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MANNOSE BINDING LECTIN, GENETIC VARIATIONS, DEFICIENCY AND DISEASE ASSOCIATIONS

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Abstract ▶ Mannose-binding lectin (MBL) is an important arm of innate immunity and plays a vital role in the first line of host defense. Genetic variation in MBL2 have been shown to associate with many infectious diseases, autoimmune and inflammatory disorders such as malaria, leishmaniasis, leprosy, tuberculosis, filariasis, HIV, rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE). MBL has been shown to bind with glycoconjugates on the surface of mannose rich microbes and deficiency of MBL has been associated with susceptibility and modulating the severity in bacterial, fungal, protozoan and viral infections. Many different approaches are being used to define 'MBL deficiency'. It is more relevant in young children in whom immune system fails to mount an effective response to carbohydrate antigens. MBL replacement therapy has been tried in the past for patients with MBL deficiency. Currently, production of recombinant MBL is underway and provides a hope for children with innate immune disorders.

Key Words: Mannose binding lectin, Genetic variation, MBL deficiency, MBL therapy.

Introduction

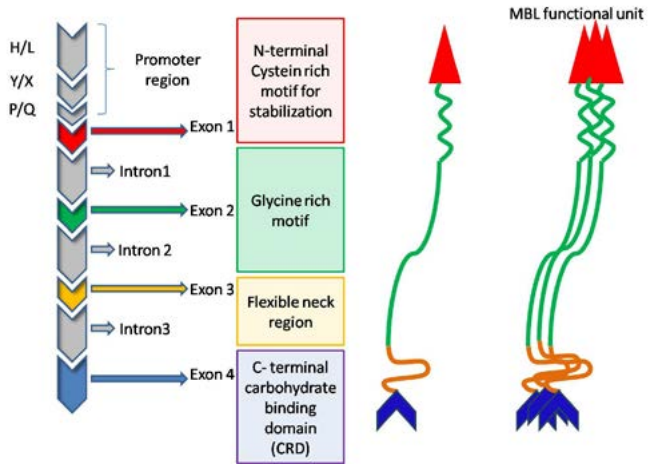
One of the fundamental components of innate immune system is the complement cascade which functions through both antibody-dependent and –independent manner providing protection against invading pathogens1. An effective immune response is mediated through the complement cascade involving interactions between cellular and humoral immunity which includes phagocytosis, chemotaxis, cell adhesion, B-cell differentiation and regulation of both B and T cell responses2. The initiation of the complement cascade is well studied and three activation mechanisms are known to be involved which include the classical, alternative and lectin pathways. The lectin pathway is the most recently discovered and is considered to be the most ancient of the three activation pathways3. The initiating complexes of lectin pathway comprise of separate recognition and enzyme components similar to the C1 complex of the classical pathway. Recognition components such as MBL and serum ficolins of the lectin pathway bind directly to carbohydrate moieties like N-acetyl glucosamine or mannose on pathogens and activate three enzymes, MBL-associated serine proteases (MASPs-1 to -3) to activate complement4.

MBL structure and function

MBL belongs to the C-type lectin family synthesized by liver and circulates in serum. Being an acute phase protein, its level rises during inflammatory conditions. As part of the collectin family characterized by collagen and lectin domains, the carbohydrate r ecognition domain (CRD) is required for binding ligand surfaces in a calcium dependent manner 5, 6. In humans, it is encoded by *MBL2* located on the long arm of chromosome 10 at 10q11.2-q21containing four exons coding four identical peptide chains of 32- kD subunit which associate to form higher oligomers (trimers-

hexamers) of a 96-kD triple helix circulating in serum. Among four exons, exon 1 encodes for a cysteine rich N-terminal region (confers stabilization) and 7 repeats of a glycine-rich motif, which contains repeated sequences of two glycines and this region is critical for the triple helix formation of the collagen structure (Figure 1).

Figure 1: Schematic representation of MBL2 and MBL subunit.

