Introduction to mannan-binding lectin

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Abstract
Mannan-binding lectin (MBL) was first discovered as a plasma opsonin for baker’s yeast and was independently characterized biochemically. It belongs to the small subfamily of collectins: C-type lectins possessing a collagen-like domain. MBL is synthesized by the liver and secreted into the bloodstream. It is believed to be an important component of innate immunity, acting as an anti-antibody and/or a disease modifier. It is thought to influence disorders as diverse as meningococcal disease, rheumatoid arthritis, cystic fibrosis and recurrent miscarriage. Lack of MBL may be most relevant in the context of a co-existing secondary immune deficiency. Replacement therapy, first carried out 30 years ago with unfractionated plasma, appears promising. The development of a recombinant product should permit the extension of MBL therapy to randomized clinical trials of sufficient size to provide clear evidence about the physiological significance of this intriguing glycoprotein.

Discovery
What we now call mannan-binding lectin (MBL; or mannose-binding lectin) was first discovered as an opsonic activity detected in vitro by the enhancement of phagocytosis of baker’s yeast. The absence of the then unknown factor was associated with diarrhoeal disease and other infections in childhood, but this opsonic defect was also found in 5–10% of the apparently healthy population. Plasma infusions were found to correct the defect, and appeared to bring about clinical improvement in some patients. MBL-replacement therapy was therefore first achieved about 30 years ago “by good clinical research using simple function tests” [1].

The plasma opsonin was found to be identical with MBL in 1989 [2]. The latter had first been detected biochemically as an impurity forming a band on SDS/PAGE containing a preparation of α-mannosidase from human liver [3]. The corresponding protein from rabbit liver was subsequently isolated and characterized by Kawasaki et al. in 1978 [4]; the partial characterization of human MBL soon followed [5,6].

Biochemistry and genetics
MBL is perhaps the best-studied collectin, the name given to a small subfamily of C-type lectins with a collagen-like domain. Along with the ficolins – in which a sugar-binding fibrinogen-type domain is combined with a collagen-like region – the collectins are part of an armoury of soluble pattern-recognition molecules that promote phagocytosis of micro-organisms, sometimes by using the lectin pathway of complement activation [7].

The product of the human MBL-2 gene is a 24 kDa polypeptide [8] to which some carbohydrate is attached by O-glycosylation. Three gene products combine to form the basic subunit consisting of a collagen-like triple helix forming a long tail with a cluster of globular lectin-like carbohydrate-recognition domains forming a head. This triplet has the capacity to combine with others to form a series of oligomers. Oligomerization increases the avidity of MBL–carbohydrate interaction and the higher the oligomeric form the greater its ability to activate complement. The monomeric triplet cannot interact effectively with MBL-associated serine proteases (‘MASPs’) and cannot activate complement [9].

The finding that low plasma MBL concentrations were associated with a point mutation in the first exon of the structural gene (codon 54) was a major breakthrough in our understanding of the genetics of MBL deficiency [10]. Although that mutation (the B variant) is the commonest cause of low MBL in European populations, it is now known that other similar mutations exist, and that there are also allelic dimorphisms in the MBL promoter region that can influence circulating protein concentration. It is a striking
consideration, however, that variant alleles conferring low MBL have been found in all ethnic groups investigated, with the possible exception of Australian Aboriginals [11]. This clearly implies some selective advantage for the heterozygous state, and suggests that high MBL concentrations may be harmful in some contexts. If so, MBL-replacement therapy may do harm under some circumstances.

Clinical significance of MBL

MBL has been shown to bind to a wide variety of microorganisms, including clinical isolates, and has been implicated in a large miscellany of disorders [11,12]. Some of these disease associations have not subsequently been confirmed by independent studies, but the long-standing belief that low MBL is a risk factor for infections in infancy (based on anecdotal evidence and small series) has been vindicated in recent years. Two large and convincing investigations, a retrospective study from London [13] and a prospective study from Greenland [14], both reached a remarkably similar conclusion: possession of variant alleles conferring low MBL concentrations is associated with a doubling of the risk of acquiring infections in early childhood.

Nevertheless, it can be contended that this association is of little clinical importance. After all, it is argued, most children with infections have normal MBL levels, and most subjects with low MBL (children or adults) seem to be healthy.

An important discovery or realization in recent years, however, has been that MBL insufficiency may be far more relevant in the context of disease than in the context of health. Many disorders cause a general impairment of the immune system (secondary immune deficiency) and the vulnerability of such patients may be amplified by a relative lack of MBL. Systemic lupus erythematosus (SLE) is a mysterious inflammatory disorder of unknown cause, but immune dysfunction is clearly one of its central features. Individuals who are homozygous (or compound heterozygous) for variant MBL alleles are slightly more at risk of developing SLE than others. In one study, however, such individuals appeared dramatically (more than 60-fold) more likely to experience pneumonia than the other SLE patients [15]. (The strength of this relationship was very much weaker in a later study of other Danish SLE patients by the same group [16].) Other examples of markedly increased susceptibility to infectious agents when low MBL is found in the context of immune compromise secondary to a particular disease are listed in Table 1. They include patients who had haematological malignancies treated with chemotherapy and/or bone-marrow or peripheral-blood stem-cell transplants. As such patients are not rare, and the treatment has a considerable mortality rate, the possibility arises that MBL-replacement therapy could be life-saving and cost-effective. (Again, one must add the caveat that another study found no association between MBL gene polymorphism and susceptibility to infection after bone-marrow transplantation [17].)

