Lectin complement pathway gene profile of the donor and recipient does not influence graft outcome after kidney transplantation

Damman, Jeffrey; Kok, Julian L.; Snieder, Harmen; Leuvenink, Henri G.D.; van Goor, Harry; Hillebrands, Jan-Luuk; van Dijk, Marcory; Hepkema, Bouke G.; Reznichenko, Anna; van den Born, Jaap

Published in:
MOLECULAR IMMUNOLOGY

DOI:
10.1016/j.molimm.2011.11.009

IMPORTANT NOTE: You are advised to consult the publisher’s version (publisher’s PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2012

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Lectin complement pathway gene profile of the donor and recipient does not influence graft outcome after kidney transplantation

Jeffrey Dammana,⁎, Julian L. Koka, Harold Snieder, Henri G. Leuvenink, Harry van Goord, Jan-Luuk Hillebrands, Marcory C. van Dijk, Bouke G. Hepkema, Anna Reznichenkob, Jaap van den Born, Martin H. de Bors, Stephan J. Bakker, Gerjan J. Navis, Rutger J. Ploeg, Marc A. Seelen

a Department of Surgery, University Medical Center Groningen, Groningen, The Netherlands
b Department of Nephrology, University Medical Center Groningen, Groningen, The Netherlands
c Department of Epidemiology, Unit of Genetic Epidemiology & Bioinformatics, University Medical Center Groningen, Groningen, The Netherlands
d Department of Pathology and Medical Biology, University Medical Center Groningen, Groningen, The Netherlands
e Department of Transplant Immunology, University Medical Center Groningen, Groningen, The Netherlands
f Department of Surgery, University of Oxford, Oxford, United Kingdom

A B S T R A C T

In kidney transplantation, complement activation was found to be induced by donor brain death, renal ischemia–reperfusion injury and allograft rejection. There are three known pathways of complement activation: the classical, lectin and the alternative pathway. The lectin complement pathway can be activated upon pattern recognition by mannose binding lectin (MBL) or ficolins (FCN). Single nucleotide polymorphisms (SNPs) in the genes encoding the lectin pathway proteins determine their functional activity and serum levels. The aim of this study was to investigate the role of the lectin gene profile of the donor and recipient on post-transplant outcome.

A total of 12 functional SNPs in the MBL2, FCN2 and MBL-associated serine proteases 2 (MASP2) genes of 1271 donor–recipient pairs were determined. Lectin genotypic variants were analyzed for association with primary non-function (PNF), delayed graft function (DGF), biopsy proven acute rejection, death-censored graft survival and patient survival.

Multivariable analyses found no association of donor and recipient MBL2 and MASP2 genotype with allograft outcome. Analysis of separate functional SNPs and haplotypes in the FCN2 gene of the donor and recipient did not reveal an association with transplant outcome. Also, the joint effect of the MBL2 and FCN2 genotype was not associated with allograft outcome. This study shows that the genetic profile of the lectin pathway of complement activation of the donor and recipient is not associated with allograft outcome after kidney transplantation.

© 2011 Elsevier Ltd. All rights reserved.

1. Introduction

The complement system is part of the innate immune system and has been shown to play an important role in the pathogenesis of renal injury inherent to kidney transplantation. Complement can be activated at different time points during transplantation namely by donor brain death, renal ischemia–reperfusion injury (IRI) and allograft rejection (Damman et al., 2011; Farrar et al., 2006; Pratt et al., 2002; Zhou et al., 2000).

There are three known pathways of complement activation: the classical, lectin and the alternative pathway. The lectin pathway is activated when liver-synthesized complement proteins, mannose-binding lectin (MBL) and/or ficolins, interact with carbohydrate structures on microbial surfaces and altered self-surfaces (Petersen et al., 2001). Subsequently, MBL-associated serine proteases 2 (MASP2) are activated which leads to cleavage of C4 and C2, thereby activating the complement cascade through generation of a C3 convertase. This ultimately leads to generation of anaphylatoxins (C3a, C5a) and formation of the membrane attack complex (MAC), which is a large pore on the target cell surface leading to cell death (Walport, 2001a, b).

