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Mannose-Binding Lectin: Clinical Implications for Infection, Transplantation, and Autoimmunity

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ABSTRACT: Mannose-binding lectin (MBL) is a recognition molecule of the lectin pathway of complement and a key component of innate immunity. MBL variant alleles have been described in the coding region of the MBL gene, which are associated with low MBL serum concentration and impaired MBL structure and function. Both high and low serum levels of functional MBL have been associated with a variety of diseases and disease complications. Functioning as double-edged sword, low MBL serum levels have been shown to enhance the risk for infections. On the other hand, high MBL serum levels and high MBL activity have been associated with inflammatory diseases, transplant rejection, and diabetic nephropathy. Underscoring the Jekyll-and-Hyde character of MBL, both high and low serum MBL levels are associated with several aspects of autoimmune diseases. This review provides a general outline of the genetic and molecular characteristics of MBL and discusses MBL–disease association and its consequence in infection, transplantation, and autoimmunity. *Human Immunology 67, 247–256 (2006).* © American Society for Histocompatibility and Immunogenetics, 2006. Published by Elsevier Inc.

ABBREVIATIONS

MBL	mannose-binding lectin
CRD	carbohydrate-recognition domain
MASP	MBL-associated serine protease
SNP	single nucleotide polymorphism
HIV	human immunodeficiency virus
I/R	ischemia/reperfusion
SLE	systemic lupus erythematosus
RF	rheumatoid factor

Mannose Binding Lectin

The ability to vastly counteract a great variety of pathogenic microorganisms is of eminent importance for immunological homeostasis. As the most rudimentary part of immunity, the innate immune system is composed of molecules that can recognize a restricted array of structures in a broad range of microorganisms, the so-called pathogen-associated molecular patterns. Mannose-binding lectin, also referred to as mannan-binding lectin or mannan-binding protein, is a recognition molecule of the lectin pathway of complement. The subsequent complement activation following the binding of MBL to its ligands is an important component of innate immunity and is proposed to be particularly important during the phase following the decay of maternal antibodies in infants [1]. However, as we will argue in this review, MBL plays an important immunological role not only during early infancy but also for the duration of life.

MBL History

The first case of an association of MBL deficiency and disease dates back to 1968. A small girl suffering from severe dermatitis, diarrhea, and recurrent bacterial infections indifferent to antibiotic and steroid therapy was reported. Hematological examination revealed a defect in the phagocytosis of yeast particles from *Saccharomyces cerevisiae,* rice starch, and *Staphylococcus aureus* by polymorphonuclear leukocytes. This defect was serum dependent. Infusion of fresh plasma corrected the phagocytic deficiency. Because the same phagocytic defect was observed in several direct relatives of the patient, it was concluded that this condition had a genetic origin [2]. This genetic defect was later identified as a polymorphism in the MBL gene [3].

To fully appreciate the implication of MBL in clinical settings, biological characteristics of MBL will be discussed prior to focusing on the association of MBL with various diseases.

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Human Immunology 67, 247–256 (2006)

