42.1 Pathogen Recognition

In pathogen recognition by C-type lectins, several levels of complexity can be distinguished; these might modulate the immune response in different ways. Firstly, the pathogen-associated molecular pattern repertoire expressed at the microbial surface determines the interactions with specific receptors (Fig. 42.1). Secondly, each immune cell type possesses a specific set of pathogen-recognition receptors. Thirdly, changes in the cell-surface distribution of C-type lectins regulate carbohydrate binding by modulating receptor affinity for different ligands. Crosstalk between these receptors results in a network of multimolecular complexes, adding a further level of complexity in pathogen recognition (Cambi and Figdor 2005; Jack et al. 2001; Thiel et al. 2006) (see Chap. 23). MBL deficiency is genetically determined and predisposes to recurrent infections and chronic inflammatory diseases. MBL deficiency has been implicated in susceptibility and course of viral, bacterial, fungal, and protozoan infection. More than 10% of the general population may, depending on definition, be classified as MBL deficient, underlining the redundancy of the immune system. MBL-disease association studies have been a fruitful area of research, which implicates a role for MBL in infective, inflammatory and autoimmune disease processes. MBL deficiency predisposes both to infection by extra-cellular pathogens and to autoimmune disease.

42.1.1 MBL Characteristics

Mannose-binding lectin is a C-type serum lectin and is primarily produced by the liver (Bouwman et al. 2005) in response to infection, and is part of many other factors termed “http://en.wikipedia.org/wiki/Acute_phase_protein” acute phase protein. MBL is made up of 96-kDa structural units, which in turn are composed of three identical 32-kDa primary subunits. The subunits consist of an N-terminal cross-linking region, a collagenlike domain, and a C-terminal carbohydrate-recognition domain (CRD) (Chap. 23; Turner and Hamvas 2000). Circulating MBL is composed of higher-order oligomeric structures, which include dimers, trimers, tetramers, pentamers, and hexamers of the structural homotrimeric unit. The oligomeric configuration of the structural units allows the MBL molecule to have multiple CRDs, facilitating multivalent ligand binding. Each CRD of MBL is structurally identical and is able to bind a range of oligosaccharides including N-acetylgalactosamine, N-acetylmuraminylpentapeptide, and L-fucose (Turner 1996). Although the various sugars are bound with different affinities, the cluster-like array of multiple binding sites allows activation of complement to be most effective. MBL is considered to play a major role in innate defense against pathogens, involving recognition of arrays of MBL-binding carbohydrates on microbial surfaces. However, recent studies have shown that MBL is also involved in the recognition of self-targets, such as apoptotic and necrotic cells (Nauta et al. 2003). In plasma, MBL is associated with MBL-associated serine proteases (MASPs). Currently, three MASPs have been identified, MASP-1, MASP-2, and MASP-3 (Chap. 23).

42.1.2 Pathogen Recognition and Role in Innate Immunity

MBL belongs to the class of collectins in the C-type lectin superfamily, whose function appears to be pattern recognition in the first line of defense in the pre-immune host. MBL recognizes carbohydrate patterns, found on the surface of a large number of pathogenic micro-organisms, including bacteria, viruses, protozoa and fungi. Mannose-binding lectin binds to the repeating sugar arrays on surfaces of pathogens through multiple lectin domains and, following binding, is able to activate the complement system via an associated serum protease, MASP-2. Importantly, MBL activates the complement system through a distinctive third pathway, independent of...
Both antibody and the C1 complex (Table 42.1). The MBL binds to neutral carbohydrates on microbial surfaces and recognises carbohydrates such as mannose, glucose, l-fucose, N-acetyl-mannosamine (ManNAc), and N-acetyl-glucosamine (GlcNAc). Oligomerisation of MBL enables high avidity binding to repetitive carbohydrate ligands, such as those present on microbial surfaces, including E. coli, Klebsiella aerogenes, Neisseria meningitides, Staphylococcus aureus, S. pneumoniae, A. fumigatus and C. albicans (cf Kerrigan and Brown 2009). However, there is also a great variation in the binding of MBL to various organisms; Candida albicans, β-haemolytic group A Streptococci and Staphylococcus aureus bind with high affinity, while Clostridium sp, Pseudomonas aeruginosa, Staphylococcus epidermidis, b-haemolytic streptococci and Streptococcus pneumoniae exhibit low or no binding (Santos et al. 2001). It is also observed that some organisms (e.g. Klebsiella sp. and Escherichia coli) show a variable pattern of binding. Later, it was shown that the absence of sialic acid from the lipopolysaccharide (LOS) of Neisseria meningitidis serogroup B, serogroup C and Neisseria gonorrhoeae permits MBL binding and presence of sialic acid on LOS results in poor or no MBL binding. In a similar study on Salmonella sp, it was found that MBL binds to rough chemotype but exhibits low or no binding with smooth chemotype (Ambrosio and De Messias-Reason 2005). In order to activate the complement system after MBL binds to its target (for example, mannose on the surface of a bacterium), the MASPs protein functions to cleave the blood protein C4 into C4a and C4b. The C4b fragments can then bind to the surface of the bacterium, and initiate the formation of a C3 convertase. The subsequent complement cascade, catalyzed by C3 convertase, results in creating a membrane attack complex, which causes the lysis of the pathogen to which MBL is bound (Worthley et al. 2005, 2006) (Fig. 42.1). Being an important component of innate immunity, acting as an ante-antibody and/or as a disease modifier, MBL is thought to influence disorders as diverse as meningococcal disease, rheumatoid arthritis, cystic fibrosis and recurrent miscarriage. Vulvovaginal candidiasis is a yeast infection of vulva and vagina; millions of women suffer from vulvovaginal candidiasis 5 worldwide. Women bearing MBL variant allele are at a higher risk for vulvovaginal candidiasis syndrome 343. The cervicovaginal lavage (CVL) MBL levels and gene mutation frequency were both higher in women suffering from vulvovaginal candidiasis than in controls (Liu et al. 2006b). On the other hand, MBL levels were low (0.30 ng/mL) in women with recurrent vulvovaginal candidiasis and were associated with a high gene mutation frequency compared to controls (1.28 ng/mL) (Ip and Lau 2004). Parenteral administration of MBL increased resistance...
of mice to hematogenously disseminated candidiasis. Thus, MBL plays an important role in innate resistance to candidiasis, suggesting a protective role of lectin in female genital tract infection (Pellis et al. 2005).

The autologous function for MBL, perhaps, is to perform a regulatory role within the immune system. The MBL interacts with human peripheral blood cells such as B lymphocytes and natural killer cells. The MBL is capable of binding to differently glycosylated ligands on several autologous cell types via its carbohydrate-recognition domain. It was speculated that this could have functional significance at extravascular sites, but perhaps only in individuals possessing MBL genotypes conferring MBL sufficiency (Downing et al. 2003, 2005).

Nevertheless, MBL genotyping of various populations has led to the suggestion that there may be some biological advantage associated with absence of the protein. In addition, the protein also modulates disease severity, at least in part through a complex, dose-dependent influence on cytokine production. Moreover, there appears to be a genetic balance in which individuals generally benefit from high levels of the protein. These findings suggest that the concept of MBL as a protein involved solely in first line defense is an over-simplification and the protein should rather be viewed as having a range of activities including disease modulation (Turner and Hamvas 2000; Dommett et al. 2006; Worthley et al. 2006). The mechanisms and signaling pathways involved in such processes remain to be elucidated. Though the deficiency of MBL is associated with increased susceptibility to infections, Roos et al. (2004) indicated that antibody-mediated classical pathway activation can compensate for impaired target opsonization via MBL pathway in MBL-deficient individuals. Lack of MBL may be most relevant in the context of a co-existing secondary immune deficiency. Replacement therapy 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. The MBL has attracted great interest as a potential candidate for passive immunotherapy to prevent infection (Gupta et al. 2008).

### 42.2 MBL Deficiency as Risk of Infection and Autoimmunity

#### 42.2.1 MBL Deficiency and Genotyping

The single point mutations in exon 1 of human MBL-2 gene appear to impair the generation of functional oligomers leading to the secondary structural abnormalities of the collagenous triple helix and a failure to form biologically functional higher order oligomers. Such deficiencies of the functional protein are common in certain populations, e.g. in sub-Saharan Africa, but virtually absent in others, e.g. indigenous Australians. There is an increased incidence of infections in individuals with such mutations and an association with the autoimmune disorders such as SLE and rheumatoid arthritis. Thus, the MBL is a potential candidate for passive immunotherapy to prevent infection.

### 42.2.2.1 Polymorphism in MBL Gene Is Associated in Exon 1 at Codon 52, 54, and 57

The concentration of MBL in plasma is determined genetically, primarily by the genetic polymorphism of the first exon of the structural gene and promoter region. The gene encoding MBL, MBL2 (MBLI is a pseudogene), is located on long arm chromosome 10q11.2–q21 and contains four exons. A number of single nucleotide polymorphisms (SNPs) have been characterized in the gene. Exon 1 harbours three missense SNPs, giving rise to amino acid exchanges in the first part of the collagenous region. Two of these SNPs: Gly54Asp, named ‘B’ and Gly57Glu, named ‘C’ exchange glycine with an acetic amino acid. The third (Arg52Cys, ‘D’) introduces a cysteine in the collagen region (the residue numbers includes the leader sequence of 20 residues) (Fig. 42.2). The wild type is denoted ‘A’. Heterozygous individuals for D, B, and C mutations have a substantial decrease in MBL serum concentration, whereas MBL is undetectable in the serum of homozygous individuals. Three structural mutations within exon 1 at codons 52, 54, and 57 have been invariably referred to as the D, B and C variants. In addition to these three variant structural alleles, the promoter region also shows a number of SNPs as well, some of which influences the expression of MBL. The three SNPs in the coding region of the MBL2 gene those are associated with abnormal polymerization of the MBL molecule, decreased serum concentrations of MBL and strongly impaired function. These MBL SNPs are associated with increased susceptibility to infections, especially in immune-compromised persons, as well as with accelerated progression of chronic diseases. Normal serum levels of MBL range from 800 to 1,000 ng/mL in healthy Caucasians, however, wide variations can occur due to point mutations in codons 52, 54 and 57 of exon 1 and in the promotor region of the MBL gene (Turner 2003). Separate point mutations in the collagenous domain of human MBL are associated with immune-deficiency, caused by reduced complement activation by the variant MBLs as well as by lower serum MBL concentrations.

MBL deficiency with B mutation is associated with 26% of Caucasian populations (Steffensen et al. 2000). In a cohort of 236 Australian blood donors, 5 MBL promoter and coding SNPs were genotyped. Significant associations were found between both coding and promoter polymorphisms and MBL antigenic and functional levels (Minchinton et al. 2002). Point mutations in exon 1 at codons 52, 54 and 57
and a promoter polymorphism at −221 bp of MBL gene were associated with increased susceptibility to various infectious diseases (Steffensen et al. 2003; Roos et al. 2006). The codon 54 mutation was frequent in both a British Caucasian and a Hong Kong Chinese population. The replacement of glycine-54 with an aspartic acid residue disrupts the fifth Gly-Xaa-Yaa repeat in the collagen-like domain of each 32 kDa MBP peptide chain and prevents the formation of the normal triple helix (Sumiya et al. 1991). Super et al. (1992) suggested that this genotype occurs in 5% of the population and encodes a functional protein. Super et al. (1992) also suggested that the Gly 54Asp allele does not account for a deficiency state, but instead suggested that MBP may have two predominant allelic forms that have overlapping function and differ only in their ability to activate the classical pathway of complement.