A tentative conclusion at present is that MBL is a disease modifier which may be of considerable importance in the context of secondary immune deficiency.

MBL-replacement therapy

The impressive list of disease associations with low MBL concentration [11,12] does not prove causation, although it is highly suggestive. In particular, all could be explained by the existence of disease susceptibility/modifier genes in linkage disequilibrium with MBL-2 on chromosome 10, in which case the concentration of plasma MBL protein would be irrelevant. The only way to obtain definite information on the function of MBL is to carry out clinical trials involving MBL-replacement therapy.

In principle, MBL-replacement therapy could be achieved in at least three ways: infusion of natural plasma MBL (as whole plasma, as Cohn fraction III or as an affinity-purified single protein); administration of recombinant MBL; and by organ or tissue transplantation. The third option could be achieved by liver transplantation (guaranteed to succeed, but rather drastic!) or by infusion of leucocytes in the form

<table>
<thead>
<tr>
<th>Feature</th>
<th>Context</th>
<th>MBL status</th>
<th>OR</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td>SLE</td>
<td>VA (homozygotes)</td>
<td>67.5</td>
<td>[15]</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>AIDS</td>
<td>VA</td>
<td>3.9</td>
<td>[28]</td>
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<tr>
<td><em>Burkholderia cepacia</em> infection</td>
<td>CF</td>
<td>VA</td>
<td>14.7</td>
<td>[29]</td>
</tr>
<tr>
<td>Cryptosporidiosis</td>
<td>AIDS</td>
<td>VA (homozygotes)</td>
<td>8.2</td>
<td>[30]</td>
</tr>
<tr>
<td>Lupus-like manifestations</td>
<td>CGD</td>
<td>VA</td>
<td>11.2</td>
<td>[31]</td>
</tr>
<tr>
<td>Early joint erosions</td>
<td>RA</td>
<td>&lt;0.15 µg/ml</td>
<td>7.4</td>
<td>[32]</td>
</tr>
<tr>
<td>Serious infections</td>
<td>Chemotherapy</td>
<td>≤0.5 µg/ml</td>
<td>25.7</td>
<td>[33]</td>
</tr>
<tr>
<td>Prolonged febrile neutropenia</td>
<td>Chemotherapy</td>
<td>≤0.5 µg/ml</td>
<td>8.3</td>
<td>[34]</td>
</tr>
<tr>
<td>Major post-transplantation infections</td>
<td>Allogeneic stem-cell transplantation</td>
<td>VA</td>
<td>2.6</td>
<td>[35]</td>
</tr>
</tbody>
</table>
of bone-marrow or peripheral-blood stem-cell preparations (not guaranteed to be successful, but monocytes and other leucocytes can synthesize MBL).

It has already been mentioned in discussing the theoretical advantage of balanced genetic polymorphisms that there ought to be some selective advantage in the heterozygous state (with its associated diminished MBL protein level). The notion that high MBL concentrations may be harmful in some circumstances is supported by studies indicating that MBL promotes the uptake of mycobacteria [18,19] and Leishmania [20]. High plasma MBL also appears to be a risk factor for primary biliary cirrhosis [21]; the reason for this is not known, but it could be related to complement-mediated inflammatory injury. This notion is supported by observations on MBL-gene-deleted mice: without MBL-A, animals were resistant to a normally lethal dose of endotoxin [22]. (In contrast, MBL-A-null mice did not have altered resistance to disseminated candidiasis or hepatic invasion by Plasmodium yoelii [23]).

Two contrasting in vitro studies indicate the complexity and microbial species-specificity of MBL–pathogen interactions. Soell et al. [24] studied the binding of streptococcal cell-wall polysaccharides to human monocytes and found that this interaction was enhanced by MBL; however, the streptococcal polysaccharide-induced release of tumour necrosis factor-α was inhibited by MBL. On the other hand, Chaka et al. [25] reported that MBL enhanced both the binding of mannanproteins from Cryptococcus neoformans to human mononuclear cells and tumour necrosis factor-α production.

It is possible that there might be an optimal concentration of MBL, and this level could vary with individuals or with the clinical context. There may also be clinical circumstances in which the monomeric triplet of MBL (non-complement fixing) may be effective; after all, monomeric MBL can neutralize influenza virus in vitro [26] and recombinant monomeric MBL was an effective anti-cancer agent in vivo in an animal model [27]. It may be, therefore, that benefit/risk ratio depends on the optimal mixture of oligomers which has to be established for each clinical indication.

**Conclusion**

MBL replacement would undoubtedly provide invaluable information about the physiological significance of MBL. Whether MBL infusion would be useful, prophylactically or therapeutically, is much less certain. Perhaps the first critical decision should be to choose the target disorder(s) in which MBL replacement can be evaluated carefully.

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**References**


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