

Review

Clinical manifestations of mannan-binding lectin deficiency

S. Thiel*, P.D. Frederiksen, J.C. Jensenius

Department of Medical Microbiology and Immunology, University of Aarhus, DK8000 Denmark

Abstract

Mannan-binding lectin (MBL) is a plasma protein of the innate immune system with the ability to initiate antimicrobial and inflammatory actions. MBL deficiency is common. More than 10% of the general population may, depending on definition, be classified as MBL deficient, underlining the redundancy of the immune system. Ongoing research attempt to illuminate at which conditions MBL deficiency may lead to disease. With examples, this review illustrates the diversity of results obtained so far.

© 2005 Elsevier Ltd. All rights reserved.

Keywords: Neutropenia; Mannose-binding lectin; Autoimmunity; Sepsis; Innate immunity

1. Introduction

The innate immune system is attracting interest, not only because of its action in the immediate defence against infections, but also because of its importance for activating an adequate specific immune response. A number of different molecules are involved in recognizing the foreign agents through structures displayed on their surface, the so-called pathogen associated molecular patterns or PAMPs. The recognition molecules may be cell-associated receptors (pathogen recognizing receptors, PRPs) or soluble pathogen recognizing molecules (PRMs). The latter group includes the collectins, among which mannan-binding lectin (MBL) attracts especial interest due to its ability to bind to microorganisms (Jack and Turner, 2003) leading to activation of the complement system. In blood, MBL is associated with MBL-associated serine proteases (MASPs). All the functions of the MASPs are not known although it is clear that MASP-2 is the enzyme of the MBL/MASP complex needed for activation of complement factor C4 (Thiel et al., 1997). Lately, it has been found that another group of PRMs, the ficolins, may activate the enzymatic cascade of complement using the same MASPs as MBL. While the involvement of MBL in the defence system has been studied extensively, little is presently known

about the function of the ficolins. For a more detailed presentation of these proteins, see Holmskov et al. (2003).

MBL was discovered as a rabbit protein binding to mannan and termed mannan-binding protein (MBP) (Kawasaki et al., 1978). Later the term mannose-binding protein was introduced, unfortunately implying a more selective reactivity than is characteristic for this protein. The burst of investigations on MBL deficiency and susceptibility to infectious diseases was roused by the seminal demonstration of low MBL levels in children deficient in opsonizing activity and suffering from unexplained sensitivity to infections (Super et al., 1989).

Initially, investigations of causal relationship between MBL and disease susceptibility relied on quantification of MBL in serum or plasma (or in some cases, of opsonizing activity). However, the finding of genetic influence on the MBL level (reviewed in Nuytinck and Shapiro, 2004) opened up for determining the MBL status by genotyping. Due to the simplicity of allotyping many papers are published based solely on genotyping. While useful conclusions may be drawn from such studies, it ought to be realized that individuals with identical genotypes for all known MBL variants may differ by 10-fold in MBL levels (Steffensen et al., 2000).

The inter-individual levels of MBL vary from approximately 5 ng (no individual completely deficient in MBL has yet been reported) to more than 10 µg/ml, but the level of MBL in each individual is quite stable throughout life. At

* Corresponding author. Tel.: +45 8942 1776; fax: +45 8619 6128.
E-mail address: st@microbiology.au.dk (S. Thiel).

birth, the level is about 2/3 of adult level, which is reached in a month, and there is a minor decline in old age (Ip et al., 2004). There is a small increase in connection with acute phase responses (Thiel et al., 1992). This increase is slow (1–2 weeks after the inducing event), and modest (up to three-fold increase) compared to the 1000-fold genetically based increase and has unfortunately led to promoting MBL as an acute phase protein, which indeed is formally right. However, we would rather focus on MBL as a protein present when needed and capable of immediate action.

2. MBL genotypes and MBL levels

The gene encoding MBL, *MBL2* (*MBL1* is a pseudogene), is located on chromosome 10q11.2-q21 and contains four exons. A number of 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 (Gly54Asp, named “B” and Gly57Glu, “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). The wild type is denoted “A”. The three variant structural alleles are associated with decreased MBL levels. The promoter region shows a number of SNPs as well, some of which influences the expression of MBL. Here we address only the polymorphisms at –550 (termed H/L), –221 (termed Y/X) and –66 (termed P/Q). Due to linkage disequilibrium only seven haplotypes are found; HYP A, LYP A, LYQA, LXPA, HYPD, LYPB and LYQC, giving a total of 28 possible genotypes (e.g., the MBL deficient genotype: LXPA/LYPB). Individuals homozygous for A show MBL levels above 1 µg/ml, except some of those homozygous for LXPA. Heterozygous people with A on one gene and B, C or D on the other mostly have MBL levels between 0.5 and 1 µg/ml, while those with variant structural allotypes on both genes (genotypes often denoted 0/0) show MBL levels below 50 ng/ml. Such low levels are also found in individuals with LXPA on one gene and B, C or D on the other.

The frequency of the haplotypes differ between ethnic groups with, e.g., LYPB being the common variant haplotype in Caucasians (12%) and Asians (22%), but very rare in Africans. In contrast, LYQC is the common variant haplotype in Africans (24%) but rarely found in Caucasian and Asian people. It is not always realized that the LXPA haplotype, with a gene frequency of 24%, is the most common cause of MBL deficiency in Caucasians, either presented as homozygous LXPA individuals (where the concentration is somewhat unpredictable), or in concert with a variant haplotype, always resulting in very low levels.

MBL genotyping methods have evolved along with inventions in molecular biology and are today fairly labour intensive, but cheap. In addition, high throughput robotized methods are now being used (methods mentioned in Skalnikova et al., 2004). A simple membrane strip genotyp-

ing kit was recently made available (Innogenetics NV, Gent, Belgium) (Nuytinck and Shapiro, 2004). A problem with genotyping is that archive patient samples often comprise plasma or serum, devoid of cells. It is possible to retrieve DNA from serum, large volumes are needed, usually 300 µl or more, and there is not 100% success. DNA can also be amplified from paraffin embedded tissue sections.

Despite the so-called acute-phase-like character discussed above, one may argue that, whatever may be the cause of low MBL, it is the actual concentration of MBL, or the level of functional activity, which is of interest.

Measuring MBL is easy and today there are several commercial suppliers of ELISA kits (Sanquin reagents, Amsterdam, the Netherlands; Hycult Biotechnology, Leiden, the Netherlands; AntibodyShop, Copenhagen, Denmark; Dobeel Corp, South Korea). In our experience (unpublished), they all work satisfactorily, measuring levels comparable to what we determine with our in-house assay based on catching MBL onto a mannan surface followed by detection of bound MBL by MAb 131-1 anti-MBL antibody (Thiel et al., 2002). This functional assay (MBL is estimated by its lectin activity) somewhat surprisingly gives exactly the same results as a sandwich assay employing coating with MAb 131-1 and development with biotinylated MAb 131-1 (Thiel et al., 2002). The presence of small amounts of non-mannan-binding, lower molecular size MBL in variant structural allotype individuals has been noted (Lipscombe et al., 1995; Garred et al., 2003a). If one wishes, this aberrant MBL may be measured in sandwich assays with suitable antibodies.