Abbreviations: IRI, Ischemia reperfusion injury; MBL, Mannose-binding lectin; MASP, MBL-associated serine proteases; FCN, Ficolin; SNP, Single nucleotide polymorphism; PNF, Primary non-function; DGF, Delayed graft function; MAF, Minor allele frequency; LP, Low producing; HP, High producing; DBD, Donation after brain death; DCD, Donation after cardiac death.

⁎ Corresponding author at: Department of Surgery, University Medical Center Groningen, University of Groningen, CMC V, Y2144, Hanzeplein 1, 9713 EZ Groningen, The Netherlands. Tel.: +31 624751035; fax: +31 684390782.
E-mail address: j.damman@umcg.nl (J. Damman).

0161-5890/—see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.molimm.2011.11.009
Within the general population, there is a large interindividual variation in serum MBL concentration and activity. Serum MBL concentration is largely determined by genetic polymorphisms within the MBL2 gene. Within the coding region, three missense mutations within the first exon (+154 C>T, +161 G>A, +170 G>A) of MBL2 significantly affect MBL function and levels. Furthermore, three polymorphisms described in the promoter region also affect serum MBL levels (–619 C>G, –290 G>C, –66 C>T). These polymorphisms impair the assembly of a monomeric MBL into functional multimeric proteins resulting in low serum level of MBL. Relative MBL deficiency occurs in almost one half of the white population (Madsen et al., 1995).

Also ficolin-2 (ficolin) serum concentrations are significantly associated with polymorphisms in the FCN2 gene. Three single nucleotide polymorphisms (SNPs) in the promoter (–986 A>G, –602 G>A, –4 A>G) and one in exon 8 (+6424 G>T) have been described, thereby significantly affecting serum ficolin-2 levels. Besides, two SNPs in exon 8 (+6359 C>T, +6424 G>T) have been shown to give respectively decreased or increased binding capacity towards N-acetylglucosamine compared to wildtype genotypes (Hummelshoj et al., 2005). Additionally, a SNP in the MAS2P2 gene significantly decreases serum levels of MASP2 (Stengaard-Pedersen et al., 2003).

MBL deficiency has been associated with a high prevalence of certain infections, especially in already immuno-compromised patients such as patients undergoing transplantation (Eisen and Minchinton, 2003; Worthley et al., 2009). On the contrary, low MBL levels might also prevent tissue injury by interrupting activation of the lectin pathway, for example in renal IRI (Castellano et al., 2010; de Vries et al., 2004; Moller-Kristensen et al., 2005). In human kidney transplantation, Berger et al. (2005, 2007) recently found an association between high recipient pre-transplant MBL level and inferior graft survival rates after transplantation. Besides, rodent studies have indicated an important role of MBL in the pathogenesis of renal IRI (de Vries et al., 2004; Moller-Kristensen et al., 2005).

We hypothesized that kidney donors or recipients with genotype-determined high lectin levels show inferior transplant outcomes compared to genotype-determined low lectin level producers. The aim of this study was to investigate the role of the lectin pathway of complement activation by association of donor and recipient MBL2, FCN2 and MASP2 genotypes on post-transplant outcome in the recipient.

2. Materials and methods

2.1. Patients and study design

Between March 7, 1993 and February 12, 2008, 1430 patients underwent kidney transplantation at the University Medical Center Groningen, The Netherlands. From this original group, 90 patients were excluded because of three or more kidney transplantations. Recipients with more than two-times allograft loss are highly sensitized patients or patients with recurrence of primary renal disease. These transplants would therefore give an overestimation of graft loss in our cohort. Consequently, this would also bias the influence of the lectin gene profile on allograft outcome. Other transplants were excluded because of simultaneous transplantation of other organs (pancreas, liver, lung and intestine) and technical problems during the operation. A total of 4 patients were lost to follow-up and of 65 transplantations no donor and recipient DNA pairs were available (Fig. 1). Informed consent was given by all patients. Donor, recipient and transplant characteristics were obtained and documented.

