mechanisms of MBL deficiency-related complications have just begun. This article will review recent findings in MBL-mediated mechanisms in infectious diseases and associated complications and will discuss potential clinical implications.

MBL in the innate immune system

The host defense system includes elements of innate and acquired immunity, which serve primarily for local containment and systemic defense, respectively (Figure 1). The first-line

of the host defense system is innate immunity, which is present throughout the entire lifespan in the healthy state. The innate immune system acts instantly upon exposure to invading pathogens, which were the first identified targets of the innate immune system. If not for the immediate response of innate immunity, any pathogen could progress into rampant systemic infection. Additionally, we now know that the innate immune system responds to abnormal self epitopes, including cells and tissues that are damaged through apoptosis, inflammation, trauma, malignant transformation or other tissue injury [1]. Millions of cells throughout the body, such as blood cells (erythrocytes, neutrophils, lymphocytes, and so on), and epithelial cells die every day. If these cells and tissues are not promptly and properly removed they may contribute to chronic inflammation and may also trigger autoimmune responses, leading to autoimmune diseases. Thus, the innate immune system acts both locally and systemically through recognition of diverse targets, all with the effect of maintaining homeostasis of the host.

The innate immune system also influences adaptive immunity. The innate immune response generates signals to activate appropriate effector molecules and cells of the adaptive immune system. Elements of adaptive immunity, such as antigen-specific antibodies, are developed by immunization and boosting with antigens that are specific to pathogens or targets, as in vaccination. Adaptive immunity requires activation and expansion of effector cells, such as memory B cells, antibody-producing B cells and helper T cells, which may require weeks to months before it is fully effective. Although adaptive immunity may last a lifetime, some antigens require repeated boosts through re-inoculation, and the adaptive immune system is unable to provide protection if antigen-specific immunity is not maintained for a specific pathogen. Despite extensive research, vaccines are not available for all infectious pathogens [32]. Additionally, effector cells of the adaptive immune system, in particular B cells, are not fully mature in infants [33]. Prior to maturity of the adaptive immune system, very young individuals are protected by maternal antibodies and glycans supplied from mother's milk, as well as by a fully formed and functional innate immune system [34,35].

Successful innate immune protection is achieved through two steps: first, identifying targets, such as pathogens and abnormal tissues and cells; second, orchestrating humoral and cellular effectors to neutralize and eliminate the identified targets. Humoral factors include pattern recognition molecules, cytokines, complement proteins and coagulation factors. Examples of the cellular elements of the innate immune system are epithelial cells and phagocytes. Activation of the innate immune system is initiated by soluble pattern recognition molecules, which may be expressed on innate immune cells, bound to the extracellular matrix, or circulating in the blood as a soluble molecule. One such soluble pattern recognition molecule is MBL, which is primarily (>95%) synthesized in the liver and secreted to circulate in the blood [1,36]. Small amounts of MBL are also synthesized in the kidney, thymus, tonsil, small intestine and vagina, where mRNA has been detected [36–38]. MBL protein has also been found in other organs, such as the skin, brain and lung, although its mRNA has not been detected in those areas [1,19,36,39]. In the lung, MBL is found in the bronchial alveolar lavage of healthy hosts and also on airway smooth muscle following infection [19,40]. In the skin and the brain, MBL is observed only following burn and trauma injury, respectively [19,39,41]. The alveolar space is surrounded by capillaries, suggesting that MBL may traverse these vessels to enter the alveoli. By contrast, MBL may be recruited to regions in the skin and the brain through local capillaries. These findings and observations suggest that MBL, as a pattern recognition molecule of the innate immune system, provides a surveillance system both locally, at points of possible contact with the external environment, as well as systemically. In this manner MBL can contribute both to immunity from pathogens as well as maintenance of tissue integrity and homeostasis.