As noted above, assays using mannan coats and development with anti-MBL antibodies estimate MBL as lectin and thus represent a functional assay for the initial step in the activation of the MBL pathway. If one wishes to estimate the activity of the MBL/MASP complex, thus measuring the MBL pathway activity, one may simply carry out the incubation with the diluted serum sample at 37 °C, and develop with anti-C4 antibody to determine the amount of C4b bound to the surface. Surprisingly, it appears that at high serum dilution (100-fold) there is little or no activation via the classical pathway through bound anti-mannan antibodies (Super et al., 1990). Nevertheless, to be on the safe side, we have chosen to take advantage of the observation that while the C1 complex is unstable at high ionic strength, the MBL/MASP complex stays intact. Thus, the sample, serum or plasma (high salt also abolishes coagulation), is diluted in a buffer containing 1 M NaCl and calcium. Following incubation in the mannan-coated wells and wash, C4 is added, and deposited C4b is estimated after incubation at 37 °C. The results of this assay correlate ($r=0.96$) with the assay for MBL as lectin or antigen (Thiel et al., 2002), except in case of MASP-2 deficiency (Stengaard-Pedersen et al., 2003). Recently, a kit for estimating the functional activity of the entire MBL pathway was made commercially available (Wieslab, Lund, Sweden). This assay measures the amount of soluble membrane attack complex deposited on the mannan surface following incubation with serum at 37 °C, and is thus sensitive to defects in any

component from MBL to C9. It may be noted that some prefer the term “lectin pathway” since L- and H-ficolin appears to function like MBL. However, L-ficolin is not a lectin but a molecule with indiscriminate activity towards acetyl groups (Krarup et al., 2004).

3. Disease associations

Numerous studies have examined relationships between infectious diseases and MBL levels and/or MBL genotypes. The limited space does not allow for mentioning all of these. Instead, we focus on illustrating different aspects of association studies. For a more comprehensive discussion of MBL and infectious diseases we refer to other recent reviews, e.g., by Nuytinck and Shapiro (2004), Eisen and Minchinton (2003) and Kilpatrick (2002). It should be stressed that the majority of individuals in the general population with low MBL levels do not suffer from this condition nor does high levels of MBL seem to be a problem. Thus, an epidemiological study encompassing almost 9245 adults failed to observe any consequences of variant allotypes (Dahl et al., 2004) while studies focussing on patients at tertiary referral hospitals (e.g., Garred et al., 1995 and Summerfield et al., 1997) covering much larger background populations find convincing associations between infections and variant allotypes.

When 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 investigations on leukaemia patients suggest a cut-off level of 500 ng/ml (Peterslund et al., 2001) or even 1 µg/ml (Neth et al., 2001), and in the cases of obstetric problems even lower levels (100 ng/ml) have been used.

3.1. Infectious complications associated with chemotherapy

Chemotherapy induces neutropenia and increased risk of infection. The chemotherapy period may thus be a unique situation for studying the role of MBL in the context of an iatrogenic immunodeficiency. Several studies have thus attempted to analyze the correlation between MBL deficiency and infections in such patients. It turns out to be fairly difficult to compare these studies due to a number of aspects: the studies include patients with a variety of underlying malignancies and thus different chemotherapy regimes have been used; some studies only examine MBL genotypes (and may in some cases disregard the strong influence of the promoter

allotype, LXPA), while others use MBL levels; the outcome measures differ between the studies (duration of fever and neutropenia—serious infection (with some variation in definition)); different combinations of antimicrobial agents and G-CSF has been used at different centers; the study period differs; some are prospective, most are retrospective, which may present difficulties due to variability in journal keeping, e.g., registration of febrile episodes. What emerges is that the indication of increased infections in patients with low MBL levels seen in some studies seems absent or much less pronounced in patients with particular strong suppression of phagocytic activity due to intensive chemotherapy, as when preparing the patient for bone marrow transplantation, or caused by the specific leukemia, as for AML. We shall first describe some reports, which find clear evidence of the importance of MBL for protection in leukemia patients, and then turn to reports where such correlation was not found.

Neth et al. (2001) examined 100 children receiving chemotherapy. MBL genotypes and MBL levels were correlated to the causes, frequency and duration of febrile neutropenic periods. The majority of children were diagnosed with acute lymphoblastic leukemia (ALL). Children with variant MBL alleles exhibited twice as many days of febrile neutropenia as children with wild type genotypes ($p = 0.014$). Each episode of febrile neutropenia was also significantly longer in the group of children with variant 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 ($p = 0.012$). No significant relationship was observed between frequency of infections, other measured clinical parameters and MBL genotypes.

Peterslund et al. (2001) described 54 adults treated with chemotherapy for a range of hematological malignancies. Within 21 days after start of chemotherapy, 16 patients developed clinically significant infections defined as bacteremia, pneumonia or both. MBL levels, measured in plasma obtained before chemotherapy, were lower in these patients than in the ones without such infections ($p < 0.0001$). All patients with the infections, except one, showed MBL levels below 0.5 µg/ml.

Spurred by these studies 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. When analysis was performed on per patient basis a higher rate of severe infections was seen in MBL deficient patients ($p = 0.008$). The impact was further increased when excluding acute leukaemic patients. Focusing on bacteremia and excluding acute leukemia and bone marrow transplant patients, MBL deficiency was associated with a greater rate of bacteremia ($p = 0.01$). The association between low MBL and infections was independent of whether or not the patients received prophylactic antibiotics or GM-CSF. When focusing on AML patients alone there was no correlation between low MBL and infections. The authors

did not see a difference in duration of febrile neutropenia for any of the groups.

Bergmann et al. (2003) studied 80 adult patients with acute myeloid leukemia (AML) and determined MBL levels. They observed no influence of MBL levels on the 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.

In line with this, 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 BMT and more than half presented with AML. Genotypes were not determined. In another study, Rocha et al. (2002) did not see any relationship between exon 1 allotypes (promoter allotypes were not determined) and post-transplant infections (follow-up period of 180 days) in donor-recipient pairs (patients with acute ($n = 39$) or chronic leukemia ($n = 68$)) after HLA-identical sibling bone marrow transplantation.

Conversely Mullighan et al. (2002) studied the MBL genotypes of allogeneic stem cell transplantation recipients ($n = 93$) and donors ($n = 90$) and recorded retrospectively the infection burden, rather than time-to-first-infection. They used a relatively long (1 year and more for some patients) follow-up period and found the presence of the haplotype HYA (in cis with wild type on the other gene, i.e., genotype encoding higher MBL levels) to be associated with reduced risk of infection, especially in the period after neutrophil recovery. Surprisingly, such association was seen for both donor and recipient haplotypes. Thus, significantly more frequent infections were seen when donors carrying an MBL mutation were used (OR 4.1). There is no theory to explain the influence of donor MBL allotypes although it has been suggested that some MBL may be synthesized from the donor cells (Mullighan and Bardy, 2004).