We calculated the number of samples needed to yield a hazard ratio of 2.00 for graft loss between MBL AO/OO versus MBL AA recipients, assuming an alpha of 0.05 and a statistical power of 95% using the PS Program (http://biostat.mc.vanderbilt.edu/wiki/Main/PowerSampleSize). According to this calculation, the sample size included in our study was large enough to reject the null hypothesis.

2.2. DNA isolation and genotyping

DNA was extracted from peripheral blood samples or splenocytes from deceased donors using a commercial kit following the manufacturer’s instructions. A total of 12 SNPs in the MBL2, FCN2 and MASP2 genes were genotyped (Supplemental Table 1). Genotyping of the selected SNPs was performed using the
Illumina VeraCode GoldenGate Assay Kit (Illumina, San Diego, CA, USA), according to the manufacturer’s instructions. Genotype clustering and calling were performed using BeadStudio software (Illumina). Because of technical reasons, three SNPs in the MBL2 gene (rs5030737, rs1800450 and rs1800451) could not be determined on the GoldenGate assay, therefore these assays were readily ordered (Applied Biosystems Assay-on-Demand) and genotyped on an ABI7900HT platform following the manufacturers’ instructions. Haplotype reconstruction was performed using the PHASE algorithm and best estimated pairs were used for analyses (Stephens et al., 2001).

2.3. Study end-points

The primary end points in this study were: primary non-function (PNF, defined as non-functioning of the allograft from transplantation on), delayed graft function (DGF, defined as the requirement for dialysis within the first week after transplantation), biopsy proven acute rejection (all biopsies were re-evaluated according to the Banff 2007 classification) during the first year after transplantation, death censored graft survival (defined as the need for dialysis or re-transplantation) and patient survival.

2.4. Statistical analysis

Statistical analyses were performed using SPSS (version 18.0) and two-sided P values under 0.05 were considered to indicate statistical significance. To compare four genotypic groups, the Kruskal–Wallis test was performed for continuous variables and the Chi-square test for categorical variables. The Mann–Whitney U test was applied to compare continuous variables between two genotypic groups. Multivariate logistic regression models were constructed to find independent risk factors for PNF, DGF and acute rejection. Furthermore, Cox proportional hazards models were used to identify independent risk factors for death-censored graft failure and patient survival.

3. Results

3.1. Study population

Donor, recipient and transplant characteristics of the 1271 kidney transplants included in our study did not differ significantly from the original group of 1430 transplants. Supplemental Table 1 shows the frequencies of the MBL2, FCN2 and MASP2 variants in our study population. Genotyping of the
12 selected SNPs was successful in >97% except for rs3124952 in the recipient (83%). The minor allele frequencies (MAFs) of all SNPs were similar to those previously reported in Caucasians and in accordance with the Hardy–Weinberg equilibrium (Chapman et al., 2007; Herpers et al., 2006; Munthe-Fog et al., 2007; Verdu et al., 2006). Since MBL and ficolin-2 serum levels are largely determined by the MBL2 genotype and FCN2 haplotype, these were reconstructed from the genotype data of the different MBL2 and FCN2 SNPs. MBL2 genotype and FCN2 haplotype frequencies were in agreement with those reported by others (Supplemental Tables 2 and 3).

3.2. MBL2 genotypes and graft outcome

Donors and recipients were divided into high, medium and low producing MBL genotypes as previously proposed by others (Supplemental Table 3) (Madsen et al., 1995). Subsequently, patients were stratified according to three models (Supplemental Table 3): in the first model (A), patients are divided based on the presence or absence of the “O” allele. The O allele represents one of the structural variants (D, B, C) in exon 1, which has shown to be highly associated with decreased plasma levels of MBL. In the second model (B), patients were divided into MBL deficient genotypes (LXA/O and O/O) and MBL sufficient genotypes (all other variants). In the third model (C), patients were divided into high producing (HP) versus low producing (LP) and deficient MBL genotypes.