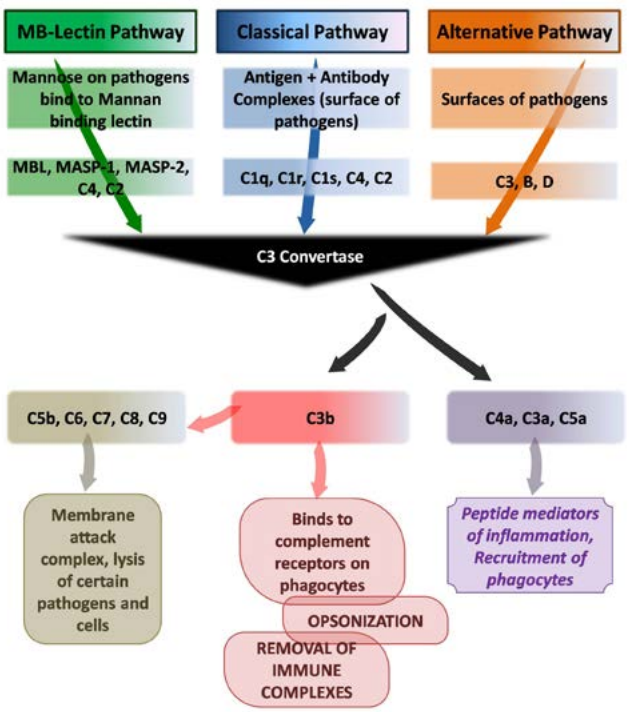


Exon 2 encodes part of the collagenous domain which contains enzyme MASP binding site and a short β-helical coiled-coil domain is encoded by exon 37. Exon 4 transcribes the most important CRD domain containing Calcium binding site and C-terminal end by which MBL binds to a wide range of pathogens such as Gram-positive and Gram-negative bacteria, parasites and viruses by recognizing D-mannose, N-acetyl-D-glucosamine and L-fructose sugar motifs on the surface of microorganisms. After post-translational modification, the final functional MBL contains subunits ranging from dimers to hexamers but the majority exists as trimers and tetramers8. Studies using high resolution force microscopy on recombinant MBL identified for the first time that the stable MBL structure was broken into an elongated state with separation of the ligand-binding domains confirming the large conformational changes happening in MBL while interacting with surface-immobilized ligands. This report highlights the importance of surface topography in immune recognition9, 10.

MBL mediated complement activation

Upon binding of the CRD complex to carbohydrate moieties present on the surface of invading pathogens, the proenzyme form of MASPs 1 and 2 is activated by cleaving the domains of complement control protein-2 (CCP-2) and serine protease producing heavy and light chains11. This activates complement (C) by MASP-2 to cleave C2 and C4 which causes the transformation of C3 into C3a and C3b12. This cleaving process then stimulates the downstream complement cascade. Thus the MBL pathway initiates complement activation in the same way as the classical pathway, forming a C3 convertase from C2b bound to C4b (Figure 2). Though both MASP-1 and MASP-2 were considered to be responsible for activation of C2, one report suggests that MASP-2 may be the major initiator of the complement pathway as evidenced by its interaction with C4. Findings also suggest that MASP-1 cleaves C2 but not C4 and MASP-1 probably helps in complement activation mediated by MBP13.

Figure 2: Overview of the main components and effector actions of complement pathway.



