**Mannose-binding lectin in innate immunity: past, present and future**

**R. M. Dommett**, **N. Klein** & **M. W. Turner**

1 Infectious Diseases and Microbiology Unit, Institute of Child Health, UCL, 30 Guilford Street, London WC1N 1EH, UK
2 Immunobiology Unit, Institute of Child Health, UCL, 30 Guilford Street, London WC1N 1EH, UK

**Key words** complement; disease associations; innate immunity; mannose-binding lectin (MBL); MBL-associated serine proteases (MASPs)

**Abstract**

The human collectin, mannose-binding lectin (MBL), is an important protein of the humoral innate immune system. With multiple carbohydrate-recognition domains, it is able to bind to sugar groups displayed on the surfaces of a wide range of microorganisms and thereby provide first-line defence. Importantly, it also activates the complement system through a distinctive third pathway, independent of both antibody and the C1 complex. Three single point mutations in exon 1 of the expressed human *MBL*-2 gene appear to impair the generation of functional oligomers. Such deficiencies of functional protein are common in certain populations, e.g. in sub-Saharan Africa, but virtually absent in others, e.g. indigenous Australians. MBL disease association studies have been a fruitful area of research and implicate a role for MBL in infective, inflammatory and autoimmune disease processes. Overall, there appears to be a genetic balance in which individuals generally benefit from high levels of the protein. However, in certain situations, reduced levels of circulating MBL may be beneficial to the host and this may explain the persistence of the deleterious gene polymorphisms in many population groups.

**Introduction**

It is now 60 years since the Australian Nobel prize winner Sir Frank Macfarlane Burnet, together with John McCrea, identified three inhibitors in serum (called α, β and γ), which were able to inactivate influenza virus (1). We now know that the β inhibitor was, in fact, a protein called mannose-binding lectin (MBL), a component of the innate immune system (2). During the past 30 years, our understanding of this protein has steadily increased as a result of extensive research activity in three main areas: (a) bio/immunochemistry (including molecular genetics), (b) microbiology and (c) immunodeficiency. Work in these areas initially proceeded independently as evidence for both an inexplicable biological function and a clinical deficiency state emerged. The isolation and characterization of the protein were necessary in order to illuminate the observations of the so-called RaRf bactericidal activity (3, 4) in the microbiology area and the opsonic deficiency reported in many paediatric populations. Some of the main developments are summarized in Table 1. This review briefly addresses issues relating to the early history of MBL, its structure, function, genetics and disease associations. Finally, future developments including the potential use of both plasma-derived and recombinant MBL are discussed.

The existence of mammalian serum lectins was first predicted in 1975 by Robinson et al. (5), and the protein was first isolated in 1978 from cytosolic fractions of rabbit liver by Kawasaki et al. (6). Subsequently, Wild et al. (7) were able to isolate MBL from both human and rat liver. More recently, extrahepatic transcription of MBL has been reported and this may have implications regarding its role in localized host defence (8).

MBL belongs to a family of proteins called the collectins, which possess both collagenous regions and lectin domains. The other major human collectins, surfactant protein A and surfactant protein D, possess structural characteristics similar to those of MBL and are found predominantly in the lung and other mucosal sites (9).
| Year | Biochemistry/immunochemistry | Microbiology | Immunodeficiency |
|------|-----------------------------|--------------|-----------------|
| 1946 | Identification of β inhibitors of heat-labile components of influenza virus in normal serum with both virus-neutralizing activity and haemagglutination-inhibiting activity (1) | | Plasma-associated phagocytic defect (28) |
| 1968 | Existence of mammalian serum lectin-like proteins specific for mannose predicted (5) | | Association of opsonic defect with frequent infections in infancy, but deficiency also present in 5% of the general population (29) |
| 1975 | MBL isolated from rabbit liver (6) | | | |
| 1976 | Plasma-associated phagocytic defect (28) | | Association of opsonic defect with frequent infections in infancy, but deficiency also present in 5% of the general population (29) |
| 1978 | MBL isolated from rabbit liver (6) | | Association of opsonic defect with suboptimal C3b deposition (31) |
| 1980 | MBL isolated from rabbit liver (6) | | Association of opsonic defect with suboptimal C3b deposition (31) |
| 1981 | MBL isolated from rabbit liver (6) | | Association of opsonic defect with suboptimal C3b deposition (31) |
| 1982 | MBL isolated from rabbit liver (6) | | Association of opsonic defect with suboptimal C3b deposition (31) |
| 1983 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1984 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1985 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1987 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1988 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1989 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1990 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1991 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1992 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1993 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1994 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1995 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1996 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1997 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1998 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 1999 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
| 2000 | Human MBL isolated from liver (7); human serum MBL isolated (121) | | Prospective study of opsonic deficiency in infancy (122) |
Structural aspects of MBL

The protein structure of MBL has been studied extensively, and aspects are presented in Figures 1 and 2. The protein consists of multimers of an identical polypeptide chain of 32 kDa. Each chain comprises four distinct regions encoded by different exons of the MBL-2 gene, as will be discussed in more detail later.

Each chain has a C-terminal, calcium-dependent carbohydrate-recognition domain (CRD); a short, α-helical, hydrophobic neck region (in the so-called coiled-coil configuration); a collagenous region containing 19 Gly-Xaa-Xaa triplets and a cysteine-rich N-terminal region. Three polypeptide chains form a triple helix within the collagenous region, stabilized by hydrophobic interactions.

Figure 1 Structure of the human MBL-2 gene and the encoded protein product. Positions of the exon 1 and promoter polymorphisms are shown. Different regions of the polypeptide are encoded by different exons of the MBL gene. Three identical 32-kDa polypeptides form a structural subunit, based on formation of a collagenous triple helix. Oligomerization of the structural subunit results in MBL molecules of differing size, but the tetrameric form shown in Figure 2 is probably the most common. MBL, mannose-binding lectin.
and interchain disulphide bonds within the N-terminal cysteine-rich region. This is the basic building block of all circulating molecular forms of MBL. In serum, MBL consists of oligomers ranging from dimers to hexamers, and X-ray crystallographic studies/electron micrographs have revealed that these oligomers have a seritiform or a bouquet-like structure due to an interruption in the collagenous region, giving rise to a kink/hinge. The ability of the protein to bind effectively to microorganisms and activate complement appears to depend on the presence of higher order oligomers (tetramers and above).

Work by Drickamer and colleagues (10, 11) and also by Ezekowitz and colleagues (12) has provided an insight into the structure of the CRD. Each CRD binds a calcium ion, enabling it to form co-ordination bonds with the 3- and 4-hydroxyl groups of specific sugars including mannose, N-acetyl-d-glucosamine, N-acetyl-mannosamine, fucose and glucose. The three CRDs in each structural subunit are separated by a constant 45-Å distance (12). Clustering of the structural subunits provides a flat platform, permitting binding of MBL to the arrays of repeating sugar groups on microbial surfaces. Although the binding affinity of each individual CRD–sugar interaction is relatively low at $10^{-3}$ M (13), the formation of higher order oligomers provides multiple CRDs, which are able to bind simultaneously with high avidity.

MBL is a major pattern-recognition molecule of the innate immune system. It primarily recognizes specific sugar groups (as above) on the surface of microorganisms, enabling it to distinguish self from non-self. It can also bind to phospholipids, nucleic acids (14) and non-glycosylated proteins. MBL has been shown to bind promiscuously to a wide range of bacteria, viruses, fungi and protozoa and some selected examples are listed in Table 2.

Neth et al. used flow cytometry to demonstrate MBL binding to clinically relevant bacterial isolates from immunocompromised children and noted differences in binding within some species such that one isolate might show strong binding, whereas another was much weaker (15). The role of specific structural features of microorganisms (e.g. the capsule), which permit or prevent binding to MBL, has been explored in several studies. The earliest work was probably by Kawakami et al. on the so-called RaRf complex (which was later identified as MBL)
and its interaction with *Salmonella enterica* serovar *Typhimurium* (3). This suggested that the structure and composition of lipopolysaccharide play a crucial role in MBL binding and function. Other mechanisms that enable microorganisms to avoid recognition and killing by MBL include lipooligosaccharide sialylation (16, 17). Despite much progress in this area, many puzzles remain to be addressed, mostly related to the exact disposition of sugars on microbial surfaces.

**Functional aspects of MBL**

Our understanding of MBL function has grown rapidly over the past three decades. It is now recognized to have a role in processes as diverse as complement activation, promotion of complement-independent opsonophagocytosis, modulation of inflammation, recognition of altered self-structures and apoptotic cell clearance.