Table 1 shows the donor, recipient and transplant characteristics when donors and recipients were stratified according to model A, with a combined evaluation of donor–recipient MBL genotype pairs. Since several baseline characteristics were significantly different between the four genotypic groups (donor sex, donor type, cold ischemia time, HLA mismatch), a multivariate model was build taken these and other variables into account. Multivariate analysis did not show a significant association between donor or recipient MBL genotype and graft outcome, except for PNF (Table 2). The significant difference in PNF was found in O allelic recipients of an AA donor kidney. However, significance was lost in a separate analysis when transplants were divided by the donor O allele (AA or AO/OO donor) or the recipient O allele (AA or AO/OO recipient). Additionally, when high producing MBL genotypes were compared with deficient MBL genotypes (“extremes”) no significant association was found with PNF or other outcome parameters (data not shown).

In addition, when patients were stratified according to model B or C, also no association was found between the donor or recipient MBL2 genotype and all transplant outcome parameters (data not shown).

3.3. FCN2 SNP analyses and graft outcome

To investigate the role of ficolin-2 in human kidney transplantation, the five functional SNPs in the FCN2 gene of the donor and recipient were separately associated with graft outcome (Supplemental Table 1). Separate analysis of the five selected SNPs of the donor and recipient revealed no association with allograft outcome for all outcome parameters (data not shown).

3.4. FCN2 haplotype analyses and graft outcome

In addition to single locus analyses of the FCN2 gene, reconstruction of the FCN2 haplotypes could reveal association with outcome. Six common haplotypes (frequencies > 1%) were reconstructed from the single FCN SNP genotype data (Supplemental Table 2). Haplotype frequencies were in agreement with previously reported findings in Caucasians (Munthe-Fog et al., 2007; Ruskamp et al., 2009). In line with the separate SNP analysis, also haplotype
association of the donor or recipient did not show any association with graft outcome (data not shown).

Until now, only diplotypes (not haplotypes) have previously been shown to be associated with increased or decreased ficolin-2 levels. Therefore, donors as well as recipients were divided into low producing (LP; GGAG and GGAT diplotypes) and high producing (HP; AAGG and AGGG diplotypes) groups, based on the publication by Munthe-Fog et al. (2007). Because of the low number of patients who were homozygous for all four SNPs, diplotype association could only be performed for donors and recipients separately. Table 3 shows the donor, recipient and transplant characteristics stratified according to LP or HP producing FCN2 diplotype. In concordance with the separate SNP and haplotype analysis, multivariate analyses showed that the FCN2 diplotype of the donor or the recipient was not associated with graft outcome (Table 4).

3.5. MASPD2 single locus analyses and graft outcome

The D105G SNP in the MASPD2 gene causes an exchange of aspartic acid with a glycine at position 105 which leads to a MASPD2 deficiency in the plasma (Stengaard-Pedersen et al., 2003). MASPD2 plays a pivotal role in lectin pathway activation and therefore we also analyzed the effect of the MASPD2 SNP for association with transplant outcome. The D105G SNP frequency was found to be 5% in donors and 4% in recipients, which is in agreement with those found by others in Caucasians. In line with the findings in the MBL2 and FCN2 genes, no association was found between the MASPD2 D105G SNP and transplant outcome (data not shown).

3.6. Lectin gene profile and graft outcome

Finally we also investigated whether the joint effects of the MBL2 and FCN2 gene variants of the donor or recipient influenced graft outcome. Donors and recipients were stratified according to the presence of both the HP FCN2 diplotype and HP MBL genotype and compared with patients having both LP FCN2 diplotype and LP MBL2 genotype. In line with our previous finding, no influence was found of the lectin profile on graft outcome (Table 5).

