The role of mannose-binding lectin in vitro and in vivo

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CHAPTER 1

Introduction
Chapter 1

Immune systems

Human immunity can be divided in the innate immune system and the adaptive immune system. There are two main differences between the two immune systems. The adaptive immune response is highly specific to an invading micro-organism and has a memory function, so that immunity to infectious microorganisms will be acquired over time. In contrast, the innate immunity is unspecific with a relatively constant role over time, and does not have a memory. The adaptive immune response can be divided in cellular immune response, which involves T lymphocytes and a humoral immune response, which involves proteins in the body fluids. In the cellular immune response T-cells get activated upon contact with an antigen and develop to activated cytotoxic T-cells, which are capable of killing micro-organisms directly. In contrast, for an optimal humoral response, the T-cells and B-cells cooperate together to generate antibodies (immunoglobulins) to the invading micro-organisms. After antigen presentation by an antigen-presenting cell (APC), B-cells develop to antibody-secreting plasma cells, with the help of T-helper (Th) cells (Figure 1A). Secreted antibodies bind to antigens on the surface of invading micro-organisms, which flags them for phagocytosis and destruction (1). The primary antibody response takes about 7-10 days to fully develop. In this first period after an infection the innate immune response is of great importance.

The innate immune response is an unspecific response that forms the first line of defense. In particular in early childhood, during the interval between the loss of passively acquired maternal antibodies and the acquisition of a mature immunologic repertoire of the adaptive immune system, innate immunity needs to function optimally. But, as mentioned above, the innate immune response also contributes to host defense during the first days after infection with new microorganisms in later life, before the adaptive immune response has been adequately induced. Therefore, the innate immune response has a critical role in the control of an infection during the first days. All innate immune cells and proteins are present in the circulation prior to an infection and will act immediately. Natural killer-cells, monocytes, macrophages and polymorphic neutrophils are leukocytes that are innate immune cells, which able to bind to micro-organisms and kill them after uptake of the bound micro-organisms by phagocytosis. The recognition and phagocytosis of invading micro-organisms by phagocytes is enhanced by marking of the microorganisms with soluble immune proteins, already present in the circulation, like complement proteins (Figure 1B).

Innate immunity and the complement system

Complement is a central component of the innate immune system, comprising at least 35 soluble proteins that collaborate in a complex manner in the elimination of microorganisms and in the removal of apoptotic cells, but complement also acts in enhancing and directing the adaptive
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The numerous plasma proteins are present not only in the circulation but also in body fluids and tissues and can interact with various cells and mediators of the immune system (5). The activation of the complement cascade can be initiated via three pathways (Figure 2). The classical pathway of the complement system is activated after formation of an antibody-antigen complex (immune complex). C1q triggers the activation process when it binds to antibodies within these immune complexes. In this way, C1q acts to bridge the innate and adaptive immune response (2-4). The numerous plasma proteins are present not only in the circulation but also in body fluids and tissues and can interact with various cells and mediators of the immune system (5). The activation of the complement cascade can be initiated via three pathways (Figure 2). The classical pathway of the complement system is activated after formation of an antibody-antigen complex (immune complex). C1q triggers the activation process when it binds to antibodies within these immune complexes. In this way, C1q acts to bridge the innate and adaptive

Figure 1: Immune response: opsonization and phagocytosis of bacteria. (A): Adaptive immune reaction. Peptide fragments of antigens present on the bacterial surface are presented to T-helper (Th) cells by antigen-presenting cells (APC). This stimulation of Th cells activates B cell (B) proliferation and differentiation to antibody forming cells (AFC). The secreted antibodies recognize the antigens on the surface of the bacteria and bind to its surface, upon formation of antibody-antigen complexes, the classical pathway of complement is activated and C3b and iC3b will be deposited on the surface of the bacteria (opsonization). (B): Innate immune reaction. Circulating complement proteins (MBL, C3b) can bind directly to repeating structures on the bacterial surface. The complement proteins cover the surface of the bacteria (opsonization). Both the adaptive and the innate immune response lead to complement proteins on the surface of the opsonized bacteria that are recognized by Complement Receptors (CR) on the surface of the phagocytic neutrophils, which leads to cell activation and phagocytosis of the bacteria.
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immune systems (4). The lectin pathway of the complement system is activated after binding of mannose-binding lectin (MBL, also known as mannan-binding lectin or mannose-binding protein) or ficolins to repeating sugar structures on the surface of micro-organisms. Upon binding, the MBL-associated serine proteases (MASP) activate the complement cascade (6). The alternative pathway of the complement system is based on auto-activation after direct binding of C3b or C3\(\cdot\)H\(\cdot\)O to the surface of micro-organisms. All three pathways lead to the cleavage of C3 as a consequence of triggered enzyme cascades. Upon activation of the classical and the lectin pathway of complement, both C4 and C2 are cleaved, thereby generating C4b2a complexes, with C3 convertase activity (7). The C3bBb complex is the C3 convertase of the alternative pathway. The alternative pathway is of importance for enhancement of both the classical and the lectin pathway, because via the amplification loop, C3b formation leads to more C3b deposition on the pathogenic surface.