**MBL and complement activation**

A role for MBL in host defence was first proposed in 1987 when Ikeda et al. observed that the protein was able to activate the classical pathway of complement (18). However, it is now clear that MBL activates a novel third pathway of complement, often termed the MBL pathway, in an antibody- and C1-independent fashion as illustrated in Figure 3.

This functional activity reflects the fact that MBL circulates in association with a group of MBL-associated serine proteases (the so-called MASPs). In 1992, Matsushita and Fujiita demonstrated the presence of a novel complement enzyme in serum, which was thought to generate the C3 convertase (C4bC2a), associated with classical pathway activation (19). However, this activity was later found to be mediated by MASP-2 (20), and the original enzyme is now known as MASP-1 and may activate C3 directly. Subsequently, a small separately synthesized fragment of MASP-2 termed sMAP or Map19 was identified (21, 22) and a third MASP (MASP-3) with no known function was also described (23).

Current understanding suggests that on binding to microorganisms, autoactivation of MASP-2 occurs, permitting cleavage of C4 and C2 to form a C3 convertase, which is indistinguishable in specificity from the convertases found in the other two activation pathways of complement (24).

It should be noted that the so-called MBL pathway is also activated by another family of proteins called ficolins. The ficolins are structurally similar to collectins, with collagenous domains linked to fibrinogen-like domains having sugar-binding properties. L- and H-ficolins are humoral factors synthesized by hepatocytes, although H-ficolin has also been observed in bronchial/alveolar fluid and in bile (25). In contrast, M-ficolin is found on peripheral blood mononuclear cells, polymorphonuclear cells and type II lung epithelial cells (26). Ficolins are also found in complexes with the MASPs and are considered to have different binding specificities compared with MBL (27).

**Opsonophagocytosis**

In 1968, Miller et al. reported a plasma-associated defect of phagocytosis in a child with severe recurrent infections, failure to thrive and diarrhoea (28). *In vitro* work revealed a failure of the child’s plasma to opsonize heat-killed bakers yeast (*Saccharomyces cerevisiae*). This defect was later detected in the sera of children with recurrent unexplained infections (29) and chronic diarrhoea of infancy (30), but, interestingly, studies in the general population also revealed a relatively high frequency of the defect (~5%). In 1981, studies linked this opsonic deficiency to the complement...
system by demonstrating that sera with the deficiency deposited less C3b on yeast surfaces (31). However, it was not until 1989 that the common opsonic defect was found to be associated with low levels of the mannose-binding protein, which we now refer to as MBL (32). In that same year, the gene for MBL was cloned (33, 34) (Genetics of Human MBL).

**Cell receptors for MBL**

In a study of MBL-coated *Salmonella montevideo*, Kuhlman et al. reported that MBL was able to interact directly with cell surface receptors and promote opsonophagocytosis (35). Subsequently, a number of putative MBL-binding proteins/receptors have been proposed including cC1qR/calreticulin (36), C1qRp (37) and CR1 (38, 39). However, it is unclear whether MBL is acting as a direct opsonin or is merely enhancing other complement pathways and/or antibody-mediated phagocytosis.

**MBL in inflammation**

The role of MBL as a modulator of inflammation appears to be complex and, accordingly, its mechanism of action remains unexplained. One possible explanation is that MBL is able to trigger proinflammatory cytokine release from monocytes (40, 41). This concept was addressed in studies by Jack et al. using *Neisseria meningitidis* incubated with increasing concentrations of MBL before being added to MBL-deficient whole blood. Release of tumour necrosis factor α, interleukin (IL)-1β and IL-6 from monocytes was enhanced at MBL concentrations below 4 μg/ml but suppressed at higher concentrations (42). Clinical studies in this area are discussed later.

**The role of MBL in the recognition of altered self and apoptosis**

A role for MBL in the clearance of apoptotic cells was first proposed by Ogden et al. in 2001 (43). MBL was found to bind directly to apoptotic cells that expose terminal sugars of cytoskeletal proteins, thereby permitting their recognition and directly facilitating their phagocytosis by macrophages. Defects in the clearance of apoptotic cells have been implicated in the pathogenesis of certain autoimmune conditions, although the precise role of MBL, if any, remains elusive. For example, in 2005, Stuart et al. reported that although MBL-deficient mice displayed defective apoptotic cell clearance, they did not develop autoimmune diseases (44).

In animal studies, MBL has been implicated in the pathophysiology of ischaemia reperfusion injury due to its ability to recognize altered self-structures. Stahl and colleagues have proposed the lectin pathway as a mediator of this process in certain organs, and the absence of MBL/MASP pathway activation appears to afford protection in these disease models (45, 46). However, the relevance of these findings to human health needs to be established.

Changes in cell surface structures during oncogenic transformation appear to promote binding of MBL to cancer cells (47) where the protein can mediate cytotoxic effects including MBL-dependent cell mediated cytotoxicity (48, 49). The relative importance of such mechanisms in tumour immunology is, at present, unknown.

**Genetics of human MBL**

There are two human MBL genes, but *MBL-1* is a pseudogene and only *MBL-2* encodes a protein product. The functional *MBL-2* gene is located on chromosome 10 (q11.2-q21) and comprises four exons as illustrated in Figure 1. Exon 1 encodes the signal peptide, a cysteine-rich region and part of the glycine-rich collagenous region. Exon 2 encodes the remainder of the collagenous region and exon 3 encodes an α-helical coiled-coil structure, which is known as the ‘neck’ region. Exon 4 encodes the CRD, which adopts a globular configuration. The promoter region of the MBL gene contains a number of regulatory elements, which affect transcription of the protein.

In 1991, the complete nucleotide sequence of all four exons of the human *MBL-2* gene was determined by Sumiya et al. in two British children with recurrent infections and low MBL levels (50). In both individuals, a point mutation was observed in codon 54, changing the codon sequence from GGC to GAC and substituting aspartic acid for glycine in the translated protein. Familial studies confirmed that the defect was inherited in an autosomal dominant fashion. In 1992, Lipscombe et al. identified a second exon 1 mutation in codon 57 (Gly → Glu), when studying a sub-Saharan African population (51), and in 1994, Madsen et al. reported a mutation in codon 52 (Arg → Cys) (52). These point mutations are now commonly referred to as variants B, C and D respectively, with variant A indicating the wild type. The B variant mutation occurs at a gene frequency of approximately 25% in Eurasian populations. In contrast, the C variant is rare in Eurasians but is commonly seen in sub-Saharan African populations, with frequencies of 50%-60%. Population studies suggest that the B variant mutation may have arisen between 50,000 and 20,000 years ago (53) since no structural gene mutations have been identified in studies of indigenous Australian populations who arrived on the continent approximately 50,000 years ago, whereas the B variant mutation was probably introduced into both North and South America at the time of the last glaciation approximately 20,000 years ago.

The effect of these exon 1 mutations on the protein product continues to be the focus of study. They are believed to impair oligomerization and lead to a functional deficiency. The B and C mutations result in the replacement of
critical axial glycines in the triple helix by dicarboxylic acids, resulting in distortion of this important part of the protein (50). In contrast, the D mutation results in the replacement of arginine with cysteine. This extra cysteine has been proposed to cause formation of adventitious disulphide bonds that hinder higher oligomer formation (54).

Several polymorphisms have also been reported in the promoter region of the gene. Studies by Madsen et al. investigating the large interindividual variation in serum MBL levels revealed three polymorphisms, H/L, X/Y and P/Q at positions −550, −221 and +4 of the MBL gene (55, 56). Subsequently, four common haplotypes were identified, namely LXP, LYP, LYQ and HYP. Of these, HYP, which is associated with medium to high levels of MBL and LXP, which is associated with low levels of the protein, appear to be most important. These promoter haplotypes are in strong linkage disequilibrium with the exon 1 mutations, resulting in seven common extended haplotypes, namely HYPA, LYPA, LYQA, LXPA, HYPD, LYPB and LYQC. Other rare haplotypes have also been described (57). Figure 4 illustrates the frequency of these various haplotypes in selected populations and highlights the degree of ethnic variation.

