Anti-microbial activities of mannose-binding lectin

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Abstract
Mannose-binding lectin (MBL; also known as mannan-binding lectin) is involved in first-line defence by binding to bacteria, viruses, protozoa and helminths through a pattern-recognition mode of detection and then initiating a range of host responses. Currently, we have been unable to extrapolate from what we know of the biochemistry of MBL binding to predict accurately the interaction of MBL with individual microorganisms; even subtle surface alterations have been shown to have an extensive impact on MBL-mediated recognition of pathogens. MBL has a major protective effect through activation of the complement system via MBL-associated serine proteases (MASPs). This can cause the lysis of Gram-negative bacteria and also opsonize a wide spectrum of potential pathogens for phagocytosis. MBL may also influence phagocytosis in the absence of complement activation through an interaction with one or more collectin receptors. This may also be the basis for a direct effect of the protein on inflammatory responses. MBL can alter the function of microbial structures, such as gp120 of HIV, to prevent infection. The protein may also interact with the components of other cascade systems such as the clotting system, which will have a role in microbial pathogenesis. An understanding of these basic mechanisms will be vital if we are to use purified or recombinant MBL in therapeutic applications.

Introduction
Mannose-binding lectin (MBL; also known as mannan-binding lectin) is a pattern-recognition molecule for which the spacing and orientation of the carbohydrate-recognition domains define what ligands the protein can target, but it is not well understood how this biochemical detail translates to the ‘real world’ of microbial interactions. The majority of our knowledge is through empirical evidence, with a number of publications identifying organisms that bind MBL and a few studies that have explored how changes in the microbial surface alter MBL binding. We cannot currently predict the microbial targets of MBL binding, other than testing organisms with very high mannose (or N-acetyl-D-glucosamine or similar) glycans on their surface.

Once it has bound, MBL is able to deploy a variety of anti-microbial activities. The protein interacts with enzymes which activate the complement system to lyse certain organisms directly or to increase phagocytosis. In addition, it can communicate with cells directly to effect changes in inflammatory response, it can modify the function of microbial structures and there is evidence that it may be able to interact with other cascade systems that are important in microbial pathogenesis.

Key words: anti-microbial activity, complement, inflammation, mannose-binding lectin (MBL), microbial pathogenesis, phagocytosis.

Abbreviations used: MBL, mannose-binding lectin; MASP, MBL-associated serine protease; LOS, lipo-oligosaccharides; TNF, tumour necrosis factor; IL, interleukin.

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Target recognition
Surveys of bacteria and individual studies have shown that MBL binds to a wide range of intact microbes (Figure 1). MBL also seems to recognize purified components of microorganisms, although we have noted previously that the relationship between binding to these isolated components does not always correlate with binding to the whole organism [1]. It is possible that these components are released as MBL decoys. The difficulty in predicting MBL binding is highlighted by organisms that contain mannose but to which MBL does not bind or where binding changes with growth phase without a change in sugar composition [2].

Further indications of which organisms may interact with MBL comes from epidemiological investigations and the study of experimental infections of MBL-knockout animals [3]. If one compares the organisms listed in Figure 1 with epidemiological reports, it is apparent that while there is some overlap, there are situations where binding to an organism has been identified with no epidemiological data and also where epidemiology shows susceptibility to a particular microbe when binding has not been described.

The recent report that MBL can bind to peptide targets expands the potential number of microbial targets to be discovered enormously [4]. Other collectins have been shown to bind to proteins [5] and our recent evidence would suggest that MBL recognizes outer-membrane proteins of Neisseria that are not thought to be glycosylated [6].

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Figure 1 | MBL binding to micro-organisms

This section includes organisms for which binding has been compared with a number of other organisms or with a standard. The group consists mostly of organisms included in the surveys in [2,16,23]. The methods used in these surveys were not identical and therefore the groupings of low, medium and high binding are arbitrary divisions based on the flow-cytometric procedure of Neth et al. [23] and extrapolated from similar organisms in each report. Organisms are subdivided further into those where only single strains have been tested or all strains cluster closely in terms of MBL binding, those where large binding differences have been noted between strains, and those where defined changes in the surface structure have been shown to affect lectin attachment. This section includes single reports of organisms exhibiting MBL binding, but where there is no quantitative relationship with other organisms. This section includes organisms where binding to the intact organism could be inferred from a measurable influence of MBL on immunological responses to intact organisms, but where binding has not been shown definitively.

Complement activation

MBL associates with at least four related proteins of the MBL-associated serine protease (MASP) system: MASP-1, -2, -3 and a truncated form of MASP-2, MAp19 [7]. It appears that it is MASP-2 which is responsible for cleaving C4 and C2 to activate the lectin pathway of complement [8], whereas the functions of the other MASPs are not entirely clear but may include the regulation of complement activation and interactions with cascade systems other than the complement system.