The complement system has three major effector functions. **Opsonization**, the process by which a pathogen is covered with proteins that facilitate its ingestion and destruction by a phagocyte, is one of the effector functions of the complement system. Immunoglobulin G (IgG), C3b and iC3b, the covalently-bound cleavage fragments of C3, are the most important complement opsonins in the defense against bacterial infection (8). Opsonins have binding sites for attachment to the cell-membrane of phagocytes and an enzymatic site for cleavage of the next component of the complement cascade. Bound B3b or iC3b reacts with C3b receptors present on the membrane of phagocytes, known as complement receptors (CR). There are 4 complement receptors, CR1-4. The CR1 and CR2 are mainly important for the humoral immune response. CR1 is the opsonin receptor on neutrophils, which activates the phagocytosis process after binding to a covalently bound C3 fragment on a micro-organism. Besides functioning as receptor for C3b or iC3b functions CR1 also as receptor for C1q and had been suggested to bind MBL (9). CR2 binding of a small C3 (C3d) fragment enhances B-cell activation and antibody secretion. Besides opsonization, **chemotaxis** (by C5a) is an effector function of complement activation in which phagocytic cells are activated and induced to move towards the site of inflammation. The last effector function of complement activation is the terminal pathway of complement, C5b-C9, which forms the so-called membrane attack complex (MAC) that causes cell lysis of the micro-organism by forming a pore in the cell membrane (10). Most micro-organisms are cleared from the circulation by phagocytes after opsonization of the micro-organisms. The only known bacterium that it is efficiently lysed via MAC insertion into the bacterial wall is *Neisseria meningitides*, which causes meningitis (11).

Although the three known ficolins, L-Ficolin (12), H-ficolin (13) and M-ficolin (14), also act at the lectin pathway of complement activation, an established role for ficolin-mediated opsonization has yet to be discovered. The ficolins will not be further discussed in this thesis, which is focused on MBL-mediated complement activation, opsonization and MBL disease association.
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Structure and function of MBL

MBL has been isolated for the first time from rabbit liver in 1978 (15) and a few years later also from rat and human liver (16). MBL belongs to a family of proteins called collectins, with both collagenous regions and lectin domains. Besides MBL, the surfactant proteins A and D (SP-A, SP-D) are other known collectins (17). The MBL molecule is built from polypeptide chains (32 kDa), consisting of four regions: the carbohydrate recognition domain (CRD), an alpha helical
hydrophobic neck, a collagenous region with 19 Glycine-X-Y repeats (X and Y indicate any amino acid) and a cysteine-rich N-terminal region. Three polypeptide chains form a triple helix (monomer subunit), stabilized by disulfide bonds between cysteine residues in the N-terminal region (18, 19). Inter-subunit disulfide bonds (S-S bridges) in the N-terminal linker region have been shown to be responsible for the association of the monomer subunits into oligomeric forms (20). MBL exists in oligomers ranging from dimers to hexamers that form tulip-like structures (Figure 3).

As a member of the innate immune system, MBL acts as soluble pattern recognition molecule, which recognizes surfaces with repeating mannan, mannose, N-acetylglucosamine (GlcNAc), pentose or fucose present on a wide variation of micro-organisms and on altered or apoptotic cells (10, 21). The structure and composition of these lipopolysaccharides play a crucial role in MBL binding and in the discrimination between self en non-self or altered-self by MBL. The binding of the CRD of MBL is calcium-dependent and with relatively low affinity. However, the formation of higher oligomeric MBL with multiple CRD’s separated from each other by a constant distance, provides a high-avidity binding when the CRD’s are bound simultaneously to repeating carbohydrate units (18).