In a retrospective study, Horiuchi et al. (2005) examined infections in 113 patients undergoing high dose therapy (HDT) followed by autologous peripheral blood stem cell transplantation (auto-PBSCT). The patients included non-Hodgkin's lymphoma ($n = 66$), acute myeloid leukemia ($n = 25$) and acute lymphoid leukemia ($n = 10$) patients. They found that carrying deficiency genotypes (B/B or B/LXA) was associated with a higher risk of contracting major bacterial infection (microbiologically confirmed systemic or disseminated infection) during the 100 days following the auto-PBSCT treatment. The patients received prophylactic antibiotics. The authors suggest that a variety of factors due to the presence of donor cells after allogeneic stem cell transplantation will influence the analyses. Thus, HDT followed by auto-PBSCT may prove a good model to analyze the contribution of MBL levels to infections after myeloablative treatments.

Studies by Aittoniemi et al. (1999) (28 patients with chronic lymphocytic leukemia) and Tacx et al. (2003) (177 patients with new onset fever admitted to an internal medicine

department) with few details did not observe any effect of MBL on infections.

As mentioned in the beginning of this section numerous differences are found between the studies conducted. A critical factor may be the differences in the intensity of the conditioning regimes used (leading to various levels and duration of neutropenia) as well as the variations in follow-up periods. Hopefully, further studies will describe the patients particularly at risk when being MBL deficient.

3.2. SIRS/sepsis

A systemic inflammatory response syndrome (SIRS) will develop in most patients after major operations (Bone, 1992). 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). These conditions present major challenges (indeed between 50 and 80% of all critically ill patients may be classified as having SIRS/sepsis) and a large number of studies have tried to identify risk markers.

Hansen et al. (2003b) studied patients randomly assigned to either conventional treatment or intensive insulin therapy at an intensive care unit (ICU). All 451 patients who needed prolonged intensive care (>5 days) were included. MBL concentrations were measured on admission, days 5, 15, and the last day in the ICU. In the group of 243 conventionally treated patients, the 49 who died (predominantly from sepsis and MOD) had significantly (three-fold) lower MBL levels upon admission than the survivors ($p = 0.04$). Intensive insulin therapy significantly reduced mortality from sepsis and MOD and the lack of significant association between MBL levels and outcome may thus be due to the small number of cases.

Another carefully conducted study investigated the MBL levels and genotypes in a total of 272 adults (197 with sepsis) prospectively admitted to the ICU (Garred et al., 2003b). 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 ($p < 0.001$), and the risk ratios for the development of “severe sepsis” and “septic shock” ranged from 1.3–3.2 times higher in patients with A/O or O/O versus A/A genotype. MBL levels were inversely related to the severity of sepsis ($p = 0.0032$). As above an increased risk of fatal outcome (83 of the patients died) was observed in patients with variant MBL allele.

Looking at adult patients admitted to a mixed medical–surgical ICU, Sutherland et al. (2005) characterized the MBL genotypes of 222 critically ill Caucasians with SIRS. The patients were divided in high or low MBL haplotype groups. Patients in the low MBL haplotype group had significantly increased prevalence of positive bacterial cultures at admission to the ICU ($p < 0.02$). Patients in the low MBL haplotype group did not have significantly increased rates of sepsis or septic shock at admission to the ICU. Sur-

vival at day 28 did not differ significantly between the low MBL haplotype and high MBL haplotype groups.

Fidler et al. (2004) analyzed the MBL genotypes and levels in a total of 100 critically ill children (50 with infectious, 50 with non-infectious insults) admitted to pediatric ICU. A seven-fold (confidence interval = 2.7–18.6, $p < 0.0001$) 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 versus A/A). A significant association was also found between severity of the systemic response to infection and the presence of an MBL mutation ($p = 0.002$). 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 1000 ng/ml had a greater chance of developing SIRS (80% versus 40%).

A study of the frequency of sepsis in very low birth weight infants (including 356 patients, 50 of which developed culture proven sepsis) 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).

The immune defense is compromised by the trauma involved in major operations. Patients are thus at increased risk of infection following major surgery. This is of particular interest in cancers since the recurrence rate is significantly higher and the survival period shorter in patients suffering post-operational infections. In a report of 156 patients undergoing major elective gastrointestinal surgery for malignant disease, Siassi et al. (2003) report that patients who developed sepsis or SIRS showed significantly lower mean post-operative MBL levels ($p = 0.013$). In addition, Ytting et al. (2005) have reported on significantly increased frequency of pneumonia after primary operation in colorectal cancer patients with low MBL levels ($p = 0.01$).

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.

3.3. Autoimmune and inflammatory diseases

The etiology of autoimmune diseases is largely unknown. Initiation by infections and the presence of inflammatory processes, and the involvement of complement components in such processes, has spurred a number of studies on association with MBL.

Studies on associations between MBL deficiency and rheumatoid arthritis (RA) have been discussed in detail before (Graudal, 2004; Barton et al., 2004) and we shall thus not go into detail. Depending on ethnic groups, type of patients and the symptoms studied associations were seen in some but not in other studies. On balance, the indications are that low

MBL levels may be linked with symptoms indicating a poor prognosis as well as an earlier debut.

From the several investigations on MBL and systemic lupus erythematosus (SLE) the consensus is emerging that low levels of MBL predisposes to development of the disease. Certainly, the connection is not like for C1q where SLE develops in almost all of the rare cases of deficiency. Rather, it may be that MBL deficiency aggravates the disease or further the development and consequently an earlier diagnosis is achieved. A summary of the studies on MBL and SLE may be found in the paper by Garred et al. (2001) who performed a meta-analysis and in, e.g., Ohlenschlaeger et al. (2004) and 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 (91 patients were included, 24 developed arterial thrombosis) (hazard ratio 7, after correction for other known risk factors) (Ohlenschlaeger et al., 2004).

Celiac disease is a multi factorial disorder with a strong allergic reaction against gluten in the small intestine. The development is linked to the HLA haplotypes, DR2 and DR8. A study by Boniotto et al. (2002) encompassing 117 patients and 130 controls indicated an association between celiac disease and the presence of variant MBL alleles. Later the same group (Boniotto et al., *in press*) investigated 149 patients and 147 controls and found the frequency of homozygosity for variant MBL alleles to be higher in the patients ($p = 0.035$). The low MBL genotypes were strongly associated with more celiac disease symptoms ($p = 0.001$) as well with increased frequency of secondary autoimmune diseases ($p = 0.01$). 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., 2003). This finding in Italy is supported from Finnish celiac disease patients. Iltanen et al. (2003) thus found association between the B allotype and MBL in a study of 88 celiac disease patients and 138 controls ($p = 0.004$). Boniotto et al. (*in press*) suggest that impaired removal of apoptotic cells due to MBL deficiency might predispose to the development of autoimmune symptoms. A recent paper now describes that mice lacking MBL exhibits less efficient removal of apoptotic cells (Stuart et al., 2005). In vitro studies have previously implicated MBL in removal of apoptotic cells (Ogden et al., 2001; Nauta et al., 2004). Another 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.