0198-8859/06/$–see front matter
doi:10.1016/j.humimm.2006.02.030

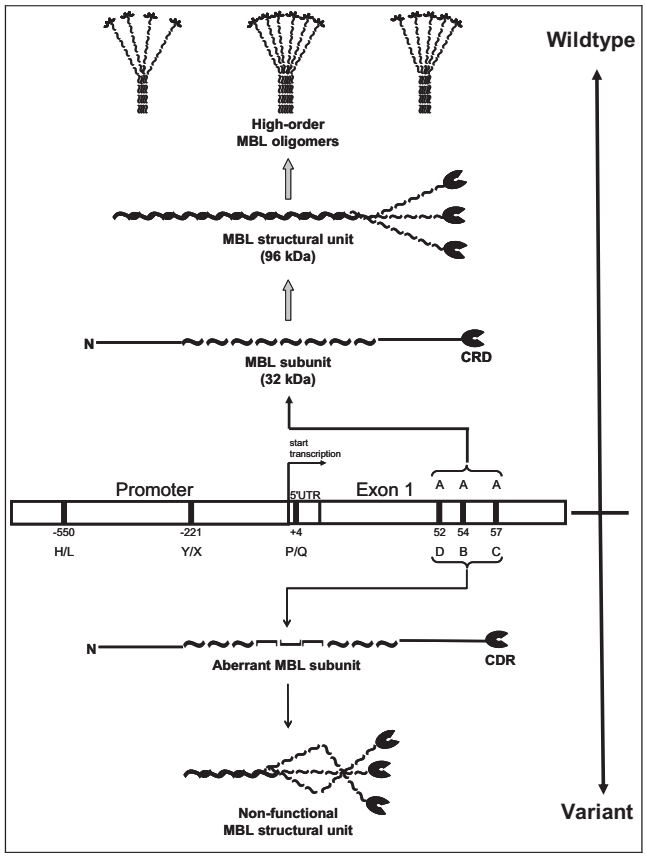

FIGURE 1 Genetic and functional buildup of MBL. Exon 1 of the *mbl-2* gene contains three known single nucleotide polymorphisms (SNPs) at codons 52, 54, and 57, referred to as alleles D, B, and C, respectively. All SNPs of exon 1 result in altered collagenous regions and, as a consequence, interfere with the formation of high-order oligomers. This impairment of polymerization causes low serum levels of high molecular weight MBL and impaired MBL function. Furthermore, H/L, Y/X, and P/Q promoter polymorphisms affect gene expression.

MBL Characteristics

Mannose-binding lectin is a C-type serum lectin and is primarily produced by the liver [4]. MBL is made up of 96-kDa structural units, which in turn are composed of three identical 32-kDa primary subunits. The subunits consist of an N-terminal cross-linking region, a collagen-like domain, and a C-terminal carbohydrate-recognition domain (CRD) [5]. Circulating MBL is composed of higher-order oligomeric structures, which include dimers, trimers, tetramers, pentamers, and hexamers of the structural homotrimeric unit. The oligomeric configuration of the structural units allows the MBL molecule to have multiple CRDs, facilitating multivalent ligand binding (Figure 1). Each CRD of MBL is structurally identical and is able to bind a range of oligosaccharides including *N*-acetylglucosamine D-mannose, *N*-acetylmannosamine, and L-fucose [1]. Although the various sugars are bound with different affinities, the cluster-like array of multiple binding sites allows activation of complement to be most effective. MBL is considered to play a major role in innate defense against pathogens, involving recognition of arrays of MBL-binding carbohydrates on microbial surfaces. However, more recent studies have shown that MBL is also involved in the recognition of self-targets, such as apoptotic and necrotic cells [6].

In plasma, MBL is associated with MBL-associated serine proteases (MASPs). Currently, three MASPs have been identified, MASP-1, MASP-2, and MASP-3 [7–9]. Although the functions of MASP-1 and MASP-3 remain subject to debate, there is a general consensus that the role of MASP-2 includes being responsible for cleavage of C4 and C2, generation of the C3 convertase C4b2a, and subsequent complement activation [10–12].

MBL Polymorphisms and Serum MBL

Exon 1 of the *mbl-2* gene, which is located on chromosome 10, contains three functional single nucleotide polymorphisms (SNPs) at codon 52 (CGT to TGT; Arg → Cys, referred to as allele "D"), codon 54 (GGC to GAC: Gly → Asp, allele "B"), and codon 57 (GGA to GAA; Gly → Glu, allele "C") (Figure 1) [13]. These SNPs of exon 1 result in altered collagenous regions and, as a consequence, interfere with the formation of high-order oligomers. This impairment of polymerization causes low serum levels of high molecular weight MBL and impaired MBL function [14]. Dependent on ethnicity, the allele frequency of variant alleles B, C, and D, commonly referred to as O alleles, may be above 40% (wildtype = A/A) [15]. In addition to the three SNPs in exon 1, there are several other polymorphic sites located in the MBL promoter region, including SNPs located at positions −550 (H/L variant) and −221 (X/Y variant), both G to C nucleotide substitutions. Furthermore, a polymorphic site is located at position +4 of the 5′-untranslated portion of the *mbl-2* gene (P/Q variant, C → T) [16–18] (Figure 1). The common allele A of exon 1 is associated with the following haplotypes: HYPA, LYPA, LYQA, and LXPA with high, high-intermediate, intermediate, and low promoter activity [17]. Although there is great variety of MBL levels in the different haplotypes, to ease interpretation it has been advocated to depict only the most significant promoter allele in position −221 (X/Y), which is found only in normal A haplotype background (YA or XA) exhibiting high and low promoter activity and serum MBL levels [18]. The structural alleles carry the following haplotypes: LYPB, LYQC, and HYPD (Table 1).