Figure 3: MBL structure. The 32-kDa polypeptide of MBL consists of 4 domains: a cross-linking region, a collagenous region, a neck region and a carbohydrate-recognition domain (CRD). Three identical polypeptides form a structural subunit, based on formation of a collagenous triple helix. Oligomerization of the structural subunits results in tulip-like molecules of MBL with different sizes, with the tetrameric form shown in this figure as the most common oligomeric form of MBL.

MBL in the circulation exists in complexes with the MASPs, a family of serine proteases (22, 23). There are 3 MASPs, MASP-1 (7, 24), MASP-2 (25), MASP-3 (26) and a truncated protein, called MAP19 or sMAP (25, 27), that is identical to the first two domains of MASP-2, without the enzymatic activity. They are generated from only two genes, the MASP1/3 gene on chromosome 3q27-q28, and the MASP2/Map19 gene on chromosome 1p36.2-6. Alternative
splicing is responsible for the generation of two different mRNAs from each gene. MASP share identical domain organizations with those of C1r and C1s, the enzymatic components of the C1 complex (28). These domains include an N-terminal C1r/C1s/sea urchin Vegf/bone morphogenic protein (CUB) domain, an epidermal growth factor (EGF)-like domain of the Ca\(^{2+}\)-binding type, which is known to mediate protein-protein interactions, a second CUB domain, two contiguous complement control proteins (CCP) modules, a linker region and a serine protease domain (29). MASP can associate in a Ca\(^{2+}\) dependent manner into homodimers via their CUB1-EGF domains. MASP are detected as zymogens in Ca\(^{2+}\)-dependent complexes with MBL, L-ficolin or H-ficolin in the circulation.

Upon binding of MBL/MASP-2 to microbial carbohydrate structures, MASP-2 is activated by promoting a proteolytic cleavage within the linker region, resulting in an A and B chain (30). The activated MASP-2 can cleave complement factor C4 and C2 to generate C4bC2a, the C3 convertase, and thus initiates the lectin pathway of the complement cascade. The other MASP have been less well investigated, and their precise role remains to be established (29). MASP-2 polymorphisms have been described, associated with low levels of MASP-2 in the circulation. No disease associations with the variant alleles of MASP-2 have been found so far (31).

MBL has been shown to bind to a wide range of Gram-positive and Gram-negative bacteria, viruses and fungi. An overview of MBL binding to a selection of micro-organisms is given in Table I (18). As indicated in the different studies, this MBL binding was categorized as low, medium or high. Some species are categorized in more than one group because studies showed conflicting results, or the binding of MBL to a micro-organism differs among different strains of the same bacterial species. This can be explained in case of some Gram-positive bacteria, in which encapsulation of the bacteria hides the carbohydrate pattern, which hinders MBL binding (32, 33). Most of the binding studies have been performed with plasma-purified MBL or recombinant human MBL (rhMBL) incubated with bacteria, instead of serum incubations. This may lead to conflicting results in the absence or presence of additional opsonizing serum proteins, modulating the MBL binding. Therefore the binding of purified MBL to micro-organisms in vitro may not be predictive for the role of MBL in the clearance of the micro-organisms within the body. Another difficulty for the in vivo interpretation of the MBL binding is that the different strains of bacteria vary substantially with regards to their binding affinity of MBL. Thus, pathogenic strains of bacteria may act in a completely different way from the bacterial strains cultured in the lab.

**MBL genotype and plasma concentration**

Human MBL is encoded by the **MBL2** gene that is located on chromosome 10q11.2-q21 (18). **MBL1** is a pseudogene that does not encode a functional protein. The **MBL2** gene consists of
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4 exons, with exon 1 encoding the signal peptide, a cysteine-rich region and seven copies of the repeated glycine-X-Y motif typical for the triple helix formation of collagen structures. Exon 2 encodes the other twelve copies of the glycine repeats. Exon 3 encodes the neck region and exon 4 the carbohydrate-binding domain (34, 35).