The combination of structural gene and promoter polymorphisms results in a dramatic variation in MBL concentration in apparently healthy individuals of up to 1000-fold (Caucasian: range <20–10,000 ng/ml). In addition, Ezekowitz and colleagues presented evidence in 1988 that MBL was an acute-phase reactant (58). In these investigations, RNA was isolated from a liver taken as part of a staging biopsy for Hodgkins disease and was compared with RNA isolated from a fresh post-mortem liver of a victim with severe trauma. The authors found that MBL messenger RNA transcripts were barely detectable in normal liver but that induction was seen in liver exposed to acute stress. Subsequent studies have shown that MBL levels can increase between 1.5 and threefold during the acute phase, but this response is variable between individuals (59). It should also be noted that even during an acute-phase response, individuals heterozygous or homozygous for MBL mutations appear unable to achieve the protein levels of those possessing a wild-type genotype. Approximately one-third of the Caucasian population possess genotypes conferring low levels of MBL, with approximately 5% having very low levels. No absolute level of MBL deficiency has been defined. Genotype and phenotype show a relatively strong correlation and studies often use just one measure to infer deficiency. However, there is ‘added value’ in performing both measures and we would strongly advocate this approach whenever possible.

**MBL gene evolution**

MBL occurs in two distinct forms in rodents and rhesus monkeys (60), but only one form is found in humans and chickens. As discussed previously, there are two human MBL genes, which are most likely due to a gene duplication event (61). However, *MBL-1* is a pseudogene and the potential mechanisms responsible for silencing the *MBL-1*
gene are under debate. In 1998, Guo et al. described an intron 1 splicing defect and two stop codons in exons 3 and 4 of the MBL-1 gene (62). More recently, Seyfarth et al. identified glycine substitutions in codon 53 of the MBL-1 gene, which bear a close resemblance to those found in codon 54 of the MBL-2 gene (63). Such substitutions were also found in other higher primates including chimpanzees and gorillas but not in more distant primates such as the rhesus monkey. The authors concluded that both the MBL-1 and the MBL-2 genes have been selectively silenced by the same molecular mechanisms, but skewed in time resulting in overall downregulation of MBL levels in the present human population.

The MBL paradox

The high frequency of variant alleles observed in certain populations was initially puzzling since it suggests that functional MBL deficiency may well be advantageous. Similarities have been proposed between the MBL genetic system and the role of the sickle cell gene in protection against malaria as occurs in carriers of the sickle cell haemoglobin allele (64). The argument runs as follows: certain intracellular parasites use C3 opsonization and C3 receptors on monocytes/macrophages to enter their host. Therefore, any reduction in complement-activating function of the host may reduce the probability of parasitization. In support of this notion is a study on patients with visceral leishmaniasis, which revealed that such patients are more likely to have high MBL levels than uninfected controls (65). A small study of Ethiopian patients with lepromatous or borderline lepromatous leprosy also found that their MBL levels were significantly higher than those of healthy blood donors (66). An alternative explanation of the unexpectedly high frequency of low MBL phenotype individuals found in many tropical regions is that excessive complement activation can result in immunopathologically mediated host damage; therefore, any mechanism that reduces complement activation may be beneficial (51).

Disease association studies

The identification of MBL deficiency as the cause of the so-called common opsonic defect has been followed by a plethora of disease association studies aimed at defining the precise role of this protein. A number of the early studies concentrated on paediatric populations and MBL was suggested to provide substitute ‘antibody’-like activity during the ‘window of vulnerability’ (approximately 6–24 months), when maternal immunoglobulin G (IgG) antibody levels have waned but the infant’s own adaptive immune response is still immature (32). Nevertheless, studies in adults suggested that there might be a role for MBL throughout life (67). Notwithstanding these reports, the majority of individuals possessing a variant MBL allele apparently suffer no ill effects and remain essentially healthy. In a study that apparently confirms this, Dahl et al. monitored 9245 adults in a Danish Caucasian population and found no evidence for significant differences in infectious disease or mortality in MBL-deficient individuals compared with controls (68). Similar findings were reported by Tacx et al. in unselected adults admitted to hospital with infections (69). Nevertheless, these studies should not be regarded as proof that MBL levels have no clinical relevance. Many groups have undertaken case-control studies, which do indeed suggest that MBL is an important immunological modulator. In some cases, there is evidence that the significance of MBL deficiency is more readily appreciated when there is another co-existing defect (70), as we first proposed in 1991 (71).

Space does not permit a comprehensive review of all the MBL clinical studies that have been undertaken to date, and the topics covered below have been selected in order to illustrate examples of possible roles for MBL in a variety of clinical situations.

MBL and infectious diseases: susceptibility and severity

Most studies have explored the role of MBL in relation to the acquisition of an infectious organism (susceptibility) and the nature of the associated clinical course (severity). In clinical practice, this distinction can be difficult. However, for the purposes of this review, we will highlight examples of infections in which MBL appears to have an influence on one or other of these two aspects of infectious diseases.

Infections in which MBL appears to have a predominant role in susceptibility to disease

Hamvas et al. have recently shown a role for MBL in mycoplasma infection (72). They studied cases of infection in patients with primary antibody deficiencies (PAD) that are known to be particularly susceptible to such organisms and compared them with a control population. More than two-thirds of PAD patients with mycoplasma infections were MBL deficient (in possession of an exon 1 variant allele) compared with one-third of the control group. In the same study, they were able to demonstrate binding of MBL to three strains of Mycoplasma using flow cytometry and proposed a role for MBL in prevention of invasive disease.

In 2003, severe acute respiratory syndrome (SARS) emerged as a highly infectious disease caused by a novel coronavirus (SARS-CoV). It provided a new challenge to previously unexposed individuals predominantly in Asia. Specific antibodies to SARS-CoV could be detected ≥10 days after the onset of symptoms, making sufferers reliant on innate immune mechanisms during the early phase of
Infections in which MBL exerts its effects on both susceptibility and severity

Hepatitis

A number of studies have addressed the role of MBL in both hepatitis B and hepatitis C infection. Yuen et al. investigated chronic carriers of hepatitis B and hepatitis C in China (77). The B variant allele was found more commonly in patients with symptomatic hepatitis B cirrhosis and in those with spontaneous bacterial peritonitis. It was also noted that MBL levels were lower in this patient cohort with chronic infection. Screening for MBL mutations in such patients was suggested in order to enable identification of those at increased risk of complications who may benefit from prophylactic antibiotic treatment. In 2005, Chong et al. also reported that MBL genotypes correlating with low protein levels were associated with the occurrence of cirrhosis and also hepatocellular carcinoma in hepatitis B carriers (78). They also demonstrated that MBL is able to bind hepatitis B surface antigen. In the same year, Thio et al. published the results of a nested case–control study of 527 patients who had either naturally recovered from hepatitis B (n = 338) or had persistent infection (n = 189). They found that MBL genotypes correlating with high serum levels were associated with recovery from infection, whereas those correlating with lower levels were associated with persistence of the virus (79). It should be noted that approximately half of the subjects were also infected with human immunodeficiency virus (HIV), but the authors concluded that this did not influence the results obtained. Matsushita et al. investigated the influence of MBL mutations in hepatitis C infection and found that sufferers who were homozygous for B variant alleles were less likely to respond to interferon treatment (80). Further work would be warranted in order to define the role of MBL in the pathogenesis of hepatitis infection.

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. These patients are rendered neutropenic by their treatment (or underlying disease process) and are subsequently at increased risk of infectious complications. In 2001, two studies were published reporting an effect of MBL deficiency in such patients. Neth et al. studied 100 children and measured MBL levels and genotype. Children in possession of MBL variant alleles spent twice as many days in hospital with febrile neutropenia during the first 6 months of their treatment compared with wild-type individuals (81). In the other study, Peterslund et al. followed 54 adults undergoing chemotherapy for various haematological malignancies and found that those who developed ‘significant’ infections (bacteraemia, pneumonia or both) in the 3-week periods post-treatment had significantly lower levels of MBL compared with those without significant infections (82). Subsequent studies have shown differing results, but drawing comparisons between them is inherently difficult. These patients are a highly heterogeneous population, with different underlying disease processes, undergoing treatment regimens of differing intensity, resulting in various degrees of immunosuppression. In one contrasting study, Bergmann et al. followed 80 adults undergoing therapy for acute myeloid leukaemia, which involves intense highly myelosuppressive treatment. They found no effect of MBL deficiency on frequency, severity or duration of fever and suggested that the nature of the treatment overwhelmed any potential influence of MBL (83). Further clinical studies in such patients are required in order to delineate the exact role of MBL.

An MBL double-knockout mouse model has been used to explore the above clinical conundrum. In 2004, Shi et al. demonstrated that MBL null mice were highly susceptible to intravenous inoculation with Staphylococcus aureus, all dying within 48 h, compared with 55% survival of MBL wild-type mice. However, when the mice were inoculated via the intraperitoneal route and rendered neutropenic (using cyclophosphamide), neutropenic MBL null mice were found to have higher accumulations of bacteria in the blood and organs compared with neutropenic wild-type mice. By day 8 post-infection, the neutropenic wild-type mice had cleared their blood, but the neutropenic MBL null mice had persistent bacteraemia. The authors were able to reverse the phenotype by treating the MBL null mice with recombinant MBL (84).

