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Low mannose-binding lectin serum levels are associated with reduced kidney graft survival

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Activation of the complement system is initiated by the alternative, the classical, or the lectin pathway. As the complement system is involved in the pathophysiology of graft rejection after kidney transplantation, we investigated the possible role of mannose-binding lectin in kidney transplantation and the influence of human leukocyte antigen (HLA) immunization on this process. In a prospective study of 544 kidney transplant patients over a follow-up period of 5 years, low serum levels of this lectin at the time of transplantation were found to be significantly associated with decreased 5-year death-censored graft survival (hazard ratio 1.68). Subanalysis showed that this association was confined to non-HLA-immunized patients (hazard ratio 1.93). The strongest association was seen in non-HLA-immunized patients receiving a kidney from a deceased donor (hazard ratio 2.93). No significant association with mannose-binding lectin levels and graft survival were found in HLA-immunized patients. Variant *MBL2* genotypes causing low mannose-binding lectin serum concentrations showed the same association pattern. Our findings demonstrate a clear protective role of mannose-binding lectin and thus innate immunity in maintaining kidney graft survival, but these are probably overruled by HLA immunization.

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If an eligible donor kidney is available, renal replacement is the therapy of choice for patients having end-stage kidney disease. During the past three decades, the kidney graft survival has increased markedly, mainly because of the introduction of new immunosuppressive treatment regimes primarily targeting components of the adaptive immune system. Several factors are known to influence graft survival, such as the underlying disease, the age of the patient and of the donor, human leukocyte antigen (HLA) match, the HLA immunization status of the patient, cytomegalovirus (CMV) infection, and whether the kidney grafts are obtained from a deceased donor or a living donor.[1] The complement system has an essential role in antibody-mediated kidney rejection via the classical C1q-dependent pathway.[2] Deposition of downstream complement component factor C4 is a prognostic marker of reduced long-term graft survival.[2]

Activation of the complement system is accomplished via three different initiation pathways: The alternative pathway, the classical pathway, and the lectin pathway.[3] In humans, four recognition molecules of the lectin pathway have been described: mannose-binding lectin (MBL), ficolin-1 (M-ficolin), ficolin-2 (L-ficolin), and ficolin-3 (H-ficolin or Hakata antigen).[4]

MBL is a pattern-recognition molecule that recognizes sugar structures on bacteria, viruses, and fungi, thereby allowing the activation of the lectin pathway of the complement via a set of serine proteases named MBL/ficolin-associated serine proteases.[5] MBL is derived from the *MBL2* gene, which is mainly expressed by hepatocytes. Following synthesis, MBL is released to the blood stream. The MBL serum level is highly related to variations in both the promoter- and protein-encoding regions of the *MBL2* gene.[6] Numerous studies have shown that the presence of *MBL2* variant alleles and low serum levels are associated with increased risk of different types of infections particularly in early childhood and in patients with different types of immunodeficiencies.[7] In addition, a role of MBL in tissue maintenance has been documented both in human and in animal models, as MBL may bind to and sequester dying host cells.[8] In line with this notion, low MBL levels and *MBL2* variant alleles have been shown to be associated with complications to cardiovascular diseases, suggesting a

cardioprotective effect of MBL.[9–12] Conversely, MBL has been shown to be an important contributor to ischemia–reperfusion injury in various animal models and in humans.[13–17] Moreover, studies have shown an important role of MBL in intestinal and renal ischemia–reperfusion injury.[18–22] Similarly, high serum levels of MBL have been reported to be associated with rejection-mediated damage of kidneys after transplantation.[23,24] However, the clinical importance of the proinflammatory role of MBL in kidney transplantation has recently been questioned, as the *MBL2* gene profile was not associated with graft survival in a large cohort of Dutch patients.[25]

Because of the possible clinical importance of MBL in kidney transplantation, and to discern whether high or low levels of MBL may be of importance for kidney survival, we investigated the possible association between MBL serum levels and *MBL2* genotypes with graft survival in conjunction with HLA immunization in a well-characterized prospective Danish regional center cohort.

RESULTS

Patients

A total of 544 patients were included; 72 patients lost their graft and 18 patients died during the study period.