The concentration of MBL in serum varies 1000-fold, from 5 ng/ml (below detection limits) to more than 5 μg/ml in different individuals, with a median concentration around 1.5 μg/ml (21, 36, 37). The level of MBL in the circulation of an individual is largely determined by single nucleotide polymorphisms (SNPs) in the MBL2 gene (Figure 4). There are three SNPs in exon 1, at codon 52 (D variant), at codon 54 (B variant) and at codon 57 (C variant, A being the wild-type) that lead to disruption of the Gly-X-Y pattern of the collagen region (38-41). The mutation is dominant, prevents oligomerization of the molecule and leads to decreased circulating levels of MBL, although the oligomerization of the D variant is less affected by the genetic alteration in exon 1 (42, 43). Besides the exon 1 mutations, three SNPs in the promoter region account for additional differences in the MBL concentration (44, 45). Two promoter variants, H and L at position -550 are in linkage disequilibrium with the X and the Y variant at position -221 and are found as three haplotypes e.g. HY, LY and LX. The HY is associated with the highest plasma levels of MBL, the

<table>
<thead>
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<th>Table I: Micro-organisms that bind MBL.</th>
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<tbody>
<tr>
<td>Bacteria</td>
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<tr>
<td><em>Actinomyces israelii</em> (82)</td>
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<tr>
<td><em>Bifidobacterium bifidum</em> (82)</td>
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<tr>
<td><em>Burkholderia cepacia</em> (83)</td>
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<td><em>Chlamydia pneumoniae</em> (84)</td>
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<tr>
<td><em>Escherichia coli</em> (33)</td>
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<td><em>Haemophilus influenzae</em> (33), (85)</td>
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<tr>
<td><em>Klebsiella aerogenes</em> (85)</td>
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<tr>
<td><em>Leptotrichia buccalis</em> (82)</td>
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<tr>
<td><em>Listeria monocytogenes</em> (33)</td>
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<tr>
<td><em>Mycobacterium avium</em> (86)</td>
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<tr>
<td><em>Mycoplasma pneumoniae</em> (87)</td>
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<tr>
<td><em>Neisseria meningitides</em> (33), (85)</td>
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<tr>
<td><em>Proprionibacterium acnes</em> (82)</td>
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<tr>
<td><em>Pseudomonas aeruginosa</em> (83)</td>
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<td><em>Salmonella montevideo</em> (88)</td>
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<tr>
<td><em>Staphylococcus aureus</em> (85)</td>
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<td><em>Streptococcus pneumoniae</em> (85)</td>
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<td>Viruses</td>
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<tr>
<td>Influenza A (89), (90), (91)</td>
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<td>HIV (89), (90), (91)</td>
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<td>Herpes simplex 2 (92), (93)</td>
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<tr>
<td>SARS-CoV (94)</td>
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<tr>
<td>Fungi</td>
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<tr>
<td><em>Aspergillus fumigatus</em> (85)</td>
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<tr>
<td><em>Candida albicans</em> (85), (95)</td>
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<tr>
<td><em>Cryptococcus neoformans</em> (96)</td>
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MBL binding: high, medium, low

HIV: human immunodeficiency virus
SARS-CoV: severe acute respiratory syndrome-coronavirus.
LY haplotypes with intermediate levels and the LX haplotype is associated with the lowest circulating plasma levels of MBL (44). The number of MBL haplotypes is further increased by a polymorphism at position +4 in the untranslated region (P/Q variants) and five additional base substitutions / deletions in the promoter region (including the variants at position -70) (40, 45). Strong linkage disequilibrium exists between the promoter region and exon 1 of the MBL2 gene, and only seven of the theoretically possible 64 MBL haplotypes are regularly found in the Caucasian population (HYPA, LYPA, LYQA, LXPA, LYPB, LYQC and HYPD) (44). Of these haplotypes, the LXPA haplotype has a reducing effect on MBL levels comparable to an exon 1 mutation. In our patient studies we mainly investigated Caucasian populations. As a control group, about 200 Dutch blood bank donors were analyzed for MBL concentrations and haplotypes. Figure 5 shows the mean MBL concentrations in relation to the MBL haplotypes (exon 1 + XY promoter). In several of our studies we used the division of high (mean 1.58 µg/ml MBL), medium (mean 0.51 µg/ml MBL) or low (mean 0.12 µg/ml MBL) expression groups (46-48), this subdivision is marked underneath the graph.