Many early reports identified MBL as a complement activator, based on the ability of the protein to activate complement to either kill Gram-negative organisms directly via the membrane-attack complex or to enhance complement-mediated phagocytosis through the increased deposition of opsonic C3 fragments [1]. Recently, much research has concentrated on the pathogenic Neisseria (in humans, N. meningitidis and N. gonorrhoeae), because these organisms are unusual in that the ability to activate complement all the way to the C9 stage is necessary for full protection against disease. The ability to deposit C3 fragments on a microbial surface is usually sufficient to protect against a pathogen, since this will stimulate uptake by phagocytes [9]. This is not the case with the pathogenic Neisseria, which are poorly...
phagocytosed by neutrophils, even when opsonized with C3 [10].

*Neisseria* are Gram-negative bacteria which contain smaller versions of the lipopolysaccharide molecules found in the outer membranes of enterobacteria such as *Escherichia coli*. These smaller lipo-oligosaccharides (LOS) are often terminated in sialic (neuraminic) acid instead of repeating O-antigens. All of the *Neisseria* that have been investigated so far are able to decrease MBL binding by the addition of sialic acid to their LOS [11–14]. It is of interest that sialylation of glycan structures is also used by HIV to reduce MBL binding [15]. *N. meningitidis* can decrease MBL binding through encapsulation [16], although we have found this to be less important than LOS sialylation [11]. In contrast, *N. gonorrhoeae* do not encapsulate.

MBL bound to the surface of *Neisseria* is able to activate complement, which leads to an increase in bacterial killing. We have shown that this is due mostly to an increase in the rate of complement activation rather than an increase in the amount of complement bound. However, this work was done by pre-incubating bacteria with the lectin and then adding a source of complement [11,12,14]. Similar experiments have shown cidal activity for *E. coli* [17] and *Salmonella* [18]. More recently, Gulati et al. [14] have shown that if MBL is added to MBL-deficient serum and then added to bacteria, there is no increase in bacterial killing due to the inactivation of MASP activity by the serum complement regulators, C1-inhibitor and α2-macroglobulin. Interestingly, others have found that α2-macroglobulin does not inhibit the C4 cleaving activity of MASP-2 [7]. This could be evidence that MBL is not effective against *N. gonorrhoeae*, although the similarity to meningococci and the epidemiology for meningococcal disease would argue against this [19,20]. The answer may lie in the way that complement is activated by MBL. Most serum- or plasma-derived MBL is purified by affinity chromatography on mannan or mannosse. During this process the MASPs will become activated, and it appears to be difficult to prevent this happening during purification [8]. Hence the MBL/MA SP used in all these experiments will contain mostly activated MASP. In the fluid phase this might combine with the fluid-phase regulators quite readily, but membrane-bound MBL/activated-MASP appears to be quite resistant to inactivation by the fluid-phase regulators [14].

For most bacteria the activation of complement to the C3 stage is sufficient for protection through increased phagocytosis. We recently described an increase in C4 and C3 deposition on *Staphylococcus aureus* in the presence of MBL [21]. This study used MBL/activated MASP added to MBL-deficient serum and then added to the bacteria. These results would suggest that the effects of MBL on *S. aureus* and *N. gonorrhoeae* are somewhat different. However, the relative amount of MBL bound by *N. gonorrhoeae* appears to be less than that bound by most *S. aureus* strains, with presumably more activated MBL/MA SP left in the fluid phase. This issue would not be trivial when the use of therapeutic MBL is being considered. If plasma-derived MBL/activated-MASP is used, there is the possibility that its complement-activating potential may be reduced by the soluble complement regulators.

Surprisingly, another report found no effect of MBL on complement activation upon *S. aureus* [22]. The difference between these reports could be due to a difference in methodology; Cunnion et al. [22] used hypo-γ-globulinaemic serum which had been affinity-depleted of MBL, whereas Neth et al. [21] used serum from adult individuals who were genetically deficient in MBL, but which would contain immunoglobulin. Bacterial strain differences could account for the differences, although MBL has been noted to bind to all *S. aureus* strains tested [23].

### Phagocytosis

Phagocytosis may be mediated by MBL in two ways: complement activation and opsonic C3 deposition, or through an intrinsic effect of MBL itself. It has been noted before that MBL deficiency led to a defect of complement-mediated opsonization of *Saccharomyces cerevisiae* [24]. The increase in complement activation on *S. aureus* led to an increase in neutrophil uptake of organisms [21]. The description of a similar effect of MBL on phagocytosis through complement activation with other, true pathogens is lacking; most reports have concentrated on the intrinsic effect of MBL. Much of this work has been performed with micro-organisms, which makes it difficult to directly compare reports from different laboratories, because pathogens have developed strategies for dealing with human phagocytes, ranging from actively promoting their own uptake to actively avoiding ingestion.

The original report by Kuhlman et al. [25] appeared to show that MBL alone attached to *Salmonella enterica* serovar Montevideo was sufficient to increase uptake of the organism by neutrophils and monocytes and enhance the function of the bacterial killing mechanisms. More recently, the same organism was shown not to be taken up by neutrophils unless they were first stimulated with fibronectin [26]. MBL was only able to increase phagocytosis of *Salmonella* by unstimulated neutrophils when the bacteria were coated with sub-optimal amounts of IgG. MBL was found to increase the uptake by macrophages of *Cryptococcus neoformans* opsonized with serum or immunoglobulin, using a slide assay of phagocytes placed on top of immobilized MBL and then offered the phagocytic target [27]. A similar slide assay was used to show the increase of phagocytosis of C4b- or Ig-coated erythrocytes by MBL [28].

