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Mannose-binding lectin and the kidney

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Introduction

The complement system is a key component of innate immunity that plays a major role in host defense against invading pathogens. This is effectuated by a direct attack of pathogens, by mediating inflammation and opsonin-dependent phagocytosis, and by induction and amplification of adaptive immunity. In recent years, it has become increasingly clear that the complement system also plays an important role in

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immune homeostasis in the healthy situation. In this respect, genetic complement deficiencies are not only associated with infectious diseases, but also with autoimmune and inflammatory diseases. Among the complement deficiencies described in humans, deficiency of the complement factor mannose-binding lectin (MBL) has the highest frequency. Due to its large genetic heterogeneity, MBL is a highly interesting and attractive object of study, and many studies have now revealed the association of a variety of human diseases with the MBL status, being either MBL-deficient or MBL-sufficient. These studies demonstrate that MBL can play both an advantageous role and an adverse role, depending on the context. In the present overview, the role of MBL in diseases of the kidney will be presented and discussed.

MBL and the lectin pathway of complement

Activation of the complement system involves three known activation pathways, i.e. the classical pathway, the alternative pathway and the lectin pathway (Figure 1) [1,2]. Recently, a fourth pathway has been described as the C2-bypass pathway [3,4]. Each of these pathways has its own mechanism of target recognition, cleavage of the key complement component C3 and activation of the common terminal complement pathway, finally resulting in formation of the membrane attack complex C5b-9.

The classical complement pathway involves binding of its recognition factor Clq to, for example, immune complexes, leading to activation of C4 and C2 involving enzymatic cleavage by the serine protease C1s, and generation of the C3 convertase C4b2a. The more recently discovered lectin pathway of complement is activated following an interaction of the plasma lectins mannose-binding lectin (MBL), L-ficolin, H-ficolin or M-ficolin with their carbohydrate ligands [5-9]. This leads to activation of MASPs (MBL-associated serine proteases) present in a pro-enzymatic complex with these lectins. Activated MASP-2, the functional homologue of C1s, generates C4b2a, followed by C3 cleavage. In contrast to the classical pathway and the lectin pathway, the alternative pathway leads to activation of C3 in a C4-independent way, involving factor B, factor D and properdin.

MBL is a multimeric C-type lectin present in human serum [10]. It consists of collagenous tails similar to those of Clq, and C-type lectin domains that can bind to certain carbohydrates, including mannose and N-acetyl glucosamine, in a calcium-dependent fashion. Three MBL gene (mbl2) polymorphisms (SNPs) have been identified that are associated with functional MBL deficiency. These SNPs are located in codon 54 (B genotype) codon 57 (C genotype), and codon 52 (D genotype) of the first exon, encoding the collagenous region of the MBL molecule [11–13]. MBL exon 1 SNPs, when present on either one or two alleles, hamper the polymerization of the MBL molecule and affect the structure and function of MBL [14,15]. Furthermore, additional SNPs in the promoter and untranslated region of the mbl2 gene modify the basal serum level of MBL [16,17]. Six SNPs of exon 1 and promoter region are in linkage disequilibrium and define seven common MBL haplotypes [17].

In the laboratory, MBL deficiency can be detected using a number of different approaches. In the first place, MBL genotyping can be performed, providing