Besides the SNP’s in the MBL2 gene, the plasma concentration MBL is also influenced by the acute-phase reaction (34). However, MBL acts as a slowly responding (after 7 days) and intermediate (increase up to three-fold) acute-phase molecule as compared to C-reactive protein (CRP), as demonstrated after surgery or malaria infection (49). The frequency of the three MBL exon-1 mutations varies considerably in various populations. The B allele (codon 54 mutation) is regularly found in Caucasian populations, whereas the C allele (codon 57) is mainly found in African populations (37). The exon-1 variant mutations occur at a frequency of approximately 25-35% in Caucasian populations (18).

![Figure 4: The MBL2 gene and polymorphisms. The MBL2 gene comprises four exons. Nucleotide (nt) positions of the exon 1 and promoter polymorphisms are shown (allele), with the nt substitution under each allele and in case of the exon 1 polymorphisms also the amino acid (aa) substitutions.](image-url)
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MBL and disease associations

General disease association

The major function of MBL might be the provision of antigen independent protection against micro-organisms. First, during the window of vulnerability following the loss of maternal antibodies and before the acquisition of an individual’s own full antibody repertoire, and later in life as ante-antibody response before antibody production against a new antigen has started (50). MBL deficiency was initially recognized as a functional opsonic defect in children with recurrent, unexplained infections (51). Since then, there has been a substantial increase in the number of publications about MBL (Figure 6), although it seems that the interest in MBL is now stabilizing. Many of the publications focus on the role of MBL deficiency in clinical disease. The studies about MBL mentioned hereafter do not provide coverage of the complete field of research, but are only examples of the many clinical association studies that have been performed.

Susceptibility and disease modulation

Several studies show an increased susceptibility to extracellular pathogens associated with MBL deficiency (52), in particular micro-organisms causing acute respiratory tract infections during early childhood (43, 53). A number of clinical studies have suggested that MBL deficiency is a risk factor for acquiring HIV infection, with a two- to eight-fold increase in risk (54, 55). But also associations of MBL deficiency and increased susceptibility to autoimmune diseases have been described, e.g. an increased frequency of mutant MBL2 alleles in patients with systemic lupus.

Figure 5: Serum MBL levels and MBL haplotypes. Serum MBL level is plotted against MBL exon 1 polymorphisms and promoter polymorphism X/Y. O indicates the presence of B, C or D variants. Haplotypes are grouped as MBL expression group, with the haplotypes associated with the highest MBL concentration at the left and those with the lowest MBL concentration at the right. The dotted line indicates an MBL level of 1 μg/ml, which is assumed to be sufficient for complement activation via the lectin pathway.
Apart from disease susceptibility, there is also evidence that MBL has a modulatory role in autoimmune diseases or inflammatory responses. In rheumatoid arthritis (RA) MBL2 variant alleles are associated with severity and early onset of the disease (58, 59), but on the other hand, the wild-type MBL2 genes were associated with persisted inflammation in affected joints of RA patients (60).

Also in patients with secondary immune deficiencies, for instance oncology patients receiving chemotherapy that induces neutropenia, a modulatory role for MBL has been suggested. Children with MBL2 variant alleles spent more days in the hospital during neutropenia compared to MBL wild-type individuals (61). In adults with chemotherapy-induced neutropenia reduced levels of MBL were found in the patients who developed infections (62). But there are also studies in which no effects of MBL deficiency were found in oncology patients (63-65).

Dual role for MBL

The majority of individuals with variant MBL2 alleles remain healthy. In a large cohort of Caucasian adults no differences were found in infectious disease or mortality in MBL-deficient individuals compared to MBL wild-type individuals (66). Also in several patient studies no differences were found between MBL-deficient patients versus MBL wild-type patients (63). Today, even though it is generally accepted that MBL is a central factor in the innate immune system, we assume that MBL deficiency alone does not increase the risk for infection, or affects the outcome of a disease, probably because other immunological activation, e.g. antibodies or ficolins (17, 67), are able to take over MBL function in most adults with MBL deficiency. Yet, MBL deficiency may still act as risk factor in disease development and outcome when there is another co-existing (immune) defect (68).

