The role of mannose-binding lectin in health and disease

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ABSTRACT

Mannose-binding lectin (MBL) is an important element of the innate immune defence system. The protein binds to the sugars present on many microbial surfaces and subsequently activates the complement system through a family of specific proteases called the MASPs (MBL-associated serine proteases). Studies of MBL binding to selected Gram-negative organisms suggest that the lipopolysaccharide (LPS) structure is of primary importance. For a range of clinically relevant organisms MBL binding leads to activation and cleavage of C4 and C3, suggesting that this is a major pathway for opsonophagocytosis. MBL deficiency, resulting from three mutations in exon 1 and polymorphisms in the promoter region of the gene, is associated with both increased susceptibility to infections and autoimmune disease. Recent evidence indicates that the protein also modulates disease severity, possibly by influencing cytokine production.

INTRODUCTION

Mannose-binding lectin (MBL) is a pattern recognition molecule of the innate immune system.[1] It belongs to a family of proteins called the collectins,[2-4] in which lectin (carbohydrate recognition) domains are found in association with collagenous structures. In man these proteins include serum MBL, lung surfactant protein A (SP-A) and lung surfactant protein D (SP-D). Each of these proteins plays an important role in innate immune defence, but MBL is of particular interest because it is able to activate the complement system in an antibody- and C1-independent manner.

MBL STRUCTURE

MBL has a bouquet-like structure with many similarities to C1q. However, unlike C1q it can exist as dimers, trimers, tetramers or hexamers. All higher-order oligomers of MBL are based on subunits comprising three identical peptide chains of 32 kDa. Each chain is characterised by a lectin domain, an α-helical coiled-coil hydrophobic neck region, a collagenous region and a cysteine-rich N-terminal region.[5,6] Three such chains interact to give a collagenous triple helix,[7] but separate at the neck region to give three independent carbohydrate recognition domains.

MBL is a C-type (calcium-dependent) lectin that is able to interact with the 3- and 4-hydroxyl groups of various sugars, including N-acetyl-D-glucosamine, mannose, N-acetyl-mannosamine, fucose and glucose.[8] The repeating arrays or patterns of sugar groups expressed on microbial surfaces make ideal targets for MBL binding, since the three sugar-binding sites of one subunit provide a flat platform with a constant distance between the individual binding sites (45 Å for human MBL).[9] Because the Kd of each separate MBL-sugar interaction is relatively low (10^{-3} M)[10] such simultaneous multiple binding is critical in order to achieve a high avidity.

MBL FUNCTION

The activation of complement by MBL represents a pathway which is independent of both the classical and alternative pathways, but which has similarities to the classical pathway. In the circulation MBL is found in association with

four structurally related pro-enzymes. These are the MBL-associated serine proteases (MASPs) 1, 2 and 3[11-13] and a truncated version of MASP-2 called MAp 19.[14,15] In serum there is a 20-fold excess of MASP-1 over MBL[16] and some evidence that the enzyme cleaves C3 directly. MASP-2, which is present at much lower concentrations than MASP-1, appears to be the more important in complement activation.[12] The available data suggest that MBL – MASP-2 complexes become activated when bound to appropriate sugar arrays on microbial surfaces.[17] The enzyme specificity of 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 either to the MBL itself or to the nearby 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 1*).

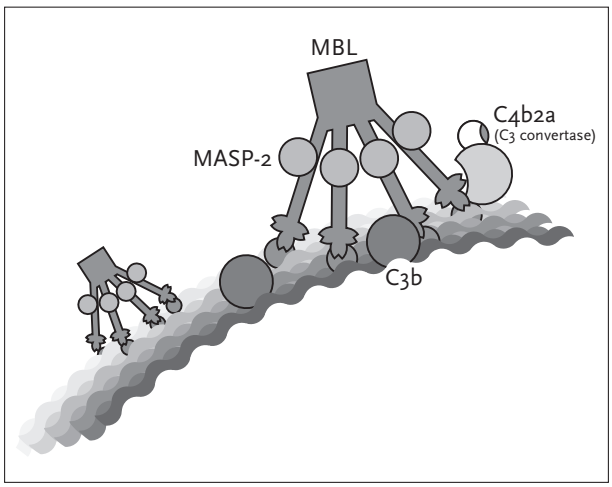

Figure 1
Mannose-binding lectin (MBL) complexed with the pro-enzyme MASP-2 is shown binding to a sugar array on the surface of a micro-organism

