REVIEW ARTICLE

Mannose-Binding Lectin: Molecular Aspect and Clinical Significance

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Introduction:

Mannose-binding lectin (MBL) is a member of the collection family of proteins which are characterised by the presence of both a collagenous region and a lectin domain. MBL was discovered and first isolated from rabbit liver cytosol in 1978 and subsequently identified in human, rabbit, bovine, rat and mouse sera[1,2]. As this lectin-like protein was originally found to be specific for binding mannose and the official gene symbol is Mbl, it is preferably named as MBL[3]. However, MBL can weakly bind a wide spectrum of oligosaccharides such as N-acetylglucosamine > N-acetylmannosamine and fucose > maltose > glucose >> galactose and N-acetylgalactosamine. As most of its sugar targets are not normally exposed on mammalian cell surfaces at high densities, it does not usually recognise self structures but is particularly well suited to interactions with microbial surfaces. Since MBL can bind to so many different sugars it is effectively a universal antibody[3]. It is now accepted that its complex with MBL-associated serine protease (MASP), "MBL-MASP", provides an antibody and Cl-independent pathway for the activation of the classical pathway of complement known as the classical pathway-II or the lectin pathway. This functional unit plays an important role in opsonophagocytic processes contributing to efficient antimicrobial immunity and protection[3]. A wide range of clinical associations with MBL deficiency are now reported[3,4,5]. Recently reported molecular, functional and clinical aspects of MBL are therefore reviewed in the present article.

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Molecular and genetic aspects:

Circulating MBL comprises higher-order oligometric structures based on the three chain subunits. Although no data are available to confirm the absolute structure, MBL is often referred to represented as a hexameric structure but dimers, trimers, tetramers, pentamers and hexamers of human protein have all been visualised by electron microscope[6]. A combination of gel filtration and socium dodecyl sulphate – polyacrylamide gel electrophoresis (SDS-PAGE) analysis of human and rabbit MBL revealed mainly trimers, tetramers and pentamers. Figure-1 shows the complement activating tetrameric human MBL based on four identical subunits and each subunit comprises three identical peptide chains of 32 KDa approximately[3,7]. In man, MBL is encoded by a gene located on the long arm of chromosome 10 and the intron-exon organisation of the human MBL gene has recently been described[8,9].

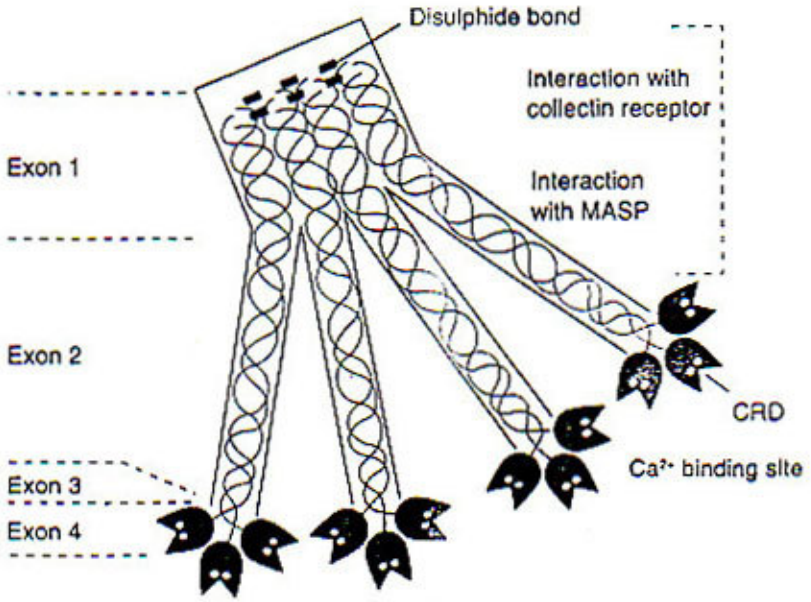

Figure-1: Complement activating tetrameric human mannose-binding lectin (MBL) based on four identical subunits. Each subunit comprises three identical peptide chains of approximately 32 kDa associated to give a disulphide linked N-terminal region, a typical collagenous triple helix based on Gly-Xaa-Yaa repeats over most of their N-terminal length, a 'neck' region and a C-terminal cluster of three lectin domains. Cysteine residues at the N-termini may provide inter-subunit stability and a Gly-Gln-Gly interruption in each chain may account for the divergence of the subunits. The chains separate in the C-terminal region to give three carbohydrate recognition domains. The collagenous regions are known to be involved in interactions with MASP, assembling MBL-MASP complexes with the ability to activate the classical pathway II of complement (see Fig. 2). In addition there is presumed to be a structural motif able to bind to collectin receptors. The four exons of the human MBL gene encode for the protein regions indicated. Abbreviations: CRD, carbohydrate recognition domain; MASP, MBL-associated serine protease[3].