MBL and human immunodeficiency virus

To date, nearly 40 million humans have been infected with HIV. The clinical consequences of viral exposure are variable. Some individuals can be repeatedly exposed to the virus but remain free from infection. Others can be infected but remain free from clinical disease. While numerous viral
and host factors will determine the fate of an individual exposed to HIV, there are data to indicate that MBL can influence both susceptibility and severity of HIV infection. The likely target for HIV binding is the heavily glycosylated glycoprotein, gp120. While MBL can be readily demonstrated to bind to purified gp120 (85), the capacity of MBL to neutralize primary HIV isolates is less convincing. Recent data indicate the MBL can opsonize HIV but does not induce neutralization at the levels at which it is normally present in serum. However, binding and opsonization of HIV by MBL may alter virus trafficking and viral antigen presentation during HIV infection. MBL may influence uptake by dendritic cells (DC), which express a cell surface lectin called ‘DC-specific intracellular adhesion molecule 3-grabbing non-integrin’ (DC-SIGN). DC-SIGN has been shown to mediate a type of infection called ‘trans’-infection, where DC bind HIV and efficiently transfer the virus to T cells. Preincubation of HIV strains with MBL prevents DC-SIGN-mediated trans-infection of T cells and indicates that at least in vitro, MBL may inhibit DC-SIGN-mediated uptake and spread of HIV (86).

Whatever the mechanism of MBL interactions with HIV, a number of clinical studies have suggested that deficiency of MBL is a risk factor for acquiring HIV infection. MBL deficiency appears to increase the acquisition of HIV infection by between three- and eightfold (87–90). There is also an increased risk of vertical transmission from infected mothers to their offspring (91). However, these findings have not been replicated in all populations, with some studies failing to demonstrate a role for MBL in HIV infection (92–94). There is even less clarity with regard to the role of MBL in HIV disease progression. Garred et al. (87) demonstrated that men with MBL variant alleles had a shorter survival time following the onset of acquired immune deficiency syndrome (AIDS) than did patients with wild-type MBL alleles. However, in a well-characterized cohort of homosexual men, variant MBL alleles had an insignificant effect on survival following the diagnosis of AIDS (95). In this latter study, there appeared to be a protective effect of MBL variant alleles, with a delay in the development of AIDS from the time of HIV seroconversion. Patients with MBL variant alleles had lower CD4 counts at the time of developing AIDS, indicating that MBL deficiency may influence the onset of AIDS for any given CD4 count. Furthermore, MBL mutations appeared to protect against the development of Kaposi sarcoma, a finding that was difficult to explain (95). In another study, Prohaszka et al. (90) found that MBL levels were lower in asymptomatic HIV-positive individuals compared with HIV-negative controls. However, the protective effect of MBL was lost in patients with an AIDS diagnosis; patients with high MBL levels had significantly lower numbers of CD4 cells. A possible explanation is that enhanced proinflammatory cytokine production in advanced HIV disease acts to increase MBL synthesis (96), elevating levels in patients with late-stage disease. Indeed, a recent study has shown in vitro that MBL can enhance proinflammatory cytokine production and viral replication (97). In the light of studies indicating a role for MBL in inflammatory modulation, it is tempting to suggest that under some circumstances, MBL may act to promote inflammatory cell activation, thereby accelerating the rate of CD4 + T-cell depletion.

Few studies have assessed the impact of MBL in the context of effective antiviral therapy. However, one study has attempted to relate MBL status and HIV-infected long-term non-progressors (LTNPs) (98). MBL levels were consistent with a wild-type genotype in the six LTNPs studied. Amoroso and colleagues had also suggested such an effect in a study showing that children with rapidly progressing disease were more likely to have MBL variant alleles (codon 54) than slower progressors (99).

**MBL in infection susceptibility and modulation of inflammation**

**MBL and cystic fibrosis**

Cystic fibrosis provides an example of a clinical condition where MBL appears to be exerting its role as an infection susceptibility gene and inflammatory modulator. Garred et al. were the first group to report that patients with MBL variant alleles have significantly impaired lung function and decreased life expectancy in comparison with wild-type individuals (100). The effect of MBL deficiency on the severity of lung disease was most apparent in patients with chronic *Pseudomonas aeruginosa* infection and it was also found that *Burkholderia cepacia* infection was more common in patients with MBL deficiency. In 2004, Davies et al. reported that an effect of MBL was only seen in adults homozygous for MBL mutations. These patients had significantly reduced lung function, more frequent hospital admissions and raised systemic inflammatory markers. However, there was no evidence of increased susceptibility to *Burkholderia cepacia* and *Pseudomonas aeruginosa* (101). Whether MBL has an effect on early colonization with *Burkholderia cepacia* and *Pseudomonas aeruginosa* or subsequent secondary viral infections or whether there is an (anti)inflammatory effect on subsequent lung damage remains unclear.

**Systemic inflammatory response syndrome and myocardial infarction**

Clinical studies of critically ill patients requiring intensive care management have shown that individuals who are MBL deficient are more likely to develop the systemic inflammatory response syndrome (SIRS) (Figure 5) and progress to septic shock and death (102, 103), findings which may well relate to the proinflammatory cytokine response.
It should also be noted that chronic inflammation is now increasingly accepted to be a risk factor for myocardial infarction (MI), and a recent study by Saevarsdottir et al. has found that patients with high MBL levels have a decreased likelihood of suffering a MI – again suggesting a potential role for MBL in modulating the inflammatory response (104).

**MBL and autoimmune disease**

As a component of the complement system with similarities to C1q, but also as a player in infectious and inflammatory processes, the structure and function of MBL have prompted studies exploring a possible role in autoimmune conditions. Systemic lupus erythematosus (SLE) has been the focus of a number of MBL genotyping studies, but the results have been somewhat inconsistent. Nevertheless, a recent meta-analysis has reviewed studies in this area and found that MBL variant alleles are indeed SLE risk factors (105). 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 (106). Studies in patients with SLE have reported that MBL deficiency also influences their risk of developing certain complications, which include arterial thromboses (107) and respiratory tract infections (108, 109).

A role for MBL in the pathogenesis of rheumatoid arthritis has also been suggested. Malhotra et al. reported that changes in IgG glycosylation secondary to the underlying disease results in MBL-associated complement activation (110). Such complement activation then contributes to chronic inflammation of the synovial membrane. However, Graudal et al. found that patients with lower MBL levels experienced earlier, more severe, symptoms and had more rapid joint destruction as visualized radiologically (111).

**MBL – the future**

Several recent research publications suggest the directions in which future work on this collectin and its associated molecules may proceed. These include therapeutic interventions, functional assays and the evaluation of the importance of MBL in disease. These are considered briefly below.

**Therapeutic potential of MBL**

MBL replacement was first attempted (without any knowledge of the deficiency) when fresh frozen plasma was given to patients and found to correct the opsonic defect (28, 29). Since then, affinity-purified, plasma-derived MBL has been safely given to many patients, resulting in normalization of enzyme-linked immunosorbent assay detectable MBL and complement-mediated opsonic activity (112). A phase 1 study showed the half-life of the protein to range between 18 and 115 h (113). The development of recombinant MBL is also at the phase 1 trial stage and such developments provide exciting prospects for the future exploration of the therapeutic potential of MBL. Exactly who would benefit from replacement therapy is under debate and the importance of targeting well-defined patient groups will be vital to its success.

**Functional investigations of the MBL and ficolin-lectin pathways**

The discovery of other components of the lectin pathway including the ficolins and the MASPs indicates that this limb of the immune system is complex and extends beyond MBL and MASP-2 alone. This knowledge enables us to question the impact of these molecules either in isolation or in combination. Functional assessment of the lectin pathway may be a far more accurate and clinically relevant measurement than MBL level and/or genotype alone. A number of different assays have been reported, which assess activity at different stages of the functional pathway; therefore, the results must be interpreted accordingly (114, 115).

The impact of deficiencies of the various adjunctive components is also the subject of much current research.
In 2003, Stengaard-Pedersen et al. reported the first identified case of MASP-2 deficiency (116). Functional analysis of the ability of MBL to activate the lectin pathway, estimating C4b deposition on a mannan surface, was performed on a group of patients with suspected immuno-deficiency. One patient was found to have deficient pathway activity despite having sufficient MBL. No MASP-2 or Map19 was found in the plasma, and genetic analysis indicated that the patient was homozygous for a point mutation in exon 3 of the gene (D105G). Clinically, the patient suffered from recurrent infections and autoimmune symptoms. Subsequently, the frequency of this mutation has been assessed in a small number of populations and values range from 1.3% to 6.3% (117). As discussed previously, the contribution of MASP-1 and MASP-3 in the pathway remains unexplained.