The MASP-2 becomes activated and is able to cleave sequentially both C4 and C2. A complex of the major fragments of these components, C4b2a, is a functionally-active C3-cleaving enzyme (or C3-convertase) and is able to generate large amounts of C3b, an important opsonin.

The C3b generated by the MBL – MASP pathway is fed into the positive feedback amplification loop of complement activation and results in the deposition of large amounts of opsonic C3b on the microbial surface.

There is some evidence to suggest that MBL is able to interact directly with cell surface receptors and promote opsonophagocytosis and other immune processes. Several putative MBL-binding proteins/receptors have been proposed, including cClqR/calreticulin,[18] $ClqR_p$[19] and CR1.[20,21]. However, it is unclear whether MBL acts as a direct opsonin for micro-organisms[22] or simply enhances other well-established pathways of complement and/or immunoglobulin receptor-mediated phagocytosis.[23]

MBL GENETICS AND POLYMORPHISMS

Within the human collecting gene cluster mapped to 10q 21-24[24] there is a single functional MBL gene comprising four exons. Exon 1 encodes the signal peptide, a cysteine-rich region and part of the glycine-rich collagen-like region. Exon 2 encodes the remainder of the collagenous region, whilst exon 3 encodes for the α-helical coiled-coil neck region. The fourth exon encodes the C-terminal carbohydrate recognition domain. Upstream of the MBL gene are a number of regulatory, promoter elements which are believed to enhance MBL transcription approximately threefold during acute-phase responses.[5,6]

MBL deficiency results predominantly from three single-point gene mutations in codons 52, 54 and 57 of exon 1 of the MBL gene.[25] These are commonly referred to as the D, B and C variants, with A indicating wild type. The B variant mutation occurs in approximately 26% of Caucasians, whereas the C variant mutation is characteristic of sub-Saharan African populations in whom it may reach frequencies of 50 to 60%. Both the B and C mutations result in the substitution of a dicarboxylic acid for an axial glycine, and this impairs correct oligomerisation.[26] In addition to the above structural gene mutations, several polymorphisms exist within the promoter region of the MBL gene. These polymorphisms are the H/L, X/Y and P/Q loci at positions -550, -221 and +4 of the MBL gene.[27] The alleles expressed at these loci are in linkage disequilibrium and four promoter haplotypes (LXP, LYP, LYQ and HYP) are commonly found. 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.[28] The HXP haplotype has never been unequivocally identified.

The three structural gene mutations are also in linkage disequilibrium with the promoter polymorphisms and every individual expresses two of the following seven possible haplotypes – HYPA, LYQA, LYPA, LXPA, LYPB, LYQC and HYPD. The frequencies of these haplotypes differ markedly between different population groups.[27] Our observations on the distribution of the B and C alleles in African and non-African populations led us to suggest that the two mutations probably arose independently after the migration of hominids out of Africa some 100,000 to 150,000 years ago.[26] It is of interest that none

of the three structural gene mutations were introduced into Australia at the time of first settlement (c. 50,000 years ago)[29] whereas the B mutation was introduced into North America at the time of the last glaciation (c. 20,000 years ago). This suggests that the B mutation may have arisen between 20,000 and 50,000 years ago on the LYP promoter background.[29]

MBL BINDING TO MICRO-ORGANISMS

We have used flow cytometry to study MBL binding to a range of clinically relevant pathogens isolated from immunocompromised children and found large differences.[30] Some organisms such as *Candida albicans*, β-haemolytic Group A *Streptococci* and *Staphylococcus aureus* have consistently exhibited high binding, whereas others such as *Clostridium* sp., *Pseudomonas aeruginosa*, *Staphylococcus epidermidis*, β-haemolytic *Streptococcus* Group B and *Streptococcus pneumoniae* appear not to bind the protein. In between are other organisms with more variable patterns of binding, such as *Klebsiella* species and *Escherichia coli*. In our present state of knowledge such heterogeneity is not readily explained, and it has prompted us to explore in more detail the determinants of MBL binding to bacteria.