Functional aspects:

MBL appears to be one of the most versatile components of the innate immune system, being functionally analogous to immunoglobulin M (IgM), immunoglobulin G (IgG) and complement Iq (CIq) (Table-I).

Table-I: Functional analogues of mannose-binding lectin

- **IgM-like functions**
  Binds to a wide range of substrates through multiple CRD binding sites.
  Each CRD binds weakly (K_d approx. 10^{-3} M)
  Achieves high functional affinity (avidity) through multi-CRD binding
- **IgG/IgA-like functions**
  Acts directly as an opsonin by coating sugar-rich microbial surfaces
  Interacts with one or more collectin receptors
- **C1q-like functions**
  Interacts with pro-serine proteases (MASP 1 and 2)
  Initiates activation of the complement system (classical pathway)
  MBL-MASP complexes associate with serine protease inhibitors, e.g. α2 macroglobulin, C1 esterase inhibitor

Yeast such as *Candida albicans* and *Cryptococcus neoformans* as well as some strains of viruses such as HIV-1, HIV-2, influenza A have been shown to bind the lectin[10,11]. More recent studies with native as well as recombinant MBL have shown that the non-encasulated bacteria such as *Listeria monocytogenes, Hemophilus influenzae, Neisseria meningitis, Neisseria cinera* and *Neisseria subflava* bind significant amount of lectin. *Streptococci, Escherichia coli* and *Neisseria meningitis* serogroup A are characterised by an intermediate MBL binding capacity whilst encapsulated *N meningitis, H influenzae*, etc exibit a low binding capacity. These results suggest that MBL binding is significantly impaired by the presence of the capsule on bacterial cells[3,12]. Studies with the parasitic protozoan Leishmania suggest that MBL has the potential to opsonize the major developmental stages of Leishmania parasites for phagocytosis and provides a possible mechanism for activation of complement on their surface leading to lysis of microbial cells[13]. Thus, MBL provides an antibody and Cl-independent pathway of complement activation, the functional unit being MBL-MASP (Figure-2).