4. Discussion

Complement activation has been shown to play a substantial role in renal injury inherent to kidney transplantation. Besides classical and alternative pathway activation, complement can be activated through the lectin pathway upon binding of ficolins and MBL with carbohydrates and subsequent activation of MASPD2.
Table 4
Subgroup analysis of low versus high producing ficolin-2 diplotypes in the donor or recipient. Multivariate logistic and Cox regression analysis for the risk of PNF, DGF, acute rejection, death-censored graft survival and patient survival.

<table>
<thead>
<tr>
<th>Lectin profile</th>
<th>PNF</th>
<th>P</th>
<th>DGF</th>
<th>P</th>
<th>Acute rejection</th>
<th>P</th>
<th>Death-censored graft survival</th>
<th>P</th>
<th>Patient survival</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP FCN2 (n=41)</td>
<td>1.39 (0.36–5.37) a</td>
<td>0.64</td>
<td>0.63 (0.30–1.30) a</td>
<td>0.21</td>
<td>0.65 (0.31–1.40) a</td>
<td>0.27</td>
<td>1.44 (0.72–2.85) a</td>
<td>0.30</td>
<td>0.83 (0.32–2.18) a</td>
<td>0.71</td>
</tr>
<tr>
<td>LP FCN2 (n=167)</td>
<td>1.05 (0.17–6.50) b</td>
<td>0.96</td>
<td>0.39 (0.14–1.08) b</td>
<td>0.07</td>
<td>0.54 (0.22–1.37) b</td>
<td>0.20</td>
<td>1.34 (0.61–2.95) b</td>
<td>0.46</td>
<td>0.70 (0.22–2.24) b</td>
<td>0.54</td>
</tr>
<tr>
<td><strong>Recipient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP FCN2 (n=134)</td>
<td>0.35 (0.09–1.26) a</td>
<td>0.11</td>
<td>1.01 (0.63–1.63) a</td>
<td>0.96</td>
<td>1.07 (0.67–1.73) a</td>
<td>0.77</td>
<td>0.78 (0.45–1.36) a</td>
<td>0.39</td>
<td>0.90 (0.50–1.62) a</td>
<td>0.73</td>
</tr>
<tr>
<td>LP FCN2 (n=177)</td>
<td>0.34 (0.08–1.38) b</td>
<td>0.13</td>
<td>1.16 (0.59–2.28) b</td>
<td>0.68</td>
<td>0.98 (0.56–1.73) b</td>
<td>0.95</td>
<td>0.81 (0.45–1.45) b</td>
<td>0.48</td>
<td>0.92 (0.47–1.80) b</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Abbreviations: PNF; primary non-function, DGF; delayed graft function, LP FCN2; Low producing ficolin-2 diplotype, HP FCN2; high producing ficolin-2 diplotype.

a Crude.
b Adjusted for donor and recipient gender and age, donor type, cold ischemia time, HLA A, B and DR mismatch, transplant number, recipient primary renal disease.

Table 5
Subgroup analysis of low versus high producing MBL2/FCN2 genotypes. Multivariate logistic and Cox regression analysis for the risk of PNF, DGF, acute rejection, death-censored graft survival and patient survival.