Mannose-binding lectin

Structurally, MBL comprises a cysteine-rich domain at the N-terminus followed by a collagen-like domain, a neck region and a carbohydrate recognition domain (CRD) at the C-terminus (Figure 2) [2]. Molecules having the collagen-like domain and CRD form the collectin family, which includes conglutinin, lung surfactant proteins (SP-A and SP-D) and collectin kidney 1 (CL-K1) [1,42,43]. Single molecules of MBL associate to form a functional trimeric subunit that can further associate to form a hexamer of trimers [2]. MBL's CRD recognizes and binds to chemical patterns, which include D-mannose, L-fucose and GlcNAc [1–5,44,45]. These carbohydrate residues are found on the surface of many pathogens, including bacteria and viruses, as well as abnormal self tissues, which contain endogenous neoepitopes that are exposed on apoptotic cells, cell debris and injured and damaged tissues [1,2,44,45].

MBL functions as an opsonin and activates complement through the lectin complement pathway, a third complement pathway in addition to the classical and the alternative complement pathways (Figure 3). The lectin pathway is also activated by ficolins, which are structurally similar to MBL and circulate in the blood, as discussed below. The lectin pathway requires activation of MASPs [1,6–8,19]. There are two genes and five gene products of MASP. MASP-1, MASP-3 and MAP44 (or MAP-1) are the alternative splice products of the *MASP-1/3* gene while MASP-2 and MAP19 (or sMAP) are the alternative splice products of the *MASP-2* gene [10,12,46,47]. MASPs form complexes with MBL [12,48], and MBL binding to carbohydrate ligand is thought to induce conformational changes that enhance proteolytic activities in the associated MASP. MASP-1 and MASP-2 have been shown to activate the alternative pathway and the lectin complement pathway [49–52]. Recent results from our laboratory provide *in vivo* evidence that MBL and MASP-1/3 complexes mediate coagulation, as mice genetically lacking either MBL or MASP-1/3 have prolonged bleeding time following tail laceration [6]. This phenotype was confirmed to be MBL-mediated because repletion of MBL in MBL null mice reduces bleeding time to the levels of MBL sufficient wild-type mice [6]. The hypocoagulation phenotype was also observed in ferric chloride (FeCl₃)-induced thrombosis assay, which is thought to mimic tissue injury as FeCl₃ generates reactive oxygen species that damage endothelial cells, causing blood clot and hemostasis [6,53,54]. Blood flow is stopped by 15 min in MBL sufficient wild-type mice whereas it is still flowing in MBL null mice even after 30 min [6]. These results support previous reports based on *in vitro* studies using recombinant MASPs, which have shown that MASPs, in complex with MBL, activate coagulation enzyme-like activities that catalyze the formation of fibrin clot (Figure 4) [8,9,55,56].

The activities of MASPs are modulated by regulatory molecules, including sMAP (MAP19), MAP and MAP44. sMAP and MAP regulate the lectin complement pathway activation [11,57]. MAP44 has been found to associate with MBL, although its effect is under investigation [12]. It has also been reported that MASPs regulate each other although the mechanisms are not fully understood.

MBL deficiency

MBL deficiency can be caused by inherited gene defects, which have been identified in 5–30% of the population, such that MBL deficiency is a common primary immunodeficiency [15,58–60]. There are three coding region SNPs, at codons 52, 54 and 57, termed the C, B and D alleles, respectively [61]. All of these SNPs are located in the collagen-like domain (all are located close to the N-terminus side of the kink, Figure 2) and produce aberrant proteins [61]. The frequency of these alleles varies among different ethnicities. While all

three alleles are observed in Caucasians, alleles C and D are very rare in Asians [13,62]. Overall, the B allele is the most common, substituting aspartic acid (D) for the glycine (G) of a wild-type A form. Additional SNPs in the promoter region cause seven known haplotypes resulting in modified secretion of MBL [13,16,17,63]. Most MBL deficiency is due to heterozygosity of these SNPs, resulting in a wide range of MBL blood concentrations, from undetectable to as high as 10 µg/ml [13,17]. While criteria for MBL deficiency have been defined by different groups, ranging from 100 ng/ml up to 1 µg/ml, it is difficult to compare results from different study groups since there is no accepted standard for measurement: there are several anti-MBL antibodies and at least two different assay methods. The sandwich method detects MBL using two antibodies, one for capturing and a second for detecting, and therefore this method measures MBL protein. By contrast, the mannan-capture method requires MBL binding to mannan, which takes the role of the capturing antibody in the sandwich method, and mannan-bound MBL is detected using an anti-MBL antibody. Consequently, this latter method assays biologically functional MBL.

