Impact of the complement lectin pathway on cytomegalovirus disease early after kidney transplantation

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Abstract

Background. This study retrospectively investigated the association between pre-transplant levels of mannose-binding lectin (MBL) plus the associated serine protease (MASP)-2 and the occurrence of cytomegalovirus (CMV) infection and symptomatic CMV disease during the first 12 weeks after kidney transplantation.

Materials and methods. Altogether 159 consecutive single kidney transplant recipients were included. The patients were screened for CMV pp65 antigenaemia every second week. No CMV prophylaxis or pre-emptive treatment was given. MBL and MASP-2 were measured in samples taken at transplantation and 10 weeks later.

Results. CMV infection, defined as at least one positive test, was found in 95 patients (59.8%). MBL and MASP-2 were measured in samples taken at transplantation and 10 weeks later.

Conclusion. Pre-transplant MBL levels do not influence the incidence of any CMV infection or symptomatic CMV disease during the first 12 weeks after kidney transplantation.

However, low MASP-2 levels may play a role in the development of symptomatic CMV disease.

Keywords: complement; cytomegalovirus infection; kidney transplantation; mannose-binding lectin; MASP-2

Introduction

Mannose-binding lectin (MBL) is a serum protein that recognizes molecular patterns on microbial surfaces and may also in certain situations bind to altered self-surfaces [1]. In serum, MBL is bound to structurally related proteins, the MBL-associated serine proteases (MASPs) 1, 2 and 3, as well as to the protein MAp19. MASP-2 circulates in plasma in complexes with MBL and with L- and H-ficolin [2]. The binding of MBL or ficolin to microbes activates MASP-2, which then cleaves C4 and C2 to generate the C3 convertase, thereby initiating the lectin pathway of complement activation. Thus, MASP-2 represents the effector component of complement activation for MBL and ficolins.

MASP-1 might also be implicated in lectin pathway activation, whereas the roles of MASP-3 and MAp19 are less well known [2].

MBL deficiency is under certain conditions associated with repeated infections both in children and adults [3,4]. The clinical significance of MASP-2 deficiency has only recently started to be studied. One case report describes MASP-2 deficiency in a patient with recurrent severe infections and autoimmune reactions [5]. Another study found MASP-2 deficiency as a risk factor for fever episodes in children undergoing chemotherapy [6].

The lectin pathway of complement activation is antibody independent and seems to be involved in several types of virus infections. For instance, studies on influenza A virus have indicated that MBL may perform direct viral
neutralization and inhibition of viral spread, in addition to an indirect mechanism through opsonization and complement activation [7]. Furthermore, human immunodeficiency virus (HIV) binds to MBL via the carbohydrate-recognition domain of MBL [8].

Herpesviruses are also recognized by MBL. Thus, MBL binds to HSV-2, and this binding leads to complement activation and increased neutralization of HSV-2 in mice [4,9].

Interestingly, low MBL levels (<500 μg/L) were found to be associated with the development of CMV infection and disease in a study consisting of 16 kidney transplant recipients with high-risk CMV serostatus (donor positive/recipient negative) [10].

The study represented here retrospectively investigated the association between pre-transplant levels of MBL and MASP-2 and the occurrence of any CMV infection and symptomatic CMV disease during the first 12 weeks after kidney transplantation in a larger patient population and investigated whether the levels of MBL and MASP-2 remained unchanged during the first 10 weeks after transplantation.

Material and methods

Patients

Originally 477 consecutive single kidney transplant recipients who were transplanted between October 1994 and July 1997 were prospectively followed for the first 3 months after transplantation to look at the natural course of CMV infection and CMV disease [11]. Of these, a subcohort of 233 recipients, who were kidney transplanted between February 1995 and June 1996, were evaluated for inclusion in a study of post-transplant diabetes mellitus. Twenty-five of these had pre-transplant diabetes mellitus and were excluded, 8 patients were excluded due to age below 18 years, 9 were excluded due to early graft loss, 7 due to early death and 11 were not available for follow-up due to transfer to a local hospital early after transplantation [12]. Thus, a total of 173 recipients were included. A post hoc analysis on osteoprotegerin, MBL and MASP-2 measured at 10 weeks after transplantation and long-term outcomes in this cohort has recently been published [13].