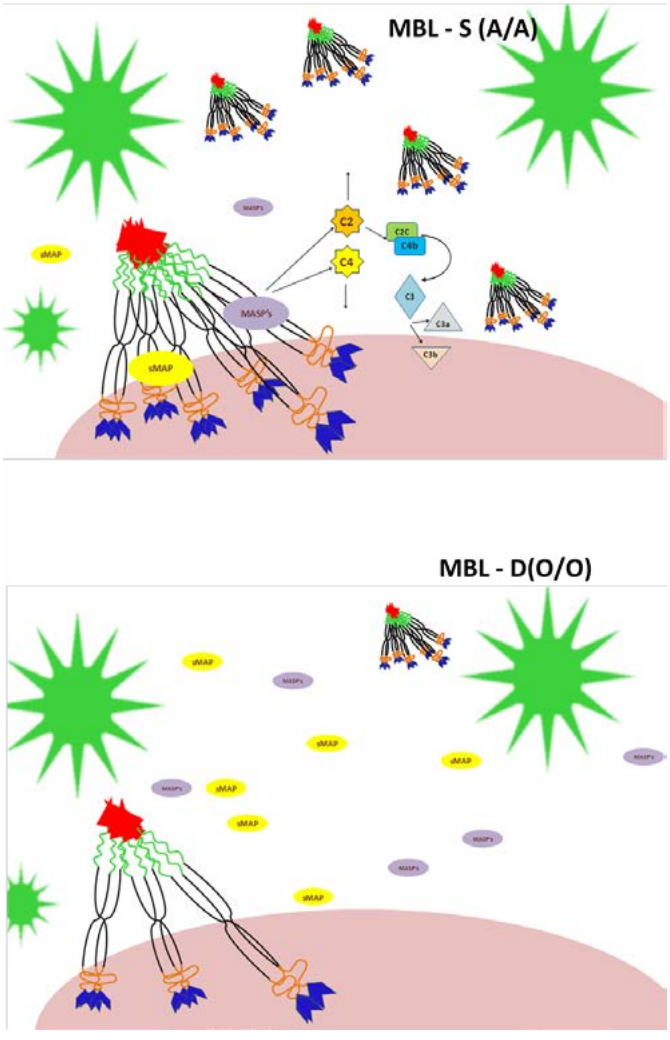
MBL structure for effective immune function of MBL

During early childhood, children deficient in MBL experience a substantial increase in infections indicating the importance of the MBL pathway in host defense. Low serum MBL levels are associated with opsonic defects and recurrent infections in infants14. Especially in the pediatric population, MBL exerts greatest influence during "window of vulnerability" between the decline in passive immunity by the mother and before the development of a fully functional adaptive immune system.

MBL2 – promoter and exon1 polymorphisms

The search for genetic variations as a cause for MBL dysfunction began when defective opsonisation was associated with low serum MBL level in an *in vitro* assay14. *MBL2* genetic polymorphisms can affect the serum levels as well as configuration and function (Figure 3).

Figure 3: Pictorial representation of normal MBL (MBL-S) from an A/A (wild type) genotype which circulates in the bloodstream complexed with sMAP and MASPs leading to opsonization and complement activation and deficient MBL (MBL-D) derived from a 0/0 (mutant) genotype which has a reduced capacity to build high order oligomers, fails to complex with MASPs and to activate complement.



The first study of genetic defects in the MBL gene was reported in three children in the UK with recurrent infections who had an opsonic defect and low serum MBL concentrations. Sequence analysis showed a mutation at base 230 of exon 1 causing a change of codon 54 from GGC to GAC resulting in replacement of glycine with an aspartic acid residue disrupting the formation of the normal triple helix, and rendering the molecule vulnerable to degradation15. Subsequently, a SNP in codon 57 identified in a Gambian population termed variant C which substitutes a glutamic acid instead of glycine (GGA to GAA) and another SNP at codon 52 termed variant D representing a substitution of arginine by cystein (CGT to TGT) were also observed8,16. Two promoter variants H and L at position -550 are in linkage disequilibrium with the X and Y variants at position -221 to produce three haplotypes HY, LY and LX. Haplotype HY is reported to be associated with the highest plasma levels of MBL, LY haplotype with intermediate and LX haplotype with the lowest plasma MBL level8.

Table 1: MBL levels in healthy controls from different ethnic background.