Some aberrant MBLs were found to be dysfunctional in activating the lectin complement pathway. Mechanisms for this are related to reduced ligand binding due to decreased oligomerization, and decreased activation of MASPs due to impaired association with mutant MBL [64,65]. Interestingly, the putative MASP bindings site is located at the C-terminus side of the kink (Figure 2) [65,66]. However, a recent study shows that MBL homologous for the B allele, the most common mutant allele, slowly activates the lectin complement pathway [15]. Even a reduced MBL activity may be sufficient for activation, as it has become clear that the alternative pathway may also be an amplification loop of the lectin complement pathway as well as for the classical complement pathway [67–69].

Compelling clinical studies show that MBL deficiency increases susceptibility to certain pathogens, as we have reviewed previously and as revealed in subsequent studies [2,70–74]. We have confirmed these clinical observations using an animal model and by comparing infectivity in MBL sufficient wild-type and mice genetically lacking MBL (MBL null) [20]. Compared with wild-type, MBL null mice (note: mice have two alleles, MBL-A and MBL-C, and MBL null mice lack both) have increased susceptibility to infection with *S. aureus*, *P. aeruginosa*, herpes simplex virus-2 and influenza A virus [19–22]. In particular, all MBL null mice die from blood infection with *S. aureus* by 48 h postinfection while 60% of MBL sufficient wild-type mice survive. Pretreatment of MBL null mice with recombinant human MBL improves mortality to the level of wild-type mice, confirming that the increased infection susceptibility is due to MBL deficiency [20]. Our work demonstrated that protective mechanisms of MBL include the efficient opsonophagocytic killing of pathogens, activation of the lectin complement pathway and induction of proinflammatory responses at an early phase of infection [20,21,75,76]. Lack of these MBL-mediated mechanisms in MBL-deficient hosts may result in systemic infection involving multiple organs, including blood (bacteremia), and uncontrolled inflammation due to cytokine storm. Such infection and subsequent cytokine release may establish an autocrine loop, further escalating complications.

Our investigations have also provided *in vivo* evidence that MBL has a role apart from activation of the lectin complement pathway. This conclusion is derived in part from the observation that mice lacking MBL and complement 3 are more susceptible to *S. aureus* infection than mice lacking only MBL or complement 3 [76]. The deficient activity may be the opsonic function of MBL, as this was discovered as a significant factor in opsonic defect patients who were suffering from recurrent infections [18,77].

MBL in coagulopathy

A delicate balance of pro-coagulant and anticoagulant activity maintains blood fluidity (Figure 4). Disruption of the balance leads to catastrophic coagulopathy, including DIC, in which coagulation and bleeding occur concurrently, a situation associated with multiple organ failure, a poor prognosis and high mortality [24,78–80]. Overt DIC is typically diagnosed by increased prothrombin time (PT) and activated partial prothromboplastin time (aPTT). Recent identification of an abnormal biphasic waveform in an aPTT assay provides earlier (~1 day, on average) and more sensitive diagnosis of DIC [25,28,81,82]. The aPTT waveform represents the change in light transmittance (T) through a plasma specimen as the aPTT reaction takes place. A biphasic waveform is characterized by an initial steep negative slope, which is called slope₁ (%T/sec) [25]. The cause of the negative slope (slope₁) in the aPTT waveform has been identified as calcium-mediated precipitation of C-reactive protein complexes with very-low-density lipoprotein [81]. MBL binding also requires calcium, as it is a C-type lectin. Our recent investigations, using a mouse model of *S. aureus* bacteremia, reveal that plasma of MBL-deficient mice present the abnormal biphasic waveform from aPTT at an early time (15 h postinfection), when bacterial counts are similar to MBL sufficient wild-type mice (Figure 5) [6]. These observations suggest that MBL deficiency is associated with developing DIC during an early phase of *S. aureus* bacteremia, and that MBL deficiency may be a risk factor for developing DIC during infection.

