Abstract

Mannose-binding lectin (MBL) is an important constituent of the innate immune defence system. The protein binds to the sugars decorating many microbial surfaces and subsequently activates the complement system through a specific protease called MASP-2. A comparison of the importance of the capsule and lipopolysaccharide (LPS) structures of selected Gram-negative organisms for the binding of MBL suggests that the LPS structure is of primary importance. For several clinically relevant organisms MBL binding leads to activation of C4 suggesting that this is a major pathway for oposonophagocytosis. MBL deficiency mainly results from three mutations in exon 1 of the gene and is associated with both increased susceptibility to infections and autoimmune disease. Recent evidence indicates that the protein also modulates disease severity, possibly through a dose dependent influence on cytokine production.

Key words

Complement activation; Innate immune defence; Mannose-binding lectin

Introduction

MBL (mannose-binding lectin, also referred to as mannan-binding lectin and mannose-binding protein) belongs to a family of proteins called the collectins in which lectin (carbohydrate recognition) domains are found in association with collagenous structures. In man, three such proteins are recognised, namely MBL, lung surfactant protein A (SP-A) and lung surfactant protein D (SP-D). Each of these proteins is believed to be of importance in innate immune defence but MBL is of particular interest because it is able to activate the complement system.

MBL Structure

MBL has a bouquet-like structure similar to C1q. However, various oligomeric structures have been visualised (dimers, trimers, tetramers, hexamers), and full functional activity, including both binding to microbial surfaces and the activation of complement, requires higher order structures such as tetramers.

All higher order oligomers of MBL are based on subunits which comprise three identical peptide chains of 32 kDa (see Figure 1). Each chain is characterised by a lectin domain, a coiled-coil hydrophobic neck region, a collagenous region and, a cysteine-rich N-terminal region. Three such chains interact to give a classical collagenous triple helix.

MBL is a calcium-dependent (or C-type) lectin that makes co-ordination bonds with the 3- and 4-hydroxyl groups of various sugars, including N-acetyl-D-glucosamine, mannose, N-acetyl-mannosamine, fucose and glucose.

The repeating arrays or patterns of sugar groups decorating microbial surfaces make appropriate targets for MBL binding since the three sugar binding sites of one subunit array offer a flat platform with a constant distance between the sites (45 Å for human MBL). Such simultaneous multiple binding is critical because the Kd of each separate MBL-sugar interaction is relatively low (10^{-3} M). All of these features facilitate interactions with non-self microbial surfaces but make self recognition less likely.
The role of MBL in health & disease

MBL Function

The activation of complement by MBL represents a third pathway independent of both the classical and alternative pathways, but with similarities to the classical pathway. In the circulation MBL is found in association with four structurally related proteins. These are the MBL-associated serine proteases (MASP)-1, 2 and 3, and a truncated version of MASP-2 called MAp19. In serum there is a 20-fold excess of MASP-1 over MBL, but MASP-2, which is present at much lower concentrations, appears to be the more important in complement activation. The available evidence suggests that MBL-MASP-2 complexes become activated when bound to appropriate sugar arrays on microbial surfaces. The enzyme activity expressed by the MASP-2 is apparently identical to that of Cl esterase and results in the sequential cleavage of C4 and C2. The C4b fragments generated bind covalently to the microbial surface and act as a focus for C2 binding/activation. The resultant C4b2a complex has C3 convertase activity and cleaves C3 in a similar manner to the C3 convertases of both the classical and alternative pathways of complement activation (see Figure 2).

There is also evidence to suggest that MBL is able to interact directly with cell surface receptors and promote opsonophagocytosis and other immune processes (Figure 2). A number of putative MBL-binding proteins/receptors have been proposed, including cClqR/calreticulin, ClqR, and CR1. However, it is unclear whether MBL is acting as a direct opsonin for microorganisms or enhancing well-established pathways of complement and/or immunoglobulin receptor-mediated phagocytosis.

MBL Genetics and Polymorphisms

The human collectin genes are clustered on chromosome 10 in the region 10q 21-24. There is a single functional MBL gene comprising four exons (see Figure 3). Exon 1 encodes the signal peptide, a cysteine-rich region and part of the lectin domain.
of the glycine-rich collagen-like region. Exon 2 encodes the remainder of the collagenous region, whilst exon 3 encodes for the "neck" region with an α-helical coiled-coil structure. The fourth exon encodes the C-terminal lectin domain. Upstream of the MBL gene are a number of regulatory, promoter elements which are believed to enhance MBL transcription during the acute-phase response.3,4