The role of ficolins is now beginning to be addressed in clinical studies. Like MBL, no absolute levels of deficiency have yet been defined. Atkinson et al. studied more than 300 children with recurrent respiratory tract infections and measured L-ficolin levels (118). An association with MBL deficiency in the same patient cohort had already been reported (119). In this study, low levels of L-ficolin were more common in patients than in controls and most common in patients with co-existing atopic disorders, suggesting a role for L-ficolin in protection from microorganisms complicating allergic disease. Polymorphisms in the ficolins have been identified, although their clinical significance is as yet unknown.

How important is MBL in human disease?

MBL is an ancient molecule, which has probably been subject to a large number of evolutionary pressures. The last 50,000 years of human evolution have been associated with major changes as hominids moved from an essentially nomadic lifestyle to increasingly crowded living arrangements in large settled communities. Associated with these changes, the spectrum of common infectious diseases would also have changed. More recently, the introduction of antibiotics, the emergence of novel infections and increasing use of immunosuppressive therapies have provided new challenges to our innate host defence system. Despite all these changing evolutionary pressures, MBL gene polymorphisms persist at high frequencies, suggesting that they offer potential advantages to the host. Thus, there exists a balance in which certain individuals benefit from the expression of high levels of the protein, whereas others (living in differing environments, eg. the tropics) may benefit from reduced levels of circulating MBL (Figure 6).

MBL status may also be either advantageous or disadvantageous when considered from the viewpoint of the severity of a particular illness. Thus, it is known that those with higher levels of MBL are better able to modulate inflammation, probably through an effect on cytokine responses. In contrast, those deficient in MBL appear to be at risk of sepsis and SIRS. For these reasons, we believe that analyses of the relevance of MBL (120) should be extended beyond its role in infectious disease and include clinical areas such as autoimmunity and inflammatory disorders.

**Figure 6** Schematic representation illustrating how both high and low serum MBL levels may impact the health of a given host. IL, interleukin; MBL, mannose-binding lectin.

---

© 2006 The Authors
Journal compilation 68 (193–209) © 2006 Blackwell Munksgaard
References

1. Burnet FM, McCrea JF. Inhibitory and inactivating action of normal ferret sera against an influenza virus strain. *Aust J Exp Biol Med Sci* 1946; 24: 277–82.

2. Anders EM, Hartley CA, Jackson DC. Bovine and mouse serum beta inhibitors of influenza A viruses are mannose-binding lectins. *Proc Natl Acad Sci U S A* 1990; 87: 4485–9.

3. Kawakami M, Ihara I, Suzuki A, Harada Y. Properties of a new complement-dependent bacterial factor specific for Rα chemotype salmonella in sera of conventional and germ-free mice. *J Immunol* 1982; 129: 2198–201.

4. Kawakami M, Ihara I, Ihara S, Suzuki A, Fukui K. A group of bacterial factors conserved by vertebrates for more than 300 million years. *J Immunol* 1984; 132: 2578–81.

5. Robinson D, Phillips NC, Winchester B. Affinity chromatography of human liver alpha-D-mannosidase. *FEBS Lett* 1975; 53: 110–2.

6. Kawasaki T, Etoh R, Yamashina I. Isolation and characterization of a mannan-binding protein from rabbit liver. *Biochem Biophys Res Commun* 1978; 81: 1018–24.

7. Wild J, Robinson D, Winchester B. Isolation of mannose-binding proteins from human and rat liver. *Biochem J* 1983; 210: 167–74.

8. Seyfarth J, Garred P, Madsen HO. Extra-hepatic binding proteins from human and rat liver. *Mol Immunol* 1983: 205: 3894–9.

9. Holmskov U, Thiel S, Jensenius JC. Collections and ficolins: humoral lectins of the innate immune defense. *Ann Rev Immunol* 2003; 21: 547–78.

10. Weis WI, Kahn R, Fourme R, Drickamer K, Hendrickson WA. Structure of the calcium-dependent lectin domain from a rat mannose-binding protein determined by MAD phasing. *Science* 1991: 254: 1608–15.

11. Weis WI, Drickamer K. Trimeric structure of a C-type mannose-binding protein. *Structure* 1994: 2: 1227–40.

12. Sherrif S, Chang CY, Ezekowitz RA. Human mannose-binding protein carbohydrate recognition domain trimertizes through a triple alpha-helical coiled-coil. *Nat Struct Biol* 1994: 1: 789–94.

13. Lobst ST, Wormald MR, Weis WI, Dwek RA, Drickamer K. Binding of sugar ligands to Ca(2+)-dependent animal lectins. I. Analysis of mannose binding by site-directed mutagenesis and NMR. *J Biol Chem* 1994: 269: 15505–11.

14. Palaniyar N, Nadesalingam J, Clark H, Shih MJ, Dodds AW, Reid KB. Nucleic acid is a novel ligand for innate, immune pattern recognition collects surfactant proteins A and D and mannose-binding lectin. *J Biol Chem* 2004: 279: 32728–36.

15. Neth O, Jack DL, Dodds AW, Holzel H, Klein NJ, Turner MW. Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition. *Infect Immun* 2000; 68: 688–93.

16. Jack DL, Dodds AW, Anwar N et al. Activation of complement by mannose-binding lectin on isogenic mutants of Neisseria meningitidis serogroup B. *J Immunol* 1998: 160: 1346–53.

17. Devyataryova-Johnson M, Rees IH, Robertson BD, Turner MW, Klein NJ, Jack DL. The lipopolysaccharide structures of Salmonella enterica serovar Typhimurium and Neisseria gonorrhoeae determine the attachment of human mannose-binding lectin to intact organisms. *Infect Immun* 2000: 68: 3894–9.

18. Ikeda K, Sannoh T, Kawasaki N, Kawasaki T, Yamashina I. Serum lectin with known structure activates complement through the classical pathway. *J Biol Chem* 1987; 262: 7451–4.

19. Matsushita M, Fujita T. Activation of the classical complement pathway by mannose-binding protein in association with a novel C1s-like serine protease. *J Exp Med* 1992: 176: 1497–502.

20. Thiel S, Vorup-Jensen T, Stover CM et al. A second serine protease associated with mannann-binding lectin that activates complement. *Nature* 1997; 386: 506–10.

21. Takahashi M, Endo Y, Fujita T, Matsushita M. A truncated form of mannose-binding lectin-associated serine protease (MASP)-2 expressed by alternative polyadenylation is a component of the lectin complement pathway. *Int Immunol* 1999; 11: 859–63.

22. Stover CM, Thiel S, Thelen M et al. Two constituents of the initiation complex of the mannann-binding lectin activation pathway of complement are encoded by a single structural gene. *J Immunol* 1999: 162: 3481–90.

23. Dahl MR, Thiel S, Matsushita M et al. MASP-3 and its association with distinct complexes of the mannann-binding lectin complement activation pathway. *Immunity* 2001; 15: 127–35.

24. Feinberg H, Uittohaag JC, Davies JM, Wallis R, Drickamer K, Weis WI. Crystal structure of the CUB1-EGF-CUB2 region of mannose-binding protein associated serine protease-2. *EMBO J* 2003: 22: 2348–59.

25. Akaiba M, Yae Y, Sugimoto R et al. Hakata antigen, a new member of the ficolin/opsonin p35 family, is a novel human lectin secreted into bronchus/alveolus and bile. *J Histochem Cytochem* 1999; 47: 777–86.

26. Liu Y, Endo Y, Iwaki D et al. Human M-ficolin is a secretory protein that activates the lectin complement pathway. *J Immunol* 2005: 175: 3150–6.

27. Lynch NJ, Roscher S, Hartung T et al. L-ficolin specifically binds to lipoteichoic acid, a cell wall constituent of gram-positive bacteria, and activates the lectin pathway of complement. *J Immunol* 2004: 172: 1198–202.

28. Miller ME, Seals J, Haye R, Levitsky LC. A familial plasma-associated defect of phagocytosis. *Lancet* 1968: 2: 60–3.

29. Soothill JF, Harvey BA. Defective opsonization. A common immunity deficiency, *Arch Dis Child* 1976; 51: 91–9.

30. Candy DC, Larcher VF, Tripp JH, Harries JT, Harvey BA, Soothill JF. Yeast opsonisation in children with chronic diarrhoeal states. *Arch Dis Child* 1980; 55: 189–93.