To this end we have studied the effect of LPS structure on MBL attachment to both *Salmonella enterica* serovar Typhimurium[31] and to the human pathogens *Neisseria gonorrhoeae*[31] and *N. meningitidis* (serogroups B and C).[32,33] In particular, we have examined the relative importance of LPS structure and capsule in determining MBL binding to the serogroup-B meningococcus. It was observed that the absence of sialic acid from the LOS of *Neisseria meningitidis* serogroup B,[32] serogroup C[33] and *Neisseria gonorrhoeae*[31] permitted MBL to bind to each of these organisms. In contrast, 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 with the smooth chemotype and expressing the O-antigen exhibited little or no MBL binding.[31] These results suggest that LPS structure exerts a major influence on MBL attachment to bacteria.

THE ROLE OF MBL IN DISEASE

The immunological significance of MBL deficiency was initially established in children,[34] 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 immune defence before the production of antibodies.[1]

There is increasing evidence that MBL – disease associations are very complex. At present the topic may be considered under the following separate headings: (a) MBL and disease susceptibility, (b) MBL and disease severity and (c) inappropriate activation of the MBL – MASP pathway.

MBL AND DISEASE SUSCEPTIBILITY

Several studies have shown that deficiency of MBL is associated with an increased overall susceptibility to infectious disease.[35,36] In terms of community health this may go some way towards explaining why some individuals suffer more infections than others.[37,38] However, in a hospital setting it also appears that MBL deficiency has an important influence on the occurrence and duration of febrile neutropenic episodes in children with malignancy.[39] In addition to such increased generalised susceptibility, other studies have identified an increased susceptibility to infection by specific pathogens in MBL-deficient individuals, including human immunodeficiency virus,[40,41] *Plasmodium falciparum*,[42] *Cryptosporidium parvum*[43] and *N. meningitides*.[44] However, in the case of intracellular parasites (e.g. *Leishmania*) it appears that MBL deficiency may actually protect against disease. It is suggested that since such parasites exploit C3b opsonisation to facilitate uptake by the C3 receptors of macrophages and thereby gain entry to those cells, any reduction in the complement-activating function of the host may help reduce the probability of infection. The most convincing evidence to date of such a mechanism is a study of patients with visceral leishmaniasis in Brazil.[45] The median MBL level of these patients was significantly higher than that of healthy individuals and MBL mutations were significantly more common in the healthy controls.

In addition to the above reports of associations with infectious disease, there have been several investigations of 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,[46] Hong Kong Chinese,[47] American Black[48] and Spanish[49] SLE patients have all shown evidence of an increased frequency of mutant MBL alleles or deficiency of the serum protein. The interpretation of these findings has usually reiterated the hypothesis proposed in relation to components of the classical complement pathway, namely that an impairment of the mechanisms involved in the removal of immune complexes may predispose to the development of autoimmune disease.

MBL AND DISEASE SEVERITY

In addition to the mounting evidence that MBL deficiency influences susceptibility to disease there have been several reports suggesting that the protein can also modulate disease severity. In the field of autoimmunity there is evidence of such a modulatory role for MBL, and recent 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.[50-53] The mechanism by which MBL exerts such effects is unclear but our recent studies on *Neisseria meningitidis*[54] suggest that one possible pathway may be through cytokine modulation. When *N. meningitidis* organisms were incubated with increasing concentrations of MBL and added to whole blood from an MBL-deficient donor, the release of the cytokines TNF-α, IL-1b and IL-6 from monocytes was enhanced at lower MBL concentrations (<4 μg/ml) but reduced at higher concentrations (>4 μg/ml). Further work is required to establish whether this complex modulation of proinflammatory cytokine release occurs with other infectious organisms.