The high frequency of variant MBL2 alleles in healthy individuals suggests that functional MBL deficiency may be advantageous (66, 69). Perhaps this deficiency during early childhood is
counterbalanced by an advantage later in life. MBL deficiency leads to a reduction in complement activation, which in turn reduced the probability of intracellular pathogen infection, e.g. *Leishmania chagasi* (70), *Mycobacterium leprae* and *Mycobacterium tuberculosis* (71). Besides reduced susceptibility to intracellular pathogens, low or intermediate levels of MBL might also prevent immuno-pathologically mediated host damage in critically ill patients (39, 72). Intermediate levels of MBL may be the most desirable phenotype (heterosis) for innate protection against a broad range of pathogens, without over-activating complement in serious ill patients (71).

**Therapeutic potential of MBL**

**History**

In 1976, Soothill described that defective serum opsonization of yeasts was frequently found in children with a range of infections and also in most of their mothers. The defective function could be corrected, *in vitro* and *in vivo*, by normal fresh frozen plasma. Sera from affected members of the same family did not correct each other (73). More than 10 years later it was reported that MBL deficiency is the cause of this opsonic defect and that purified MBL, in an *in vitro* assay, could restore the opsonization of *Saccharomyces cerevisiae* in the sera lacking MBL (51). Another 9 years later, the first *in vivo* substitutions in two MBL-deficient individuals with plasma-purified MBL were reported (74). After infusion of the MBL, the opsonic capacity in these sera was normalized, and no adverse effects of the MBL infusion were encountered. One of the two recipients, a two-year old girl with a low IgA level as well, was suffering from recurrent infections from the age of 4 months. She received 6 infusions with MBL, after which she did not encounter any infections for a period of three years. To demonstrate clinical efficacy associated with MBL infusions, double-blind randomized trials in MBL-deficient individuals are necessary. A suitable target patient group would preferentially consist of patients with two co-existing minor immuno-deficiencies (one of them being MBL deficiency), together predisposing to disease (75).

**Plasma-purified MBL versus recombinant MBL**

The *in vitro* and *in vivo* obtained results with MBL substitution suggest that MBL substitution might be a beneficial treatment of patients with MBL deficiency. For establishing production of MBL as therapeutic product, access to adequate starting material is needed.

There are two ways of producing MBL. Plasma-purified MBL has been generated by the Statens Serum Institute (SSI) in Copenhagen, Denmark, from a Cohn fraction III-like paste, a waste fraction from the ethanol fractionation of plasma for the production of intravenous immunoglobulin (IVIG). This fraction was solubilized in Tris-buffered saline (TBS), whereby MBL is extracted. After several ultrafiltrations the MBL extract was solvent/detergent-treated. The
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following step was affinity chromatography on a Superdex 200 pg column in the presence of CaCl₂. MBL was eluted from the column with mannose with a purity of about 60%. Another virus-inactivation step was performed, followed by a second chromatography step, to wash out the solvent and detergent. The eluted MBL product is a liquid product, containing mainly high oligomeric MBL (≥ 290 kDa), which is at least 3 years stable under cold room storage. The purity is at least 65%, with IgM, serum amyloid P (SAP), α2-macroglobulin and IgA as major contaminants (76). A second generation plasma-purified MBL procedure contained an extra virus-removal step during the purification process, by nanofiltration of the product. The product, referred to as MBL-SSI is stabilized with albumin and is well tolerated (77).

Recombinant human MBL (rhMBL) production is developed by NatImmune, Copenhagen, Denmark. The synthesis of rhMBL has been accomplished in several mammalian cell lines. However, rhMBL structurally and functionally similar to natural MBL was only obtained through synthesis in human embryonic kidney cells, followed by selective carbohydrate affinity chromatography. The expression construct was prepared by cloning the four protein-encoding exons and the three intervening introns of the human \textit{MBL2} gene into the vector. The yield of rhMBL varied between 300 and 600 ng rhMBL/ml of serum-free culture supernatant. (78). MBL oligomerization was similar to that of natural MBL and also C4 activation on a mannan-coated surface by rhMBL was similar to plasma-derived MBL (78, 79).