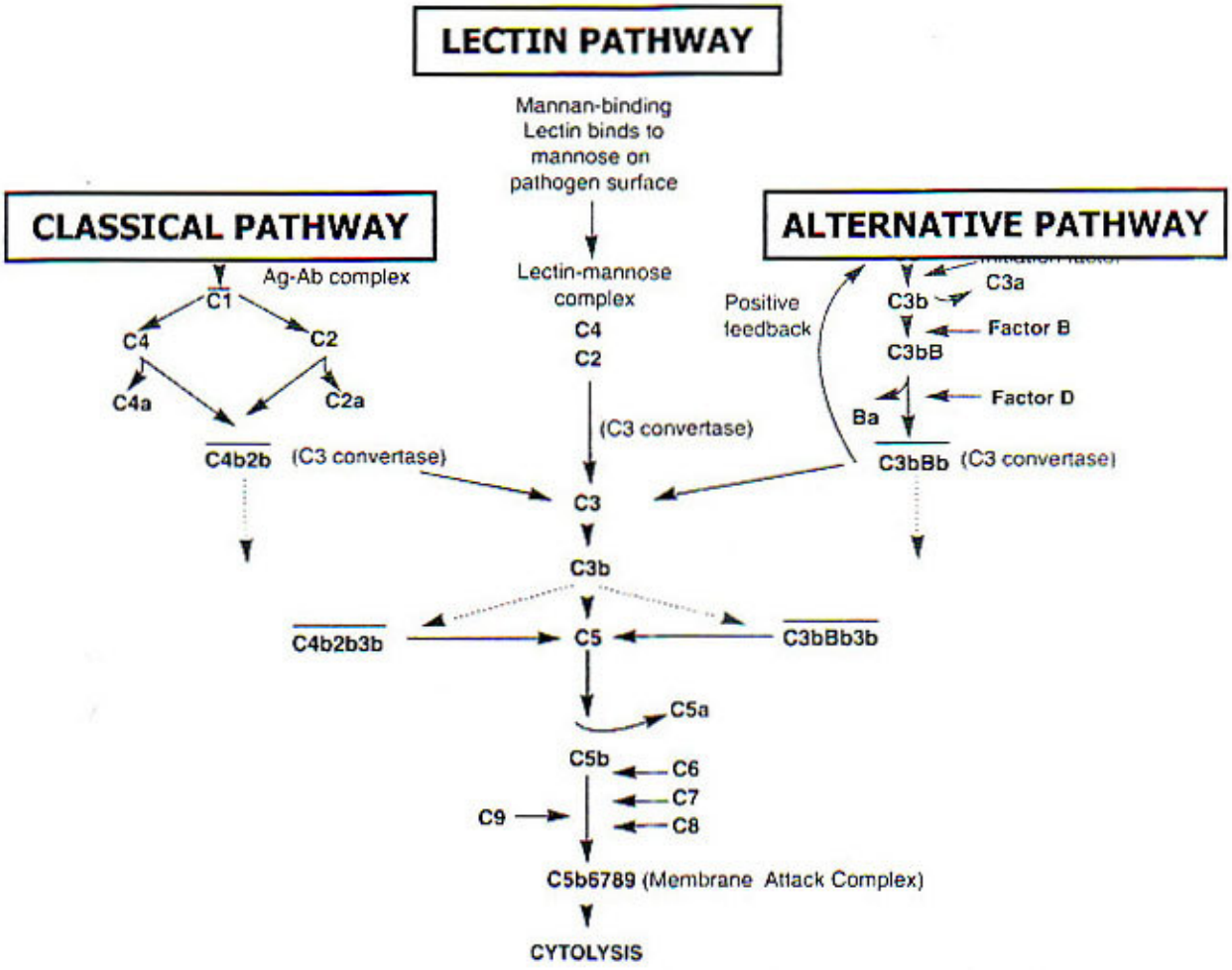

Figure-2: Complement activation pathways with focus on the mannan-binding lectin activation pathway.

On specific binding to microbial surface carbohydrates, MBL activates the complement system by means of its own lectin pathway, depending on the MBL-associated serine proteases (MASPs). This eventually leads to phagocytosis or lysis of pathogenic microorganisms, including bacteria, viruses, protozoa and fungi. Only the normally oligomerized forms of MBL are functional and therefore capable of associating with the MASPs and binding efficiently to the microbial carbohydrates.

For maximal efficacy it is important that proteins of the innate immune system should be present at physiologically significant levels at birth or shortly afterwards. In two small studies MBL was shown to be present in the serum at birth and reached maximal (adult) levels in the first weeks of life. In a study of 885 longitudinally collected serum samples from 168 pre-term Chinese infants, MBL levels were shown to rise from a mean of 500 ng/ml at 25 weeks of gestation to 1780 ng/ml at 20 weeks post-full-term[3,14]. The significance of a particular MBL serum level will depend on numerous factors and consideration must be given to ethnicity, age and promoter region polymorphisms. However, the most important determinant of serum level is the presence of a structural gene mutation. As a guide, the median serum concentrations of MBL for British Caucasoids were found to be: wildtype, 1630 ng/ml; heterozygous for codon 54 mutation, 358 ng/ml and homozygous for codon 54 mutation, ~10 ng/ml[3,15].

Clinical significance:

Several studies have now shown an association between low serum MBL levels and an increased risk of recurrent infection in man [3,16,17,18]. Large studies with paediatric patients have yielded highly significant associations for both homozygosity and heterozygosity of MBL mutant allies and increased risk of infections [3,19,20,21,22]. Paediatric patients with MBL gene polymorphisms associated with low levels of the functional protein (MBL) have an increased risk of progression from infection to sepsis and systemic inflammatory response syndrome (SIRS)[4]. It seems likely that any individual with low levels of MBL will be at risk of infection particularly in early life, but such risk might only manifest itself in association with other coexisting immunological abnormalities. As shown in Figure-3, there is an association between low serum MBL levels or the presence of structural gene mutations and an increased risk of infection and many other factors determine the success of the immune response to a particular pathogen [3,4,5,16,17,21].