MBL in complex with MASP mediates coagulation at the site of injury (tail laceration and FeCl₃-induced vascular injury, in our experimental models), as described above. There are routine clinical coagulation assays, which include fibrinogen concentration, platelet count, PT and aPTT, to screen for primary coagulation disorders (such as congenital coagulation factor deficiencies, including factors VII and VIII). Results of these routine clinical coagulation assays are comparable between MBL sufficient and deficient mice, suggesting that MBL deficiency alone does not alter the coagulation in the normal state, in the absence of injury or infection [6]. However, MBL deficiency may contribute to coagulopathy and other complications in the setting of tissue injury and infection.

Although DIC frequently arises subsequent to infection, some DIC patients are negative for bacterial culture, suggesting that traumatic tissue injury itself may also induce DIC [25]. The details of DIC development are not fully understood, and risk factors and biological markers to predict DIC have not been identified [25,83–86]. Not all patients with similar clinical presentations and clinical testing results develop DIC, suggesting that genetic factors are involved. Our latest investigations provide evidence that deficiency of MBL and/or MASPs is a contributing factor in DIC development in infection [6]. Clinical studies investigating these factors in DIC patients are required to further elaborate and characterize these phenomena.

MBL in tissue injury

Studies from our laboratory have also investigated organ damage, which is associated with DIC and can be assayed by organ-specific assays. Organ injury in infection with DIC may arise from invading bacteria, inflammation, hypoperfusion and physical injury. Injury to certain organs, such as the liver and kidney, may be assessed by routine clinical blood tests that measure metabolites or organ-specific enzymes. Tests for liver damage include blood levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Kidney function, specifically glomerular filtration, can be followed by measurements of blood urea nitrogen (BUN) and creatinine. Our results show that, compared with MBL sufficient wild-type mice, MBL-deficient mice have increased blood levels of the liver enzymes ALT and AST [6] at an early stage of infection. By contrast, blood levels of creatinine and BUN are

not affected by MBL deficiency, suggesting that renal complications are less dependent on MBL function [6]. These results show that MBL-deficient mice develop organ damage earlier in bacteremic infection and that there are different organ sensitivities to MBL insufficiency [6].

The liver is an organ of particular interest, as it plays a large role in the innate immune system, particularly macrophage function in the reticulo–endothelial system, and it is the primary site of MBL synthesis. Studies have demonstrated increased liver cirrhosis (replacement of hepatocytes with fibrotic tissues) in the setting of MBL deficiency [87,88]. This association and our own experimental findings suggest that the liver is particularly affected in MBL deficiency [36].

MBL is also detected in other organs, as mentioned above. In the skin, following UVB exposure, MBL is most likely recruited from local capillaries into damaged sub-epithelial tissue [1,36,39]. In this setting, MBL aids in clearing apoptotic keratinocytes [39]. Our animal burn model studies provide *in vivo* evidence of MBL involvement in the skin. In a model of burn injury, using a sublethal burn injury to 5% of total body surface area, eschar (dead skin) was sloughed off in MBL sufficient wild-type mice whereas it remained attached in MBL null mice [89]. Of note, mice deficient in complement proteins, such as C1q, C3 or C4, were similar to wild-type mice, suggesting that the phenotype does not require complement activation and is not complement dependent. We further investigated the effects of MBL deficiency in infection following burn, as infection in burn units is a major problem and is associated with high mortality [90–94]. *P. aeruginosa*, a common pathogen in burn units, was not pathogenic in even MBL-deficient mice without burn. However, all MBL null mice died when *P. aeruginosa* was infected following burn. Once again, the increased mortality was reduced to the level (60% survival) of wild-type mice with repletion of MBL to wild-type levels. These results suggest that MBL deficiency itself is not a problem with burn or *P. aeruginosa* infection alone, but the combination of burn and infection is deadly.