Of 123 healthy Danish individuals investigated, 93 were homozygous (74.3%) for GGC, 28 heterozygous (22.8%), and 2 homozygous for GAC (1.6%). The gene frequency of the GAC allele was found to be 0.13. DNA sequencing of the cloned exon 1 from one GAC homozygous individual revealed no other substitution. The median MBP concentration in the group containing the GAC allele was 43.4 times lower than in the GGC homozygous group. However, the range of MBP in plasma was wide and overlapping between the groups. MBP protein was detected in both the GAC homozygotes. This study suggested that the GAC allele is able to produce a functional MBP protein which may be detected in serum at low concentrations (Garred et al. 1992).

The point mutation (GGA to GAA) involving codon 57 of exon 1 has been reported in Gambians from West Africa. In the Gambians the codon 57 mutation was remarkably common whereas the codon 54 mutation was very rare. In contrast, the codon 54 mutation was frequent in both the British Caucasian and the Hong Kong Chinese population. It was predicted that both homozygous and heterozygous individuals would have profoundly reduced serum levels of the protein and this was confirmed by immunoassay complement activation through lectin pathway. These two mutations have arisen independently because of the divergence of African and non-African populations.

**Codon 52 Polymorphism Increases Risk of Premature Delivery:** MBL2 gene polymorphisms are associated with an increased risk of neonatal infections. A relation between the maternal MBL2 genotype and the risk of premature delivery has been indicated. Bodamer et al. (2006) suggested that the frequency of the codon 52 polymorphism was higher in the pre-term group compared to the term group (10.8% vs. 4.9%), while the frequency of codon 54 polymorphism was equal in both groups (11.3% vs. 11.8%). Data suggest that the fetal MBL2 genotype might be an additional genetic factor contributing to the risk of premature delivery.

**SNPs at Codon 54 in MBL Are Associated with Increased Prevalence of Respiratory Infection:** The SNPs of the innate immunity receptors CD14, MBL, and Toll-like receptor-2 with clinical phenotype in critically ill patients with systemic
inflammatory response syndrome are associated with increased prevalence of positive bacterial cultures and sepsis but not with altered prevalence of septic shock or decreased 28-day survival. Furthermore, CD14 SNPs were associated with Gram-negative bacteria and Toll-like receptor-2 with Gram-positive bacteria, whereas MBL was not associated with a particular organism class. The prevalence of the codon 54 mutation of the MBL gene in patients having repeated respiratory infections as well as the prevalence of the MBL mutant genotype among patients with diffuse panbronchiolitis was further supported (Gomi et al. 2004).

MBL Gene Polymorphisms in Gestational Diabetes Mellitus: Insulin resistance is a feature of gestational diabetes mellitus (GDM). A genetic predisposition to a pro-inflammatory state could favor the appearance of GDM during pregnancy. An association has been found between G54D and GDM. GDM patients carrying the G54D mutation require insulin therapy more frequently and have heavier infants than GDM women homozygous for the wild-type allele. An inverse correlation in GDM patients between neonatal weight and plasma MBL levels has been reported. Thus, pregnant women bearing the G54D MBL allele have a greater risk for developing GDM and having heavier infants (Megia et al. 2004).

MBL Genotypes in Acute Lymphoblastic Leukemia: Epidemiological studies show that acute lymphoblastic leukemia (ALL) can be induced by interactions between the immune system and early childhood infections. Since, certain types of childhood acute lymphoblastic leukemia develop as a multiple step process involving both pre- and postnatal genetic events, the MBL may play a critical role in the immune response in early childhood before specific immune protection develops. Schmiegelow et al. (2002) indicated that low-level MBL genotypes is associated with an increased risk of childhood ALL, particularly with early age at onset. Childhood ALL may often be initiated in utero. The prenatal origin of childhood leukemia was ascertended in children with B-precursor acute lymphoblastic leukemia carrying the chromosomal translocation t(12;21), the most common subtype of all childhood ALL. Study provided evidence that the development of t(12;21) B-precursor ALL may be initiated in utero. However, age at leukemia may be inversely correlated with the burden of cells with leukemia clonal markers, i.e. leukemia predisposed cells at birth (Hjalgrim et al. 2002).

MBL Genotypes in Viral Hepatitis: The prevalence of mutations in MBL gene was assessed in patients of hepatitis B. A mutation in codon 52 of the MBP gene was present in two (11%) of 19 Caucasian patients with acute hepatitis B and nine (27%) of 33 Caucasian patients with chronic hepatitis B, compared with four (4%) of 98 Caucasian controls. By contrast the prevalence of the mutation was similar in Asian patients with chronic hepatitis B and in Asian controls (one [5%] of 20 vs. two [2%] of 117). Mutations in codon 54 and codon 57 were found in similar proportions of patients and controls. These findings showed in Caucasian, but not Asian, patients an association of the codon 52 mutation of the MBL gene with persistent hepatitis B virus (HBV) infection. They suggest an important role for this gene, or a gene in linkage disequilibrium with MBL, in determining outcome after HBV infection in adult but not neonatal life (Thomas et al. 1996).

Bellamy et al. (1998) investigated the association between variant MBP alleles and malaria, tuberculosis, and HBV in adults and children in Gambia. Of the 2,041 Gambians screened for MBP mutations, 944 (46%) were homozygous for the wild-type allele, 922 (45%) were carriers of a single variant allele, and 175 (8.6%) possessed 2 mutant alleles. The most common mutation in Africans – the codon 57 variant allele – was weakly associated with resistance to tuberculosis in both patients and controls. Although MBP deficiency may predispose to recurrent infections, this study failed to provide evidence that such a deficiency is a major risk factor for infectious diseases (Bellamy et al. 1998).

The hepatitis C virus (HCV) envelope glycoprotein E2 binds the DC-SIGN and the related liver endothelial cell lectin through high-mannose N-glycans. Several high-mannose N-glycans are not associated with a positive bacteria, whereas MBL was not associated with a particular organism class. The prevalence of the codon 54 mutation of the MBL gene in patients having repeated respiratory infections as well as the prevalence of the MBL mutant genotype among patients with diffuse panbronchiolitis was further supported (Gomi et al. 2004).

The hepatitis C virus (HCV) envelope glycoprotein E2 binds the DC-SIGN and the related liver endothelial cell lectin through high-mannose N-glycans. Several high-mannose N-glycans are not associated with a positive bacteria, whereas MBL was not associated with a particular organism class. The prevalence of the codon 54 mutation of the MBL gene in patients having repeated respiratory infections as well as the prevalence of the MBL mutant genotype among patients with diffuse panbronchiolitis was further supported (Gomi et al. 2004).

42.2.1.2 Loss of Carbohydrate Binding and MASP-2 Auto-Activation in Mutated MBL

The mutations Gly25→Asp and Gly28→Glu disrupt the disulfide-bonding arrangement of the protein and cause at least a fivefold increase in the half-time of secretion of MBP compared with wild-type rat serum MBP. A similar phenotype, including a threefold increase in the half-time of
secretion, disruption of the disulfide bonding arrangement, and inefficient complement fixation, was observed when nearby glucosylgalactosyl hydroxylsine residues at positions 27 and 30 were replaced with arginine residues. The results suggest that defective secretion resulting from structural changes in the collagen-like domain is likely to be a contributory factor for MBP immunodeficiency (Heise et al. 2000).

To investigate the molecular defects associated with heterozygosity, rat serum MBP polypeptides (MBP-A: 56% identical in sequence to human MBP) and rat MBP polypeptides containing mutations associated with human immunodeficiency were co-expressed in mammalian expression system. The resulting proteins are secreted almost exclusively as heterooligomers that were defective in activating the complement cascade. Functional defects were caused by structural changes to the N-terminal collagenous and cysteine-rich domains of MBP, disrupting interactions with associated serine proteases. These mutations demonstrated how a SNP gives rise to the molecular defects that lead to the disease phenotype in heterozygous individuals (Wallis 2002). Wallis et al. (2005) analyzed the molecular and functional defects associated with two variant proteins of lectin pathway. Mutations Gly25→Asp and Gly28→Glu created comparable structural changes in rat MBL but the G28E variant activated complement >10-fold less efficiently than the G25D variant, which in turn had approximately sevenfold lower activity than wild-type MBL. Analysis of mutant MBL-MASP-2 complexes formed from recombinant proteins showed that reduced complement activation by both mutant MBLs was due to failure of activation of MASP-2 efficiently on binding to a mannan-coated surface. Disruption of MBL-MASP-2 interactions as well as to changes in oligomeric structure and reduced binding to carbohydrate ligands compared with wild-type MBL probably account for the intermediate phenotype of the G25D variant. However, carbohydrate binding and -MASP-2 activation are ostensibly completely decoupled in complexes assembled from the G28E mutant, such that the rate of MASP-2 activation is no greater than the basal rate of zymogen MASP-2 autoactivation. Analogous molecular defects in human MBL probably combine to create the mutant phenotypes of immunodeficient individuals (Wallis et al. 2005).

Since it is difficult to evaluate MBL in patients blood on the only basis of protein contents, or in combination with MBL genotyping due to possible association of altered oligomeric state of MBL, Dumestre-Perard et al. (2002) purified MBL from human plasma and showed the presence of MBL in two different oligomeric forms. Data on the specific activity of these forms showed that the higher oligomeric forms of MBL had the ability to induce C4 cleavage more efficiently than the corresponding lower oligomers (Dumestre-Perard et al. 2002).

How to Define Abnormal MBL Pathway and Disease Associations: Although, the association between MBL deficiency and risk of infection with other common diseases and death during years of follow-up has established the role of MBL in innate immune system, yet, in a large ethnically homogeneous Caucasian population, there was no evidence for significant differences in infectious disease or mortality in MBL-deficient individuals versus controls and suggested that MBL deficiency is not a major risk factor for morbidity or death in the adult Caucasian population (Dahl et al. 2004; Eisen and Minchinton 2003; Kilpatrick 2002; Summerfield et al. 1997). While addressing possible correlation between MBL levels and clinical conditions an issue is how to define MBL deficiency. The physiologically relevant MBL level leading to clinical manifestations is likely to differ in different diseases. In the examples given below, a number of different levels have been used as cut-off values defining MBL deficiency. Judged from clinical trials it appears that at least 200 ng MBL/mL plasma is needed for reconstituting in vitro functional activity (C4b deposition) after MBL infusion in MBL deficient individuals (Valdimarsson et al. 2004). On the other hand, in leukaemic patients a cut-off level of 500 ng/mL or more has been suggested (Peterslund et al. 2001; Neth et al. 2001), and in the cases of obstetric problems even lower levels (100 ng/mL) have been indicated.