The present study is a post hoc analysis of the same 173 recipients. Frozen sera obtained immediately before transplantation and at 10 weeks post-transplantation were available for measurement of high sensitivity (hs) CRP, MBL and MASP-2 in 159 of the recipients, who were included in the study. CRP, MBL and MASP-2 were retrospectively analysed. Adequate data for prospective investigation of any CMV infection, symptomatic CMV disease and acute rejection episodes during the first 12 weeks after transplantation were also available for the same 159 recipients. Demographic data are summarized in Table 1.

No patient in this study received prophylaxis against CMV or other herpes viruses. The study was approved by the Datainspectorate, Oslo, Norway, and by the regional Ethics Committee.

Immunosuppressive treatment

Cyclosporin (CsA), steroids and azathioprine (AZA) constituted the standard immunosuppressive therapy. All patients received prednisolone, and all but two patients initially received CsA: two patients initially received tacrolimus. Initially 141 (88.7%) of the patients received AZA. Mycophenolate mofetil (MMF) was usually given as a substitute to those who did not receive AZA. In some cases of rejection or side effects from the standard medication a small percentage of the patients were switched to MMF or tacrolimus during the study. During the first 100 days after transplantation five patients received MMF, four of them being retransplants. As the intention with the original study was to investigate the natural course of cytomegalovirus infection after kidney transplantation, the study population had a quite homogenous standard immunosuppressive drug regimen. With the introduction of mycophenolate and tacrolimus into the standard immunosuppressive drug therapy, the patient inclusion in the study was stopped to avoid differences in standard drug therapies influencing the incidence of CMV infection.

Table 1. Baseline characteristics and risk factors (n = 159)

<table>
<thead>
<tr>
<th></th>
<th>CMV infection +</th>
<th>CMV infection –</th>
<th>P</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient-related factors</td>
<td>99 (62%)</td>
<td>60 (38%)</td>
<td>0.001</td>
<td>47 (16)</td>
</tr>
<tr>
<td>Recipient age</td>
<td>50 (16)</td>
<td>42 (15)</td>
<td>0.001</td>
<td>48 (30.2%)</td>
</tr>
<tr>
<td>Female</td>
<td>28 (28.3%)</td>
<td>20 (33.3%)</td>
<td>0.60</td>
<td>48 (30.2%)</td>
</tr>
<tr>
<td>Cold ischaemia</td>
<td>8.2 (1.2 to 17.3)</td>
<td>2.6 (&lt;1 to 14.1)</td>
<td>0.21</td>
<td>8.6 (8.5)</td>
</tr>
<tr>
<td>Time (h)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute rejection</td>
<td>75 (44%)</td>
<td>24 (15%)</td>
<td>&lt;0.001</td>
<td>99 (62%)</td>
</tr>
<tr>
<td>MBL at tx (μg/L)</td>
<td>2755 (526 to 4862)</td>
<td>2389 (529 to 5002)</td>
<td>0.68</td>
<td>2597 (526 to 4939)</td>
</tr>
<tr>
<td>MASP-2 at tx (μg/L)</td>
<td>256 (142 to 394)</td>
<td>241 (160 to 366)</td>
<td>0.94</td>
<td>252 (148 to 382)</td>
</tr>
<tr>
<td>hs CRP at tx (mg/L)</td>
<td>0.24 (0.10 to 0.53)</td>
<td>0.12 (0.10 to 0.50)</td>
<td>0.18</td>
<td>0.17 (0.10 to 0.52)</td>
</tr>
<tr>
<td>Donor-related factors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Donor age</td>
<td>47 (15%)</td>
<td>41 (16)</td>
<td>0.015</td>
<td>44 (16)</td>
</tr>
<tr>
<td>Living</td>
<td>37 (37.4%)</td>
<td>31 (51.7%)</td>
<td>0.10</td>
<td>68 (42.8%)</td>
</tr>
</tbody>
</table>