31. Turner MW, Mowbray JF, Roberton DR. A study of C3b deposition on yeast surfaces by sera of known opsonic potential. *Clin Exp Immunol* 1981; 46: 412–9.
Mannose-binding lectin in innate immunity

32. Super M, Thiel S, Lu J, Levinsky RJ, Turner MW. Association of low levels of mannan-binding protein with a common defect of opsonisation. *Lancet* 1989: 2: 1236–9.

33. Sastry K, Herman GA, Day L et al. The human mannos-binding protein gene. Exon structure reveals its evolutionary relationship to a human pulmonary surfactant gene and localization to chromosome 10. *J Exp Med* 1989: 170: 1175–89.

34. Taylor ME, Brickell PM, Craig RK, Summerfield JA. Structure and evolutionary origin of the gene encoding a human serum mannos-binding protein. *Biochem J* 1989: 262: 763–71.

35. Kuhlman M, Joiner K, Ezekowitz RA. The human mannos-binding protein functions as an opsonin. *J Exp Med* 1989: 169: 1733–45.

36. Malhotra R, Thiell S, Reid KB, Sim RB. Human leukocyte C1q receptor binds other soluble proteins with collagen domains. *J Exp Med* 1990: 172: 955–9.

37. Tenner AJ, Robinson SL, Ezekowitz RA. Mannose binding protein (MBP) enhances mononuclear phagocyte function via a receptor that contains the 126,000 Mr component of the C1q receptor. *Immunity* 1995: 3: 485–93.

38. Ghiran I, Barbashov SF, Klickstein LB, Tas SW, Jensenius JC, Nicholson-Weller A. Complement receptor 1/CD35 is a receptor for mannos-binding lectin. *J Exp Med* 2000: 192: 1797–806.

39. Klickstein LB, Barbashov SF, Liu T, Jack RM, Nicholson-Weller A. Complement receptor type 1 (CR1, CD35) is a receptor for C1q. *Immunity* 1997: 7: 345–55.

40. Soell M, Lett E, Holvaek F, Scholler M, Wachsmann D, Klein JP. Activation of human monocytes by streptococcal rhamnose glucos polymers is mediated by CD14 antigen, and mannan binding protein inhibits TNF-alpha release. *J Immunol* 1995: 154: 851–60.

41. Chaka W, Verheul AF, Vaishnav VV et al. Induction of TNF-alpha in human peripheral blood mononuclear cells by the mannosprotein of Cryptococcus neoformans involves human mannos binding protein. *J Immunol* 1997: 159: 2979–85.

42. Jack DL, Read RC, Tenner AJ, Frosch M, Turner MW, Klein NJ. Mannose-binding lectin regulates the inflammatory response of human professional phagocytes to Neisseria meningitidis serogroup B. *J Infect Dis* 2001: 184: 1152–62.

43. Ogden CA, deCathelineau A, Hoffmann PR et al. C1q and mannos binding lectin engagement of cell surface calreticulin and CD91 initiates macrophagocytosis and uptake of apoptotic cells. *J Exp Med* 2001: 194: 781–95.

44. Stuart LM, Takahashi K, Shi L, Savill J, Ezekowitz RA. Mannose-binding lectin-deficient mice display defective apoptotic cell clearance but no autoimmune phenotype. *J Immunol* 2005: 174: 3220–6.

45. Hart ML, Ceonzo KA, Shaffer LA et al. Gastrointestinal ischemia-reperfusion injury is lectin complement pathway dependent without involving C1q. *J Immunol* 2005: 174: 6373–80.

46. Walsh MC, Bourcier T, Takahashi K et al. Mannose-binding lectin is a regulator of inflammation that accompanies myocardial ischemia and reperfusion injury. *J Immunol* 2005: 175: 541–6.

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

48. Ma Y, Uemura K, Oka S, Kozutsumi Y, Kawasaki N, Kawasaki T. Antitumor activity of mannan-binding protein in vivo as revealed by a virus expression system: mannan-binding protein-dependent cell-mediated cytotoxicity. *Proc Natl Acad Sci U S A* 1999: 96: 371–5.

49. Nakagawa T, Kawasaki N, Ma Y, Uemura K, Kawasaki T. Antitumor activity of mannan-binding protein. *Methods Enzymol* 2003: 363: 26–33.

50. Sumiya M, Super M, Tabona P et al. Molecular basis of opsonic defect in immunodeficient children. *Lancet* 1991: 337: 1569–70.

51. Lipscombe RJ, Sumiya M, Hill AV et al. High frequencies in African and non-African populations of independent mutations in the mannos binding protein gene. *Hum Mol Genet* 1992: 1: 709–15.

52. Madsen HO, Garred P, Kurtzhalts JA et al. A new frequent allele is the missing link in the structural polymorphism of the human mannos-binding protein. *Immunogenetics* 1994: 40: 37–44.

53. Turner MW, Dinan L, Heatley S et al. Restricted polymorphism of the mannos-binding lectin gene of indigenous Australians. *Hum Mol Genet* 2000: 9: 1481–6.

54. Wallis R, Drickamer K. Molecular determinants of oligomer formation and complement fixation in mannos-binding proteins. *J Biol Chem* 1999: 274: 3580–9.

55. Madsen HO, Garred P, Thiel S et al. Interplay between promoter and structural gene variants control basal serum level of mannan-binding protein. *J Immunol* 1995: 155: 3013–20.

56. Madsen HO, Satz ML, Hogh B, Svejgaard A, Garred P. Different molecular events result in low protein levels of mannos-binding lectin in populations from southeast Africa and South America. *J Immunol* 1998: 161: 3169–75.

57. Boldt AB, Petzl-Erler ML. A new strategy for mannos-binding lectin gene haplotyping. *Hum Mutat* 2002: 19: 296–306.

58. Ezekowitz RA, Day LE, Herman GA. A human mannos-binding protein is an acute-phase reactant that shares sequence homology with other vertebrate lectins. *J Exp Med* 1988: 167: 1034–46.

59. Thiel S, Holmskov U, Hvid L, Laursen SB, Jensenius JC. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. *Clin Exp Immunol* 1992: 90: 31–5.

60. Mogues T, Ota T, Tauber AI, Sastry KN. Characterization of two mannos-binding protein cDNAs from rhesus monkey (Macaca mulatta): structure and evolutionary implications. *Glycobiology* 1996: 6: 543–50.

61. Sastry R, Wang JS, Brown DC, Ezekowitz RA, Tauber AI, Sastry KN. Characterization of murine mannos-binding protein genes Mbl1 and Mbl2 reveals features common to other collectin genes. *Mamm Genome* 1995: 6: 103–10.
62. Guo N, Mogues T, Weremowicz S, Morton CC, Sastry KN. The human ortholog of rhesus mannose-binding protein-A gene is an expressed pseudogene that localizes to chromosome 10. *Mamm Genome* 1998; 9: 246–9.

63. Seyfarth J, Garred P, Madsen HO. The ‘involution’ of mannose-binding lectin. *Hum Mol Genet* 2005; 14: 2859–69.

64. Allison AC. Protection afforded by sickle-cell trait against subtertian malareal infection. *BMJ* 1954: 4857: 290–4.

65. Santos IK, Costa CH, Krieger H et al. Mannose-binding lectin enhances susceptibility to visceral leishmaniasis. *Infect Immun* 2001; 69: 5212–5.

66. Garred P, Harboe M, Oettinger T, Koch C, Svejgaard A. Dual role of mannose-binding protein in infections: another case of heterosis? *Eur J Immunogenet* 1994; 21: 125–31.

67. Summerfield JA, Ryder S, Sumiya M et al. Mannose binding protein gene mutations associated with unusual and severe infections in adults. *Lancet* 1995: 345: 886–9.

68. Dahl M, Tybjaerg-Hansen A, Schnoehr P, Nordestgaard BG. A population-based study of morbidity and mortality in mannose-binding lectin deficiency. *J Exp Med* 2004: 199: 1391–9.

69. Tacx AN, Groeneveld AB, Hart MH, Aarden LA, Hack CE. Mannan binding lectin in febrile adults: no correlation with microbial infection and complement activation. *J Clin Pathol* 2003: 56: 956–9.

70. Aittomierri J, Baer M, Soppi E, Vesikari T, Miettinen A. Mannan binding lectin in the prevention of Mycoplasma infection. *Infect Immun* 2005: 73: 5238–40.