It has been thought that lung surfactant proteins, such as SP-A and SP-D, provided the primary innate immune protection in the lung. Mice genetically lacking SP-A or SP-D have increased susceptibility to infection with influenza A virus [95–98]. SP-D-deficient mice, within a few weeks after birth, develop emphysematous-like lungs that have enlarged, lipid-filled air spaces [99]. In a similar fashion, MBL null mice, upon aging for 20 months, also develop a lung condition characterized by pneumonia with acidophilic macrophages, reflecting accumulation of apoptotic cells [100]. Moreover, in primary lung infection with influenza A virus, there is a significantly higher virus titer in lungs of MBL null mice [19]. These observations provide *in vivo* evidence that MBL contributes to innate immune protection in the lung and that MBL deficiency also increases susceptibility to influenza A virus infection [19]. This infection can be reduced by pretreating MBL null mice with recombinant human MBL (rhMBL), further confirming that the increased infection susceptibility is due to lack of MBL and that treatment with exogenous rhMBL can correct MBL deficiency [19]. MBL also has effects on the inflammatory response in the lung during viral infection, as it was seen that white blood cell recruitment is delayed in MBL null mice, and neutrophils are detected in MBL null mice at a time when almost no neutrophils are seen in wild-type mice [19].

We have also learned through these investigations that macrophages obtained from MBL null mice are more prone to apoptosis in tissue culture media *in vitro*, compared with wild-type. This may reflect an MBL-related vulnerability specific to the *in vitro* environment, such as physical, mechanical or chemical stress, or possibly an overall predisposition to apoptosis. Either of these possibilities suggests that MBL-deficient cells may be more susceptible to injury *in vivo*.

To investigate the molecular mechanisms involved with the MBL-deficient phenotypes, we utilized high throughput analysis using arrays of antibodies for soluble factors. These assays were performed with bronchioloalveolar lavage fluid of infected lungs. Our investigations show that several factors are increased in bronchioloalveolar lavage fluid of virus-infected MBL null mice, including Axl (tyrosine kinase), IFN- γ , insulin-like growth factor-binding protein-6, IL-1 α , leptin, leptin receptor, P-selectin, platelet factor 4 and VCAM-1 [19]. All of these factors may be involved with tissue injury, in particular, IFN- γ , IL-1 α , leptin and leptin receptors are associated with lung injury [101–109]. IFN- γ has been linked to lung fibrosis [101,109] while IL-1 α and leptin have been causally associated with acute lung injury [105,108]. The injury prone phenotype in the lung of MBL null mice may contribute in part to the clinical observation that MBL deficiency significantly shortens life expectancy among patients with cystic fibrosis and acute respiratory distress syndrome [110,111]. The idea that MBL deficiency is associated with tissue injury may also be related to the findings of increased mortality in infective endocarditis and liver cirrhosis in hepatitis virus infection related to MBL deficiency [73,87,88]. In both of these two conditions there is the phenomenon of tissue injury in the setting of infection, and it is possible that this combination of stresses in the absence of sufficient MBL results in exacerbation of tissue complications.

Most past investigations have focused on MBL deficiency, related both to low protein levels as well as dysfunctional protein, and susceptibility to infection. More recent studies have found that high blood levels of MBL may also be pathological [112–117]. This is difficult to discern, however, since MBL blood levels may fluctuate, and in particular have been found to be elevated following surgery, and this increase, among other factors, may trigger tissue injury [118].

The pathophysiology may be explained, in part, by the finding that MBL mediates complications in ischemia–reperfusion injury. In animal models of reperfusion injury, MBL null mice have significantly less tissue damage in the heart, the gut and the kidney [119–121]. In these investigations, MBL is deposited on damaged myocardium and that injury requires activation of the alternative complement pathway, as mice lacking factor D, a component of the alternative complement pathway are protected (Figure 3) [119]. These findings contribute to the understanding of the complicated physiology of MBL, which may be beneficial and limit tissue injury in infection but may mediate injury and complication in other inflammatory states. For instance, it may be that the atherosclerotic complications in diabetes associated with high MBL levels are related to an MBL-mediated innate immune function [113].