Serum level ranges of high, intermediate, and low MBL-producing genotypes have not been defined within the current literature. Major problems in developing an

TABLE 1 MBL genotypes and haplotypes

Haplotype	Common reference	Phenotype (MBL production)
HYA	A	High
LYA	A	High/intermediate
LXA	A	Low
HYD	D	Deficient
LYB	B	Deficient
LYC	C	Deficient

Genotype	Common reference	Phenotype (MBL production)
HYA/HYA HYA/LYA HYA/LXA LYA/LXA	HP	High
LXA/LXA HYA/O LYA/O	LP	Low
LXA/O O/O	DF	Deficient

A: Ranking according to MBL production of the different MBL haplotypes. Variant alleles D, B, and C are commonly referred to as O alleles.

B: Ranking according to MBL production of the different MBL genotypes.

MBL serum classification system are the various different MBL serum concentration assays that detect different molecular forms of MBL. It could be advocated that the correct way to evaluate MBL in serum is to functionally assess MBL activity by standardized assays [19, 20]. Correctly quantifying MBL by means of complement activation via the lectin pathway in future studies will enable us to develop new standard ranges for functional serum MBL [21].

MBL Epidemiology

A great variety of allele frequencies in various ethnic groups worldwide has been described. B allele frequencies have been reported as high as 0.80 in certain South American Indian groups with C allele frequencies as high as 0.32 in West Africans. In contrast, no variant alleles were found in the Aboriginal Australian population and C or D alleles were absent in Eskimos and in certain South American populations (reviewed in [21]). The high frequency of MBL variant alleles in different ethnic groups and demographic areas suggests that the obvious immunological disadvantages of low MBL serum levels somehow have a beneficial counterweight.

Currently, there are two main hypotheses suggesting positive pressure for variant MBL alleles. One hypothesis suggests that low MBL levels are beneficial in children because MBL-mediated complement activation could facilitate mitigation of harmful tissue damage by priming or promoting aggressive immune responses. The other prevailing hypothesis suggests that MBL enhances the uptake of intracellular microorganisms; thus low MBL levels would be protective.

MBL and Associated Diseases

MBL has been studied in a great diversity of diseases. Both decreased and elevated serum levels of MBL and different SNPs of the *mbl2* gene and its promoter have been associated with a variety of diseases, reflecting the Jekyll-and-Hyde character of MBL. To structure the discussion of this double-edged sword phenomenon, involvement of MBL in different diseases will be discussed according to the etiology.

MBL and Infection

When the adaptive immune response is either immature or compromised, the innate immune system constitutes the principle defense against infection. A logical consequence of impaired MBL function would be an enlarged susceptibility to infectious disease. The phenomenon of an increased incidence of infectious disease in MBL-deficient patients has been shown in pediatric patients and in immune-compromised patients. However, it also has been shown that adult patients with recurrent infectious disease are more likely to have insufficient serum MBL levels [22–24].

MBL and bacterial infections. The adaptive immune system of children is in the developmental stage and relies to a great extent on the innate immune system to counteract infectious pathogens. In support of the theory that MBL has an important protective role in early childhood is a British study of 266 pediatric patients (mean age 3.5 years) suffering from meningococcal disease [25]. Damonstrating a clinical association between MBL variant alleles and meningococcal disease, the authors suggested that genetic variants of the MBL gene might account for one third of all meningococcal disease patients. Patients undergoing myeloablative bone marrow transplantation or cytotoxic chemotherapy are severely immune compromised. MBL deficiency has been shown to be associated with severe bacterial infections after chemotherapy and major infections following allogeneic hemopoietic stem cell transplantation [26, 27].