<table>
<thead>
<tr>
<th>Lectin profile</th>
<th>PNF</th>
<th>P</th>
<th>DGF</th>
<th>P</th>
<th>Acute rejection</th>
<th>P</th>
<th>Death-censored graft survival</th>
<th>P</th>
<th>Patient survival</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Donor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP MBL2/FCN2 (n=23)</td>
<td>1.79 (0.31–10.43) a</td>
<td>0.52</td>
<td>0.68 (0.25–1.84) a</td>
<td>0.44</td>
<td>0.72 (0.25–2.04) a</td>
<td>0.54</td>
<td>1.04 (0.38–2.84) a</td>
<td>0.94</td>
<td>0.57 (0.13–2.58) a</td>
<td>0.47</td>
</tr>
<tr>
<td>LP MBL2/FCN2 (n=78)</td>
<td>1.26 (0.14–11.05) b</td>
<td>0.83</td>
<td>0.21 (0.04–1.05) b</td>
<td>0.06</td>
<td>0.50 (0.17–1.50) b</td>
<td>0.22</td>
<td>0.82 (0.29–2.27) b</td>
<td>0.70</td>
<td>0.54 (0.12–2.47) b</td>
<td>0.42</td>
</tr>
<tr>
<td><strong>Recipient</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HP MBL2/FCN2 (n=74)</td>
<td>0.25 (0.03–2.32) a</td>
<td>0.23</td>
<td>0.81 (0.41–1.58) a</td>
<td>0.53</td>
<td>1.08 (0.54–2.16) a</td>
<td>0.83</td>
<td>0.56 (0.25–1.25) a</td>
<td>0.16</td>
<td>1.20 (0.48–2.97) a</td>
<td>0.70</td>
</tr>
<tr>
<td>LP MBL2/FCN2 (n=78)</td>
<td>0.36 (0.03–4.00) b</td>
<td>0.40</td>
<td>1.05 (0.42–2.64) b</td>
<td>0.92</td>
<td>0.82 (0.45–1.89) b</td>
<td>0.83</td>
<td>0.55 (0.24–1.27) b</td>
<td>0.16</td>
<td>1.36 (0.53–3.46) b</td>
<td>1.00 (reference)</td>
</tr>
</tbody>
</table>

Abbreviations: PNF; primary non-function, DGF; delayed graft function, LP MBL2/FCN2; Low producing mannose binding lectin genotype and ficolin-2 diplotype, HP; high producing mannose binding lectin genotype and ficolin-2 diplotype.

a Crude.
b Adjusted for donor and recipient gender and age, donor type, cold ischemia time, HLA A, B and DR mismatch, transplant number, recipient primary renal disease.
There is growing evidence that the lectin pathway is significantly involved in the course of renal IRI and allograft rejection (Berger et al., 2005; de Vries et al., 2004; Moller-Kristensen et al., 2005). In contrast, the present study shows that genetic variation in the donor and recipient MBL2, FCN2 and MASP2 genes, thereby significantly affecting their protein functions and levels, is not associated with allograft outcome after kidney transplantation.

Renal IRI is an inevitable consequence of kidney transplantation and several groups have clearly demonstrated a crucial role for complement activation in the course of renal IRI (Zhou et al., 2000). Mouse knock-out models have revealed an involvement of the alternative pathway, whereas the classical pathway is unlikely to play a role in renal IRI (Lin et al., 2006; Zhou et al., 2000). Besides alternative pathway activation, also the lectin pathway is involved in renal IRI since MBL-A and MBL-C deficient mice are partially protected against renal IRI (Moller-Kristensen et al., 2005). In addition to the findings in rodents, renal MBL deposition was also demonstrated in a swine model of IRI (Castellano et al., 2010). In humans, MBL deposition has been demonstrated in patients suffering from DGF and PNF after kidney transplantation (de Vries et al., 2004).