Data are means (SD), medians (interquartile range). CMV: cytomegalovirus; CMV infection: positive CMV pp65 antigen test during the first 12 weeks after transplantation; tx: transplantation. Chi-squared tests are used to compare categorical data and unpaired t-tests are used to compare normally distributed data between different groups. For non-normally distributed data the Mann–Whitney test is applied.
Rejection

Rejection episodes were diagnosed clinically by a >20% rise in serum creatinine in the absence of urinary tract obstruction and renal graft artery stenosis (excluded by an ultrasonographic method and duplex Doppler examination of the renal graft). Dehydration, infection and nephrotoxic medication (including CsA) were also excluded. Clinical rejection episodes were recorded during the first 12 weeks after transplantation and were treated with boluses of methylprednisolone. If no significant decrease in serum creatinine was observed on the fifth day after the start of standard doses of methylprednisolone, treatment with antithymocyte globulin or OKT3 was given. The first rejection episode was confirmed by subsequent response to rejection treatment and in 35% of the patients with suspected rejection also by an ultrasound-guided core biopsy. Recurrent rejection episodes and steroid-resistant rejections were normally biopsy confirmed.

CMV infection and disease

CMV pp65 lower matrix protein was detected in leukocytes from ethylenediaminetetraacetic acid (EDTA) blood samples. The method was a previously described modification of a reported procedure [14]. CMV infection was defined as a result of one or more cells positive for CMV pp65 antigen per 10^5 leukocytes. Screening for CMV pp65 antigenaemia was performed every second week during the first 12 weeks after transplantation until the test became positive. Thereafter, the test was performed once or twice a week. No CMV prophylaxis or pre-emptive therapy was given.

Symptomatic CMV disease was defined as the detection of CMV in a clinical specimen accompanied either by CMV syndrome with fever, muscle pain, leucopenia and/or thrombocytopenia (other causes excluded), or by organ involvement such as hepatitis, gastrointestinal ulceration, pneumonitis or retinitis. Symptomatic CMV disease was treated with intravenous ganciclovir.

None of the patients had their immunosuppressive treatment reduced, and no anti-CMV therapy was given with asymptomatic CMV infection.

Laboratory analyses

Blood samples were drawn in the fasting state immediately before transplantation and 10 weeks post-transplantation, followed by centrifugation. The serum was removed and stored at −70°C, and hsCRP, MBL and MASP-2 were analysed in June 2006. Serum MBL and MASP-2 levels were measured by immunoassays as described previously [15,16]. Serum MBL concentrations were measured using an in-house time-resolved immunofluorometric assay with a lower detection level of 10 µg/L. In brief, microtiter wells were coated with monoclonal anti-MBL antibody followed by incubation with samples diluted 200-fold. After washing, monoclonal anti-MBL antibody labelled with europium was added, and after incubation and washing, the amount of bound, labelled antibody was assessed by time-resolved fluorometry. A number of control serum samples covering different MBL levels were included in all assays. The assay for MASP-2 is also a sandwich-type europium-based assay using a combination of two monoclonal anti-MASP-2 antibodies, one directed against the N-terminal part of MASP-2 and the other against its C-terminal part. hsCRP was measured as previously described with antibodies and standard from DakoCytomation (Glostrup, Denmark) [17].

Statistics

Student t-tests were used for comparing normally distributed data and Chi-squared tests were used to compare categorical data between groups. For non-normally distributed data Mann–Whitney tests were used for unpaired data and Wilcoxon tests were applied for paired data. To test the effect of acute rejection and of low MASP-2 levels on the development of CMV disease during the first 12 weeks a multiple Cox proportional hazard model that allows time-dependent covariates was applied.

Results

Association between MBL, MASP-2, hsCRP and the occurrence of any CMV infection and symptomatic CMV disease

The overall incidences of any CMV infection (positive CMV pp65 antigen test) and symptomatic CMV disease in all patients during the first 12 weeks after transplantation were 99 (62.3%) and 35 (22%), respectively. During the same period 99 patients (62.3%) experienced at least one acute rejection episode.