The possibility of MBL involvement has also been considered in two other inflammatory bowel diseases (IBD), Ulcerative colitis (UC) and Cohn's disease (CD). Their pathogenesis is unknown, but genetic as well as environmental factors, e.g., microorganisms, are implicated. Rector et al. (2001) examined MBL genotypes of 431 IBD patients (142 UC and 287

CD and 2 with indeterminate colitis) and compared this with 112 affected and 141 non-affected first-degree relatives and 308 healthy controls. The number of individuals with low MBL variant haplotypes was significantly lower in UC as compared to CD ($p=0.01$) and to controls ($p=0.02$), while no difference was found between CD and controls. It was suggested that MBL may be responsible for the more extensive complement-mediated mucosal damage in UC compared to CD and low MBL levels could thus protect somewhat against the development of the disease.

Seibold et al. (2004), conducting a smaller study, measured MBL levels in 74 CDs, 22 UCs and 32 healthy controls, and MBL genotyped 58 CDs, 18 UCs and 47 controls (excluding patients with active disease from the study). 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 ($p < 0.01$). If only CD patients and controls were considered, the significance increased further ($p=0.005$). Antibodies against mannan from *Saccharomyces cerevisiae* are present in many patients with CD (this is a routine clinical parameter). In this study, 47% in the patients with CD and in 0% of the controls. It was found that more CD patients with anti-mannan antibodies had low MBL levels ($p < 0.0001$) as was also true when looking at T-cell proliferation in response to mannan ($p < 0.0001$). It would appear that the immune reactivity against mannan in CD is regulated by the MBL concentration.

Studies on MBL and IBD are few and it seems likely that profound (and potentially useful) information may be gained by extending these studies.

3.4. The possible role of MBL in 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. A few examples are given below.

Kawasaki disease is a systemic vasculitis in childhood possibly caused by infections. The vasculitis has a predilection for the coronary arteries and in the developed world Kawasaki disease is the most common cause of acquired heart disease in children (Royle et al., 2005). Based on the idea of MBL as an initiator of inflammation, Biezeveld et al. (2003) studied the frequency of MBL genotypes in 90 Dutch patients with Kawasaki disease. They found a higher frequency of MBL mutations as compared to the genotypes in 88 controls ($p=0.03$). In children younger than 1 year, those with mutations were at higher risk of development of coronary artery lesions (OR 16, $p=0.026$). Kawasaki disease occurs more frequent in oriental children (roughly 50/100,000, 10 times more frequent than in Caucasians) (Royle et al., 2005). Studying Kawasaki disease among Hong Kong Chinese patients, Cheung et al. (2004) included 71 patients and 41 matched controls and determined MBL genotypes and MBL levels. They did not see a difference between the MBL genotypes of patients and controls. When analyzing for brachioradial arte-

rial stiffness, which is an important cardiovascular risk factor in the children, they found this to be associated with low MBL genotypes (multiple linear regression analysis, $p=0.03$), concluding that MBL genotypes may modulate the disease. The recent finding of an association between a novel human coronavirus and Kawasaki disease (Esper et al., 2005) may fit with the many indications of MBL having anti-viral activity.

Plaque material may be removed from inside of the carotid artery (e.g., by endarterectomy) to avoid cerebral attack. Restenosis is often occurring after such a procedure (in typically 10% of the patients after the first year). In a prospective study of 123 patients by Rugonfalvi-Kiss et al. (2005), it was 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 38 heart transplant recipients, Fiane et al. (2005) recorded transplant-associated coronary artery disease and observed an association with MBL deficiency ($p=0.02$). 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 ($p=0.016$). Assuming that MBL may interact with the transplanted tissue and initiate complement activation, this study thus add to the list of studies suggesting that complement activation is harmful for the endothelium in general, and possibly for the allograft endothelium in particular.

Atherosclerosis is a main cause of morbidity in the western world, and since inflammatory reactions are involved in the damage to the endothelium studies have also been aimed at examining a possible correlation to MBL. 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 (relative risk 4.9, $p=0.017$). Saevarsdottir et al. (2005) found in a cohort study in Iceland (including a cross-sectional group of 987 and a nested control sample of 1309 individuals) that the risk of developing myocardial infarction was higher in MBL deficient individuals.

It is known that type 1 diabetic patients are at high risk for micro- and macro-vascular complications, possibly due to inflammatory reactions. 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, 2005; Saevarsdottir et al., 2005). Normoalbuminuric type 1 diabetics 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., 2003a). No correlation between MBL and C-reactive protein levels (a generally used marker for inflammatory reactions) was seen. Another study showed that 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 (OR = 1.52)

(Hansen et al., 2004). Saraheimo et al. (2005) confirmed the elevated serum MBL levels in type 1 diabetic patients with diabetic nephropathy. In a prospective 18-years 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 $\mu\text{g/ml}$ 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). The data above suggest 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.

3.5. Obstetrics

There are indications for a link between immunological disorders with increased risk of in utero infections and recurrent spontaneous abortion (RSA). In line with this, Kilpatrick et al. (1995) reported an association between low MBL levels and unexplained RSA. Surprisingly they found the frequency of MBL deficiency to be higher not only in females but also in male partners of couples with RSA (16 and 14%, respectively) compared to controls (<5%). In another study from Kilpatrick et al. (1999) once more both female and male MBL deficiency levels were over-represented in Scottish RSA couples. Christiansen et al. (1999) (including 146 RSA females and 41 male partners and 444 controls) found an increased frequency of MBL deficiency (15%) only in the female partners as compared to 9% of the control group. The prevalence of MBL deficiency increased with increasing number of abortions ($p < 0.01$).

In another study, MBL levels was measured in 217 women with unexplained RSA and 111 of their husbands and were compared with corresponding measurements in 104 couples with uncomplicated reproductive histories and 210 healthy blood donors (Kruse et al., 2002). Among women with RSA 19% of the women had low MBL levels as compared to 12% of the control group ($p = 0.02$). Association was only seen with maternal MBL deficiency. Patients with MBL levels below 100 ng/ml had a higher abortion rate than patients with normal MBL levels ($p < 0.05$). As in a study by Baxter et al. (2001), who investigated 76 RSA couples, Kruse et al. (2002) did not see a difference when analyzing the MBL genotypes. Also, Baxter et al. (2001) who included 76 couples with RSA and 69 control couples found the frequencies of MBL genotypes to be similar in the two groups. It appears that measuring MBL levels and not genotyping is most relevant in this group of patients.