MBL deficiency is one of the most common immunodeficiencies. Three single point gene mutations in codons 52, 54 and 57 of exon 1 of the MBL gene have been described23 and are referred to as the D, B and C variants, with A indicating wild type. The B variant mutation occurs in approximately 26% of Caucasians and 22% of the southern Chinese whereas the C variant mutation is characteristic of sub-Saharan African populations in whom it may reach frequencies of 50-60%. The frequencies of the B, C and D variant alleles in selected populations are shown in Table 1. Both the B and C mutations result in the substitution of a dicarboxylic acid for an axial glycine and it is believed that this impairs correct oligomerisation.24 In addition to the above structural gene mutations, several polymorphisms exist within the promoter region of the MBL gene. These polymorphisms, which were identified by Madsen et al.,25 are the H/L, X/Y and P/Q loci at positions -550, -221 and +4 of the MBL gene. Four promoter haplotypes (LXP, LYP, LYQ and HYP) are commonly found and of these the HYP haplotype is associated with
high MBL levels whereas the LXP haplotype is found in association with low levels of the protein. In approximately 12% of the Caucasian population the combined effects of the exon 1 mutations and the LXP promoter polymorphisms result in profoundly reduced MBL levels (< 500 ng/ml).

The three structural gene mutations are in linkage disequilibrium with the promoter polymorphisms and every individual expresses two of the following seven possible haplotypes – HYP A, LYQA, LYP A, LXP A, LYPB, LYQC and HYPD. The frequencies of these haplotypes differ markedly between different population groups (see Figure 4). Our original observations on the distribution of the B and C alleles in African and non-African populations led us to suggest that the two mutations had probably arisen independently after the migration of hominids out of Africa some 100,000-150,000 years ago. A later study of indigenous Australian populations showed that none of the three structural gene mutations was introduced into Australia at the time of first settlement (50,000 years ago) whereas the B mutation was introduced into North America at the time of the last glaciation (~20,000 years ago). This suggests that the B mutation may have arisen 20,000-50,000 years ago on the LYP background.

### MBL Binding to Microorganisms

MBL deficiency has been implicated in susceptibility to viral, bacterial, fungal and protozoal infections. We have developed a simple flow cytometric method for measuring MBL attachment to microorganisms and have used this procedure to survey a variety of microbial groups and some individual pathogens.

We have studied MBL binding to a range of clinically relevant pathogens isolated from immunocompromised children and found large differences. Some organisms such as *Candida albicans*, *β-haemolytic Group A Streptococci* and *Staphylococcus aureus* consistently...
exhibited high binding whereas others such as Clostridium sp., Pseudomonas aeruginosa Staphylococcus epidermidis, β-haemolytic Streptococcus Group B and Streptococcus pneumoniae apparently did not bind the protein. Between these extremes we observed other organisms with more variable patterns of binding e.g. Klebsiella species and Escherichia coli. Such heterogeneity has prompted us to explore in more detail the determinants of MBL binding to bacteria.

We have studied the effect of LPS structure on MBL attachment to both Salmonella enterica serovar Typhimurium and the human pathogen Neisseria gonorrhoeae and N. meningitidis (serogroups B and C). In particular, we have examined the relative importance of LPS structure and capsule in determining MBL binding to the serogroup B meningococcus.

We found that the absence of sialic acid from the LOS of Neisseria meningitidis serogroup B, serogroup C and Neisseria gonorrhoeae allowed MBL to bind to each of these organisms. MBL appeared to bind very poorly, or not at all, to organisms with sialylated LOS. In the case of Salmonella species, organisms of the rough chemotype (not expressing the O-antigen) showed MBL binding whereas organisms having the smooth chemotype and expressing the O-antigen exhibited little or no MBL binding. The results obtained lead us to conclude that LPS structure exerts a major influence on MBL attachment to bacteria.

**MBL-disease Associations**

Initially, the immunological significance of MBL was established in studies of children with MBL deficiency, but there are now numerous studies indicating a role for the lectin in later life and supporting the notion that it should be considered as an ante-antibody, a humoral factor playing a critical role in first line defence before the production of antibodies.

Increasingly there is evidence that the role of MBL in disease is a complex issue. At present it is convenient to consider the topic under the following separate headings: (a) MBL and disease susceptibility (b) MBL and disease severity and (c) inappropriate activation of the MBL-MASP pathway. These categories are summarised in Table 2.

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**Figure 4** MBL haplotype frequencies in selected populations. Four promoter haplotypes are characteristic of A variant (wild type) exon 1 sequences i.e. HYPA, LYQA, LYP A and LXPA. The three exon 1 mutations (B, C and D alleles) are each in linkage disequilibrium with a different promoter haplotype (LYPB, LYQC and HYPD). There is, to date, no published data for any Chinese population but the frequency profile may be similar to that of the Caucasian population but with lower frequencies of LYPB, LYQC and HYPD.
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MBL and Disease Susceptibility

Several studies have presented evidence that deficiency of MBL increases the generalised susceptibility of an individual to infectious disease, but a particularly striking association is that with acute respiratory tract infections during early childhood. Many MBL deficient children benefit from prophylactic use of antibiotics suggesting that many of the infections are bacterial. Other studies have identified an increased susceptibility to infection by specific pathogens in MBL-deficient individuals, including human immunodeficiency virus, Plasmodium falciparum, Cryptosporidium parvum, and N. meningitidis. However, there are some striking exceptions to these reports, and in the case of intracellular parasites (e.g. Leishmania) it seems that MBL deficiency may actually protect against disease. It is suggested that since such parasites use C3 opsonisation and C3 receptors to enter cells, any reduction in the complement-activating function of the host may help to reduce the probability of parasitisation. The most persuasive evidence to date of such a mechanism is a study of patients with visceral leishmaniasis in Brazil. The median MBL level of these patients was significantly higher than that of healthy individuals and, as expected, MBL mutations were significantly more common in the healthy controls.