71. Turner MW, Super M, Levinsky RJ, Summerfield JA. The molecular basis of a common defect of opsonization. In: Chapel HM, Levinsky RJ, Webster ADB, eds. *Progress in Immunodeficiency III*, International Congress and Symposium Series. London and New York: Royal Society of Medicine Services Limited, 1991, 171–83.

72. Hamvas RM, Johnson M, Vlieger AM et al. Role for mannose binding lectin in the prevention of Mycoplasma infection. *Infect Immun* 2005: 73: 1399–404.

73. Rota PA, Oberste MS, Monroe SS et al. Characterization of a novel coronavirus associated with severe acute respiratory syndrome. *Science* 2003: 300: 1394–9.

74. Marra MA, Jones SJ, Astell CR et al. The genome sequence of the SARS-associated coronavirus. *Science* 2003: 300: 1399–404.

75. Ip WK, Chan KH, Law HK et al. Mannose-binding lectin in severe acute respiratory syndrome coronavirus infection. *J Infect Dis* 2005: 191: 1697–704.

76. Zhang H, Zhou G, Zhi L et al. Association between mannose-binding lectin gene polymorphisms and susceptibility to severe acute respiratory syndrome coronavirus infection. *J Infect Dis* 2005: 192: 1355–61.

77. Yuen MF, Lau CS, Lau YL, Wong WM, Cheng CC, Lai CL. Mannose binding lectin gene mutations are associated with progression of liver disease in chronic hepatitis B infection. *Hepatology* 1999: 29: 1248–51.

78. Chong WP, To YF, Ip WK et al. Mannose-binding lectin in chronic hepatitis B virus infection. *Hepatology* 2005: 42: 1037–45.

79. Thio CL, Mosbruger T, Astemborski J et al. Mannose binding lectin genotypes influence recovery from hepatitis B virus infection. *J Virol* 2005: 79: 9192–6.

80. Matsuhashi M, Hijiakata M, Ohta Y et al. Hepatitis C virus infection and mutations of mannose-binding lectin gene MBL. *Arch Virol* 1998: 143: 645–51.

81. Neth O, Hann I, Turner MW, Klein NJ. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. *Lancet* 2001: 358: 614–8.

82. Peterslund NA, Koch C, Jensensuc JC, Thiel S. Association between deficiency of mannose-binding lectin and severe infections after chemotheraputry. *Lancet* 2001: 358: 637–8.

83. Bergmann OJ, Christiansen M, Laursen I et al. Low levels of mannose-binding lectin do not affect occurrence of severe infections or duration of fever in acute myeloid leukaemia during remission induction therapy. *Eur J Haematol* 2003: 70: 91–7.

84. Shi L, Takahashi K, Dundee J et al. Mannose-binding lectin-deficient mice are susceptible to infection with Staphylococcus aureus. *J Exp Med* 2004: 199: 1379–90.

85. Ezekowitz RA, Kuhlman M, Groopman JE, Byrn RA. A human serum mannose-binding protein inhibits in vitro infection of the human immunodeficiency virus. *J Exp Med* 1989: 169: 185–96.

86. Ying H, Ji X, Hart ML et al. Interaction of mannose-binding lectin with HIV type 1 is sufficient for virus opsonization but not neutralization. *AIDS Res Hum Retroviruses* 2004: 20: 327–35.

87. Garred P, Madsen HO, Balslev U et al. Susceptibility to HIV infection and progression of AIDS in relation to variant alleles of mannose-binding lectin. *Lancet* 1997: 349: 236–40.

88. Garred P, Richter C, Andersen AB et al. Mannan-binding lectin in the sub-Saharan HIV and tuberculosis epidemics. *Scand J Immunol* 1997: 46: 204–8.

89. Nielsen SL, Andersen PL, Koch C, Jensenius JC, Thiel S. The level of the serum opsonin, mannan-binding protein in HIV-1 antibody-positive patients. *Clin Exp Immunol* 1995: 100: 219–22.

90. Prohaszka Z, Thiel S, Ujhelyi E, Szlak J, Banhegyi D, Fust G. Mannan-binding lectin serum concentrations in HIV-infected patients are influenced by the stage of disease. *Inmunol Lett* 1997: 58: 171–5.

91. Boniotti M, Crovella S, Pirulli D et al. Polymorphisms in the MBL2 promoter correlated with risk of HIV-1 vertical transmission and AIDS progression. *Genes Immun* 2000: 1: 346–8.

92. Malik S, Arias M, Di Flumeri C, Garcia LF, Schurr E. Absence of association between mannose-binding lectin gene polymorphisms and HIV-1 infection in a Colombian population. *Immunogenetics* 2003: 55: 49–52.

93. McBride MO, Fischer PB, Sumiya M et al. Mannose-binding protein in HIV-seropositive patients does not contribute to disease progression or bacterial infections. *Int J STD AIDS* 1998: 9: 683–8.

94. Senaldi G, Davies ET, Mahalingam M et al. Circulating levels of mannose binding protein in human immunodeficiency virus infection. *J Infect* 1995: 31: 145–8.
Mannose-binding lectin in innate immunity

R. M. Dommett et al.

95. Maas J, Roda Husman AM, Brouwer M et al. Presence of the variant mannose-binding lectin alleles associated with slower progression to AIDS. Amsterdam Cohort Study. AIDS 1998: 12: 2275–80.

96. Araki T, Tabona P, Summerfield JA. Human mannose-binding protein gene is regulated by interleukins, dexamethasone and heat shock. Q J Med 1993: 86: 575–82.

97. Heggelund L, Molinnes TE, Espevik T et al. Modulatory effect of mannose-binding lectin on cytokine responses: possible roles in HIV infection. Eur J Clin Invest 2005: 35: 765–70.

98. Hundt M, Heiken H, Schmidt RE. Low mannose-binding lectin serum concentrations in HIV long-term nonprogressors? AIDS Res Hum Retroviruses 2000: 16: 1927.

99. Amoroso A, Berrino M, Boniotto M et al. Polymorphism at codon 54 of mannose-binding protein gene influences AIDS progression but not HIV infection in exposed children. AIDS 1999: 13: 863–4.

100. Garred P, Pressler T, Madsen HO et al. Association of mannose-binding lectin gene heterogeneity with severity of lung disease and survival in cystic fibrosis. J Clin Invest 1999: 104: 431–7.

101. Davies JC, Turner MW, Klein N. Impaired pulmonary status in cystic fibrosis adults with two mutated MBL-2 alleles. Eur Respir J 2004: 24: 798–804.

102. Garred P, Strom J, Quist L, Taaning E, Madsen HO. Association of mannose-binding lectin polymorphisms with sepsis and fatal outcome, in patients with systemic inflammatory response syndrome. J Infect Dis 2003: 188: 1394–403.

103. Fidler KJ, Wilson P, Davies JC, Turner MW, Peters MJ, Klein NJ. Increased incidence and severity of the systemic inflammatory response syndrome in patients deficient in mannose-binding lectin. Intensive Care Med 2004: 30: 1438–45.

104. Saevarsdottir S, Oskarsdottir OO, Aspelund T et al. Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. J Exp Med 2005: 201: 117–25.

105. Lee YH, Witte T, Motom T et al. The mannose-binding lectin gene polymorphisms and systemic lupus erythematosus: two case-control studies and a meta-analysis. Arthritis Rheum 2005: 52: 3966–74.

106. Davies EJ, Teh LS, Ordi-Ros J et al. A dysfunctional allele of the mannose binding protein gene associates with systemic lupus erythematosus in a Spanish population. J Rheumatol 1997: 24: 485–8.

107. Ohlenschlaeger T, Garred P, Madsen HO, Jacobsen S. Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. N Engl J Med 2004: 351: 260–7.

108. Garred P, Voss A, Madsen HO, Junker P. Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients. Genes Immun 2001: 2: 442–50.

109. Takahashi R, Tsutsumi A, Ohtani K et al. Association of mannose binding lectin (MBL) gene polymorphism and serum MBL concentration with characteristics and progression of systemic lupus erythematosus. Ann Rheum Dis 2005: 64: 311–4.

110. Malhotra R, Wormald MR, Rudd PM, Fischer PB, Dwek RA, Sim RB. Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. Nat Med 1995: 1: 237–43.

111. Graudal NA, Homann C, Madsen HO et al. Mannan binding lectin in rheumatoid arthritis. A longitudinal study. J Rheumatol 1998: 25: 629–35.

112. Valdimarsson H, Stefansson M, Vikingsdottir T et al. Reconstitution of opsonization activity by infusion of mannan-binding lectin (MBL) to MBL-deficient humans. Scand J Immunol 1998: 48: 116–23.