42.2.2 MBL and Viral Infections

42.2.2.1 MBL and HIV Interaction

A broad range of proteins binds high-mannose carbohydrates found on the surface of the envelope protein gp120 of the HIV and thus interfere with the viral life cycle, providing a new method of controlling HIV infection. While glycosylation of HIV gp120/gp41 provides a formidable barrier for development of strong antibody responses to the virus, it also provides a potential site of attack by the innate immune system through MBL/MBP. The MBL that binds to HIV depends on the high-mannose glycans present on gp120 while host cell glycans incorporated into virions do not contribute substantially to this interaction. The MBL has been shown to interact with all tested HIV strains. However, drugs that alter processing of carbohydrates enhance neutralization of HIV primary isolates by MBL. Complement activation on gp120 and opsonization of HIV due to MBL binding have also been observed but these immune mechanisms have not been studied in detail. MBL has also been shown to block the interaction between HIV and DC-SIGN. Clinical studies show that levels of MBL, an acute-phase protein, increase during HIV disease. Because of apparently universal reactivity with HIV strains, MBL clearly represents an important mechanism for recognition
of HIV by the immune system. However, further studies are needed to define the in vivo contribution of MBL to clearance and destruction of HIV (Botos and Wlodawer 2005; Ji et al. 2005). MBL that binds to high mannose glycans on HIV-1 (gp120), has been shown to neutralize the cell line-adapted strain HIV (IIIB). But HIV primary isolates (PI) are generally more resistant to neutralization by antibodies. Considering that PI are produced in primary cells that could alter the number of high mannose glycans on HIV relative to cell lines, Ying et al. (2004) showed that both PI and cell line-adapted HIV, despite binding of MBL, are relatively resistant to neutralization by levels of MBL normally present in serum. However, binding and opsonization of HIV by MBL may alter virus trafficking and viral-antigen presentation during HIV infection (Ying et al. 2004).

In search of the effect of MBL-2 polymorphisms on susceptibility and progression of HIV-1 infection in children, Dzwonek et al. (2006) observed MBL deficiency more frequently in patients with severe disease. The study suggested that MBL-2 variants may be less frequent in children classified as long-term non-progressors (LTNPs) and hence MBL analysis can be useful in identifying children with slow disease progression and, consequently, may not require immediate antiretroviral treatment (Dzwonek et al. 2006). Production of HIV in the presence of the mannosidase I inhibitor deoxymanojirimycin (dMM) significantly enhanced binding of HIV to MBL and increased MBL neutralization of an M-tropic HIV primary isolate. In contrast, HIV cultured in presence of alpha-glucosidase I and II inhibitors, castanospermine and deoxynojirimycin showed only slight effect on virus binding and neutralization by MBL. The study suggested that specific alterations of the N-linked carbohydrates on HIV gp120/gp41 can enhance MBL-mediated neutralization of virus by strengthening the interaction of HIV-1 with MBL (Hart et al. 2003).

### 42.2.2.2 Susceptibility to RSV, CoV and HTLV

Respiratory syncytial virus (RSV) is the most important microbiological cause of lower respiratory tract infection (LRTI) in infants. MBL deficiency is the most common immunodeficiency on the African Continent. MBL deficiency has an impact on the hospitalization for LRTI caused by RSV in infants from Soweto, South Africa. But in contrary to expectations, results suggested no association between low levels of MBL or carriage of variant alleles and LRTI caused by RSV (Kristensen et al. 2004).

**Corona Virus Infection and Acute Respiratory Syndrome:**
Little is known about the innate immune response to severe acute respiratory syndrome (SARS) corona virus (CoV) infection. The MBL plays an important role in SARS-CoV infection. The distribution of MBL gene polymorphisms was significantly different between patients with SARS and normal subjects, with a higher frequency of haplotypes associated with low or deficient serum levels of MBL in patients with SARS. There was, however, no association between MBL genotypes, which are associated with low or deficient serum levels of MBL, and mortality related to SARS. MBL could bind SARS-CoV in calcium-dependent and mannan-inhibitable fashion in vitro, suggesting that binding is through the carbohydrate recognition domains of MBL. Furthermore, deposition of complement C4 on SARS-CoV was enhanced by MBL. Inhibition of the infectivity of SARS-CoV by MBL in fetal rhesus kidney cells suggested that MBL contributes to the first-line host defense against SARS-CoV and that MBL deficiency is a susceptibility factor for acquisition of SARS (Ip et al. 2005).

**Susceptibility to T-cell Lymphotropic Virus:** Pontes et al. (2005) investigated the association between MBL gene polymorphism and the susceptibility to human T-cell lymphotropic virus (HTLV) infection in 83 HTLV-infected asymptomatic subjects. Detection of MBL*A, MBL*B, and MBL*C was performed by amplifying a fragment of 349 bp (exon 1) and restriction fragment length polymorphism analysis with BanI and MboII endonucleases. A strong association has been demonstrated between MBL polymorphism and HTLV infection. Presence of genotype BB may be associated with the susceptibility to HTLV. Though further studies, with a larger number of individuals, are necessary, MBL polymorphism could have a possible impact on diseases associated with HTLV infection (Pontes et al. 2005).

### 42.3 Autoimmune and Inflammatory Diseases

Studies demonstrating the binding of MBL to the endothelium and causing excessive complement activation and subsequent tissue damage are known (Jordan et al. 2003), where as MBL deficiency may be advantageous in some circumstances since MBL may lead to an increased cytokine secretion by macrophages (Takahashi et al. 2002). There seems to be a delicate balance as to when MBL levels may be involved in harmful or in beneficial inflammation such as in the cardiovascular and other systems.

#### 42.3.1 MBL Gene in Rheumatoid Arthritis

The etiology of autoimmune diseases is largely unknown. Studies on associations between MBL deficiency and rheumatoid arthritis (RA) have been discussed in reviews (Graudal 2004; Barton et al. 2004). Results depend on ethnic groups, type of
patients and the symptoms studied. Low levels of serum MBL are associated with a higher erythrocyte sedimentation rate (ESR), joint swelling score, limitation of joint motion score, and annual increase in radiographic destruction score. Despite this, indications are that low MBL levels may be linked with symptoms indicating a poor prognosis as well as an earlier debut. Whether variant alleles of the MBL gene causing low serum concentrations of MBL, are associated with increased susceptibility to RA and erosive outcome in an inception cohort of patients with early polyarthritis was studied by Jacobsen et al. (2001). Jacobsen et al. (2001) suggested that MBL variant alleles appear to be weak susceptibility markers for RA, and patients with early polyarthritis and homozygous for MBL structural variant alleles have a higher risk of developing early erosive RA. These findings, together with the positive association between MBL variant alleles and the increased serum levels of IgM RF and CRP, point at the MBL gene as a relevant locus in the pathophysiology of RA. Graudal (2004) indicated that MBL-deficient patients have a relative risk of a severe radiographic event of 3.1 compared with the MBL competent group. The relative risk (RR) of early IL-1α auto-antibodies positive patients developing serious radiographic joint destruction was significantly lower than for IL-1β auto-antibodies-negative patients. Perhaps MBL and IL-1β auto-antibodies are predictors of prognosis of RA and play important roles in the pathogenesis of RA (Graudal 2004; Lee et al. 2005). Low levels of the protein have been related to a poor prognosis in rheumatoid arthritis perhaps due to the modulatory action that MBL of the protein have been related to a poor prognosis in rheumatopathogenesis of RA (Graudal 2004; Lee et al. 2005). Low levels predictors of prognosis of RA and play important roles in the exerts on the secretion of tumor necrosis factor.

Genotypes related to a lower production of MBL have also been linked to the development of systemic lupus erythematosus (Villarreal et al. 2001; Davies et al. 1995). MBL Deficiency as Risk of Infection and Autoimmunity

42.3.2 Systemic Lupus Erythematosus

Genetic factors play a major role in the development of SLE. More than 5% of cases are familial and the concordance rate between identical twins is 40%. Genetic studies in mice suggest a complex mechanism of transmission involving interactions among several susceptibility genes and, probably, protective genes. Genetic studies in humans have identified nearly 50 chromosomal areas possibly involved in lupus transmission. Significant linkage has been found for at least six regions, two on chromosome 1, one near the HLA region on chromosome 6, and three on chromosomes 2, 4, and 16, respectively. The genetic polymorphism of cytokines and, perhaps, of the T-cell receptor (TCR) may contribute to deregulate lymphocyte activity. The polymorphism of the Fc receptors of immunoglobulins may affect immune complex clearance, thereby promoting tissue damage. Further genetic studies are needed to enrich the fund of knowledge on lupus and to identify new targets for treatment (Perdriger et al. 2003).

From the several investigations on MBL and SLE the consensus is emerging that low levels of MBL predispose to development of the disease. However, the connection is not like for C1q where SLE develops in almost all of the rare cases of deficiency. Rather, it seems that MBL deficiency aggravates the disease or the development (Ohlenschlaeger et al. 2004; Takahashi et al. 2005). In SLE patients, MBL deficiency increase the risk for respiratory tract infections (Garred et al. 2001; Takahashi et al. 2005) as well as the risk of developing arterial thromboses (Ohlenschlaeger et al. 2004).

As with infectious disease, there is some evidence that the risk of pathology increases if there is another co-existing immune defect. For example, in a cohort of Spanish patients, the odds ratio for developing SLE was 2.4 for individuals with MBL deficiency, but this increased to 3.2 when there was also a co-existing partial C4 deficiency (Davies et al. 1997). Studies in patients with SLE have reported that MBL deficiency also influences their risk of developing certain complications, which include arterial thromboses (Ohlenschlaeger et al. 2004) and respiratory tract infections (Garred et al. 2001; Takahashi et al. 2005).

42.3.2.1 MBL Polymorphisms in SLE

Whether dysfunctional or deficient MBP variants are found with increased frequency in black patients with SLE was determined (Sullivan et al. 1996). Two structural polymorphisms of MBP, associated with low serum levels of MBP, were found with significantly increased frequency in the SLE patient population. In contrast, a promoter haplotype associated with particularly high serum levels of MBP was negatively associated with SLE. Thus, it seemed that deficiencies of MBP predispose individuals to SLE (Sullivan et al. 1996). The distribution of promoter variants of the MBL gene and correlations between the promoter variants and serum MBL concentrations in Chinese patients with SLE were investigated. Significant differences in the distribution of the two pairs of promoter polymorphisms, H/L and Y/X, between SLE patients and controls, were observed. Analysis of the correlation between promoter haplotypes and serum MBL levels revealed HY as the highest-producing, LY as the intermediate-producing, and LX as the lowest-producing haplotypes. The LX haplotype was present at a frequency of 0.259 in SLE patients and 0.154 in controls and was significantly associated with SLE. The low-producing promoter polymorphism of the MBL gene is associated with SLE, and a low serum MBL level is a risk factor for SLE (Ip et al. 1998). Whether occurrence, characteristics, and progression of SLE are associated with polymorphism of the MBL gene and with serum MBL concentration, Takahashi et al. (2005) reported that MBL gene...
polymorphism influences susceptibility to SLE, but has no
direct effect on disease characteristics. Serum MBL levels
fluctuate during the course of SLE in individual patients.
MBL genotyping may be useful in assessing the risk of infec-
tion during treatment of SLE (Takahashi et al. 2005). MBL and
FcγRII (CD32) polymorphisms have both been implicated as
candidate susceptibility genes in SLE. These patients carried
MBL codon 54 mutant allele more frequently than controls and
the haplotype HY W52 W54 W57 was significantly lower in
cases compared with controls. The MBL gene codon 54 mutant
allele appears to be a risk factor for SLE, whilst haplotypes
encoding for high levels of MBL are protective against the
disease. However, differences between controls and patients
were not significant when FcγRIIa polymorphisms were con-
sidered (Villarreal et al. 2001).