Since chorioamnionitis is usually the result of pathogenic organisms Annells et al. (2005) examined 181 women with spontaneous preterm birth (between 20 and 35 weeks of pregnancy), divided them into 69 with and 112 without chorioamnionitis and further established a number of SNPs in immunologically relevant genes. They found that the

presence of the MBL B allele was positively associated with chorioamnionitis (multivariable odds ratio 2.0, $p = 0.04$). Annells et al. (2004) also found the presence of the B allele to be significantly (multivariable odds ratio 2.3, $p = 0.02$) associated with preterm birth before 29 weeks ($n = 202$) as compared to children born at term ($n = 185$).

Babula et al. (2003) observed a higher frequency of MBL variant allele in women with vulvovaginal candidiasis (VVC) when examining 42 patients and 43 controls. MBL was shown to be present in cervicovaginal lavage as was also found by Pellis et al. (2005). Babula et al. (2004) also studied 122 patients with vestibulitis and compared with 99 controls. The variant MBL B allele was more frequent ($p < 0.01$) and the MBL levels were lower ($p < 0.0001$) in the patients. On the other hand Pellis et al. (2005) studied 22 women with bacterial vaginosis and 25 patients with vulvovaginal candidiasis and 23 women with no symptoms as controls group and observed no influence of MBL allotypes.

With a link to the above mentioned studies on correlation with MBL genotypes or levels and vaginal infections (fungal and bacterial), and as animal results has indicated an antiviral activity of MBL, Gadjeva et al. (2004) studied a cohort of 70 patients with genital HSV-2 infection (symptomatic and asymptomatic). A higher frequency of MBL-deficient patients was seen in the symptomatic group when compared to the asymptomatic group, suggesting that MBL may be involved in the clearance of HSV-2 infection.

While more investigations are surely needed, it seems that low MBL levels may be a risk factor for RSA and obstetric complications.

4. MBL for therapy

We have been interested in the possibility of therapy with MBL in clinical situations. An early trial with MBL purified from donor plasma indicated absence of adverse effects (possibly even beneficial effect) (Valdimarsson et al., 1998) when reconstituting MBL deficient individuals, and the safety of such MBL infusion was born out in a phase I study (Valdimarsson et al., 2004). A number of considerations led us to exploit the possibility of producing clinical grade recombinant MBL (rMBL), and promising results led to establishing a company having this aim (Jensenius et al., 2003). Phase I trials with rMBL have now been successfully concluded. While many of the MBL-deficiency-associated clinical conditions mentioned above would theoretically be candidates for reconstitution treatment, one must initially aim at investigating small defined patient groups with relatively short follow-up periods of immunodeficiency, such as selected patients with chemotherapy-induced neutropenia or SIRS/sepsis patients (but obviously a better definition of the patients may be needed). The treatment of chronic disorders may possibly also be considered on the longer term.

5. Animal studies

The creation of MBL knock-out mice has 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 a sepsis model where *Staphylococcus aureus* was injected via the tail vein (Shi et al., 2004), lack of MBL led to significantly increased mortality. 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 it was found that 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 Møller-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, de Vries et al. (2004) found MBL-A and -C deposited in the kidneys after ischemia/reperfusion in MBL wild type mice.

6. Conclusions

When bound to microorganisms the MBL/MASP complex is an efficient activator of complement with ensuing killing

of the foreign agent. One would thus a priori expect significant manifestations of MBL deficiency. Accordingly, there is abundant evidence for association between susceptibility to infections and MBL deficiency. But this appears to surface only in certain clinical situations where other elements of the immune system are compromised. The immune defence is abundant, or redundant, and survival of the individual is ensured by manifold defence mechanisms. A more subtle, regulatory role of MBL is indicated by the prevalence of MBL deficiency in autoimmune disorders, suggesting new avenues of investigation.

References

- Ahrens, P., Kattner, E., Kohler, B., Hartel, C., Seidenberg, J., Segerer, H., Moller, J., Gopel, W., 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., Miettinen, A., Laine, S., Sinisalo, M., Laippala, P., Vilpo, L., Vilpo, J., 1999. Opsonising immunoglobulins and mannan-binding lectin in chronic lymphocytic leukemia. *Leuk. Lymphoma* 34, 381–385.
- Annells, M.F., Hart, P.H., Mullighan, C.G., Heatley, S.L., Robinson, J.S., McDonald, H.M., 2005. Polymorphisms in immunoregulatory genes and the risk of histologic chorioamnionitis in Caucosoid women: a case control study. *BMC Pregnancy Childbirth* 5, 1–9.
- Annells, M.F., Hart, P.H., Mullighan, C.G., Heatley, S.L., Robinson, J.S., Bardy, P., McDonald, H.M., 2004. Interleukins-1, -4, -6, -10, tumor necrosis factor, transforming growth factor-[beta], FAS, and mannose-binding protein C gene polymorphisms in Australian women: risk of preterm birth. *Am. J. Obstet. Gynecol.* 191, 2056–2067.
- Babula, O., Danielsson, I., Sjoberg, I., Ledger, W.J., Witkin, S.S., 2004. Altered distribution of mannose-binding lectin alleles at exon I codon 54 in women with vulvar vestibulitis syndrome. *Am. J. Obstet. Gynecol.* 191, 762–766.
- Babula, O., Lazdane, G., Kroica, J., Ledger, W.J., Witkin, S.S., 2003. Relation between recurrent vulvovaginal candidiasis, vaginal concentrations of mannose-binding lectin, and a mannose-binding lectin gene polymorphism in Latvian women. *Clin. Infect. Dis.* 37, 733–737.
- Barton, A., Platt, H., Salway, F., Symmons, D., Lunt, M., Worthington, J., Silman, A., 2004. Polymorphisms in the mannose binding lectin (MBL) gene are not associated with radiographic erosions in rheumatoid or inflammatory polyarthritis. *J. Rheumatol.* 31, 442–447.
- Baxter, N., Sumiya, M., Cheng, S., Erlich, H., Regan, L., Simons, A., Summerfield, J.A., 2001. Recurrent miscarriage and variant alleles of mannose binding lectin, tumour necrosis factor and lymphotoxin alpha genes. *Clin. Exp. Immunol.* 126, 529–534.
- Bergmann, O.J., Christiansen, M., Laursen, I., Bang, P., Hansen, N.E., Ellegaard, J., Koch, C., Andersen, V., 2003. 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.* 70, 91–97.
- Biezeveld, M.H., Kuipers, I.M., Geissler, J., Lam, J., Ottenkamp, J.J., Hack, C.E., Kuijpers, T.W., 2003. Association of mannose-binding lectin genotype with cardiovascular abnormalities in Kawasaki disease. *Lancet* 361, 1268–1270.
- Bone, R.C., 1992. Toward an epidemiology and natural history of SIRS (systemic inflammatory response syndrome). *JAMA* 268, 3452–3455.
- Boniotto, M., Braida, L., Baldas, V., Not, T., Ventura, A., Vatta, S., Radillo, O., Tedesco, F., Percopo, S., Montico, M., Amoroso, A., Crovella, S., 2005. Evidence of a correlation between mannose binding lectin and celiac disease: a model for other autoimmune diseases. *J. Mol. Med.* 83 (4), 308–315 [Epub 2005, Jan 6].