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**Fig. 1.** The three pathways of the complement system. Complement can be activated via the classical pathway, involving Clq as a recognition molecule, via the lectin pathway, involving MBL, L-ficolin, H-ficolin or M-ficolin as recognition molecules, or via the alternative pathway, involving the binding of factor B to spontaneously hydrolysed C3 [C3 (H2O)], cleavage of factor B by factor D, and stabilization of the alternative pathway C3 convertase by properdin (P) and a protective surface. Both the classical pathway and the lectin pathway lead to cleavage of C4 and C2 and formation of the C3 convertase C4b2a. Cleavage of C3 by C3 convertase initiates the terminal complement pathway, finally resulting into formation of the membrane attack complex (MAC) C5b-9. Other functional products of complement activation include C3b as a major opsonin and the chemotactic factors C3a and C5a, being important pro-inflammatory mediators.
clear and basic information on the genetic status. The disadvantage of this approach is that MBL function might still be highly variable within the seven haplotypes that can be defined with the most frequent SNPs, most likely explained by additional genetic variation [14]. Secondly, MBL serum concentrations can be measured by ELISA. Most of such ELISA’s have a strong preference for measurement of high molecular weight (wildtype) MBL and, therefore, provide an excellent correlate to MBL function (Figure 2). Since MBL serum levels are stable and hardly affected by for example, serum storage, and since a recent study also shows that these serum levels are extremely constant over time in the same individual [18], the measurement of MBL serum concentration is highly useful in clinical studies to determine the MBL status. By ELISA, concentrations of functional MBL may vary up to 1000-fold in the human population. Finally, MBL status can be measured functionally, using e.g. MBL complex assays to determine the activity of the MBL–MASP complex, or MBL pathway assays to measure activation of the complement pathway initiated by MBL [19,20]. These assays are the first choice in a clinical diagnostic setting, but in a research setting their use might be hampered, since they require high-quality and well-stored material.

Depending on ethnicity, the total allele frequency of the B, C and D alleles in exon 1 of the MBL gene may be above 40% [17]. Since these polymorphisms apparently are not subject to a high negative selection pressure, it has been proposed that the polymorphisms are associated with host protection in certain situations [21]. Furthermore, the clinical effect of MBL deficiency is strongly dependent on the immune status and the pathogen pressure of the individuals tested, and most MBL-deficient individuals are apparently healthy. Therefore, although MBL gene polymorphisms do have important functional consequences for activation of the lectin pathway of complement, most affected persons have alternative mechanisms for target recognition to reach a sufficient level of anti-microbial protection [14]. However, MBL deficiency may confer a strongly increased risk for infectious disease in immune-compromised individuals [22,23].

MBL and IgA nephropathy

IgA nephropathy (IgAN) is a common renal disease, characterized by mesangial deposition of IgA and complement. In vivo and in vitro evidence has been presented for involvement of the alternative pathway, whereas the classical pathway is unlikely to contribute. However, several studies have shown glomerular deposition of C4, as well as circulating C4 activation products in a subpopulation of IgAN patients [24,25], suggesting a contribution of the lectin pathway of complement. Indeed, deposition of MBL in association with IgA in glomeruli was reported in a subpopulation of IgAN patients by several authors [26–28]. Whereas Matsuda et al. [26] reported MBL deposition in association with deposition of C2 and C4, proteinuria, subnormal creatinine clearance and mesangial proliferation, these findings could not be reproduced by Lhotta et al. [27] and by Endo et al. [28].

Interestingly, MBL deposition was also observed in glomeruli from patients with Henoch Schönlein purpura nephritis [29,30]. Although in the first publication [29], a relation with complement activation or clinical disease characteristics could not be observed, Hisano et al. [30] presented a relation between MBL deposition and clinical and histological parameters of disease, including haematuria, proteinuria, mesangial matrix expansion and glomerular sclerosis in children with Henoch Schönlein purpura nephritis.

On the basis of in vitro experiments with purified molecules, our group provided evidence for a calcium-dependent interaction between human polymeric but not monomeric IgA and the lectin domain of MBL, suggesting that MBL does bind to IgA-associated carbohydrates [31]. These studies strongly suggest that human IgA exposes carbohydrates that serve as a ligand for MBL. We hypothesize that such an
interaction takes place in glomeruli from IgAN patients.

In line with this hypothesis, we recently confirmed, in an Italian–Dutch collaboration, the presence of IgA in the mesangial area of glomeruli from IgAN patients [32]. We divided our population of IgAN patients in two groups based on the complement deposition profile. About 75% of IgAN patients showed negative glomerular staining for MBL, L-ficolin, MASP, C4d and C4-binding protein, indicating that C3 and C5b-9 activation in these patients most likely occurs via the alternative pathway. In contrast, 25% of IgAN patients show glomerular deposition of MBL, L-ficolin, MASP, C4d but not C1q, which is strongly indicative for activation of complement via the lectin pathway of complement. Importantly, the clinical and histological data clearly indicate that activation of the lectin pathway of complement is associated with more severe renal damage, as demonstrated by proteinuria, the presence of renal failure, and histological findings of mesangial proliferation, extracapillary proliferation, glomerular sclerosis and interstitial fibrosis [32].