42.3.2.2 Lectin Pathway in Murine Lupus
Nephritis
In SLE, hypocomplementaemia and complement deposition
have been described both in man and in experimental models.
In mice, MBL is expressed in two forms, MBL-A and
MBL-C. In young and old MRL-lpr and control MRL+/+,
the declining levels of MBL-A and MBL-C showed a
high degree of correlation. In aged MRL-lpr mice in which
autoimmunity is most pronounced, high auto-Ab titers and
strong deposition of glomerular immune complexes were
associated with deposition of C1q, C3, MBL-A and
MBL-C. Thus, in addition to the classical pathway and the
alternative pathway, the lectin pathway of complement
activation is also involved in murine lupus nephritis (Trouw et al.
2005). SLE patients were associated with a reduced func-
tional activity of the MBL pathway of complement, in rela-
tion to expression of MBL variant alleles, increased levels of
autoantibodies against cardiolipin and C1q, but not against
MBL. The enhanced production of autoantibodies may be
related to disturbed clearance of apoptotic material due to
impaired MBL function (Seelen et al. 2005). Cohorts of
MRL-lpr mice, which are known to develop age-dependent
SLE-like disease showed that at 2 months of age all mice
already had elevated levels of anti-C1q autoantibodies, and
elution of kidneys confirmed the presence of these antibodies
in renal immune deposits in MRL-lpr mice and not in control
MRL+/+ mice. Thus, anti-C1q antibodies are already present
in serum and immune deposits of the kidney early in life and
therefore can play a role in nephritis during experimental
SLE-like disease in mice (Trouw et al. 2004).

**Effect of (S)-Armepavine on Autoimmune Disease of
MRL/MpJ-lpr/lpr Mice:** (S)-Armepavine (C19H23O3N;
MW313) from *Nelumbo nucifera* suppresses T cells prolifera-
tion. (S)-armepavine prevents lymphadenopathy and elongated
life span of MRL/MpJ-lpr/lpr mice, which have disease
features similar to human SLE. The action seemed to be
mediated by inhibition of splenocytes proliferation, suppres-
sion of IL-2, IL-4, IL-10, and IFN-γ gene expressions, reduct-
on of glomerular hypercellularity and immune complexes
deposition, and decrease of urinary protein and anti-double
stranded DNA autoantibody production. It has been suggested
that (S)-armepavine is an immunomodulator for the manage-
ment of autoimmune diseases like SLE (Liu et al. 2006a).

**42.3.2.3 Autoantibodies to C1q and MBL in SLE**
In SLE patients, there is an association between the occur-
rence of autoantibodies to C1q and MBL and renal involve-
ment. The presence of autoantibodies to MBL, analogous to
autoantibodies to C1q in patients with SLE, contributes to
the disease development. Anti-MBL autoantibodies were of
the IgG isotype and the binding site of IgG anti-MBL was
located in the F(ab′)2 portion. Anti-MBL are present in sera
from SLE patients and influence the functional activity of
MBL (Seelen et al. 2003). More SLE patients have IgG anti-
MBL antibodies than normal controls. However, in SLE,
these antibodies are neither sensitive nor specific for this
condition. They occur more frequently in (proliferative)
lupus nephritis, particularly during active disease. Further-
more, levels of anti-C1q rise, in many cases, prior to a
relapse of lupus nephritis, suggesting a pathogenic role for
the autoantibodies in immune complex-mediated renal dis-
ease. In addition, anti-C1q may interfere with the clearance
of apoptotic cells, so influencing induction and expression of
autoimmunity (Kallenberg 2008; Mok et al. 2004).

**Cardiovascular Disease and SLE:** Cardiovascular
disease is an important complication in patients with SLE. Variant
alleles of the MBL gene are associated with SLE as well as
with severe atherosclerosis. Ohlenschlaeger et al. (2004)
determined whether *MBL* variant alleles were associated
with an increased risk of arterial thrombosis among Danish
patients with SLE and suggested that among patients with
SLE, homozygosity for *MBL* variant alleles is associated
with an increased risk of arterial thrombosis. The risk of
venous thrombosis is not increased, indicating that MBL has
a specific role in providing protection against arterial throm-
bus (Ohlenschlaeger et al. 2004).

**42.3.3 Systemic Inflammatory Response
Syndrome/Sepsis**
A systemic inflammatory response syndrome (SIRS) is well
known in patients after major surgery (Bone 1992). Clinical
studies of critically ill patients requiring intensive care man-
agement have shown that individuals who are MBL deficient
are more likely to develop the SIRS and progress to septic
findings which may well relate to the proinflammatory cytokine response. In some cases, SIRS occurs in response to infection and “sepsis” is then used to describe the symptoms. More severely a septic shock may develop with multi-organ dysfunctions (MOD). The MBL levels and genotypes were investigated in 272 adults (197 with sepsis) prospectively admitted to the ICU. No difference was seen between genotype frequencies in patients with SIRS as compared to healthy controls. But the frequency of MBL variant genotypes was significantly higher in patients with sepsis compared with the patients without sepsis, and the risk ratios for the development of “severe sepsis” and “septic shock” ranged from 1.3 to 3.2 times higher in patients with A/O or O/O versus A/A genotype. MBL levels were inversely related to the severity of sepsis (Garred et al. 2003a, b). The SNP of MBL with clinical phenotype in critically ill Caucasians with SIRS are associated with increased prevalence of positive bacterial cultures at admission to the ICU. Patients in low MBL haplotype group did not have significantly increased rates of sepsis or septic shock at admission to the ICU. Survival at day 28 did not differ significantly between the low MBL haplotype and high MBL haplotype groups. Furthermore, MBL was not associated with a particular organism class. The prevalence of the codon 54 mutation of MBL gene in patients having repeated respiratory infections as well as the prevalence of MBL mutant genotype among patients with diffuse panbronchiolitis was further supported (Gomi et al. 2004).

In contrast, the prevalence of the MBL mutant genotype among patients with nontuberculous mycobacteria or Aspergillus chronic infection was not different from that in control subjects. Thus, SNPs in innate immunity receptors may alter recognition and clearance of bacteria without changing outcomes of critically ill adults with systemic inflammatory response syndrome (Sutherland et al. 2005). Polymorphisms of both codon 54 allele and promoter variants of the mannan MBL gene in patients with primary Sjogren’s syndrome (SS) was suggested as one of the genetic factors that determines susceptibility to SS (Wang et al. 2001).

Fidler et al. (2004) analyzed the MBL levels and genotypes levels in 100 critically ill children admitted to ICU. A sevenfold greater risk of developing SIRS within 48 h of admission (60% of the patients) was observed for those carrying MBL variant alleles than those with wild type alleles (A/O + O/O vs. A/A). A significant relation was also found between severity of the systemic response to infection and the presence of an MBL mutation. If the severity of illness among the patients admitted with infections was divided into localized infection, sepsis, and septic shock, the median MBL levels were inversely related to severity, and the children with MBL levels below 1,000 ng/mL had a greater chance of developing SIRS. A study of the frequency of sepsis in very low birth weight infants did not reveal statistical significance in clinical data between infants with and without specific mutations in a number of genes, including MBL genotypes (Ahrens et al. 2004). Following surgery of 156 patients undergoing major elective gastrointestinal surgery for malignant disease, Siassi et al. (2003) reported that patients who developed sepsis or SIRS showed significantly lower mean post-operative MBL levels. In colorectal cancer patients, postoperative infection is associated with poor prognosis. Ytting et al. (2005) have reported significantly increased frequency of pneumonia after primary operation in colorectal carcinoma patients who were having low MBL levels. MBL deficiency appears to play an important role in susceptibility of critical ill patients to the development and progression of sepsis and septic shock, and confers a substantially increased risk of fatal outcome. There is clearly a need for improvement in defining which patient groups and which clinical data are relevant to examine.

### 42.3.4 MBL and Inflammatory Bowel Diseases

Inflammatory bowel disease (IBD) is a pathological spectrum encompassing ulcerative colitis (UC), Crohn’s disease (CD), and indeterminate colitis. The resultant IBD phenotype is the consequence of multiple interactions between environmental factors, particularly enteric flora, and the host response to this environment, determined by immunogenetic, epithelial, and other non-immune genetic factors. MBL, as an important component of innate immunity, has engendered considerable research interest. In an early study of 340 unrelated patients with IBD genotyped for MBL2 exon 1 coding mutations, the frequency of deficient alleles was significantly lower in patients with UC than either the control group, or those with CD (Rector et al. 2001). The study by Rector et al. (2001) suggests that MBL deficiency could be protective against UC; alternatively, it could be interpreted that MBL deficiency, in individuals otherwise predisposed to IBD, may skew the phenotype away from the UC spectrum of disease towards CD. This concept is supported by another study that genotyped MBL2 in patients with CD, UC, or healthy controls (Seibold et al. 2004). The study also assessed anti-Saccharomyces cerevisiae antibody (ASCA) and MBL levels within the same subsets of patients, albeit slightly different numbers within each group. CD patients with MBL deficiency were significantly more likely to be positive for ASCA and for their lymphocytes to proliferate in response to mannan. Thus, it appears that MBL deficiency could impair normal processing of mannan-expressing microbial antigens, such as those found on the cell surface of many common microorganisms. The accumulated antigens could then stimulate the immune system, and contribute to the production of ASCA and possibly the pathogenesis of Crohn’s disease. Thus, MBL deficiency
might act primarily to influence IBD-specific phenotype in these patients. It should be noted, however, that a follow-up study, testing a larger cohort of CD patients \( n = 241 \), failed to confirm the significant association between variant MBL genotypes and ASCA positivity (Joossens et al. 2006). Seibold et al. (2004) suggested that the frequency of homozygous and compound heterozygous for variant exon 1 alleles differed significantly between patients suffering from CD or UC and the healthy controls.