- Boniotto, M., Braidia, L., Spano, A., Pirulli, D., Baldas, V., Trevisiol, C., Not, T., Tommasini, A., Amoroso, A., Crovella, S., 2002. Variant mannose-binding lectin alleles are associated with celiac disease. *Immunogenetics* 54, 596–598.
- Boniotto, M., Radillo, O., Braidia, L., Pirulli, D., Citta, A., Not, T., Amoroso, A., Crovella, S., 2003. Detection of MBL-2 gene expression in intestinal biopsies of celiac patients by in situ reverse transcription polymerase chain reaction. *Eur. J. Histochem.* 47, 177–180.
- Cheung, Y.F., Ho, M.H., Ip, W.K., Fok, S.F., Yung, T.C., Lau, Y.L., 2004. Modulating effects of mannose binding lectin genotype on arterial stiffness in children after Kawasaki Disease. *Pediatr. Res.* 56, 591–596.
- Christiansen, O.B., Kilpatrick, D.C., Souter, V., Varming, K., Thiel, S., Jensenius, J.C., 1999. Mannan-binding lectin deficiency is associated with unexplained recurrent miscarriage. *Scand. J. Immunol.* 49, 193–196.
- Dahl, M., Tybjaerg-Hansen, A., Schnohr, P., Nordestgaard, B.G., 2004. A population-based study of morbidity and mortality in mannose-binding lectin deficiency. *J. Exp. Med.* 199, 1391–1399.
- de Vries, B., Walter, S.J., Peutz-Kootstra, C.J., Wolfs, T.G., van Heurn, L.W., Buurman, W.A., 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.
- Eisen, D.P., Minchinton, R.M., 2003. Impact of mannose-binding lectin on susceptibility to infectious diseases. *Clin. Infect. Dis.* 37, 1496–1505.
- Esper, F., Shapiro, E.D., Weibel, C., Ferguson, D., Landry, M.L., Kahn, J.S., 2005. Association between a novel human coronavirus and Kawasaki disease. *J. Infect. Dis.* 191, 499–502.
- Fiane, A.E., Ueland, T., Simonsen, S., Scott, H., Endresen, K., Gullestad, L., Geiran, O.R., Haraldsen, G., Heggelund, L., Andreassen, A.K., Wergeland, R., Froland, S., Aukrust, P., Mollnes, T.E., 2005. Low mannose-binding lectin and increased complement activation correlate to allograft vasculopathy, ischaemia, and rejection after human heart transplantation. *Eur. Heart J.*, Apr 8; [Epub ahead of print].
- Fidler, K.J., Wilson, P., Davies, J.C., Turner, M.W., Peters, M.J., Klein, N.J., 2004. Increased incidence and severity of the systemic inflammatory response syndrome in patients deficient in mannose-binding lectin. *Int. Care Med.* 30, 1438–1445.
- Gadjeva, M., Paludan, S.R., Thiel, S., Slavov, V., Ruseva, M., Eriksson, K., Lowhagen, G.B., Shi, L., Takahashi, K., Ezekowitz, A., Jensenius, J.C., 2004. Mannan-binding lectin modulates the response to HSV-2 infection. *Clin. Exp. Immunol.* 138, 304–311.
- Garred, P., Larsen, F., Madsen, H.O., Koch, C., 2003a. Mannose-binding lectin deficiency-revisited. *Mol. Immunol.* 40, 73–84.
- Garred, P., Madsen, H.O., Hofmann, B., Svejgaard, A., 1995. Increased frequency of homozygosity of abnormal mannan-binding-protein alleles in patients with suspected immunodeficiency. *Lancet* 346, 941–943.
- Garred, P., Strom, J.J., Quist, L., Taaning, E., Madsen, H.O., 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., Voss, A., Madsen, H.O., 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.
- Graudal, N., 2004. Natural history and prognosis of rheumatoid arthritis: association of radiographic outcome with process variables, joint motion and immune proteins. *Scand. J. Rheumatol.* 118 (Suppl.), 1–38.
- Hansen, T.K., 2005. Mannose-binding lectin (MBL) and vascular complications in diabetes. *Horm. Metab. Res.* 37 (Suppl. 1), 1–4.
- Hansen, T.K., Tarnow, L., Thiel, S., Steffensen, R., Stehouwer, C.D., Schalkwijk, C.G., Parving, H.H., Flyvbjerg, A., 2004. Association between mannose-binding lectin and vascular complications in Type 1 diabetes. *Diabetes* 53, 1570–1576.
- Hansen, T.K., Thiel, S., Knudsen, S.T., Gravholt, C.H., Christiansen, J.S., Mogensen, C.E., Poulsen, P.L., 2003a. Elevated levels of mannan-binding lectin in patients with Type 1 Diabetes. *J. Clin. Endocrinol. Metab.* 88, 4857–4861.
- Hansen, T.K., Thiel, S., Wouters, P.J., Christiansen, J.S., Van den Berghe, G., 2003b. Intensive insulin therapy exerts antiinflammatory effects in critically ill patients and counteracts the adverse effect of low mannose-binding lectin levels. *J. Clin. Endocrinol. Metab.* 88, 1082–1088.
- Holmskov, U., Thiel, S., Jensenius, J.C., 2003. Collectins and ficolins: humoral lectins of the innate immune defense. *Annu. Rev. Immunol.* 21, 547–578.
- Horiuchi, T., Gondo, H., Miyagawa, H., Otsuka, J., Inaba, S., Nagafuji, K., Takase, K., Tsukamoto, H., Koyama, T., Mitoma, H., Tamimoto, Y., Miyagi, Y., Tahira, T., Hayashi, K., Hashimura, C., Okamura, S., Harada, M., 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.
- Hovind, P., Hansen, T.K., Tarnow, L., Thiel, S., Steffensen, R., Flyvbjerg, A., Parving, H.H., 2005. Mannose-binding lectin as a predictor of microalbuminuria in type 1 diabetes – An inception cohort study. *Diabetes* 54, 1523–1527.
- Iltanen, S., Maki, M., Collin, P., Mustalahti, K., Kaukinen, K., Partanen, J., Hulkkonen, J., Hurme, M., Aittoniemi, J., 2003. The association between mannan-binding lectin gene alleles and celiac disease. *Am. J. Gastroenterol.* 98, 2808–2809.
- Ip, W.K., To, Y.F., Cheng, S.K., Lau, Y.L., 2004. Serum mannose-binding lectin levels and mbl2 gene polymorphisms in different age and gender groups of southern Chinese adults. *Scand. J. Immunol.* 59, 310–314.
- Jack, D.L., Turner, M.W., 2003. Anti-microbial activities of mannose-binding lectin. *Biochem. Soc. Trans.* 31, 753–757.
- Jensenius, J.C., Jensen, P.H., McGuire, K., Larsen, J.L., Thiel, S., 2003. Recombinant mannan-binding lectin (MBL) for therapy. *Biochem. Soc. Trans.* 31, 763–767.
- Jordan, J.E., Montalto, M.C., Stahl, G.L., 2001. Inhibition of mannose-binding lectin reduces postischemic myocardial reperfusion injury. *Circulation* 104, 1413–1418.
- Kawasaki, T., Etoh, R., Yamashina, I., 1978. Isolation and characterization of a mannan-binding protein from rabbit liver. *Biochem. Biophys. Res. Commun.* 81, 1018–1024.
- Kilpatrick, D.C., 2002. Mannan-binding lectin: clinical significance and applications. *Biochim. Biophys. Acta* 1572, 401–413.
- Kilpatrick, D.C., Bevan, B.H., Liston, W.A., 1995. Association between mannan binding protein deficiency and recurrent miscarriage. *Hum. Reprod.* 10, 2501–2505.
- Kilpatrick, D.C., McLintock, L.A., Allan, E.K., Copland, M., Fujita, T., Jordanides, N.E., Koch, C., Matsushita, M., Shiraki, H., Stewart, K., Tsujimura, M., Turner, M.L., Franklin, I.M., Holyoake, T.L., 2003. No strong relationship between mannan binding lectin or plasma ficolins and chemotherapy-related infections. *Clin. Exp. Immunol.* 134, 279–284.
- Kilpatrick, D.C., Starrs, L., Moore, S., Souter, V., Liston, W.A., 1999. Mannan binding lectin concentration and risk of miscarriage. *Hum. Reprod.* 14, 2379–2380.
- Krurup, A., Thiel, S., Hansen, A., Fujita, T., Jensenius, J.C., 2004. L-ficolin is a pattern recognition molecule specific for acetyl groups. *J. Biol. Chem.* 279, 47513–47519.
- Kruse, C., Rosgaard, A., Steffensen, R., Varming, K., Jensenius, J.C., Christiansen, O.B., 2002. Low serum level of mannan-binding lectin is a determinant for pregnancy outcome in women with recurrent spontaneous abortion. *Am. J. Obstet. Gynecol.* 187, 1313–1320.
- Lipscombe, R.J., Sumiya, M., Summerfield, J.A., Turner, M.W., 1995. Distinct physicochemical characteristics of human mannose binding protein expressed by individuals of differing genotype. *Immunology* 85, 660–667.
- Ma, Y., Uemura, K., Oka, S., Kozutsumi, Y., Kawasaki, N., Kawasaki, T., 1999. Antitumor activity of mannan-binding protein in vivo as