Additional opsonic factors do not always seem to be necessary for MBL to increase phagocytosis. MBL alone enhances influenza A uptake by neutrophils [29] and mycobacterial uptake by macrophages [30], although the latter could be part of the infection strategy of this group of organisms. We have shown a direct effect of MBL on the uptake of *N. meningitidis* by neutrophils, monocytes and macrophages [31], although such uptake by neutrophils is unlikely to be significant in terms of organism killing [32]. In our further investigation of neisserial phagocytosis we
have found that MBL improves the uptake of these bacteria by enhancing the ability of macrophages to internalize bacteria bound to the macrophage membrane. MBL does not appear to be involved in the initial binding of organisms to the phagocyte membrane [33]. This may explain some of the discrepancies apparent in the literature regarding an effect of MBL alone and MBL acting in concert with other opsonins, since if a phagocyte-organism-binding mechanism is absent, then the presence of opsonins which do improve binding would be required for an MBL-mediated effect on internalization.

Inflammation

There have been a number of reports that have described modifications of the inflammatory response by MBL. The first, by Soell et al. [34], showed inhibition of tumour necrosis factor (TNF) output by monocytes in response to streptococcal rhamnose polymers, a molecule related to the lipopolysaccharide of Gram-negative bacteria. Another report showed that MBL decreased TNF release in response to a cryptococcal membrane glycoprotein [35], and yet another showed that MBL enhanced TNF release after monocyte exposure to an entire organism, Candida albicans [36]. Interestingly, MBL has been shown to reduce phagocytosis of Candida [37]. Similarly, increasing concentrations of MBL caused increased release of TNF and interleukin 6 (IL6) from monocytes responding to promastigotes of Leishmania chagasi [38].

One aspect of these studies is that they have all used MBL concentrations of 5 µg/ml or above, which is towards the very top of the normal physiological range of MBL concentration. In the case of Leishmania this seems justified, since the authors found an increased risk of infection in individuals with higher MBL concentrations. We have investigated the capacity of MBL to regulate cytokine responses to N. meningitidis in the range of 0–8 µg/ml. When increasing concentrations of MBL were added to whole blood, there was an initial increase in cytokine production by monocytes which was most marked for IL1β and IL6, but at higher MBL concentrations (>4 µg/ml) TNFa, IL1β and IL6 were suppressed [31]. Changes in cytokine production were associated with a change in the adhesion-molecule profile of neutrophils [31]. The importance ascribed to changes in inflammation is controversial. In meningococcal sepsis, mortality correlates with high levels of the pro-inflammatory cytokine, TNF [39], probably as a consequence of increased LOS levels in these patients [40]. High TNF levels could be simplistically linked with high mortality, but when relatives of patients who died of meningococcal disease were tested for the ability to produce cytokines when whole blood was challenged with meningococcus, these relatives, and therefore probably the dead patients, produced low amounts of TNF compared with IL10 [41]. An initial brisk TNF response may actually be beneficial, for instance by limiting initial bacterial proliferation. It is only when the organisms are releasing LOS leads to high levels of pro-inflammatory cytokines. The presence of meningococci also lead to changes in adhesion molecule expression associated with impaired vascular endothelial function.

These studies would suggest that MBL is able to modulate the inflammatory response, but it is likely that the nature of the response will be different for different organisms or groups of organisms. Caution will be required when MBL therapy is considered for certain patient groups, since the history of agents designed to influence the inflammatory system has been disappointing [42].

MBL binding modifies the function of the target

MBL may not necessarily have to engage its effector functions to achieve host protection from disease. There are a number of epidemiological reports showing that MBL deficiency predisposes to HIV infection (e.g. [43]). This is supported by evidence that MBL binds to and inhibits HIV-1 infection in vitro [44]. The mechanism appears to be through binding to mannose residues on gp120. This glycoprotein mediates the binding of the virus to CD4 on human T-cells, and the presence of MBL is able to inhibit this binding. MBL activates complement on this protein, but this is in addition to its blocking role [45]. This process may be enhanced by blocking of the ability of HIV to infect dendritic cells, which can infect T-cells in trans, via dendritic cell-specific intercellular adhesion molecule-3 grabbing nonintegrin (‘DC-SIGN’) [46].

Other functions

It is clear that our understanding and knowledge of the MBL/MASP system are incomplete. It has recently been suggested that MASP-1 may have thrombin-like activity, cleaving fibrinogen and activating plasma transglutaminase (Factor XIII) [47]. Blood coagulation can have major impacts on the pathogenesis of disease, ranging from preventing the spread of organisms to the pathology of disseminated intravascular coagulation seen in sepsis syndrome [40]. This link to another major cascade system suggests that there may be further functions of MBL/MASP yet to be discovered.

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