### 42.3.4.1 Celiac Disease and MBL

Celiac disease is a multi-factorial/auto-immune disorder caused in genetically susceptible patients, by the ingestion of dietary gluten. Though very little is known about the genetic factors, but there is a strong association of two HLA haplotypes (DQ2 or DQ8) with celiac disease. DQ2 or DQ8, the non-

The study also analyzed apoptosis within small intestinal biopsy specimens, and showed that MBL tended to aggregate to areas of apoptosis within the epithelium. MBL has been implicated in the normal clearance of apoptotic bodies (Nauta et al. 2003; Ogden et al. 2001). The authors postulated that the association between MBL and celiac disease, and indeed other autoimmune conditions, could relate to impaired apoptosis, whereby MBL deficiency impairs the normal removal and clearance of apoptotic cells that may subsequently reveal previously hidden self-antigen, causing loss of self-tolerance, and spreading of autoimmunity (Boniotto et al. 2005). The association between variant \( MBL2 \) alleles and coeliac disease has also been confirmed within the Finnish population (Iltnan et al. 2003) (Worthley et al. 2006). The low MBL genotypes were strongly associated with more celiac disease symptoms as well with increased frequency of secondary autoimmune diseases. By immunohistochemistry MBL was found to be present, together with apoptotic cells, in the basal lamina under the intestinal epithelium, where they had previously found mRNA for MBL. Boniotto et al. suggested that impaired removal of apoptotic cells due to MBL deficiency might predispose to the development of autoimmune symptoms. Mice lacking MBL have been shown to be less efficient in removal of apoptotic cells (Stuart et al. 2005). In vitro studies have also implicated MBL in removal of apoptotic cells (Ogden et al. 2001; Nauta et al. 2004). Alternative explanation could be that increased susceptibility to intestinal infections and diarrhea, associated with low MBL, may change the intestinal epithelia thus allowing for abnormal stimulation of anti-gliadin immune responses and triggering of the cascade leading to celiac disease. A role for MBL like in celiac disease could be easily applied to other autoimmune disorders.

### 42.3.4.2 MBL and Gastrointestinal Infection

Despite the well-established role of MBL in innate immunity, there have been relatively few studies describing the clinical effect of MBL deficiency in enteric infections. One notable exception is the association between MBL deficiency and risk of \textit{Cryptosporidium parvum} enteritis. This study indicated that patients with biallelic coding mutations \((O/O)\) had a significantly greater chance of cryptosporidiosis compared to those who were either wild-type or heterozygous for \( MBL2 \) mutation (Kelly et al. 2000). The association between MBL deficiency and cryptosporidiosis was confirmed in young Haitian children by Kirkpatrick et al. (2006). However, the two combined studies present compelling evidence for the role of MBL in the host defense against \textit{Cryptosporidium spp.} infection. However, MBL deficiency was not associated with an increased risk of \textit{Escherichia coli} 0157: H7 colitis nor the complication of HUS (Proulx et al. 2003). \textit{H. pylori} is one of the most common human bacterial infections, affecting approximately 50% of humans. Several immunogenetic polymorphisms are associated with clinical outcomes in \textit{H. pylori} infection, as well as with the risk of infection itself. \textit{H. pylori} activates MBL in vitro (c/t Worthley et al. 2006). Studies demonstrated that \textit{H. pylori}-related chronic gastritis causes an increase in gastric mucosal MBL expression, but no association was found between \( MBL2 \) genotype and risk of chronic gastritis (Bak-Romaniszyn et al. 2006; Worthley et al. 2007).

MBL has been implicated in mediating gastrointestinal ischemia/reperfusion injury in mice (Hart et al. 2005). But MBL-null mice (deficient in the murine genes encoding MBL) developed only minor gut injury after induced ischemia/reperfusion insult compared to the wild-type mice. On the contrary, MBL has been implicated as a mediator of ischemia/reperfusion injury in both the myocardium (Walsh et al. 2005) and the kidney (Møller-Kristensen et al. 2005).

### 42.3.5 Rheumatic Heart Disease

Whereas MBL deficiency has been associated with rheumatic disorders, high MBL levels associated to disease has been reported by Hansen et al. (2003). Rheumatic fever (RF) is the most common cause of acquired valvular disease in
children and young adults. The pathogenic mechanisms responsible for the development of RF/RHD are associated to an abnormal host immune response (both at humoral and cellular level) to cross-reactive streptococcal antigens. The significantly elevated circulating MBL levels in patients with RHD together with the greater prevalence of MBL deficiency in controls suggest that MBL may cause undesirable complement activation contributing to the pathogenesis of RHD (Schafranski et al. 2004). Probably, MBL deficiency may represent an advantage against the development of rheumatic mitral stenosis and that increased MBL levels may be related to the development of RHD. Under normal conditions, MBL does not bind to the organism’s own tissues, but in situations of cellular hypoxia, glycosylation of cell surfaces may occur, leading to the deposition of MBL followed by complement activation. The significantly elevated levels of MBL observed in chronic RHD suggest that MBL may represent a pathogenic factor in the complex physiopathology of the disease, whereas MBL deficient individuals might be less susceptible to develop chronic RHD. Studies demonstrating the binding of MBL to the endothelium and causing excessive complement activation and subsequent tissue damage are known (Jordan et al. 2003), where as MBL deficiency may be advantageous in some circumstances since MBL may lead to an increased cytokine secretion by macrophages (Takahashi et al. 2002). Under some other conditions, MBL is associated to disease severity in both infectious and autoimmune disease (Garred et al. 1997, 1999). Although MBL is an acute-phase protein produced by the liver, its level only shows a moderate increase and determined genetically in inflammatory diseases. The elevated MBL levels in patients with chronic RHD might corroborate the chronic inflammatory activity present in these individuals and contribute to valve injury through complement activation. In addition, MBL may act as an immunomodulatory molecule, inducing a higher secretion of cytokines by macrophages (Turner and Hamvas 2000).

42.3.6 MBL in Cardio-Vascular Complications

There seems to be a delicate balance as to when MBL levels may be involved in harmful or in beneficial inflammation in the cardiovascular system. For example, Kawasaki disease is a systemic vasculitis in childhood possibly caused by infections and in the developed world Kawasaki disease is the most common cause of acquired heart disease in children (Royle et al. 2005). MBL as an initiator of inflammation, Biezeveld et al. (2003) while studying the frequency of MBL genotypes with Kawasaki disease in Dutch patients found a higher frequency of MBL mutations as compared to the genotypes in controls. Children younger than 1 year were at higher risk of development of CAD. Kawasaki disease occurs more frequent in oriental children (10 times more frequent than in Caucasians) (Royle et al. 2005). In Chinese, Cheung et al. (2004) did not see a difference between the MBL genotypes of patients and controls. The recent observation of an association between a human coronavirus and Kawasaki disease (Esper et al. 2005) fits with the many indications of MBL having antiviral activity. Plaque material may be removed from inside of the carotid artery (e.g., by endarterectomy) to avoid cerebral attack. Rugonfalvi-Kiss et al. (2005) indicated that female patients with genotypes associated with lower MBL levels had a slower rate to early restenosis, suggesting that a high level of MBL may be part of the pathophysiology of this condition.

In a study of 76 patients with severe atherosclerosis, Madsen et al. (1998) found that there were more patients with myocardial infarcts among Norwegians with low MBL allotypes than in controls. Saevarsdottir et al. (2005) found in a cohort study in Iceland that the risk of developing myocardial infarction was higher in MBL deficient individuals. The relationship between markers of innate immunity and clinical outcomes in patients with heart failure (HF) after acute myocardial infarction (AMI) suggested that atherogenesis and heart failure are associated with the altered control of inflammation by innate immune defenses, which include TLRs and MBL. Circulating levels of MBL and sTLR2 may reflect different aspects of the innate immune response and the involvement of innate immunity responses in the pathogenesis of post-MI heart failure (Ueland et al. 2006).

Lectin Pathway in Myocardial Ischemia-Reperfusion: MBL plays a dual role in modifying inflammatory responses to sterile and infectious injury. Although complement is widely accepted as participating in the pathophysiology of ischemia-reperfusion injury, the specific role of the lectin pathway has been addressed by Jordan et al. (2001). Since, blockade of the lectin pathway with inhibitory mAbs protects the heart from ischemia-reperfusion by reducing neutrophil infiltration and attenuating pro-inflammatory gene expression, it appears that the lectin complement pathway is activated after myocardial ischemia-reperfusion and leads to tissue injury.

Mice devoid of MBL-dependent lectin pathway activation but fully active for alternative and classical complement pathways, are protected from cardiac reperfusion injury with resultant preservation of cardiac function. Significantly, mice that lack a major component of the classical complement pathway initiation complex (C1q) but have an intact MBL complement pathway are not protected from injury. Thus, the MBL-dependent pathway of complement activation is the key regulator of myocardial reperfusion ischemic injury (Walsh et al. 2005).
Diabetic Patients at High Risk for Diabetic-Nephropathy: Whether lectin pathway of complement activation plays a role in the pathogenesis of human glomerulonephritis, is not well known. It has been proposed that MBL may bind to altered self components, which may possibly be found in diabetic patients, and MBL could thus be a potential pathogenic factor for diabetic cardiovascular complications, e.g., nephropathy (Hansen et al. 2005; Saevarsdottir et al. 2005). Normo-albuminuric type 1 diabetes have been found to have higher MBL levels than non-diabetic controls, with a stepwise increase in circulating MBL levels with increasing levels of urinary albumin excretion (Hansen et al. 2003). In another study, a significantly larger proportion of patients with diabetic nephropathy presented a MBL genotype associated with higher MBL level, when compared to the group with MBL genotypes associated with low MBL levels (Hansen et al. 2004). The elevated serum MBL levels in type 1 diabetic patients with diabetic nephropathy were confirmed by Saraheimo et al. (2005). In a long follow up study on 270 type 1 diabetic patients, it was found that for patients with type 1 diabetes and MBL- levels below the median of 1.6 µg/mL the risk of developing micro or macro-albuminuria was 26%, while the patients with MBL- levels above the median had a risk of 41% of developing micro or macro-albuminuria (Hovind et al. 2005). These studies suggested that a high MBL geno- and phenotype is associated with an increased risk of developing diabetic kidney disease and that assessing MBL status may prove beneficial in identifying patients at risk for micro- and macro-vascular complications (c/r Thiel et al. 2006).

MBL has been detected in the glomeruli of patients with lupus nephropathy, membranous nephropathy, membranoproliferative glomerulonephritis type I and anti-GBM nephritis. It was proposed that MBL binds to agalactosyl oligosaccharides of IgG that terminate in N-acetylglucosamine (Lhotta et al. 1999). Elevated serum MBL levels of MBL were implicated in the pathogenesis of renal manifestations of Henoch-Schönlein purpura (Endo et al. 2000), in IgA nephropathy (Endo et al. 1998), in other forms of human glomerulonephritis (Lhotta et al. 1999), and in vascular complications of diabetes mellitus type 1 (Hansen et al. 2003).