- revealed by a virus expression system: mannan-binding protein-independent cell-mediated cytotoxicity. *Proc. Natl. Acad. Sci. U.S.A.* 96, 371–375.
- Madsen, H.O., Videm, V., Svejgaard, A., Svennevig, J.L., Garred, P., 1998. Association of mannose-binding-lectin deficiency with severe atherosclerosis. *Lancet* 352, 959–960.
- Møller-Kristensen, M., Wang, W., Ruseva, M., Thiel, S., Nielsen, S., Takahashi, K., Shi, L., Ezekowitz, A., Jensenius, J.C., Gadjeva, M., 2005. Mannan-binding lectin recognizes structures on ischemic reperfused mouse kidneys and is implicated in tissue injury. *Scand. J. Immunol.* 61, 426–434.
- Mullighan, C.G., Heatley, S., Doherty, K., Szabo, F., Grigg, A., Hughes, T.P., Schwarzer, A.P., Szer, J., Tait, B.D., Bik To, L., Bardy, P.G., 2002. Mannose-binding lectin gene polymorphisms are associated with major infection following allogeneic hemopoietic stem cell transplantation. *Blood* 99, 3524–3529.
- Mullighan, C.G., Bardy, P.G., 2004. Mannose-binding lectin and infection following allogeneic hemopoietic stem cell transplantation. *Leuk. Lymphoma* 45, 247–256.
- Nauta, A.J., Castellano, G., Xu, W., Woltman, A.M., Borrias, M.C., Daha, M.R., van Kooten, C., Roos, A., 2004. Opsonization with C1q and Mannose-Binding Lectin Targets Apoptotic Cells to Dendritic Cells. *J. Immunol.* 173, 3044–3050.
- Neth, O., Hann, I., Turner, M.W., Klein, N.J., 2001. Deficiency of mannose-binding lectin and burden of infection in children with malignancy: a prospective study. *Lancet* 358, 614–618.
- Nuytinck, L., Shapiro, F., 2004. Mannose-binding lectin: laying the stepping stones from clinical research to personalized medicine. *Personalized Med.* 1, 35–52.
- Ogden, C.A., deCathelineau, A., Hoffmann, P.R., Bratton, D., Ghebrehwet, B., Fadok, V.A., Henson, P.M., 2001. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. *J. Exp. Med.* 194, 781–796.
- Ohlschlaeger, T., Garred, P., Madsen, H.O., Jacobsen, S., 2004. Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. *N. Engl. J. Med.* 351, 260–267.
- Pellis, V., De Seta, F., Crovella, S., Bossi, F., Bulla, R., Guaschino, S., Radillo, O., Garred, P., Tedesco, F., 2005. Mannose binding lectin and C3 act as recognition molecules for infectious agents in the vagina. *Clin. Exp. Immunol.* 139, 120–126.
- Peterslund, N.A., Koch, C., Jensenius, J.C., Thiel, S., 2001. Association between deficiency of mannose-binding lectin and severe infections after chemotherapy. *Lancet* 358, 637–638.
- Rector, A., Lemey, P., Laffut, W., Keyaerts, E., Struyf, F., Wollants, E., Vermeire, S., Rutgeerts, P., Van Ranst, M., 2001. Mannan-binding lectin (MBL) gene polymorphisms in ulcerative colitis and Crohn's disease. *Genes. Immun.* 2, 323–328.
- Rocha, V., Franco, R.F., Porcher, R., Bittencourt, H., Silva Jr., W.A., Latouche, A., Devergie, A., Esperou, H., Ribaud, P., Socie, G., Zago, M.A., Gluckman, E., 2002. Host defense and inflammatory gene polymorphisms are associated with outcomes after HLA-identical sibling bone marrow transplantation. *Blood* 100, 3908–3918.
- Royle, J., Burgner, D., Curtis, N., 2005. The diagnosis and management of Kawasaki disease. *J. Paediatr. Child Health* 41, 87–93.
- Rugonfalvi-Kiss, S., Dosa, E., Madsen, H.O., Endresz, V., Prohaszka, Z., Laki, J., Karadi, I., Gonczol, E., Selmeci, L., Romics, L., Fust, G., Entz, L., Garred, P., 2005. High rate of early restenosis after carotid eversion endarterectomy in homozygous carriers of the normal mannose-binding lectin genotype. *Stroke* 36, 944–948.
- Saevarsdottir, S., Oskarsson, O.O., Aspelund, T., Eiriksdottir, G., Vikingsdottir, T., Gudnason, V., Valdimarsson, H., 2005. Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. *J. Exp. Med.* 201, 117–125.
- Saraheimo, M., Forsblom, C., Hansen, T.K., Teppo, M., Fagerudd, J., Pettersson-Fernholm, K., Thiel, S., Tarnow, L., Ebeling, P., Flyvbjerg, A., Groop, H., on behalf of the Finn Diane Study Group, 2005. Increased levels of mannan-binding lectin in type 1 diabetic patients with incipient and overt nephropathy. *Diabetologia* 48, 198–202.
- Seibold, F., Konrad, A., Flogerzi, B., Seibold-Schmid, B., Arni, S., Jnliger, S., Kun, J.F.J., 2004. Genetic variants of the mannan-binding lectin are associated with immune reactivity to mannans in Crohn's disease. *Gastroenterology* 127, 1076–1084.