Multiple organ failure resulting from systemic inflammation reminds the predominant course of morbidity and mortality in paediatric intensive care unit (PICU) patients. Targeted early resuscitation, careful glycaemic regulation, judicious use of steroids and administration of activated protein C have all proven beneficial. However, the reasons for success of such strategies are unclear and suggest further improvements are likely to stem from a greater understanding of pathogenesis of critical illness i.e. ICU patients [4,5,19,23,24].

Numerous studies have shown that individuals with MBL deficiency are also susceptible to infections [17,18,19,20]. MBL may also influence cytokine production and therefore the host inflammatory response. So, it may be

hypothesized that MBL deficiency would increase the risk of SIRS and severity of sepsis in children admitted to PICU. Gene to molecular studies also showed that human MBL deficiency is caused by point mutation within axon-1 of MBL-2 gene at codons 52, 54 or 57 resulting amino acid substitution [3,4].

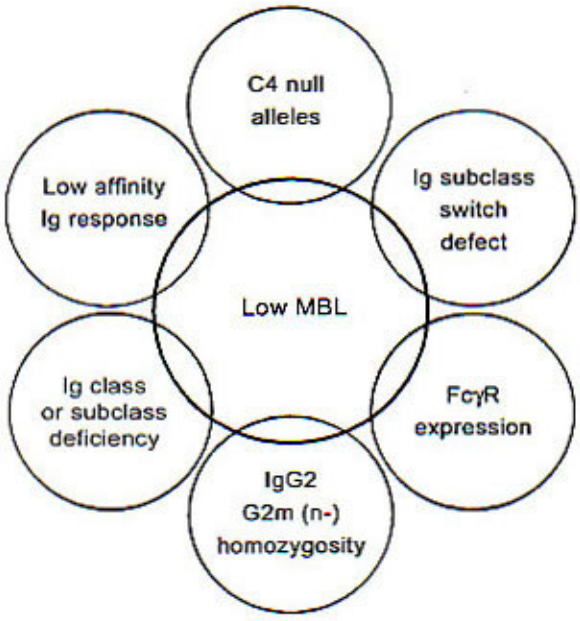

Figure-3: Several studies have now shown an association between low serum MBL levels or the presence of MBL structural gene mutations and an increased risk of infection. Many different factors determine the success of an immune response to a particular pathogen and we measure very few of these. It is possibly rare for low serum MBL levels to be solely responsible for clinical illness but pathology might be more common when an MBL-deficient individual also manifests some other immune defect. Such coexisting partial immuno-deficiencies are not rare. For example 8% of Caucasian populations lack two of the four possible functional C4 genes and might have a reduced capacity for complement activation. If 5% of the population are considered to have a profoundly low serum MBL concentration, approximately 1/250 individuals would present with both low levels of MBL and low levels of C4. Abbreviations: Ig, immunoglobulin; MBL, mannose-binding lectin[3].

Conclusions:

MBL appears to play an important role in opsonophagocytic processes. Together with other components of the innate immune system, it contributes to efficient antimicrobial immunity and protection. These are not only during the physiological window of vulnerability following the decay of maternal antibody, but also in the early phase of every primary contact with a sugar-rich pathogen.

Critically ill children with MBL mutated gene are at greatly (seven fold) increased risk of developing SIRS within 48 hours of presentation to PICU. Therefore, MBL status in PICU patients as well as in newborns with sepsis and recurrent infection will be of great help for paediatricians to initiate treatment for neonatal sepsis, a common cause of neonatal morbidity in developing countries. Also many other clinical associations of MBL deficiency have been reported such as SLE, rheumatoid arthritis, respiratory infections, cystic fibrosis and recurrent miscarriage. However, further confirmatory studies are required in these newer emerging fields [3,25].

So it is hypothesized that MBL deficiency would result systemic insult that may lead to development of SIRS and increased severity of sepsis in children admitted to PICU. MBL status plays a critical role in determining which children developed systemic inflammation and influence the clinical severity of that response irrespective of underlying illness.

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