Taken together, the available data suggest that activation of the lectin pathway of complement in glomeruli from IgAN patients, involving not only MBL but also L-ficolin [32], plays an adverse role in this disease. Increased disease progression in association with glomerular lectin pathway activation may possibly be ascribed to the increased production of pro-inflammatory activation products of the complement system.

**MBL and diabetic nephropathy**

Although the pathogenesis of type I and type II diabetes is different, both diseases may show similar complications upon disease progression, as a consequence of elevated blood glucose levels. Vascular complications of type I and type II diabetes are common and a leading cause for renal failure, frequently leading to dialysis and transplantation. A number of studies have, up to now, investigated the potential role of MBL in diabetes. Type I diabetic patients have increased plasma levels of MBL [33,34]. This is also found at disease onset [35]. The increased levels of circulating MBL in type I diabetics as compared to healthy aged-matched controls are most apparent in carriers of alleles that promote a high production of MBL [35]. However, the difference could not be explained by genetic differences, indicating that the increased levels of MBL are probably not directly related to susceptibility to the disease.

It has been suggested that the high production of MBL in type I diabetics is related to impaired insulin production and subnormal concentrations of insulin in the portal vein [34]. The latter fact would also be apparent during insulin substitution therapy, due to systemic administration. In line with this hypothesis, treatment of severely ill patients with insulin resulted in less production of MBL and suppressed the acute phase response as assessed by CRP production [36]. Alternatively, MBL production could be increased as a result of hyperglycaemia, as can be deduced from the reported correlation between MBL levels and HbA1c [37]. Interestingly, type II diabetic patients were reported to have normal levels of circulating MBL [38], possibly supporting a role for insulin in MBL regulation.

In a population of albuminuric type I diabetic patients without overt albuminuria, MBL serum levels were correlated with the urinary albumin excretion [33]. In a follow-up study, these authors showed that type I diabetic patients with diabetic nephropathy showed higher levels of circulating MBL than diabetic controls without proteinuria [34]. Furthermore, this association could be confirmed by MBL genotyping: diabetic nephropathy was clearly associated with the presence of MBL alleles that promote high MBL production [34]. MBL levels measured early in the disease were demonstrated to be an independent risk factor for the later development of persistent micro- or macro-albuminuria [39].

In a recent analysis of type II diabetic patients, high plasma levels of MBL were shown to predict albuminuria only when present in combination with high levels of CRP [38]. Importantly, in type II diabetics, a high MBL level is a significant and independent risk factor for death, probably mainly explained by cardiovascular disease [38].

Recently, the role of MBL in diabetic injury has been addressed experimentally, using streptozotocin-induced diabetes in mice. In this experimental model, a role for MBL in development of renal injury could not yet be unequivocally demonstrated [40].

Taken together, MBL plays an unfavourable role in diabetic patients, most likely involving a pro-inflammatory role of MBL and complement activation at the vascular level. At present, to our knowledge, no data are available concerning the binding of MBL to proteins modified by high glucose. Basic studies in this direction would contribute significantly to the understanding of the role of MBL in diabetic vascular injury.

**MBL and I/R injury**

Renal ischaemia/reperfusion (I/R) injury is a major issue in nephrology, both in the situation of renal transplantation and in other diseases characterized by acute renal failure. A large number of experimental studies have provided evidence that complement is involved in I/R injury (reviewed in [41]). The complement activation pathway involved could be the classical pathway, the alternative pathway, and/or the lectin pathway, depending on the model systems studied. For experimental renal I/R injury, it has been shown that the terminal complement pathway and the membrane attack complex are involved, whereas C4 is not required [42–45], suggesting a role for the alternative pathway.
A number of studies provide evidence that activation of the lectin pathway can be responsible for complement activation and related inflammation in I/R injury [46]. Cultured endothelial cells that were subjected to oxidative stress bind MBL and show deposition of C3 upon their exposure to human serum [47]. In an in vivo model for myocardial infarction [48], treatment of rats with a blocking antibody directed against rat MBL showed significantly less myocardial damage upon occlusion of a coronary artery than controls. Inhibition of MBL resulted also in reduced inflammation and expression of pro-inflammatory genes [48].