42.3.7 Other Inflammatory Disorders

42.3.7.1 Sarcoidosis and MBL Variants

Sarcoidosis is a chronic granulomatous disease of unknown aetiology. The causative agent may be an infectious microorganism. MBL variants predisposed to sarcoidosis by increasing their susceptibility to micro-organisms among sarcoidosis patients showed that the frequencies of variants were similar regardless of severity of disease outcome. MBL gene variants did not indicate to influence susceptibility to sarcoidosis, age of disease onset, or severity of disease. The average patient ages at time of diagnosis were similar for all MBL genotypes (Foley et al. 2000). However, a 57-year-old woman patient with pulmonary sarcoidosis suggested interstitial nephritis without proteinuria and hematuria, whereas a renal biopsy showed granulomatous interstitial nephritis and mild mesangial proliferative glomerulonephritis. From this case of renal sarcoidosis, it was hypothesized that P. acnes might be involved in pathogenesis of granulomatous interstitial nephritis and that it plays a role in glomerular complement activation via the lectin pathway (Hagiwara et al. 2005).

42.3.7.2 Behcet’s Disease

Behcet’s disease (BD) is a multisystemic, recurrent inflammatory disease caused by the combination of genetic and environmental factors. The haplotypes of the MBL2 gene can influence therapeutic response in BD, thus affecting the clinical symptoms in BD patients. The promoter region, MBL2-550*C/*C (L/L) homozygote was found to have a lower frequency in BD patients than that in controls. No difference was observed in the allele frequencies of G-221 C (Y/X), C + 4 T (P/Q) or Gly54Asp (A/B) of the MBL2 gene in BD patients and in controls. The HYPA haplotype contributed to BD occurrence, whereas the LYP A haplotype was negatively associated with BD. BD patients with several symptoms and with an earlier disease-onset age had a higher HYPA haplotype frequency. BD patients showing poor response (S) to therapy had a higher HYPA frequency than those showing good response (M). It appeared that possessing HYPA increases the risk of BD and that the MBL2 HYPA haplotype plays a role in MBL levels and increases the susceptibility to BD (Park et al. 2005).

42.3.7.3 Cystic Fibrosis (CF) Lung Disease

The MBL deficiency has been associated with poor outcome in cystic fibrosis (CF) lung disease. A mutation in MASP-2 and higher serum levels of MBL than healthy controls belonging to the MBL pathway in serum have been described. Thus, MBL pathway function is affected both by MBL and by MASP-2 genotypes (Carlsson et al. 2005). Patients undergoing abdominal aortic aneurysm (AAA) repair are exposed to an ischaemia-reperfusion injury (IRI), which is in part mediated by complement activation. During IRI, the patients undergoing AAA repair experience a mean decrease in plasma MBL level of 41% representing significant lectin pathway activation. This indicated that consumption of MBL occurs during AAA repair, which suggested an important role for lectin pathway in IRI. Hence, specific transient inhibition of lectin pathway activity can be of significant therapeutic value in patients undergoing open surgical AAA repair (Norwood et al. 2006).
In Guillain-Barre syndrome (GBS), complement activation plays a crucial role in the induction and extent of the post-infectious immune-mediated peripheral nerve damage. The MBL2 genotype, serum MBL level, and MBL complex activity are associated with the development and severity of GBS. The MBL2 B allele was associated with functional deficiency and relatively mild weakness. Studies support the hypothesis that complement activation mediated by MBL contributes to the extent of nerve damage in GBS, which is codetermined by the MBL2 haplotype (Geleijns et al. 2006).

### 42.3.7.4 Experimental Polymicrobial Peritonitis
Peritonitis is the most common and major complication in the treatment modality of peritoneal dialysis (PD) for uraemic patients. The contribution of the different complement activation pathways was studied in the host defense against experimental polymicrobial peritonitis induced by cecal ligation and puncture by using mice deficient in either C1q or factors B and C2. The C1q-deficient mice lack the classical complement activation pathway. Mice with a deficiency of both factors B and C2 lack complement activation via the classical, the alternative, and the lectin pathways and exhibited the maximum mortality of 92%, indicating a significant contribution of the lectin and alternative pathways of complement activation to survival (Windbichler et al. 2004). While examining the role of serum MBL concentration and point mutations in MBL gene in PD-related peritonitis, Lam et al. (2005) found that both homozygous and heterozygous patients had profoundly reduced serum level of MBL. Thus, dialysis patients having lower MBL levels may increase the susceptibility of infection.

### 42.4 Significance of MBL Gene in Transplantation

**MBL Replacement Therapy Following Stem Cell Transplantation:** Life-threatening complications such as graft versus host disease and infection remain major barriers to the success of allogeneic hematopoietic stem cell transplantation (SCT). Among various factors, MBL deficiency is a risk factor for infection in other situations where immunity is compromised. MBL2 coding mutations were associated with an increased risk of major infection following transplantation. MBL2 promoter variants were also associated with major infection. The high-producing haplotype HYA was associated with a markedly reduced risk of infection. Donor MBL2 coding mutations and recipient HYA haplotype were independently associated with infection in multivariate analysis. Thus, these results suggest that MBL2 genotype influences the risk of infection following allogeneic SCT and that both donor and recipient MBL2 genotype are important (Mullighan et al. 2002).

A retrospective study examining associations between polymorphisms in the gene encoding MBL, MBL2 and risk of major infection post-SCT was conducted in 96 related myeloablative transplants. The study showed that “low-producing” MBL2 coding alleles, when present in the donor, were significantly associated with increased risk of major infection in the recipient following neutrophil count recovery. Furthermore, a “high-producing” MBL2 haplotype, HYA, when present in the recipient, was protective against infection. Since MBL is under development as a therapeutic agent, findings suggest that administration of MBL may reduce the risk of infection post-transplant. Further work is required to confirm these results. These results indicate a report of a genetic determinant of risk of infection post-SCT, and highlight the importance of non-HLA genetic factors in determining the risk of transplant complications (Mullighan and Bardy 2004).

**Complement Activation Is Harmful for the Allograft Endothelium:** In heart transplant recipients, Fiane et al. (2005) recorded transplant-associated coronary artery disease and observed an association with MBL deficiency. They also recorded that acute rejection of the transplant was seen in 6 out of 6 with MBL deficiency as compared to 15 out of 32 with higher MBL levels. Assuming that MBL may interact with the transplanted tissue and initiate complement activation, this study added to the list of studies, which suggested that complement activation is harmful for the endothelium in general, and possibly for the allograft endothelium in particular.

**MBL Pathway and SPKT Graft Survival:** Simultaneous pancreas-kidney transplantation (SPKT) is the treatment of choice for patients with type 1 diabetes and renal failure. However, this procedure is characterized by a high rate of postoperative infections, acute rejection episodes, and cardiovascular mortality. The lectin pathway of complement activation contributes to cardiovascular disease in diabetes and may play an important role in inflammatory damage after organ transplantation. MBL serum levels and MBL genotypes in patients who received an SPKT from 1990 through 2000 and related graft survivals revealed that survival was significantly better in recipients with MBL gene polymorphisms associated with low MBL levels. Thus,
MBL is a potential risk factor for graft and patient survival in SPKT. It is hypothesized that MBL contributes to the pathogenesis of inflammation-induced vascular damage both in the transplanted organs and in the recipient’s native blood vessels (Berger et al. 2007). To address further the role of MBL deficiency, Verschuren et al. (2008) showed that high levels of serum MBL are associated with protection against urinary tract infections and, more specifically, against urosepsis after SPKT. These results indicate an important role for the lectin pathway of complement activation in antimicrobial defense in these transplant recipients (Verschuren et al. 2008).

42.5 MBL in Tumorigenesis

42.5.1 Polymorphisms in the Promoter

In paediatric oncology patients with febrile neutropenia, MBL levels are correlated to clinical and laboratory parameters. Structural exon-1 MBL2 mutations and the LX promoter polymorphism were related to deficient MBL levels. The capacity to increase MBL concentrations during febrile neutropenia was associated with MBL2 genotype. Infectious parameters did not differ between MBL-deficient and MBL-sufficient neutropenic children. However, most patients (61%) were severely neutropenic, compromising the opsonophagocytic effector function of MBL. MBL substitution might still be beneficial in patients with phagocytic activity (Frakking et al. 2003).

Five polymorphisms in the promoter and first exon of the MBL2 gene alter the expression and function of MBL in humans and are associated with inflammation-related disease susceptibility. These five polymorphisms create six well-characterized haplotypes that result in lower (i.e., LYB, LYC, HYD, and LXA) or higher (i.e., HYA and LYA) serum MBL concentrations. A statistically significant association was found between the X allele of the promoter Y/X polymorphism (which results in a lower serum MBL concentration) and improved lung cancer survival among white patients but not among African American patients. The functional Y/X polymorphism of the innate-immunity gene MBL2 and MBL2 haplotypes and diplotypes appear to be associated with lung cancer survival among white patients (Pine et al. 2007). A significantly higher incidence of MBL deficiency/insufficiency-associated genotypes was found among patients with malignant disease. Findings reflecting anti-tumorigenic activity of MBL protein suggest potential therapeutic application. However, it cannot be excluded that mbl-2 mutant alleles may be in linkage disequilibrium with an unidentified tumor susceptibility gene(s) (Swierzko et al. 2007).

42.5.2 MBL Binding with Tumor Cells

Changes in cell surface structures during oncogenic transformation appear to promote binding of MBL to cancer cells (Hakomori 2001) where the protein can mediate cytotoxic effects including MBL-dependent cell mediated cytotoxicity (Ma et al. 1999; Nakagawa et al. 2003). Experimental studies suggest that MBL (both wild-type and the mutant B allele) may possess anti-colorectal cancer tumor activity (Ma et al. 1999). The MBP/MBL binds specifically to oligosaccharides expressed on the surface of human colorectal carcinoma cells, SW1116. MBL binding occurs in colon adenocarcinoma cell lines (Colo205, Colo201 and DLD-1), but not in any of the leukemic cell lines. The binding of MBL to these cell lines was sugar-specific and calcium-dependent. The degree of MBL binding was correlated with the expression of Lewis A and Lewis B antigens on these cell lines (Muto et al. 1999). Intra-tumoral administration of the recombinant vaccinia virus carrying a MBL2 gene significantly reduced tumor size as compared to controls, along with prolonged survival of mice (Ma et al. 1999). However, these results were not reflected in clinical trials. In fact, patients with colorectal cancer have increased activation of the lectin-complement pathway and increased levels of serum MBL (Ytting et al. 2004). In patients undergoing surgery for colorectal cancer, however, low pre-operative levels of serum MBL have been linked to an increased risk of developing post-colectomy pneumonia (Ytting et al. 2005). Further studies may clarify the role of the lectin-complement pathway in colorectal cancer.