In addition to the above reports of associations with infectious disease, there have been numerous investigations focusing on possible associations between MBL deficiency and susceptibility to autoimmune disease. There is strong evidence of such an association in the case of systemic lupus erythematosus (SLE). Cohorts of British, Hong Kong Chinese, American Black and Spanish SLE patients have all shown evidence of an increased frequency of mutant MBL alleles or deficiency of the protein. These observations are similar to earlier work on components of the classical complement pathway and suggest that impaired mechanisms for removal of immune complexes may be the common underlying aetiological link.

MBL and Disease Severity

In addition to the studies showing that MBL deficiency influences susceptibility to disease there are several reports suggesting that the protein can also modulate disease severity. There have been several studies of Asian patients with hepatitis which strongly suggests that MBL may have a modulatory role in this disease. One investigation of 93 Japanese patients with chronic hepatitis C found that those patients responding poorly to interferon therapy had a significantly higher frequency of homozygosity for the B variant allele. Subsequently, the same group found that both the LYPB haplotype and the low promoter LXP A haplotype were more common in the interferon resistant patients. On the basis of these observations the authors suggested that determination of the MBL haplotype of patients with hepatitis C will help to identify those most likely to benefit from interferon treatment.

MBL levels and exon 1 mutations have also been investigated in a cohort of 190 Chinese patients who were chronic carriers of hepatitis B or C. The authors found an increased frequency of the B variant allele in patients with symptomatic hepatitis B cirrhosis and in patients with spontaneous bacterial peritonitis (SBP). The authors suggested that the screening of Asian hepatitis B carriers for MBL variant alleles would identify those individuals at risk of developing symptomatic cirrhosis and spontaneous bacterial peritonitis. Moreover, it was argued that patients with symptomatic cirrhosis and the B variant allele should be offered prophylactic antibiotic therapy.

In the field of autoimmunity there is particularly persuasive evidence of a modulatory role for MBL. Recent

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<td><strong>MBL deficiency and protection against disease:</strong></td>
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studies from two centres have indicated that MBL variant alleles are associated with both severity and early onset of disease in patients with rheumatoid arthritis. The mechanism by which MBL exerts such effects is unclear but extrapolating from our recent studies on *Neisseria meningitidis* we would suggest that one possible pathway is cytokine modulation. We found that when *N. meningitidis* was incubated with increasing concentrations of MBL and added to whole blood the release of the cytokines TNF-α, IL-1β and IL-6 from monocytes was enhanced at lower MBL concentrations (<4 µg/ml) but reduced at higher concentrations (>4 µg/ml).

**Inappropriate Activation of the Lectin Pathway**

Pathology associated with unregulated or inappropriate activation of the classical/alternative pathways of complement is well documented and it is to be expected that similar reports involving the MBL-MASP pathway will appear. To date these fall into two areas, namely renal disease and reperfusion injury.

Endo and colleagues concluded that MBL-MASP activation contributed to the glomerular damage observed in a significant number of patients with IgA nephropathy. However, in another study of renal biopsies from several patients with different forms of glomerulonephritis Lhotta and co-workers concluded that the MBL deposition observed was of minor importance. Subsequent studies have described MBL deposition in the glomeruli of a patient with post-streptococcal glomerulonephritis and in ten patients with Henoch-Schonlein Purpura Nephritis. Further work is required to evaluate the role of the MBL-MASP system in these disorders.

In a recent report MBL depletion and anti-human MBL monoclonal antibodies were used to establish a role for the MBL-MASP pathway in initiating the complement activation which occurs following hypoxia-reoxygenation of human endothelial cells. In a follow up study from the same group the MBL-MASP pathway was shown to be activated in rats following myocardial ischaemia-reperfusion suggesting that it is implicated in the subsequent tissue injury. Blockade of the lectin pathway with inhibitory monoclonal antibodies protected the heart from ischaemia-reperfusion, and may represent a promising therapeutic approach for this common surgical complication.

**Conclusions**

MBL is one of several pattern recognition molecules involved in innate immune defence. Like IgG and IgM of the adaptive immune system the major effector function of MBL is complement activation. Whilst many clinically relevant organisms bind MBL, others avoid recognition by LOS sialylation e.g. *Neisseria*. In contrast, some intracellular parasites such as *Leishmania* may actively seek recognition in order to facilitate pathogenicity. Although MBL deficiency is associated with an increased susceptibility to infections involving extracellular pathogens, there is also increasing evidence of a role for MBL in modulating disease severity through pro-inflammatory cytokines. Further dissection of both these roles is required in a range of diseases.

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**References**