Whereas humans have one functional MBL gene, mice and rats produce two MBL species, named MBL-A and MBL-C. Recently, experimental studies became available, using mice that are genetically deficient for both MBL-A and MBL-C, showing that these mice were protected against intestinal and myocardial I/R injury [49,50]. In renal I/R injury in the mouse, clear deposition of MBL was demonstrated in tubular and endothelial cells [51,52]. Furthermore, mice deficient for MBL-A and MBL-C were partially protected against renal I/R injury [52].

Together, these studies confirm that MBL and the lectin pathway of complement are involved in I/R injury. With respect to renal I/R injury, several pathways are probably involved, including MBL-mediated complement activation. A recent study suggests that MBL may bind to natural IgM antibodies that recognize auto-antigens in ischaemic tissue, thereby activating the lectin pathway [53]. Further studies are required for the definition of the mechanisms involved.

**MBL and renal transplantation**

Renal transplantation involves early immunological and non-immunological processes including ischaemia and reperfusion, leading to tissue damage. Lessons from patients and experimental models have indicated that early graft damage determines the long-term outcome of a graft. Therefore, we hypothesize that complement contributes to early and late graft damage upon renal transplantation.

Several studies point to a role for complement in renal transplantation. Inhibition of complement by soluble complement receptor-1 prolonged graft survival and decreased complement activation and tissue injury in a model of renal allotransplantation in the rat [54,55]. A more recent study showed a critical role for local complement production in the graft, using an animal model for allotransplantation. Kidneys from C3-deficient mice showed long-term survival when transplanted in MHC-incompatible C3+/− recipients without immune suppression, whereas C3+/− kidneys from the same strain were subject to rapid acute allograft rejection [56]. Moreover, in a recent study in human renal transplantation patients, the donor C3 allotype was shown to be a risk factor for renal transplant loss [57]. These studies provide evidence that locally produced C3 contributes to renal allograft rejection, but they do not provide insight into the mechanism of C3 activation.

A recent study in a mouse renal transplantation model indicates that, in contrast to previous studies in C3-deficient mice [56], deficiency of C4, a key complement factor of both the classical and the lectin pathway of complement, does not modify the survival of mouse renal allografts [58]. These studies suggest that C3, but not C4, is involved in acute allograft rejection in this mouse model, and may suggest a role for the alternative pathway in C3 activation. To the best of our knowledge, studies directly addressing the role of components of the lectin pathway in experimental transplantation have not yet been reported.

Based on the adverse role of MBL in inflammatory diseases, and on the contribution of MBL to I/R injury, we hypothesized that MBL might play an unfavourable role in human renal transplantation via its contribution to complement activation. To test this hypothesis, we analysed the pre-transplantation MBL status of 266 recipients of renal allografts [59]. Patients were divided on basis of their serum MBL concentration into two groups, i.e. high MBL (> 400 ng/ml) and low MBL (< 400 ng/ml). Transplantation patients with high MBL levels turned out to have a significantly worse death-censored graft survival than patients with a low MBL level. We could establish that the excess graft loss in the high-MBL group was largely due to treatment-resistant transplant rejection (Figure 3).

In follow-up to these studies, we performed a similar analysis in diabetic patients treated with simultaneous pancreas/kidney transplantation. In this study, we were able to confirm the protective effect of low MBL levels, not only with respect to renal transplant survival, but similarly for pancreas transplant survival. Moreover, MBL proved to be a major determinant in overall survival of these patients, and high levels of MBL (above 400 ng/ml) were associated with a hazard.
ratio of 6 for patient death. The adverse effect of MBL was largely explained by cardiovascular death in this high-risk patient population [60].

In order to further understand the mechanisms and interactions in the local environment of a renal allograft, early analysis of complement deposition in human allograft biopsies is required, both following early I/R injury as during rejection processes. In a preliminary study, MBL deposition was shown in early biopsies of kidneys from non-heart-beating donors [51]. Renal deposition of C4d, a marker for acute and chronic humoral rejection, could not be associated with MBL deposition [61,62], but recently an association with deposition of H-ficolin, another recognition molecule of the lectin pathway, has been presented [62].