42.6 Complications Associated with Chemotherapy

42.6.1 Neutropenia

Secondary immunodeficiencies due to disease or treatment have provided interesting patient populations within which to study the role of MBL. One such group comprises those receiving chemotherapy for malignancy. In these patients,
chemotherapy induces neutropenia and increased risk of infection. Studies have thus attempted to analyze the correlation between MBL deficiency and infections in such patients. It is difficult to compare these studies since these studies include patients with a variety of underlying malignancies and variety of chemotherapy, different combinations of antimicrobial agents and various other factors (Klein and Kilpatrick 2004). However, there is clear evidence of the importance of MBL for protection in leukemia patients. MBL genotypes and MBL levels were correlated to the causes, frequency and duration of febrile neutropenic periods in children receiving chemotherapy (Neth et al. 2001). The majority of children were patients of acute lymphoblastic leukemia (ALL). Children with variant MBL alleles exhibited twice as many days of febrile neutropenia as children with wild type genotypes. Analysis by MBL quantification supported this as children with less than 1 μg MBL/mL had a higher number of days with febrile neutropenia. Peterslund et al. (2001) described infections defined as bacteremia, pneumonia or both in hematological malignancies of 54 adults treated with chemotherapy. All patients with the infections, except one, showed MBL levels below 0.5 μg/mL. Vekemans et al. (2005) conducted a prospective observational study focusing on assessment of MBL as a risk factor for infection during chemotherapy induced neutropenia in adult hematological cancer patients. They included 255 patients and determined MBL levels as well as MBL genotypes. A higher rate of severe infection was seen in MBL deficient patients. The impact was further increased when acute leukaemic patients were excluded. In a contrasting study, Bergmann et al. (2003) followed 80 adults undergoing therapy for acute myeloid leukaemia (AML), which involves intense highly myelo-suppressive treatment. They found no effect of MBL deficiency on frequency, severity or duration of fever and suggested that the severe immunosuppression induced by the combination of the myeloid cancer and chemotherapy may obscure the normal effector functions of MBL, though, Kilpatrick et al. (2003) failed to see anything but a modest effect of MBL levels below 100 ng/mL in a retrospective study on 128 patients, most of whom were prepared for bone marrow transfer and more than half presented with AML. Results cast doubt on the potential value of MBL replacement therapy in this clinical context (Kilpatrick et al. 2003). A growing body of evidence indicates that genetic factors are involved in an increased risk of infection. MBL gene polymorphisms that cause low levels of MBL are associated with the occurrence of major infections in patients, mainly bearing hematological malignancies, after high-dose chemotherapy (HDT) rescued by autologous peripheral blood stem cell transplantation (auto-PBSCT). A retrospective examination of 113 patients treated with HDT and auto-PBSCT revealed that the low-producing genotypes, B/B and B/LXA, were associated with major bacterial infection. A nation-wide study, conducted to assess the allele frequency of the MBL coding mutation in a total of 2,623 healthy individuals in Japan, revealed the frequency of allele B as 0.2, almost the same in seven different areas of Japan. This common occurrence suggested that MBL deficiency may play an important role in the clinical settings of immune-suppression (Horiuchi et al. 2005). Studies by Aittoniemi et al. (1999) in patients with chronic lymphocytic leukemia did not observe any effect of MBL on infections. A possible association between MBL genotypes and severe infections in patients with multiple myeloma receiving moderate strength induction chemotherapy has been studied. From the MBP genotypes, identified in bone marrow biopsies, the study concluded that during induction chemotherapy in patients with multiple myeloma, a general protective effect of wild-type MBL2 against chemotherapy-related infections was not apparent. However, indications were there of a reduced occurrence of sepsicaemia in patients with wild-type compared with variant MBL2. Further studies in larger cohorts of patients are relevant (Molle et al. 2006). Thus, further studies are required to describe the patients particularly at risk when being MBL deficient.

### 42.6.2 Animal Studies

The MBL knock-out mice have made possible experimental investigations of the effect of MBL deficiency. The mouse has two genes encoding different MBL molecules (MBL-A and -C) compared to one in humans. Both MBLs in mice are able to bind to carbohydrate surfaces and activate the complement system. A slight difference in carbohydrate specificity has been reported for the two mouse MBLs. Mice with only MBL-A knocked-out were first produced, but only mice with both MBL-A and -C knocked out (MBL DKO) are suitable as animal model of human MBL deficiency. In 2004, Shi et al. demonstrated that MBL DKO sepsis model mice were highly susceptible to intravenous inoculation via tail vein with Staphylococcus aureus, all dying within 48 h, compared with 55% survival of MBL wild-type mice. Infusion of recombinant MBL reversed the phenotype. No difference was seen when the bacteria were injected intra-peritoneally. However, if the mice were treated with cyclophosphamide, simulating chemotherapy-induced neutropenia, before the intra peritoneal infection, the MBL DKO had more abscesses than the wild type. The MBL DKO mice were also more susceptible to challenge with herpes simplex virus type 2 (Gadjeva et al. 2004). In line with the suggested involvement of MBL in autoimmune diseases the MBL DKO mice were examined for autoimmune symptoms when 18-month-old (Stuart et al. 2005). No such signs were observed. On the other hand, the ability to clear apoptotic
cells was less efficient in the MBL knock-outs. It has been hypothesized that while MBL does not bind significantly to healthy tissue, changes due to abnormal conditions might reveal MBL ligands. Indeed, MBL is expressed by some tumor cell lines, and gene therapy with an MBL-vaccinia construct was found protective in nude mice transplanted with a human colorectal cancer cell line (Ma et al. 1999). In vitro studies have indicated binding of MBL to cells exposed to hypoxia-reoxygenation (simulating ischemia/reperfusion) and subsequently it was shown that infusion of a blocking anti-MBL antibody would protect against myocardial destruction following ischemia/reperfusion in a rat model (Jordan et al. 2001). Using MBL DKO mice Möller-Kristensen et al. (2005) found, in a model of kidney ischemia reperfusion (I/R) injury, that the MBL DKO were partially protected as evidenced by a better kidney function in these mice after ischemia/reperfusion. Increased deposition of the complement factor C3 was seen in wild type mice, and binding of MBL to sections of kidney could be inhibited with mannose. In agreement with this, deVries et al. (2004) found MBL-A and -C deposited in the kidneys after ischemia/reperfusion in MBL wild type mice. The recombinant vaccinia virus carrying human MBP gene possesses a potent growth-inhibiting activity against human colorectal carcinoma cells transplanted in KSN nude mice. The treatment resulted into a prolonged life span of tumor-bearing mice. Local production of MBP had a cytotoxic activity, which was proposed as MBP dependent cell-mediated cytotoxicity (MDCC). This study offers a model for the development of an effective and specific host defense factor for cancer gene therapy (Ma et al. 1999; Thiel et al. 2006).

42.7 MBL: A Reconstitution Therapy

Since genetically determined MBL deficiency is very common and can be associated with increased susceptibility to a variety of infections, the potential benefits of MBL reconstitution therapy need to be evaluated. In a phase I trial on 20 MBL-deficient healthy adult volunteers receiving a total of 18 mg of MBL in three 6 mg doses given (i.v.), once a week for a period of 3 weeks did not show adverse clinical changes or any sign of infusion-associated complement activation. Study suggested that infusion of purified MBL as prepared by Statens Serum Institut (SSI) is safe. However, adults have to be given at least 6 mg twice or thrice weekly for maintaining protective MBL levels assumed to be about 1,000 ng/mL (Valdimarsson et al. 2004). Considerations of MBL genotyping and association with infection opens the possibility of producing clinical grade recombinant MBL that resulted in establishing a company having this aim (Jenseni 2003). The treatment of chronic disorders may possibly also be considered on the longer term. The invention led to use of at least MBL oligomer comprising at least one MBL subunit, for the manufacture of a medicament for prophylaxis and/or treatment of infection (Thiel and Jenseni 2007).

References

Ahrens P, Kattner E, Kohler B et al (2004) Mutations of genes involved in the innate immune system as predictors of sepsis in very low birth weight infants. Pediatr Res 55:652–656

Aittoniemi J, Miittenen A, Laine S et al (1999) Opsonising immunoglobulins and mannanbinding lectin in chronic lymphocytic leukemia. Leuk. Lymphoma 34:381–385

Ambrosio AR, De Messias-Reason IJ (2005) Leishmania (Viannia) braziliensis: interaction of mannos-binding lectin with surface glycoconjugates and complement activation. An antibodindependent defence mechanism. Parasite Immunol 2:333–340

Bak-Romaniszyn L, Cedzyński M, Szmekj J et al (2006) Mannanbinding lectin in children with chronic gastritis. Scand J Immunol 63:131–5

Barton A, Platt H, Salway C et al (2004) Polymorphisms in the mannos binding lectin (MBL) gene are not associated with radiographic erosions in rheumatoid or inflammatory polyarthritis. J Rheumatol 31:442–447

Bellamy R, Ruwende C, McAdam KP et al (1998) Mannose binding protein deficiency is not associated with malaria, hepatitis B carriage nor tuberculosis in Africans. Q J Med 91:13–18

Berger SP, Roos A, Mallat MJ et al (2005) Association between mannos-binding lectin levels and graft survival in kidney transplantation. Am J Transplant 5:1361–1366

Berger SP, Roos A, Mallat MJ et al (2007) Low pretransplantation mannos-binding lectin levels predict superior patient and graft survival after simultaneous pancreas-kidney transplantation. J Am Soc Nephrol 18:2416–2422

Bergmann OJ, Christiansen M, Laursen I et al (2003) Low levels of mannos-binding lectin do not affect occurrence of severe infections or duration of fever in acute myeloid leukaemia during remission induction therapy. Eur J Haematol 70:91–97

Bernig T, Boersma BJ, Howe TM et al (2007) The mannos-binding lectin (MBL2) haplotype and breast cancer: an association study in African-American and Caucasian women. Carcinogenesis 28:828–836

Beievezel MH, Kuipers IM, Geissler J et al (2003) Association of mannos-bindng lectin genotype with cardiovascular abnormalities in Kawasaki disease. Lancet 361:1268–1270

Bodamer OA, Mitterer G, Maurer W et al (2006) Evidence for an association between mannos-binding lectin 2 (MBL2) gene polymorphisms and pre-term birth. Genet Med 8:518–524

Bone RC (1992) Toward an epidemiology and natural history of SIRS (systemic inflammatory response syndrome). JAMA 268: 3452–3455

Bonito M, Braida L, Spano A et al (2002) Variant mannos-binding lectin alleles are associated with celiac disease. Immunogenetics 54:596–598

Bonito M, Braida L, Baldass V et al (2005) Evidences of a correlation between mannos binding lectin and celiac disease: a model for other autoimmune diseases. J Mol Med 83:308–316

Botos I, Wlodawer A (2005) Proteins that bind high-mannose sugars of glycoconjugates and complement activation. An antibody-dependent defence mechanism. Parasite Immunol 2:333–340

Bouwman LH, Roos A, Terpstra OT, de Knijff P et al (2005) Mannose binding lectin gene polymorphisms confer a major risk for severe infections after liver transplantation. Gastroenterology 129:408
Cambi A, Figdor CG (2005) Levels of complexity in pathogen recognition by C-type lectins. Curr Opin Immunol 17:345–351

Carlsson M, Sjoholm AG, Eriksson L et al (2005) Deficiency of the mannose-binding lectin pathway of complement and poor outcome in cystic fibrosis: bacterial colonization may be decisive for a relationship. Clin Exp Immunol 139:306–313