Pre-transplant levels of MBL and hsCRP were the same in the recipients who developed any CMV infection during the first 12 weeks and in those without CMV infection, 2755 (526–4862) µg/L versus 2389 (529–5002) µg/L (P = 0.68) for MBL and 0.24 (0.10–0.53) mg/L versus 0.12 (0.10–0.50) mg/L (P = 0.18) for CRP, respectively (Table 1).

Furthermore, the pre-transplant levels of MBL were the same in those who developed symptomatic CMV disease and in those with no CMV disease, 2420 (361–4698) µg/L versus 2723 (565–4996) µg/L, respectively (P = 0.70) (Table 2). Pre-transplant CRP levels were also the same in these two groups.

The frequency of patients with pre-transplant MBL levels <500 µg/L (a level previously used by others as an indicator of low levels) was compared in recipients who developed any CMV infection and those who did not, and low

<table>
<thead>
<tr>
<th>Parameters at tx</th>
<th>CMV disease +</th>
<th>CMV disease –</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL (µg/L)</td>
<td>2420 (361–4698)</td>
<td>2723 (565–4996)</td>
<td>0.70</td>
</tr>
<tr>
<td>MASP-2 (µg/L)</td>
<td>195 (117–327)</td>
<td>258 (160–394)</td>
<td>0.034</td>
</tr>
<tr>
<td>Hs CRP (mg/L)</td>
<td>0.23 (0.10–0.53)</td>
<td>0.16 (0.10–0.52)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Data are median (interquartile range). Since data are non-normally distributed the Mann–Whitney test is applied. CMV: cytomegalovirus; tx: transplantation.
CMV: cytomegalovirus; tx: transplantation. The frequency of low levels of MBL and of MASP-2 are compared between patients with and without CMV infection (positive CMV pp65 antigen test) and between patients with and without symptomatic CMV disease during the first 12 weeks after transplantation. The categorical data are compared between the two groups by means of the Fisher exact test.

**Table 3.** Low levels of MBL and MASP-2 and the risk of CMV infection and symptomatic CMV disease during the first 12 weeks after tx (n = 159)

<table>
<thead>
<tr>
<th>Parameters at tx</th>
<th>CMV infection +</th>
<th>CMV infection −</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL &lt; 500 (µg/L)</td>
<td>23 (23.2%)</td>
<td>13 (21.7%)</td>
<td>0.49</td>
</tr>
<tr>
<td>MBL ≤ 148 (µg/L)</td>
<td>27 (27.3%)</td>
<td>13 (21.7%)</td>
<td>0.46</td>
</tr>
<tr>
<td>MBL &lt; 500 (µg/L)</td>
<td>9 (25.7%)</td>
<td>27 (21.8%)</td>
<td>0.39</td>
</tr>
<tr>
<td>MBL ≤ 148 (µg/L)</td>
<td>14 (40%)</td>
<td>26 (21.0%)</td>
<td>0.028</td>
</tr>
</tbody>
</table>

Parameters at tx CMV infection + CMV infection − P

MBL < 500 (µg/L) 23 (23.2%) 13 (21.7%) 0.49
MBL ≤ 148 (µg/L) 27 (27.3%) 13 (21.7%) 0.46
MBL < 500 (µg/L) 9 (25.7%) 27 (21.8%) 0.39
MBL ≤ 148 (µg/L) 14 (40%) 26 (21.0%) 0.028

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**Table 4.** CMV infection and symptomatic CMV disease during the first 12 weeks after tx in CMV seronegative recipients of seropositive donors (n = 30)