113. Valdimarsson H, Vikingsdottir T, Bang P et al. Human plasma-derived mannose-binding lectin: a phase I safety and pharmacokinetic study. Scand J Immunol 2004: 59: 97–102.

114. Petersen SV, Thiels S, Jensen L, Steffensen R, Jensenius JC. An assay for the mannose-binding lectin pathway of complement activation. J Immunol Methods 2001: 257: 107–16.

115. Seelen MA, Roos A, Wieslander J et al. Functional analysis of the classical, alternative, and MBL pathways of the complement system: standardization and validation of a simple ELISA. J Immunol Methods 2005: 296: 187–98.

116. Stengaard-Pedersen K, Thiels S, Gadjeva M et al. Inherited deficiency of mannan-binding lectin-associated serum protease 2. N Engl J Med 2003: 349: 554–60.

117. Carlsson M, Sjoholm AG, Eriksson L et al. Deficiency of the mannan-binding lectin pathway of complement and poor outcome in cystic fibrosis: bacterial colonization may be decisive for a relationship. Clin Exp Immunol 2005: 139: 306–13.

118. Atkinson AP, Cedzynski M, Szemraj J et al. L-ficolin in children with recurrent respiratory infections. Clin Exp Immunol 2004: 138: 517–20.

119. Cedzynski M, Szemraj J, Swierok AS et al. Mannan-binding lectin insufficiency in children with recurrent infections of the respiratory system. Clin Exp Immunol 2004: 136: 304–11.

120. Casanova JL, Abel L. Human mannose-binding lectin in immunodeficiency predisposing to infection and atopy in infancy. Arch Dis Child 1983: 58: 799–802.

121. Kawasaki N, Kawasaki T, Yamashina I. Isolation and characterization of a mannan-binding protein from human serum. J Biochem (Tokyo) 1983: 94: 937–47.

122. Richardson VF, Larcher VF, Price JF. A common congenital immunodeficiency predisposing to infection and atopy in infancy. Arch Dis Child 1983: 58: 799–802.

123. Kawasaki N, Yamashina I. Mannan-binding protein and conglutinin in bovine serum. J Biochem (Tokyo) 1985: 98: 1309–20.

124. Turner MW, Seymour ND, Kazatchkine MD, Mowbray JF. Suboptimal C3b/C3bi deposition and defective yeast opsonization. I. Evidence for the absence of essential co-factor activity. Clin Exp Immunol 1985: 62: 427–34.

125. Oka S, Ikeda K, Kawasaki T, Yamashina I. Isolation and characterization of two distinct mannan-binding proteins from rat serum. Arch Biochem Biophys 1988: 260: 257–66.

126. Drickamer K. Two distinct classes of carbohydrate-recognition domains in animal lectins. J Biol Chem 1988: 263: 9557–60.
127. Kawasaki N, Kawasaki T, Yamashina I. A serum lectin (mannan-binding protein) has complement-dependent bactericidal activity. *J Biochem (Tokyo)* 1989: **106**: 483–9.

128. Super M, Levinsky RJ, Turner MW. The level of mannann-binding protein regulates the binding of complement-derived opsonins to mannann and zymosan at low serum concentrations. *Clin Exp Immunol* 1990: **79**: 144–50.

129. Sastry K, Zahedi K, Leilas JM, Whitehead AS, Ezekowitz RA. Molecular characterization of the mouse mannann-binding proteins. The mannann-binding protein A but not C is an acute phase reactant. *J Immunol* 1991: **147**: 692–7.

130. Weis WI, Drickamer K, Hendrickson WA. Structure of a C-type mannann-binding protein complexed with an oligosaccharide. *Nature* 1992: **360**: 127–34.

131. Matsushita M, Takahashi A, Hatsue H, Kawakami M, Fujita T. Human mannann-binding protein is identical to a component of Ra-reactive factor. *Biochem Biophy Res Commun* 1992: **183**: 645–51.

132. Summerfield JA, Sumiya M, Levin M, Turner MW. Association of mutations in mannann binding protein gene with childhood infection in consecutive hospital series. *BMJ* 1997: **314**: 1229–32.

133. Matsushita M, Endo Y, Fujita T. Cutting edge: complement-activating complex of ficolin and mannann-binding lectin-associated serine protease. *J Immunol* 2000: **164**: 2281–4.

134. Jack DL, Jarvis GA, Booth CL, Turner MW, Klein NJ. Mannann-binding lectin accelerates complement activation and increases serum killing of Neisseria meningitidis serogroup C. *J Infect Dis* 2001: **184**: 836–45.

135. Matsushita M, Kuraya M, Hamasaki N, Tsujimura M, Shiraki H, Fujita T. Activation of the lectin complement pathway by H-ficolin (Hakata antigen). *J Immunol* 2002: **168**: 3502–6.

136. Townsend R, Read RC, Turner MW, Klein NJ, Jack DL. Differential recognition of obligate anaerobic bacteria by human mannann-binding lectin. *Clin Exp Immunol* 2001: **124**: 223–8.

137. Davies J, Neth O, Alton E, Klein N, Turner M. Differential binding of mannann-binding lectin to respiratory pathogens in cystic fibrosis. *Lancet* 2000: **355**: 1885–6.

138. Swanson AF, Ezekowitz RA, Lee A, Kuo CC. Human mannann-binding protein inhibits infection of HeLa cells by *Chlamydia trachomatis*. *Infect Immun* 1998: **66**: 1607–12.

139. van Emmerik LC, Kuijper EJ, Fijen CA, Dankert J, Thiell S. Binding of mannann-binding protein to various bacterial pathogens of meningitis. *Clin Exp Immunol* 1994: **97**: 411–6.

140. Polotsky VY, Belisle JT, Mikusova K, Ezekowitz RA, Joiner KA. Interaction of human mannann-binding protein with *Myobacterium avium*. *J Infect Dis* 1997: **175**: 1159–68.

141. Saifuddin M, Hart ML, Gewurz H, Zhang Y, Spear GT. Interaction of mannann-binding lectin with primary isolates of human immunodeficiency virus type 1. *J Gen Virol* 2000: **81**: 949–55.

142. Hart ML, Saifuddin M, Uemura K et al. High mannann glycan and sialic acid on gp120 regulate binding of mannann-binding lectin (MBL) to HIV type 1. *AIDS Res Hum Retroviruses* 2002: **18**: 1311–7.

143. Ji X, Gewurz H, Spear GT. Mannann binding lectin (MBL) and HIV. *Mol Immunol* 2005: **42**: 145–52.

144. Fischer PB, Ellermann-Eriksen S, Thiel S, Jensenius JC, Mogensen SC. Mannann-binding protein and bovine conglutinin mediate enhancement of herpes simplex virus type 2 infection in mice. *Scand J Immunol* 1994: **39**: 439–45.

145. Gadjeva M, Paludan SR, Thiel S et al. Mannann-binding lectin modulates the response to HSV-2 infection. *Clin Exp Immunol* 2004: **138**: 304–11.

146. Tabona P, Mellor A, Summerfield JA. Mannann binding protein is involved in first-line host defence: evidence from transgenic mice. *Immunology* 1995: **85**: 153–9.

147. Schelenz S, Malhotra R, Sim RB, Holmskov U, Bancroft GJ. Binding of host collectins to the pathogenic yeast CRYPTOCoccus neoformans: human surfactant protein D acts as an agglutinin for acapsular yeast cells. *Infect Immun* 1995: **63**: 3360–6.

148. Kelly P, Jack DL, Naem A et al. Mannann-binding lectin is a component of innate mucosal defense against Cryptosporidium parvum in AIDS. *Gastroenterology* 2000: **119**: 1236–42.

149. Klabunde J, Uhlemann AC, Tebo AE et al. Recognition of plasmidium falciparum proteins by mannann-binding lectin, a component of the human innate immune system. *Parasitol Res* 2002: **88**: 113–7.

150. Kahn SJ, Wlekinski M, Ezekowitz RA, Coder D, Aruffo A, Farr A. The major surface glycoprotein of Trypanosoma cruzi amastigotes are ligands of the human serum mannann-binding protein. *Infect Immun* 1996: **64**: 2649–56.

151. Lozano F, Suarez B, Munoz A et al. Novel MASP2 variants detected among North African and Sub-Saharan individuals. *Tissue Antigens* 2005: **66**: 131–5.

152. Lee SG, Yum JS, Moon HM et al. Analysis of *M. avium*-infected LXPA and LYPB with interferon-resistant hepatitis C virus infection in Japanese patients. *J Hepatol* 1998: **29**: 695–700.