Together, these studies indicate a role for complement and for the lectin pathway in human renal allograft injury. The mechanisms of complement activation and the contribution of complement to the rejection process are largely unexplored and experimental in vivo and in vitro studies are required. However, it is likely that early graft injury, either induced by the transplantation procedure (including I/R injury) or induced by early rejection processes, may trigger the binding of MBL and other innate immune molecules to injured cells, and subsequently cause complement activation and production of inflammatory mediators. These processes may lead to enhanced inflammation and antigen presentation, as well as amplification of graft recognition by the adaptive immune system. Furthermore, local production of complement molecules including C3 contributes to early inflammation, antigen presentation and injury [63].

**MBL and the kidney: conclusions**

The kidney is an organ that is prone to immunological injury, and complement plays a well-recognized role in many renal disease processes [59,64]. The present review proposes that MBL, a recognition molecule of a more recently discovered complement activation pathway, plays an adverse role in a number of renal inflammatory diseases (IgA nephropathy, diabetic nephropathy, renal I/R injury, renal transplantation, possible post-streptococcal glomerulonephritis [65]). This adverse role of MBL is in contrast to the favourable role of MBL in protection against pathogens and infectious disease. In this context, we recently reported that a high-MBL status is strongly protective against severe infections in recipients of liver transplants [23].

We hypothesize that the contribution of MBL to renal injury is largely due to the deposition of circulating MBL in the injured kidney, followed by activation of the complement cascade and production of pro-inflammatory molecules. Recent data demonstrate that circulating MBL is produced by the liver, whereas extrahaepatic production of circulating MBL could not be established [23]. The kidney does not show mRNA for MBL under resting conditions [66]. However, we do not exclude that MBL might be produced in the kidney under inflammatory conditions, for example by resident cells in response to inflammatory stimuli, or by infiltrating inflammatory cells. The latter option is supported by data suggesting low level production of MBL in the spleen and by monocyte-derived cells [66]. Such local MBL, as has now been proposed for locally produced C3, may contribute to the inflammatory process.

Further studies are required to identify the different pathogenetic mechanisms involved in MBL-mediated renal injury. The recent availability of MBL-knockout mice will hopefully facilitate exploration of the role of MBL and the lectin pathway in experimental renal disease. It is anticipated that detailed insight into the role of the lectin pathway of complement in renal inflammatory disease will lead to development of novel and well-targeted therapeutic strategies.

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**Conflict of interest statement.** A previous review on this subject was published in French in the Proceedings of the Actualités Néphrologiques Jean Hamburger, Hôpital Necker, 2007.

**References**


Towards the prevention of bone fractures in dialysed patients?

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Epidemiology of bone fractures in dialysed patients

Incidence

Alem et al. [1] have compared the incidence of hip fractures in US dialysis patients [based on United States Renal Data System (USRDS) Registry data] with that of the general population, based on data from Olmstead County (Minnesota). They report a four times higher incidence in dialysed patients matched for age, gender and race (the study being limited to Caucasians).

Very recently, the incidence of hip fractures and all fractures has been studied in the 12 countries of the Dialysis Outcomes and Practice Patterns Study (DOPPS, phase 2). The yearly incidence is 0.89% for hip fractures and 2.6% for all fractures (for a mean age around 60 years), without major differences between countries after adjustment for demographics and comorbidity [2]. This study extends to the worldwide haemodialysis (HD) community the evidence of a much higher incidence of hip fracture in HD patients than in the general population. Indeed, at around 60–65 years, the yearly incidence ranges from 0.07% to 0.22% in the general population (reviewed in [16]), vs 0.49–1.57% in HD patients from DOPPS 2 countries (Table 1). The incidence of hip fractures in HD patients is actually similar to or higher than that of the general population aged 10 to 20 more years! The reasons for this much higher incidence in HD patients are discussed below.

Risk factors

Stehman-Breen et al. [3] identified multiple independent risk factors for hip fracture in dialysed