(A) In adults:

Country/Ethnicity	Age group (years)	Serum MBL level (ng/ml)	Reference
Hungary	50-60	Median (IQR) - 1027 (253-2120)	(17)
Australian	not given	Mean (range) - 1940 (0-8810)	(18)
Finnish	44-60	Mean – 3970	(19)
Swedish	50-60	Mean – 1680	(20)
Caucasians,		Mean (Caucasians) - 1672;	(21)
African-American	60-70	Mean (African Americans) – 1158	
Egyptians	30-38	Mean=619	(22)
Chinese	16-20	Mean = 2050	(23)
	31-40	Mean = 2160	
	41-57	Mean = 1466	

(B) In children:

Country/Ethnicity	Age group (years)	Serum MBL level (ng/ml)	Reference
Chinese	0 to 6	Median (p2.5-p97.5)=2536 (161-5070)	(24)
Han Chinese	0 to 2	Cord blood (median)=1462	(25)
		Newborns (median)=1597	
		Children (median)=2536	
Turkish	3 to 11	Median (range) = 2950 (1.5-4048.5)	(26)
Hungary	1 to 15	Mean±SD = 1411 ± 576	(27)
Dutch Caucasian	13 to 16	Median = 1650	(28)
Chinese	2 to 3	Median (IQR)=1065 (862-1452)	(29)
Polish	9 to 10	Median (range) = 1762 (41-8544)	(30)
Netherlands	0 to 18	Median (IQR)= 1090 (420-2700)	(31)

The promoter polymorphisms and three point mutations in codon 52, 54 and 57 exhibit a pattern of linkage disequilibrium and thus combination of SNPs at both promoter and exon1 occur in a nonrandom fashion. Though 28 possible combinations can occur due to two SNPs linked as haplotypes, only seven haplotypes have been reported till

now. These include HYPA, LYQA, LYPA, LXPA, HYPD, LYQC and LYPB32. Studies in various populations and age groups indicate that MBL serum levels largely depend on the *MBL2* genotype18.

Factors other than genetic variation, which influence serum MBL, include age, hormonal status and immune activation. MBL levels vary with age, increasing in the first months of life and falling at about the age of 12 due to hormonal changes33. MBL levels also vary hugely based on the ethnicity both in children and adults, as described in table 1.a and 1.b. Lower MBL levels have been found in preterm neonates with comparable levels in cord blood34. A cohort study of 95 patients with autoimmune thyroid disorders suggested that MBL levels were found to negatively correlate with thyroid stimulating hormone (TSH) in patients with autoimmune hypo- or hyperthyroidism, irrespective of the genotype35.

MBL deficiency

Though there have been a number of studies describing the genotype/phenotype or serum levels / disease association, a clear understanding of MBL deficiency is still lacking. *MBL2* genotype and corresponding serum MBL levels show a wide range making it difficult to correlate genotype/phenotype associations. Cut-off levels to define an MBL 'deficient' state and 'MBL deficient genotype' hugely vary ranging from <50 ng/ml to <1000 ng/ml depending on the age and ethnicity. In pediatric populations, low MBL or 'MBL deficiency' seem to be an important predisposing factor for infectious diseases. A recent study indicates *MBL-2* polymorphisms were associated with increased risk for bacterial infections in children with B acute lymphoblastic leukemia36. In severe meningococcal or pneumococcal infections, 'MBL deficient' children with serum MBL < 500 ng/mL were found to a have a higher risk of death 37.

Future directions - recombinant MBL therapy

The first MBL product for therapeutic use was isolated from plasma of Danish blood donors by the Statens serum institute (SSI) Copenhagen, Denmark 38. The first patient to receive MBL replacement therapy was a two year old girl who had suffered debilitating and recurrent infection from the age of 4 month. She had opsonic defect and very low MBL level. The girl was given daily infusion (2 mg) of MBL for 3 consecutive days and this treatment was repeated after 10 days. The MBL concentration in her blood reached normal values after each infusion and the opsonic activity of her plasma was temporally restored to normal. She remained free from recurrent or abnormal infection during the 8 yr since she received this treatment 39. Jensenius and his group first produced recombinant MBL at the University of Aarhus Denmark. A human endothelial kidney cell line was transiently transfected and cultured in protein-free medium40.

MBL replacement therapy to help patients with MBL deficiency has undergone phase I clinical trials41. Phase II and III trials and production of recombinant MBL for replacement therapy are currently underway. Thus MBL replacement therapy should be carefully chosen and will be restricted to a few carefully selected patients until proof of efficacy is established by more controlled clinical trials.

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