Phase I

To assess the safety, tolerability, and pharmacokinetics of MBL as therapeutic product, phase-I clinical trials have been performed both for plasma-purified MBL and for rhMBL. The phase-I clinical trial with plasma-purified MBL was carried out with 20 MBL-deficient healthy adult volunteers (80). They received a total of 18 mg of MBL prepared by the SSI in Copenhagen, Denmark. The MBL was administered intravenously in three doses of 6 mg once a week. Serum levels of MBL increased up to normal levels of 1.2 – 4.5 μg/ml after each infusion. The half-life of the infused MBL was highly variable, with a mean half-life of 70 hours (range 18-115 hour). There was no complement activation upon MBL infusion, measured as C3a levels in the blood of the volunteers. No antibodies to MBL, human immunodeficiency virus (HIV) or hepatitis virus were observed. Thus MBL-SSI infusion in MBL-deficient individual is a safe procedure. However, the applied doses were not sufficient to maintain protective levels of MBL (> 1.0 μg/ml) (80).

The phase-I clinical trial with rhMBL was designed as placebo-controlled double-blinded study in 24 MBL-deficient healthy male subjects. The subjects were divided into 4 groups. Within each group 4 subjects received infusion with rhMBL while 2 subjects received placebo. rhMBL was administered as both single intravenous (i.v.) infusions (0.01, 0.05, 0.1, and 0.5 mg/kg) and repeated i.v. infusions (0.1 or 0.3 mg/kg given at 3-day intervals). There was no difference in incidence and type of adverse events reported in the study between the groups of subjects.
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receiving rhMBL or placebo. All adverse events reported as drug-related were mild and no serious adverse events were recorded. There were no clinically significant changes in laboratory evaluations and no anti-MBL antibodies were detected following rhMBL administration. After single i.v. doses of rhMBL, the maximal plasma levels increased in a dose-dependent manner, reaching a geometric mean of 9.7 µg/ml ± 10.5% in the highest dose group (0.5 mg/kg), with an elimination half-life of approximately 30 h. Administration of rhMBL restored the ability to activate the lectin pathway of the complement system without non-specific activation of the complement cascade. In conclusion, no safety or tolerability concern was raised following rhMBL administration. No signs of immunogenicity were detected, and an rhMBL plasma level sufficient to achieve therapeutic benefit (>1.0 µg/ml) can be achieved (81).

An MBL substitution study with plasma-purified MBL in children is described in this thesis, while phase-IB/II clinical trials with rhMBL are ongoing at the moment. Exactly which individuals would benefit from MBL replacement therapy is under debate, and the importance of targeting well-defined patient groups will be vital to the success of MBL as therapeutic drug.

Aim of the study

The research described in this thesis was designed to unravel the role of MBL in complement-mediated opsonization of different micro-organisms. We also report about the circulating MBL levels in different patient cohorts and finally describe the effect of plasma-purified MBL substitution in MBL-deficient children with cancer.

*Chapters 2 and 3* describe the role of MBL in complement activation during the opsonization of various micro-organisms *in vitro*. With the use of several MBL-sufficient sera, MBL-deficient sera as well as sera deficient in one or more complement components, we developed an opsonophagocytosis assay. With this assay, we investigated the role of MBL in opsonization and the complement pathways activated upon MBL binding to various micro-organisms.

*Chapters 4 and 5* report about the role of MBL deficiency during a very vulnerable period in childhood. In a cohort of premature neonates admitted to the neonatal intensive care unit, we determined the prevalence of MBL deficiency and the reliability of umbilical cord blood to measure the MBL concentration in the child. We further investigated whether MBL deficiency is associated with neonatal pneumonia and sepsis during the first 72 h and during the first month after birth.

*Chapters 6 and 7* focus on the role of MBL in a cohort of pediatric oncology patients, who often develop fever and infectious complications during chemotherapy-induced neutropenia. MBL genotype was correlated to clinical and laboratory parameters and MBL concentrations were measured longitudinally in time during febrile neutropenia.
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Chapter 8 reports about an MBL substitution trial in which we investigated the pharmacokinetics and safety of MBL substitution in 12 pediatric oncology patients who received neutropenia-inducing chemotherapy. Chapter 9 provides insight into the in vitro opsonization and complement activation capacity of the in vivo substituted MBL in the above-mentioned patients.

Chapter 10 summarizes and discusses the findings in this thesis and defines the questions raised during the research presented here that may be interesting for future research.

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Introduction