INAPPROPRIATE ACTIVATION OF THE LECTIN PATHWAY

Pathology associated with unregulated or inappropriate activation of the classical and alternative pathways of complement has been well documented over many years and it is to be expected that similar reports involving the MBL – MASP pathway will appear. At the time of writing there have been a small number of such studies in the fields of renal disease and reperfusion injury.

In one of the first studies in this area Endo and colleagues reported that MBL – MASP activation contributed to the glomerular damage observed in a significant number of patients with IgA nephropathy.[55] However, in another study of renal biopsies from several patients with different forms of glomerulonephritis, Lhotta and co-workers claimed that the MBL deposition observed was of minor importance.[56] Subsequent studies have described MBL deposition in the glomeruli of a patient with poststreptococcal glomerulonephritis[57] and in ten patients with Henoch-Schönlein purpura nephritis.[58] Much further work is required to evaluate the role of the MBL – MASP system in these disorders.

Recently MBL depletion and anti-human MBL monoclonal antibodies have been used to establish a role for the MBL – MASP pathway in initiating the complement activation which occurs following hypoxia-reoxygenation of human endothelial cells.[59] In a separate 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.[60] Blockade of the lectin pathway with inhibitory monoclonal antibodies protected the heart from ischaemia reperfusion, and suggests that this therapeutic approach should be explored in human patients.

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Discussion following lecture by M.W. Turner

Verbrugh: This topic is now open for discussion. I would first like to discuss the binding of mannose-binding lectin (MBL) to the surfaces of micro-organisms, one of the first observations that you talked about. There was quite some heterogeneity in binding between species of micro-organisms and within species. You did not say much about that, except that there was some focal site of binding, but is there anything else that you or anybody else in this room may add on that phenomenon? Because that seems to be important for MBL's mode of action.

Turner: Well, we have looked at both *Neisseria meningitidis* B and C and a range of isogenic mutants of salmonella. The bulk of the work that has been published has been done in those organisms. There is a consistent story that the presence of sialic acid strongly inhibits the binding of MBL. But there are problems with some organisms regarding the expression of sugars. Sometimes there will be a terminal sugar accessible that ought to bind MBL and yet we do not see any binding. So I must say that there is still an awful lot of work to be done. We cannot simplistically take the sugar structure that has been published for a particular mutant and say with any certainty, without doing the experiments, that MBL will bind or not. The fact is that sugars are not fixed in space. They are waving around and there are all sorts of stereo configurations that need to be taken into account. It is more complicated than simple linear sequences would suggest. Not a very satisfactory answer, but I am afraid that is the way it is.

Verbrugh: Because microbes are also rather dynamic structures where cell walls are broken down and synthesised, and you do show some binding at certain sites of the cell surface, but not at other sites, that may have to do with the structuring of the cell wall at the time in that organism.

Turner: I should also say, by the way, that the flow cytometry is not that sensitive, because when we came to do the immuno gold staining, we found that the sialylated parent organism of *Neisseria meningitidis* serogroup B did show some foci of binding but in much lower numbers and with the serogroup C, the 8026 mutant, which we can exogenously sialylate. We have data showing that a little bit of binding does occur. So obviously, whatever one says about the flow-cytometry results, one has to bear in mind that there is a sensitivity issue there and that one can push that a little bit further by using better techniques.

Van Strijp: One thing that would be far more sensitive is looking at it functionally. Is there complement activation, yes or no? Is there a correlation between the binding assay and complement activation in all of these organisms that you showed?