The presence of MBL variant alleles in patients with cystic fibrosis is associated with poor prognosis and a reduction of 8 years in the estimated predicted age of survival [28]. It has been demonstrated that the shortened life span in carriers of variant alleles results primarily from the more aggressive course of lung disease caused by chronic *Pseudomonas aeruginosa* infection and an increased risk of acquiring *B. cepacia* infection which in turn is often associated with an even greater mortality than chronic *P. aeruginosa* colonization. Although MBL can bind only weakly to whole *P. aeruginosa* bacteria *in vitro,* it is suggested that the protective role of MBL is a result of clearance or neutralization of *P. aeruginosa.* Al-

ternatively, it is suggested that MBL may have a protective role against the viral infections suggested to precede *P. aeruginosa* colonization and exacerbation, which in turn may slow the progression of the disease.

In Caucasians, it has been suggested that individuals homozygous for MBL exon 1 codon variants could have an increased risk of invasive pneumococcal disease [29–31]. However, a concomitant illness is an independent risk factor for acquiring invasive pneumococcal infection. Patients and controls have not been matched according to these concomitant illnesses in these studies.

Patients with low serum MBL levels undergoing elective gastrointestinal resections for malignant disease of the gastrointestinal tract show significantly more postoperative infections [32, 33]. As postoperative infections are a major cause of morbidity and mortality, identification of patients prone to them would be of great clinical value.

In marked contrast to the protective properties of MBL against extracellular bacterial infections is the observation that mycobacterial infections (*Mycobacterium tuberculosis* and *M. leprae*) occur more frequently in patients with increased serum MBL levels. Complement-mediated enhanced phagocytosis as a result of opsonization has been suggested to facilitate these intracellular infections [34].

MBL and virus infections. MBL has been studied in relation to various viruses. Persistent hepatitis B virus infection has been reported to be associated with the variant alleles located at codons 52 and 54 of the MBL gene, responsible for low MBL serum levels [35, 36]. Furthermore it has been suggested that high MBL serum levels are associated with increased survival rates among Japanese patients with hepatitis B [37].

In contrast to hepatitis B, the association of MBL and hepatitis C appears less conclusive. Several studies have suggested that low-MBL producing genotypes are associated with a poor response to interferon treatment in Japanese chronic hepatitis patients [31, 38–40]. However, these results could not be confirmed in European patients [41].

The role of MBL in HIV infection has been studied to a great extent in recent years and has recently been extensively reviewed Several clinical studies have shown that MBL serum levels increase during HIV infection, indicating a role for MBL in the pathogenesis of HIV infection and progression [42, 43]. It has been suggested that MBL is involved in the recognition of HIV. The envelope protein gp120 of the HIV-1 virus is highly glycosylated with N-linked carbohydrates, enabling MBL to bind, opsonize, and neutralize the HIV-1 virus [44–47]. The finding that the variant MBL B allele is more frequent among HIV patients with high viral loads underscores the involvement of MBL in HIV progression This antiviral quality of MBL could enrich the current therapeutic arsenal in HIV treatment by MBL infusion. Although infections with common pediatric viruses, including respiratory syncytial virus and Epstein–Barr virus, lack association with MBL [48–50], it has been shown that MBL is able to neutralize and inhibit the spread of the influenza A virus [51, 52]. This inhibitory quality of MBL was independent from complement, suggesting that human MBL can affect innate immunity by direct viral neutralization and inhibition of viral spread and by indirect opsonization and complement activation.

MBL AND TRANSPLANTATION

Tissue damage and impaired organ function resulting from ischemia/reperfusion (I/R) injury still remain enormous predicaments in solid-organ transplantation. The hypoxic state to which an organ is subjected during organ harvesting, transport, and implantation activates various immunological events [53–57]. The complement system plays an important role in mediating tissue injury after oxidative stress. Activation and deposition of complement on the vascular endothelium following oxidative stress have been demonstrated [58–60] and, more interestingly, tissue injury after I/R is significantly reduced by complement inhibition [58, 61–63]. Complement activation via the lectin pathway following oxidative stress has been demonstrated, indicating that inhibition of MBL could be a novel approach in reducing ischemia/reperfusion damage [64, 65]. Indeed, recent experiments in MBL-knockout mice support a role for MBL in the pathogenesis of I/R injury *in vivo* [66, 67].