- Shi, L., Takahashi, K., Dundee, J., Shahroor-Karni, S., Thiel, S., Jensenius, J.C., Gad, F., Hamblin, M.R., Sastry, K.N., Ezekowitz, R.A., 2004. Mannose-binding Lectin-deficient Mice Are Susceptible to Infection with *Staphylococcus aureus*. *J. Exp. Med.* 199, 1379–1390.
- Siassi, M., Hohenberger, W., Riese, J., 2003. Mannan-binding lectin (MBL) serum levels and post-operative infections. *Biochem. Soc. Trans.* 31, 774–775.
- Skalnikova, H., Freiburger, T., Chumchalova, J., Grombirikova, H., Sediva, A., 2004. Cost-effective genotyping of human MBL2 gene mutations using multiplex PCR. *J. Immunol. Methods* 295, 139–147.
- Steffensen, R., Thiel, S., Varming, K., Jersild, C., Jensenius, J.C., 2000. Detection of structural gene mutations and promoter polymorphisms in the mannan-binding lectin (MBL) gene by polymerase chain reaction with sequence-specific primers. *J. Immunol.* 261, 33–42.
- Stengaard-Pedersen, K., Thiel, S., Gadjeva, M., Møller-Kristensen, M., Sorensen, R., Jensen, L.T., Sjøholm, A.G., Fugger, L., Jensenius, J.C., 2003. Inherited deficiency of mannan-binding lectin-associated serine protease 2. *N. Engl. J. Med.* 349, 554–560.
- Stuart, L.M., Takahashi, K., Shi, L., Savill, J., Ezekowitz, R.A., 2005. Mannose-binding lectin-deficient mice display defective apoptotic cell clearance but no autoimmune phenotype. *J. Immunol.* 174, 3220–3226.
- Summerfield, J.A., Sumiya, M., Levin, M., Turner, M.W., 1997. Association of mutations in mannose binding protein gene with childhood infection in consecutive hospital series. *BMJ* 314, 1229–1232.
- Super, M., Levinsky, R.J., Turner, M.W., 1990. The level of mannan-binding protein regulates the binding of complement-derived opsonins to mannan and zymosan at low serum concentrations. *Clin. Exp. Immunol.* 79, 144–150.
- Super, M., Lu, J., Thiel, S., Levinsky, R.T., Turner, M.W., 1989. Association of low levels of mannan-binding protein with a common defect of opsonisation. *Lancet* 334, 1236–1239.
- Sutherland, A.M., Walley, K.R., Russell, J.A., 2005. Polymorphisms in CD14, mannose-binding lectin, and Toll-like receptor-2 are associated with increased prevalence of infection in critically ill adults. *Crit. Care Med.* 33, 638–644.
- Tacx, A.N., Groeneveld, A.B.J., Hart, M.H., Aarden, L.A., Hack, C.E., 2003. Mannan binding lectin in febrile adults: no correlation with microbial infection and complement activation. *J. Clin. Pathol.* 56, 956–959.
- Takahashi, R., Tsutsumi, A., Ohtani, K., Muraki, Y., Goto, D., Matsumoto, I., Wakamiya, N., Sumida, T., 2005. Association of mannose binding lectin (MBL) gene polymorphism and serum MBL concentration with characteristics and progression of systemic lupus erythematosus. *Ann. Rheum. Dis.* 64, 311–314.
- Thiel, S., Møller-Kristensen, M., Jensen, L., Jensenius, J.C., 2002. Assays for the functional activity of the mannan-binding lectin pathway of complement activation. *Immunobiology* 205, 446–454.
- Thiel, S., Holmskov, U., Hviid, L., Laursen, S.B., Jensenius, J.C., 1992. The concentration of the C-type lectin, mannan-binding protein, in human plasma increases during an acute phase response. *Clin. Exp. Immunol.* 90, 31–35.
- Thiel, S., Vorup-Jensen, T., Stover, C.M., Schwaebler, W., Laursen, S.B., Poulsen, K., Willis, A.C., Eggleton, P., Hansen, S., Holmskov, U., Reid, K.B., Jensenius, J.C., 1997. A second serine protease associated with mannan-binding lectin that activates complement. *Nature* 386, 506–510.
- Valdimarsson, H., Stefansson, M., Vikingsdottir, T., Arason, G.J., Koch, C., Thiel, S., Jensenius, J.C., 1998. Reconstitution of opsonizing activity by infusion of mannan-binding lectin (MBL) to MBL-deficient humans. *Scand. J. Immunol.* 48, 116–123.

- Valdimarsson, H., Vikingsdottir, T., Bang, P., Saevarsdottir, S., Gudjonsson, J.E., Oskarsson, O., Christiansen, M., Blou, L., Laursen, I., Koch, C., 2004. Human plasma-derived mannan-binding lectin: a phase I safety and pharmacokinetic study. *Scand. J. Immunol.* 59, 97–102.
- Vekemans, M., Georgala, A., Heymans, C., Muanza, F., Paesmans, M., Klastersky, J., Barette, M., Meuleman, N., Huet, F., Robinson, O.J., Marchetti, O., Calandra, T., Costantini, S., Ferrant, A., Petersen, K., Axelsen, M., Aoun, M., 2005. Influence of mannan 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.
- Ytting, H., Christensen, I.J., Jensenius, J.C., Thiel, S., Nielsen, H.J., 2005. Preoperative mannan-binding lectin pathway and prognosis in colorectal cancer. *Cancer Immunol. Immunother.* 54, 265–272.