Crosstalk among complement activation, coagulation & inflammation

It has been recognized that there is significant interaction among the regulatory and effector systems of inflammation, complement activation and coagulation [122,123]. Further, discoordination of these systems can have important pathologic consequences, such as chronic inflammation, chaotic coagulopathy and tissue injury [83,84,124]. In the setting of infection, multisystem abnormalities have been identified in MBL-deficient hosts, including DIC, organ damage and systemic inflammation (indicated by elevated blood IL-6 levels, among other findings). It is overly simplistic, but it may be considered that an absence of MBL leads to insufficient coordination between these three systems, and so MBL deficiency may be a risk factor for complications during infection. Additionally, these results suggest that blood IL-6 levels may be an early predictor of developing complications and may explain the previous findings that anti-IL-6 antibody treatment reduced coagulation activation during sepsis [125].

In infected hosts, endothelial cells may also be involved with the pathophysiology of coagulopathy and organ failure. For example, MBL binds to damaged endothelial cells (hypoxia treated) that express cytokeratin 1 (CK-1), an MBL ligand [126–130]. MBL binding to CK-1 increases expression of VCAM-1, which is induced through the NF- κ B pathway [131–133]. More recent studies have demonstrated that MASP-1 activates protease activated receptor 4 (PAR4) on endothelial cells via p38 MAPK pathway, further confirming roles that MBL binding to the damaged endothelial cells is a trigger of local inflammation via activation of MASP [134].

MBL plays a central role in inflammation, coagulation and immunity, and is a pivotal point of intersection for these systems (Figure 6). Their coordination is essentially to maintain blood fluidity and preserving homeostasis throughout the body, and dysregulation may result from both excessive as well as insufficient levels of MBL. Infection poses an especially serious risk to these systems, and complications may include systemic inflammation and sepsis, coagulopathy such as DIC, and failure of immunity leading to unchecked infection.

Hypothesis & model for MBL-mediated host protective mechanisms

Results from our investigations show that: MBL activates the lectin complement pathway and initiates coagulation through a thrombin-like activity, resulting in a productive immune response and without coagulopathy; during bacterial infection, MBL null mice develop a coagulopathy, including DIC, and suffer endorgan complications; pretreatment with exogenous MBL corrects these impairments. Based on these observations, our novel hypothesis is that MBL plays a central regulatory and effector role in hemostasis, immunity and inflammation, which ultimately serves to maintain tissue homeostasis and to protect vital organs. In consequence, MBL deficiency may be a predictive risk factor for the complications of organ failure and DIC (Figure 3).

Expert commentary & five-year view

The collectin family has expanded since MBL was discovered as a serum factor in the 1970s and then identified as a risk factor for infection in the 1980s. The collectin family now also includes CL-K1 (collectin kidney 1), CL-P1 (P for placenta) and CL-L1 (L for liver) [42,135,136]. There are also three ficolins: H-ficolin (Hakata antigen), liver (L)-ficolin and monocytes (M)-ficolin [46,137]. The ficolins are structurally similar to MBL in that they all possess a collagen-like domain, while a fibrinogen-like domain replaces the CRD of the collectins. Ficolins bind to acetylated compounds, which are typically acetylated sugars (e.g., N-acetyl-glucosamine) found on the surface of the pathogens [138]. As with MBL, there are multiple ficolin gene polymorphisms resulting in a wide range of ficolin blood levels. Generally, however, ficolin blood levels are substantially greater compared with MBL, with several fold higher L-ficolin levels and more than tenfold higher H-ficolin levels [46]. In notable contrast, M-ficolin appears to be secreted and sequestered into solid tissues and is not found at high levels in the blood. Like collectins, ficolins also form a complex with MASPs that result in activation of complement [46]. It is possible that these other several types of collectins play similar and complementary roles in innate immune defense, perhaps with differing target preferences and possibly different downstream effects in terms of regulating and activating host defense systems. Clinical studies on collectins, ficolins and MASPs will provide new data about their relative roles in different diseases, and these studies should be supplemented with laboratory investigations of the underlying biology and biochemical mechanisms involved in their function.