<table>
<thead>
<tr>
<th>Parameters at tx</th>
<th>CMV infection + (n = 18)</th>
<th>CMV infection − (n = 12)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL (µg/L)</td>
<td>3443 (423–5643)</td>
<td>837 (343–1372)</td>
<td>0.16</td>
</tr>
<tr>
<td>MASP-2 (µg/L)</td>
<td>181 (116–323)</td>
<td>239 (142–356)</td>
<td>0.35</td>
</tr>
<tr>
<td>hsCRP (µg/L)</td>
<td>0.13 (0.10–0.47)</td>
<td>0.41 (0.10–0.85)</td>
<td>0.47</td>
</tr>
<tr>
<td>MBL (µg/L)</td>
<td>2591 (166–4862)</td>
<td>1636 (476–3802)</td>
<td>0.66</td>
</tr>
<tr>
<td>MASP-2 (µg/L)</td>
<td>208 (123–327)</td>
<td>216 (134–333)</td>
<td>0.79</td>
</tr>
<tr>
<td>hsCRP (µg/L)</td>
<td>0.11 (0.10–0.42)</td>
<td>0.38 (0.10–0.89)</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Data are medians (interquartile range). CMV: cytomegalovirus; CMV infection: positive CMV pp65 antigen test. tx: transplantation. When comparing parameters between groups the Mann–Whitney test is used.

MBL levels were equally distributed in these two groups (P = 0.49) (Table 3). Similarly, low MBL levels were not found more often in the recipients who developed symptomatic CMV disease (P = 0.46).

Using an alternative cut-off level of, e.g., 400 µg/L between normal and low MBL did not change the non-significant difference between the groups.

Six of the patients had no detectable MBL before transplantation. Only three of these patients developed CMV infection, comparable to ~60% in the overall cohort.

In the CMV serostatus high-risk recipients (donor seropositive/recipient seronegative) (n = 30), the pretransplant levels of MBL and MASP-2 were the same in those who developed any CMV infection compared to those without CMV infection (P = 0.16 and P = 0.35, respectively) as well as in those with symptomatic CMV disease compared to those with no CMV disease (P = 0.66 and P = 0.79, respectively) (Table 4).

Similar to MBL and hsCRP, pre-transplant MASP-2 levels were also the same in recipients who developed any CMV infection during the first 12 weeks and in those without CMV infection, 256 (142–394) µg/L versus 241 (160–366) µg/L (P = 0.94) (Table 1).

However, MASP-2 levels measured at transplantation were significantly lower in patients who developed symp-

**Table 5.** Risk factors of symptomatic CMV disease during the first 12 weeks after tx (n = 159)

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Risk ratio</th>
<th>95% CI</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute rejection</td>
<td>2.84</td>
<td>1.35–5.97</td>
<td>0.006</td>
</tr>
<tr>
<td>MASP-2 ≤ 148 µg/L</td>
<td>1.93</td>
<td>0.98–3.81</td>
<td>0.058</td>
</tr>
</tbody>
</table>

tx: transplantation; CMV: cytomegalovirus. The effects of clinical acute rejection during the first 12 weeks and of low MASP-2 levels at tx are estimated by a multiple Cox proportional hazard model that allows time-dependent covariates.

**Table 6.** Parameters measured at transplantation and 10 weeks later (n = 159)

<table>
<thead>
<tr>
<th>At tx</th>
<th>10 weeks after tx</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBL (µg/L)</td>
<td>2597 (526–4939)</td>
<td>1520 (270–3069)</td>
</tr>
<tr>
<td>MASP-2 (µg/L)</td>
<td>252 (148–382)</td>
<td>380 (302–492)</td>
</tr>
<tr>
<td>hsCRP (mg/L)</td>
<td>0.17 (0.10–0.52)</td>
<td>0.8 (0.26–2.17)</td>
</tr>
</tbody>
</table>

Data are medians (interquartile range). The Wilcoxon test is used to compare non-normally distributed data between the two time points.

Low MASP-2 levels were defined as the lower quartile (148 µg/L) for the whole study population. Low MASP-2 levels occurred as frequent in the recipients who developed any CMV infection as in those who did not (P = 0.39) (Table 3). However, low MASP-2 levels were found more frequently in recipients who developed symptomatic CMV disease than in those without CMV disease (P = 0.028) (Table 3).

Of the CMV serostatus high-risk recipients (n = 30), 11 had low pre-transplant MASP-2 levels (MASP-2 ≤ 148 µg/L). Eight of these developed CMV infection, and three had no CMV infection (P = 0.44). Six of them developed symptomatic CMV disease and five had no CMV disease (P = 1.00).