In support of the involvement of MBL in transplant-related I/R injury is the fact that MBL depositions were observed early after transplantation of ischemically injured kidneys [68]. Moreover, high MBL levels are associated with significantly decreased renal allograft survival, linked to therapy-resistant rejection [69]. Apart from I/R damage, MBL may also be involved in graft failure by other MBL-mediated mechanisms. Damage caused by acute rejection may be enhanced in the presence of high levels of circulating MBL by interaction of MBL with damaged tissue. MBL can bind to necrotic and late apoptotic cells, resulting in enhanced phagocytosis of these cells by macrophages and dendritic cells. Phagocytosis of necrotic cells in turn may induce dendritic cell maturation and macrophage activation. It is conceivable that high MBL levels may increase immune reactivity and cell damage via binding to damaged tissue and enhancing activation of antigen-presenting cells.

Studying MBL activity and MBL serum levels in liver transplantation, we recently demonstrated that transplantation of a liver genetically mismatched for MBL

genotype results in MBL serum conversion. This finding corroborates the notion that the liver is the pivotal site of MBL production. Furthermore, patients receiving donor liver with an MBL-variant genotype have an approximately fourfold increased risk of acquiring a life-threatening infection within the first year after transplantation [70]. As infection is the primary cause of death at all time points after liver transplantation, it is of great clinical value to identify high-risk patients.

MBL and Autoimmunity

The role of the adaptive immune system in autoimmunity is well established and interest in the role of the innate immune system in the immunopathogenesis of autoimmune diseases is mounting. Evidence that the innate immune system could lead to autoimmunity, either by priming or by promoting aggressive immune responses, is growing [71, 72]. A major current pathophysiological concept of autoimmunity is impaired apoptotic cell clearance. MBL has been demonstrated to facilitate the clearance of apoptotic cells *in vitro* [73–75] and *in vivo* [76]. A result of cells going into apoptosis is alteration of membrane carbohydrates leading to increased expression of fucose and *N*-acetyl-glucosamine [77, 78]. Redistribution or clustering of glycoproteins has been suggested to enable MBL to bind to these carbohydrates expressed on apoptotic cells, thereby facilitating clearance [6, 75]. Alternatively, it can be argued that an increased serum MBL concentration could facilitate and propagate a cellular immune response by lectin pathway complement activation, after initial tissue damage (Figure 2).

In systemic lupus erythematosus (SLE), MBL alleles were demonstrated in several studies to predispose to disease development [79]. This association has been underscored by a recent meta-analysis which incorporated all available published results of MBL genotyping in SLE and demonstrated that MBL variant alleles such as MBL exon 1 codon 54 B, promoter −550 L, and promoter −221 × are SLE risk factors. Interestingly, SLE patients with MBL deficiency manifest more frequent renal involvement, increased infection rate, and strongly increased risk for arterial thrombosis [79, 80]. Furthermore, MBL-deficient SLE patients manifest increased levels of autoantibodies against molecules associated with apoptotic cells, such as C1q and cardiolipin [81].

Studies of the association between MBL and rheumatoid arthritis have demonstrated that MBL is able to bind to rheumatoid factor (RF) complexes and as a consequence could assist RF clearance by the reticuloendothelial system [82, 83]. The observations that MBL insufficiency is associated with elevated IgM RF, increased joint erosions, inflammation, and early disease onset support the MBL RF clearance theory [84–88].