Turner: That is a good question. First of all, two things we always do are to worry a bit about the possibility that the binding we see may be going in the opposite direction and that there is some lectin on the surface of the organism recognising a sugar on the MBL, because it is a sugar-bearing molecule itself. So we always do an EDTA chelation experiment to show that the binding is a calcium-dependent one. And we always do an inhibition with an inositol glucosamine or mannose and show that that competes. So we have two controls in all these systems to help us confirm that it really is a C-type lectin interaction. Whenever we looked for C4 binding, which we can do with purified C4, we always found an absolute correlation. If it binds MBL, we can go on. We have got data to show those strains which bind MBL, bind C4, and there is a strong correlation between the levels of binding, so the answer is, yes, there is a correlation. We have yet to see an exception to that.

Van Strijp: There are people stating that the old-fashioned alternative pathway may no longer exist and that most of it is mediated by MBL. Do you think that is true?

Turner: The thing about the alternative pathway, the real important point about it, is the amplification loop, which certainly exists. I think the only point at issue is whether there is circulating this small amount of precleaved C3 which will pick up factor B. Even Peter Lackman, who was very positive about that concept in the 1980s, was very happy to think that it might well be MBL causing some of that entrée into the system. I do not have a strong view on that, but I think the fact is that both MBL-associated serine proteases (MASP) and MBL have been shown in quite primitive organisms, and even in urochordates all the MASP proteins are present. So it is an ancient system, there is no doubt about it. Whether or not there is a separate mechanism whereby organisms can become coated by alternative pathway components without MBL playing a role, I do not know.

Degener: In your experiments you have probably worked with MBLs in a solution with a bacterial inoculum, but in the human body, or in the animal experiment, where does the binding take place then? Is that at the level of mucous membranes or is there anything more needed before the binding to MBLs takes place, for instance adherence of bacteria to a membrane?

Turner: We can say that the protein is essentially an intravascular protein most of the time, but in the presence of inflammation it certainly gets extravascular. It is present in the synovial fluids of rheumatoid arthritis patients for example, it is present in jejunal secretions of AIDS patients with cryptosporidial diarrhoea. We made Western blots of such aspirates and proved MBL to be there. So it gets to some mucosal surfaces when there is inflammation, but I do not think it would normally be present at such sites. Two other members of the same family, the collectin family, are lung surfactant protein A and protein D. Many like to think of them as innate immune equivalents of IgA on mucosal surfaces. The other area where there has been some work suggesting it might get into mucosal surfaces is with cystic fibrosis, because again you would not expect it to play any role at all in that disease. But there are data to suggest that it does get into alveolar fluids.

De Vries: You said that there might be some discussion about the suggested selective advantage for the mutations. And you had two possible explanations. One was the decreased complement-mediated immunopathology and the other was the decreased invasion of intracellular parasites. Those are quite testable hypotheses. Is there any data on the subject?

Turner: Well, on the question of the parasites there was a paper in *Infection and Immunity* last year from Alan Ezekowitz collaborating with a group in Brazil. MBL binding to *Leishmania* has been directly demonstrated to the premastigotes. But population studies in Brazilians with and without *Leishmania* support the concept that having the mutation protects against leishmaniasis. There is a small population study with that disease which does support it.

De Vries: That is the outcome. But is there any evidence in that study or in any other related study that the mutation decreases the invasion by *Leishmania* parasites for instance of macrophages?

Turner: Not that I am aware of.

De Vries: And about the complement-mediated immunopathology?

Turner: There is no direct data there, but there is with C6 deficiencies some very good work from South Africa. In the Cape C6 deficiency is very common among the coloured population. These people are more susceptible to Neisserial infections, but they are far less likely to die of those infections. Many years ago Ross and Denton suggested that this is a possible explanation of the fact that they are not activating the complement system through to the end of the lytic pathway, and therefore not releasing the mediators which are so damaging, and that this might be an advantage for them in their particular environment. So it is by analogy with that C6 deficiency story that this suggestion has been put forward. Obviously, MBL should be regarded as a complement component, there is no question about that. So it is not an outrageous extrapolation to think of it in the same sort of way, but there is no more direct supportive data.