Cheung YF, Ho MH, Ip WK et al (2004) Modulating effects of mannose-binding lectin genotype on arterial stiffness in children after Kawasaki disease. Pediatr Res 56:591–596

Dahl M, Tybaerg-Hansen A, Schnorr P et al (2004) A population-based study of morbidity and mortality in mannose-binding lectin deficiency. J Exp Med 199:1391–1399

Davies EJ, Snowden N, Hillarby MC et al (1995) Mannose-binding protein gene polymorphism in systemic lupus erythematosus. Arthritis Rheum 38:110–114

Davies EJ, Teh LS, Ordi-Ros J et al (1997) A dysfunctional allele of the mannose binding protein gene associates with systemic lupus erythematosus in a Spanish population. J Rheumatol 24:485–488

deVries B, Walter SJ, Peutz-Kootstra CJ et al (2004) The mannose-binding lectin-pathway is involved in complement activation in the course of renal ischemia-reperfusion injury. Am J Pathol 165:1677–1688

Dommert RM, Klein N, Turner MW (2006) Mannose-binding lectin in innate immunity: past, present and future. Tissue Antigens 68:193–209

Downing I, Koch C, Kilpatrick DC (2003) Immature dendritic cells possess a sugar-sensitive receptor for human mannann-binding lectin. Immunology 109:369–364

Downing I, MacDonald SL, Turner ML et al (2005) Detection of an autologous ligand for mannose-binding lectin on human B lymphocytes. Scand J Immunol 62:507–514

Dumestre-Percard C, Ponard D, Arlaud GJ et al (2002) Evaluation and clinical interest of mannann-binding lectin function in human plasma. Mol Immunol 39(7–8):465–473

Dzowneak A, Novelli V, Bajaj-Elliott M et al (2006) Mannose-binding lectin in susceptibility and progression of HIV-1 infection in children. Antivir Ther 11:499–506

Eisen DP, Minchinton RM (2003) Impact of mannose-binding lectin on susceptibility to infectious diseases. Clin Infect Dis 37:1496–1506

Endo M, Ohi H, Ohwasa I et al (1998) Glomerular deposition of mannose-binding lectin (MBL) indicates a novel mechanism of complement activation in IgA nephropathy. Nephrol Dial Transplant 13:1984–1990

Endo M, Ohi H, Ohwasa I et al (2000) Complement activation through the lectin pathway in patients with Henoch-Schönlein purpura nephritis. Am J Kidney Dis 35:401–719

Esper F, Shapiro ED, Weibel C et al (2005) Association between a novel human coronavirus and Kawasaki disease. J Infect Dis 191:499–502

Fiane AE, Ueland T, Simonsen S et al (2005) Low mannose-binding lectin and increased complement activation correlate to allograft vasculopathy, ischaemia, and rejection after human heart transplantation. Eur Heart J 26(16):1660–1665

Fidler KJ, Wilson P, Davies JC et al (2004) Increased incidence and severity of the systemic inflammatory response syndrome in patients deficient in mannose-binding lectin. Int Care Med 30:1438–1446

Foley PJ, Mullighan CG, McGrath DS et al (2000) Mannose-binding lectin promoter and structural gene variants in sarcoidosis. 30:549–552

Frakking F, van de Wetering M et al (2003) The role of mannose-binding lectin (MBL) in paediatric oncology patients with febrile neutropenia. Eur J Cancer 42:909–916

Gadjeva M, Paludan SR, Thiel S et al (2004) Mannan-binding lectin modulates the response to HSV-2 infection. Clin Exp Immunol 138:304–311

Garred P, Thiel S, Madsen HO et al (1992) Gene frequency and partial protein characterization of an allelic variant of mannann binding protein associated with low serum concentrations. Clin Exp Immunol 90:517–521

Garred P, Madsen HO, Baslev U et al (1997) Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose binding lectin. Lancet 349:236–240

Garred P, Madsen HO, Halberg P et al (1999) Mannose-binding lectin polymorphism and susceptibility to infection in systemic lupus erythematosus. Arthritis Rheum 42:2145–2152

Garred P, Voss A, Madsen HO, Junker P (2001) Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. Genes Immun 2:442–450

Garred P, Larsen F, Madsen HO, Koch C (2003a) Mannose-binding lectin deficiency-revisited. Mol Immunol 40:73–84

Garred P, Strom JJ, Quist L et al (2003b) Association of mannose-binding lectin polymorphisms with sepsis and fatal outcome, in patients with systemic inflammatory response syndrome. J Infect Dis 188:1394–1403

Garred P, Larsen F, Seyfarth J, Fujita R, Madsen HO (2006) Mannose-binding lectin and its genetic variants. Genes Immun 7:85–94

Geleijns K, Roos A, Houwing-Duistermaat JJ et al (2006) Mannose-binding lectin contributes to the severity of Guillain-Barre syndrome. J Immunol 177:4211–4217

Gomi K, Tokue Y, Kobayashi T et al (2004) Mannose-binding lectin gene polymorphism is a modulating factor in repeated respiratory infections. Chest 126:95–99

Graudal N (2004) The natural history and prognosis of rheumatoid arthritis: association of radiographic outcome with process variables, joint motion and immune proteins. Scand J Rheumatol Suppl 118:1–38

Graudal NA, Madsen HO, Tarp U et al (2000) The association of variant mannose-binding lectin genotypes with radiographic outcome in rheumatoid arthritis. Arthritis Rheum 43:515–521

Gupta K, Gupta RK, Hajela K (2008) Disease associations of mannose-binding lectin & potential of replacement therapy. Indian J Med Res 127:431–440

Hagisawa S, Ohi H, Eishi Y et al (2005) A case of renal sarcoidosis with complement activation via the lectin pathway. Am J Kidney Dis 45:580–587

Hakomori S (2001) Tumor-associated carbohydrate antigens defining tumor malignancy: basis for development of anti-cancer vaccines. Adv Exp Med Biol 491:369–402

Hansen TK (2005) Mannose-binding lectin (MBL) and vascular complications in diabetics, Horm Metab Res 37(suppl 1):1–4

Hansen TK, Thiel S, Knudsen ST et al (2003) Elevated levels of mannose-binding lectin in patients with type 1 diabetes. J Clin Endocrinol Metab 88:4857–4861

Hansen TK, Tarnow L, Thiel S, Parving HH, Flyvbjerg A et al (2004) Association between mannose-binding lectin and vascular complications in type 1 diabetes. Diabetes 53:1570–1576

Hart ML, Saifuddin M, Spear GT (2003) Glycosylation inhibitors and neureaminidase enhance human immunodeficiency virus type 1 binding and neutralization by mannose-binding lectin. J Gen Virol 84(Pt 2):353–360

Hart ML, Ceonzo KA, Shaffer LA et al (2005) Gastrointestinal ischemia-reperfusion injury is lectin complement pathway dependent without involving C1q. J Immunol 174:6373–6380

Heise CT, Nicholls JR, Leamy CE, Wallis R (2000) Impaired secretion of rat mannose-binding protein resulting from mutations in the collagen-like domain. J Immunol 165:1403–1409

Hjalgrim LL, Madsen HO, Melbye M et al (2002) Presence of clone-specific markers at birth in children with acute lymphoblastic leukaemia. Br J Cancer 87:994–999

Horiuchi T, Gondo H, Miyagawa H et al (2005) Association of MBL gene polymorphisms with major bacterial infection in patients treated with high-dose chemotherapy and autologous PBSCT. Genes Immun 6:162–166
Turner MW, Hamvas RM (2000) Mannose-binding lectin: structure, function, genetics and disease associations. Rev Immunogenet 2:305–322
Ueland T, Espevik T, Kjekshus J et al (2006) Mannose binding lectin and soluble Toll-like receptor 2 in heart failure following acute myocardial infarction. J Card Fail 12:659–663
Valdimarsson H, Vikingsdottir T, Bang P et al (2004) Human plasma-derived mannose-binding lectin: a phase I safety and pharmacokinetic study. Scand J Immunol 59:97–102
Vekemans M, Georgala A, Heymans C et al (2005) Influence of mannann binding lectin serum levels on the risk of infection during chemotherapy-induced neutropenia in adult haematological cancer patients. Clin Microbiol Infect 11(suppl 2):20
Verschuren JJ, Roos A, Schaapherder AF et al (2008) Infectious complications after simultaneous pancreas-kidney transplantation: a role for the lectin pathway of complement activation. Transplantation 85:75–80
Villarreal J, Crosdale D, Ollier W et al (2001) Mannose binding lectin and FcγRIIa (CD32) polymorphism in Spanish systemic lupus erythematosus patients. Rheumatology 40:1009–1012
Wallis R (2002) Dominant effects of mutations in the collagenous domain of mannose-binding protein. J Immunol 168:4553–4558
Wallis R, Lynch NJ, Roscher S et al (2005) Decoupling of carbohydrate binding and MASP-2 autoactivation in variant mannose-binding lectins associated with immunodeficiency. J Immunol 175:6846–6851
Walsh MC, Bourcier T, Takahashi K et al (2005) Mannose-binding lectin is a regulator of inflammation that accompanies myocardial ischemia and reperfusion injury. J Immunol 175:541–546
Wang ZY, Morinobu A, Kanagawa S et al (2001) Polymorphisms of the mannose binding lectin gene in patients with Sjögren’s syndrome. Ann Rheum Dis 60:483–486
Werth VP, Berlin JA, Callen JP et al (2002) Mannose binding lectin (MBL) polymorphisms associated with low MBL production in patients with dermatomyositis. J Invest Dermatol 119:1394–1399
Windbichler M, Echtenacher B, Hehlgs T et al (2004) Involvement of the lectin pathway of complement activation in antimicrobial immune defense during experimental septic peritonitis. Infect Immun 72:5247–5252
Worthley DL, Bardy PG, Mullighan CG (2005) Mannose-binding lectin: biology and clinical implications. Intern Med J 35:548–556
Worthley DL, Bardy PG, Gordon DL et al (2006) Mannose-binding lectin and maladies of the bowel and liver. World J Gastroenterol 12:6420–6428
Worthley DL, Mullighan CG, Dean MM et al (2007) Mannose-binding lectin deficiency does not increase the prevalence of Helicobacter pylori seropositivity. Eur J Gastroenterol Hepatol 19:147–152
Ying H, Ji X, Hart ML, Gupta K et al (2004) Interaction of mannose-binding lectin with HIV type 1 is sufficient for virus opsonization but not neutralization. AIDS Res Hum Retroviruses 20:327–336
Ytting H, Jensenius JC, Christensen IJ, Thiel S, Nielsen HJ (2004) Increased activity of the mannann binding lectin complement activation pathway in patients with colorectal cancer. Scand J Gastroenterol 39:674–679
Ytting H, Christensen IJ, Jensenius JC et al (2005) Preoperative mannose-binding lectin pathway and prognosis in colorectal cancer. Cancer Immunol Immunother 54:265–272