MBL

The median MBL concentration in the whole patient group was measured to be 1137 μg/l (range: 0–9031 μg/l, 25 and 75%: 420 and 2346 μg/l, respectively). To find the best cutoff level between low and high MBL, receiver-operating characteristic analysis of the MBL concentration in relation to the 5-year kidney graft survival was performed (Supplementary Figure S1 online). The sensitivity of this analysis was found to be 74.2, the specificity 34.7, and the criterion >454.049. Thus, the optimal MBL cutoff level was 454 μg/l. This level was used for further analyses to discriminate between low and high MBL levels throughout. The low-MBL group counted 145 patients (26.7%) with a median concentration of 179 μg/l, and the high MBL group counted 399 patients (73.3%) with a median concentration of 1738 μg/l. There was a very good correlation between MBL2 genotypes and serum concentrations (Figure 1) ($P<0.00001$).

After genotyping, the patients were grouped according to variations in the promoter and in the structural part of the *MBL2* gene. Two groups were constructed, *YA/YA + YA/XA + XA/XA + YA/YO* versus *XA/YO + YO/YO*, according to previous experience,[26] corresponding to medium/high level of functional MBL and low level of functional MBL. In the group of patients with the medium/high-expression haplotypes, the median MBL concentration was 1484 μg/l ($n=432$), and in the low *MBL2*-expressing haplotypes the median MBL concentration was 75 μg/l ($n=80$).

Demographics of the MBL groups

No significant differences were observed between the group of patients with a MBL level ⩽454 μg/l and patients with >454 μg/l with regard to age at transplantation, recipient gender, donor age, donor gender, donor type (deceased or living), number of HLA-DR mismatches, cold ischemia time, delayed graft function, treatment with mycophenolate mofetil, or the number of deaths during the 5-year follow-up (Table 1). A small difference was observed between the two groups in relation to donor CMV status: 55.7% of the donors were CMV-positive in patients with a MBL level ⩽454 μg/l vs. 66.6% in patients with MBL level >454 μg/l ($P=0.04$) and in relation to number of HLA-A, B mismatches: 1.77 mismatches in patients with a MBL level ⩽454 μg/l vs. 1.99 in patients with MBL level >454 μg/l ($P=0.04$) (Table 1).

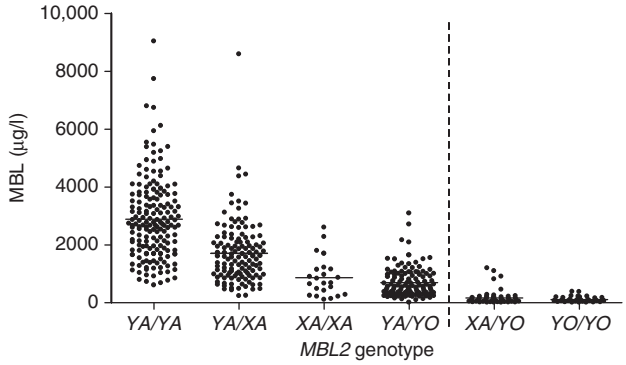

Figure 1 | Serum levels of mannose-binding lectin (MBL) in kidney transplant patients according to *MBL2* genotypes ($P<0.00001$). The genotypes *YA/YA, YA/XA, XA/XA,* and *YA/YO* corresponding to medium/high levels of functional MBLs are grouped and the genotypes *XA/YO* and *YO/YO* corresponding to low levels of functional MBLs are grouped.

Kidney graft survival according to MBL serum concentration

The 5-year kidney graft survival censored for death with a functioning graft was 79.0% in patients with an MBL level ⩽454 μg/l (low-MBL group, $n=145$) and 85.4% in patients with an MBL level >454 μg/l (high-MBL group, $n=399$) ($P=0.0345$, Figure 2a). If the patients were divided according to whether they were HLA-immunized or not, the 5-year death-censored graft survival in non-HLA-immunized patients was 81.6% in the low-MBL group ($n=129$) compared with 88.5% in the high-MBL group ($n=335$) ($P=0.0176$, Figure 2b), and in HLA-immunized patients the graft survival was 68.1% in the high-MBL group ($n=64$) and in the low-MBL group ($n=16$) four patients lost the graft and the last observed patient lost the graft ($P=0.4019$, Figure 2c).

After transplantation with a kidney from a deceased donor, the graft survival was 71.5% in the low-MBL group ($n=105$) and 86.1% in the high-MBL group (276), respectively ($P=0.0007$, Figure 2d), and after transplantation with a kidney from a living donor the opposite pattern was observed, although not significant: 96.7% in the low-MBL group ($n=40$) and 84.2% in the high-MBL group ($n=123$) ($P=0.0777$, Figure 2e). Only one patient in the low-MBL group of patients transplanted with a living donor lost the graft.