Vandenbroucke: The mutation changes the structure of the protein, you said. I was wondering when you said MBL deficiency, because you also said that neutropenic patients have lower levels. What does this mean? Does it mean a less active MBL, or does it mean lower levels of protein? In other words, if you want to study MBL deficiency, should we look at the genes, should we look at active protein or should we look at protein levels?

Turner: Good question. We believe that it is advantageous to both genotype and measure the protein levels. There is detectable protein in individuals with the mutations. But what there is not, is higher-order oligomers, which is absolutely critical for MBL function. If you cannot build it up to those higher-order structures, then you have a functional deficiency. But if you analyse what is circulating

by SDS page, for example, from someone who is homozygous for a mutation compared with my own MBL, which shows normal levels, you can see that there is a spike in the monomeric region in the person with the homozygous mutation and then a series of higher-order oligomers in my serum. So there is detectable protein there, but it is functionally lousy protein.

Appelmelk: The protein is evidently rather promiscuously binding to a variety of sugars. We as humans are full of high-mannose chains. So why are we not killed by MBL? When there is flexibility, you only need mannose. Why do we stay alive?

Turner: I think there are two possible answers to that. First of all, we tend to cap sugars with sialic acid on most cell surfaces. As a generalisation, I think you would agree that most oligosaccharide chains on human cell surfaces, protein oligosaccharides and so on, are terminating with sialic acid. Secondly, the sorts of characteristic repeating sugars that one sees on *Candida* for example, with mannan particularly, are much less commonly seen on mammalian cell surfaces. So the fact of the matter is, you have to have a repeating array of sugar groups. It is about this position and separation between them that the MBL is beautifully organised to respond to.

Kullberg: I am still intrigued by the *Leishmania* issue, because when there is a 40% incidence of polymorphism, one would think that there would be a strong selection pressure to keep these people in the population. Leishmaniasis obviously is not a disease that occurs everywhere, which would lead to this selection. So would not the *Leishmania* story act as a paradigm for other intracellular organisms? And the first one you would think of is *M. tuberculosis*, and maybe *Salmonella* may have a large impact. Are there any studies on that?

Turner: The reason why I have not discussed other organisms is because it is at this stage a rather confusing story. There are groups working on both *Mycobacterium tuberculosis* and leprosy, and initially the data seemed to be supporting the hypothesis, but I have recently examined a thesis from the University of Hong Kong. There was a large cohort of Chinese patients with tuberculosis, and instead of those individuals having the expected high frequency of wild-type MBL genotypes, in fact there was an increased frequency of mutations. It was against the hypothesis. I am again not going to put my neck on the block and say, I am totally wedded to the concept, because there are enough worrying stories coming through to make me a bit reserved on that front. Obviously, other diseases have also been considered: malaria was a candidate very early on because of its high incidence in Africa. The frequency obviously varies enormously around the world, as you saw, but what is very striking is that it is in the tropical regions of the world that we get the highest frequencies. I personally have not worked on this at all. I am just acting as a mouthpiece for some of the work of others. It is an extremely interesting question, because clearly the mutations have arisen independently of each other, the 57 and the 54 mutation, in different parts of the world and then both have risen to high frequencies in the population. It is quite a striking thing that that should have happened. It does not seem very logical that it is simply genetic drift, and that is really about as far as I can go at this stage. The malaria story is equally confused. There is one study by Bellamy in The Gambia which came to the conclusion that there was no strong association.[1] Another study by a German group came to the contrary view. But that was in a much smaller cohort.

Kullberg: The other question is about the pathophysiology of this mechanism. Do we really know that the binding of a micro-organism like *Leishmania* and the subsequent invasion is the cause, or might it be that the macrophage activation is playing a role through differences in cytokine induction, which also would explain this fact?

Turner: I agree with you there.

REFERENCE

1. Bellamy R. Identifying genetic susceptibility for tuberculosis in Africans: a combined approach using a candidate gene and a genome-wide screen. Clin Sci (Lond) 2000;98:245-50.