Low MASP-2 levels were tested as predictor for symptomatic CMV disease when adjusting for acute rejection in a multiple Cox proportional hazard model allowing time-dependent covariates (Table 5). Low pre-transplant MASP-2 levels were found to be associated with CMV disease of borderline statistical significance (RR = 1.93) (95% CI 0.98–3.81, P = 0.058).

In CMV seropositive recipients (n = 113) low pre-transplant MASP-2 levels increased the risk of symptomatic CMV disease in a multiple Cox proportional hazard model adjusting for acute rejection, but still only of borderline statistical significance (RR = 2.35) (95% CI 0.956–5.794, P = 0.063).

**Change in parameters from before transplantation to 10 weeks post-transplantation**

When comparing the levels of MBL, MASP-2 and CRP measured at transplantation with the levels of the same parameters measured 10 weeks later, there was a significant change in all parameters (Table 6). For MASP-2 and CRP
the levels increased significantly from transplantation to 10 weeks later. On the other hand, the levels of MBL decreased significantly during the same time period. The levels of all parameters measured at 10 weeks after transplantation were the same in recipients of living donor kidneys as in recipients of deceased donor kidneys (data not shown). Furthermore, the average changes in the levels of MBL, MASP-2 and CRP from transplantation to 10 weeks later were the same in patients with and without rejection episodes.

### Discussion

In this study no association between pre-transplant MBL levels and any CMV infection (positive CMV pp65 antigen test) during the first 12 weeks after kidney transplantation was found, but low pre-transplant MASP-2 levels seemed to be associated with the development of symptomatic CMV disease.

A previous study by Spiller et al. showed that MBL-deficient serum did not inhibit classical complement activation by CMV-infected fibroblasts [18]. Spiller et al. also found that CMV-infected cells activate complement independent of anti-CMV antibody as the presence of anti-CMV antibody had no effect on the degree of complement activation. In that study the authors indicate the presence of unknown CMV or host-encoded proteins that bind directly to C1q. Whereas the in vitro study by Spiller et al. demonstrates no role for the MBL pathway in complement activation by CMV, the present study suggests that low MASP-2 concentrations predispose to symptomatic CMV disease.

Nevertheless, Manuel et al. found an association between pre-transplant MBL levels <500 µg/L and development of late CMV infection in a group of 16 kidney graft recipients who were high-risk CMV serostatus (donor positive/recipient negative) [19]. Eleven of the recipients developed CMV infection, and nine of these patients had low MBL levels. Two of the 16 patients developed acute rejection, one of them experienced both rejection and CMV infection. It is not mentioned whether the rejection in this patient preceded the CMV infection or not. If the rejection occurred first this may explain the CMV infection in this patient. Previous studies have shown that acute rejection is a strong predictor of CMV infection [11]. However, the study of Manuel et al. is far too small to adjust for acute rejection when evaluating risk factors for CMV infection. The patient population in the present study consists of 159 kidney graft recipients, implying a substantially stronger statistical power, and we therefore suggest that pre-transplant levels of MBL do not predict CMV infection.

However, low pre-transplant levels of MASP-2 were found to be associated with the development of symptomatic CMV disease. This may indicate that MASP-2 may not prevent subclinical CMV infection but may play a role in preventing symptomatic CMV disease. Moreover, when tested as a predictor for symptomatic CMV disease in a multiple Cox proportional hazard model adjusting for acute rejection, low MASP-2 levels increased the risk of CMV disease, albeit with borderline statistical significance. This may indicate that the lectin pathway of complement activation may be involved in the prevention of symptomatic CMV disease. However, only 35% of the first rejection episodes were biopsy proven, suggesting overdiagnosis of acute rejection in the present study. The multiple analysis should therefore be regarded with some caution. Nevertheless, whether low pre-transplant MASP-2 levels would reach statistical significance in a larger study population remains to be shown.