Development of MBL replacement therapy has started experimentally, using recombinant human MBL to supplement MBL-deficient patients, particularly in the setting of infectious

diseases. A clinical study on recombinant human MBL has passed a Phase I trial [139]. However, this study was completed prior to the report of our finding that MBL interferes with the coagulation system *in vivo* [6], an unwanted side effect for a therapeutic agent. In an attempt to reduce complications with the coagulation system, MBL derivatives have been produced. One such agent is recombinant chimeric lectin 4 (RCL4), a derivative of MBL in which the collagen-like domain of MBL is replaced with that of L-ficolin [7,140]. RCL4 is at least tenfold more efficient in activating the lectin complement pathway while it is significantly weaker in promoting thrombin-like activity [7]. Recent investigations in our laboratory demonstrate that RCL4 is more efficient than MBL in reducing infection with viruses, including influenza A, Ebola and Nipah [7,140]. Further research in this direction will improve our understanding of the interaction and coordination of the complement and coagulation systems and may yield agents that could have roles in protection from infection and possibly in correction of coagulopathy.

Diagnosis of MBL deficiency has largely relied upon analysis of genotypes and/or measurement of blood levels at the time of presentation during an acute episode. Recent studies show that in some patients, blood MBL levels drastically increase following surgery, correlating with increased systemic and local inflammation [119,141]. It is reasonable to speculate that such fluctuations of blood MBL levels, and corresponding inflammatory stimulation, may occur in the setting of infectious diseases. These derangements may be mechanistically related to the development of complications such as DIC, systemic inflammation, sepsis and organ failure in many conditions, including infectious and non-infectious diseases. Understanding the sequence of these events may help to elucidate the mechanisms underlying these complications, and this knowledge would aid in the development of better diagnostic tools and therapeutic agents.

Key issues

- The first-line of host defense is the innate immune system, including mannose-binding lectin (MBL), a serum pattern recognition molecule.
- The innate immune system is biologically primitive, and functions as an element of other homeostatic mechanisms, including blood coagulation and inflammation.
- Derangements of innate immune function, including those related to MBL, may result in complications of infection, inflammation and coagulation, which may result in multifactorial organ damage.
- There are no reliable biomarkers, including genetic markers, to predict complications related to innate immune system dysfunction.
- Discovery of biomarkers of innate immune dysfunction would be a great aid to basic science research and clinical care.
- Only a subset of patients presenting with similar clinical symptoms develop complications, suggesting involvement of genetic factors.
- While MBL deficiency related to gene polymorphisms is a common immunodeficiency and has been associated with susceptibility to infection, high MBL blood levels may be also problematic.
- Animal model studies provide *in vivo* evidence that MBL deficiency predisposes to complications, including disseminated intravascular coagulation and organ damage, in the setting of bacterial and viral infection.

- MBL status, including both excess and deficiency, may be a risk factor in developing complications related to innate immune dysfunction.
- Clinical studies are warranted to examine how soluble pattern recognition lectins, including MBL, are interacting with coagulation and inflammation and their outcome.

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Papers of special note have been highlighted as:

• of interest

•• of considerable interest

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Figure 1. Overview of the host defense system

Figure 2. The molecular structure of mannose-binding lectin

Figure 3. Simplified overview of complement pathways depicting essential components
 B: Factor B; D: Factor D; MAC: Membrane attack complex; MASP: Mannose-binding
 lectin-associated serine protease; MBL: Mannose-binding lectin.

Figure 4. Coagulation cascade

Simplified cascades are presented to show actions of MBL–MASP complex.

MASP: MBL-associated serine proteases; MBL: Mannose-binding lectin; tPA: Tissue plasminogen activator; UK: Urokinase.

Figure 5. Waveform from activated partial thromboplastin time of infected wild-type and mannose-binding lectin null mice

Numbers and arrows indicate slope_1 (%T/sec). The slope_1 normal range is -0.10 – $+0.05$. In MBL null mice, aPTT waveform is biphasic with slope_1 of -0.421 . Citrated plasma from five mice were pooled for aPTT assay. For more information see [6]. aPTT: Activated partial prothromboplastin time; MBL: Mannose-binding lectin.

Mammalian lectins are central to the host defense

Figure 6. Summary of mannose-binding lectin-mediated host defense mechanisms