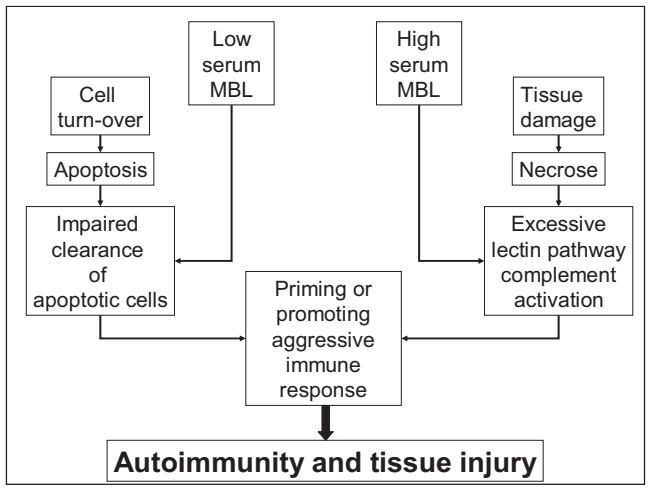

FIGURE 2 MBL acting as double-edged sword in autoimmunity. Low serum MBL levels could result in impaired clearance of apoptotic cells, facilitating an aggressive immune response leading to autoimmunity. High serum MBL levels could cause excessive complement activation via the lectin pathway following tissue damage. This in turn could prime and promote an immune response resulting in autoimmunity and tissue damage.

We recently demonstrated that both MBL serum concentration and MBL complex activity were significantly higher in new-onset diabetic patients than in healthy controls [89]. We hypothesize that MBL is involved in the pathogenesis of diabetes by assisting the autoimmune process of insulitis, pathognomonic for early stages of type 1 diabetes [89].

The above-mentioned association of MBL and autoimmunity again underscores the Jekyl-and-Hyde character of MBL, as both high and low serum MBL levels are associated with several aspects of autoimmune diseases.

A major source of mortality and morbidity in diabetes is caused by microvascular complications, as a substantial portion of diabetic patients develop diabetic nephropathy and retinopathy. MBL has been demonstrated to be associated with diabetic microvascular complications. Several studies have now characterized the association between the increased risk of developing renal complications and the presence of high-MBL producing genotypes in diabetic patients [90–92]. The involvement of MBL in the pathogenesis of diabetic nephropathy now appears to be appreciated; however, the exact immunological process involved remains to be studied. In contrast to microvascular diabetic complications, low MBL has been reported to be associated with macrovascular pathology. High MBL serum levels predict a decreased likelihood of myocardial infarction in diabetic patients, possibly indicating a role for MBL in the clearance of atherogenic agents [93]. However, it can be hypothesized that, in a patient with high serum MBL, once a myocar-

dial infarction has occurred, the sustained injury could be greater due to the I/R damage facilitated by MBL.

Concluding Remarks

Since the first report of the clinical implications of MBL deficiency almost four decades ago [2], our knowledge of the lectin pathway has expanded tremendously. At present, MBL replacement therapy is being studied in phase I, II, and III studies [94–96]. Infusing serum MBL in MBL-deficient subjects could potentially induce negative effects, for example autoimmune processes. However, no adverse clinical or laboratory changes have been reported upon repetitive MBL infusion. Furthermore, no antibodies directed against MBL were found. Unfortunately, the half-life of infused MBL is short, varying from 18 to 115 hours. Thus, to maintain sufficient MBL serum levels, MBL should be administered twice or three times weekly, rendering MBL substitution therapy costly and arduous. Several other therapeutic interventions can be put forward to compensate for MBL deficiency in immunocompromised patients, including intensified clinical follow-up and preemptive antimicrobial therapy. Intravenous and subcutaneous immunoglobulin administration might also be alternative therapies to counteract a malfunctioning lectin pathway [14, 97, 98].

Assessment of high MBL serum levels by evaluating the risk for graft loss prior to transplantation may be beneficial to patients with renal failure. Furthermore, as MBL appears to be associated with I/R damage, preoperative assessment of serum MBL levels could be advocated prior to surgical procedures as abdominal aortic aneurysm repair or iliaco-femoro-popliteal bypass. It can be hypothesized that, in these cases, blockage of MBL could be beneficial. To the best of the authors knowledge, however, this has never been studied.

Nonetheless, swift clinical implementation of the current MBL knowledge may have a vast impact on patient care, especially in patients struggling to uphold their immune response.

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