In CMV serostatus high-risk recipients, no association was found between pre-transplant levels of the parameters and any CMV infection or symptomatic CMV disease. High-risk CMV serostatus is in itself such a strong risk factor for symptomatic CMV disease that low MASP-2 levels may not increase the risk further in this recipient group. Since MASP-2 represents the effector component of complement activation for MBL and ficolins, it may not be surprising that low MASP-2 levels represent a stronger risk factor than low MBL levels for symptomatic CMV disease.

A previous study has shown that MBL and MASP-2 are only marginally influenced by surgery in relation to colon cancer treatment as measured 30 days after surgery [20]. The present study demonstrates that this is not the case when measuring the parameters 10 weeks after kidney transplantation. The levels of MASP-2 and CRP increased significantly and those of MBL decreased significantly from transplantation to 10 weeks later. The changes in MBL and MASP-2 were the same in those who experienced an acute rejection episode during the follow-up time as in those without acute rejection.

Satomura et al. found significantly higher serum MBL levels in patients with chronic renal failure compared to normal controls [21]. They also found that serum MBL levels were significantly higher in haemodialysis patients than in predialytic patients. The authors found no relationship between serum MBL levels and glomerular filtration rate, and they speculate whether increased production could account for the elevated serum MBL levels in patients with chronic renal failure. Improved renal function after kidney transplantation may account for the reduction in serum MBL levels from transplantation to 10 weeks later in the present study. On the other hand, it cannot be excluded that the medical treatment selectively could reduce the synthesis of MBL.

Sund et al. showed in a previous study of living donor kidney recipients that the presence of the complement markers C4d, C3 and MASP-1 in kidney graft biopsies were associated with acute rejection [22]. In that study MBL was not present in kidney graft biopsies from living donor recipients. However, other authors have observed MBL depositions in post-transplant biopsies of transplanted deceased donor kidneys [23]. In the present study MBL and MASP-2 were not measured in kidney biopsies, and whether the decrease in serum MBL levels after kidney transplantation could be explained by ‘trapping’ of this marker in the transplanted kidney has to be investigated in future studies.

With regard to the regulation of MBL and MASP-2 levels, the promoter region of the gene encoding MBL has been described in detail, and the promoter region of the gene encoding MASP-2 is currently being studied by several laboratories [24–26]. A recent study showed that a
transcription factor called signal transducer and activator of transcription 3 (Stat3) is an important MASP-2 promoter activator [26]. Whether upregulation of Stat3 is involved in the increase of MASP-2 levels after transplantation remains unknown. More than 98% of mRNA encoding MBL and MASP-2 are, in humans, found in the liver [27]. Thus, although both proteins are primarily produced in hepatocytes it is clear from these studies that different signalling factors will stimulate up or down regulation of these two genes. It is important to note that MASP-2 is a protein that is known to be in association with several different pattern recognition molecules, including MBL and the ficolins. Although the fine balance between the distribution of MASP-2 on these molecules is not known, a lower concentration of MBL would thus in principle result in more MASP-2 on the ficolins and vice versa. The result in the present report would thus indicate that more MASP-2 associated with the ficolins is an advantage in the anti-viral defence found in kidney-transplanted patients.

The increase in the levels of MASP-2 that were seen after kidney transplantation in the present study may also at least partly be explained by acute phase response. Ytting et al. found a minor but significant increase in mean MASP-2 levels on the 8th postoperative day in 60 patients undergoing colectomy compared to the preoperative levels [28].

Berger et al. showed significantly higher death censored graft survival in 266 deceased donor kidney transplant recipients 10 years after transplantation in those with pre-transplant MBL levels of ≤400 μg/L compared to those with higher MBL levels [29]. The authors assume that higher MBL levels may lead to a more severe form of rejection resulting in graft loss. The present study was not designed to look at long-term graft survival.

In conclusion, the pre-transplant serum levels of MBL do not influence the incidence of any CMV infection or symptomatic CMV disease during the first 12 weeks after kidney transplantation. Low pre-transplant MASP-2 levels seem to be involved in the development of symptomatic CMV disease, but this has to be confirmed in future larger studies.

Conflict of interest statement. None declared.

References


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