Berger et al. (2005) found a significant association between high pre-transplant serum MBL levels in the recipient and rejection-associated graft loss after kidney transplantation. In their study, patients were stratified according to a cut-off of 400 ng/ml since levels below have earlier been shown to be closely related to MBL variant alleles A/O and O/O, whereas MBL levels above 400 ng/ml were related to MBL wild type A/A. Unfortunately, Berger et al. did only determine serum MBL levels, whereas no genotyping was performed due to the lack of DNA. They were able to confirm their results in patients undergoing simultaneous pancreas–kidney transplantation. Although the recipient MBL genotype was an independent predictor for patient survival, the association between graft survival and the MBL genotype was not shown (Berger et al., 2007). The disparity between our findings and the study by Berger et al. might be explained by the fact that Berger et al. did not determine functional serum MBL levels. To determine functional MBL levels, an enzyme-linked immunosorbent assay (ELISA) is used in which MBL is captured by a mannann-coated plate. In this assay, only high order MBL oligomers, capable of binding to mannann, will be detected whereas low order MBL oligomers will not bind mannann. Instead, Berger et al. determined serum MBL concentrations by an ELISA using the mAb 3E7 capture antibody. This assay is designed to measure whole MBL (low and high order oligomers) concentration, instead of functional MBL levels. In support of their findings, their group has clearly demonstrated a direct association of their assay with the MBL genotype and function in healthy individuals. However, it is unlikely that this can be extrapolated to renal transplant recipients since functional serum MBL levels are severely reduced in patients undergoing dialysis compared to healthy controls (Lam et al., 2005). In fact, Satomura et al. (2010) have found even higher MBL levels in dialysis patients compared to controls using an ELISA technique similar to Berger et al. Serum MBL levels in dialysis patients are still largely determined by the MBL genotype and therefore MBL genotyping is likely to be a more suitable approach to investigate the role of MBL in kidney transplantation. Moreover, our study comprises a much larger transplant cohort (n = 1271) compared to the study by Berger et al. (n = 266). Notably, the association of Berger et al. was only found in deceased donor grafts. However, in separate analyses of only deceased donors, we also found no association of the lectin gene profile with graft outcome (data not shown).

The lack of association between MBL genotypes and graft outcome is strengthened by a recent report in 669 renal transplant recipients. Although the donor genotype was not taken into account, their study also found no association between recipient MBL2 genotype and graft survival up to 10 years after transplantation (Bay et al., 2011). In addition to MBL genotypes, we also found no association of FCN2 polymorphisms on graft outcome. Ficolin deposition has previously been demonstrated in C4d positive kidney biopsies of ABO-incompatible transplants and during acute rejection episodes (Imai et al., 2006). In contrast, we found no association between five functional SNPs in the FCN-2 gene with acute rejection or other transplant parameters. 1-Ficolin serum levels are closely related to four functional SNPs in the FCN2 gene. However, haplotype reconstruction has previously revealed a significant contributing effect of the +6424 SNP on the –986 SNP thereby severely decreasing 1-ficolin serum concentrations. Haplotype analysis reflects the natural situation in individuals much better and is therefore likely to be a more useful tool for association studies compared to FCN2 single locus analysis. Besides separate analysis of the FCN2 SNPs, also haplotype and diplotype analysis did not reveal any association with graft outcome. Moreover, also no difference in graft outcome was found between donor and recipients with a combined high MBL2 and FCN2 genotype compared to patients with both a low MBL2 and FCN2 genotype.

Previous studies have indicated a relation between MBL and the prevalence of renal diseases, for instance IgA and diabetic nephropathy (Endo et al., 1998; Hansen et al., 2004; Matsuoka et al., 1998). In our study population, the MAFs of the determined SNPs in the MBL gene, as well as the haplotype and genotype frequencies were similar in donors as well as recipients. Therefore it is unlikely that the MBL genotype has a major impact on the development of end-stage renal disease.

In conclusion, this study shows that the genetic profile of the lectin pathway of complement activation of the donor and recipient does not influence allograft outcome after kidney transplantation. Our large study cohort and the lack of association of both MBL and ficolin genotypes with graft outcome suggests a minor role for the lectin pathway in allograft outcome.

Funding source

This project has been supported by the Foundation: “De Drie Lichten” in The Netherlands.

Authors’ contributions

Dammann J, Kok J.L., Snieder H., Seelen M.A. designed and performed experiments, analyzed data and wrote the paper, van Dijk M.C.R.F. provided biopsy data, edited and approved the final manuscript, Leuvenink H.G.D., van Goor H., Hillebrands J.H. Hepkema B.G., Renzienchenko A., van den Born J., de Borst M.H., Bakker, S.J.L., Navis G.J. and Ploeg R.J. edited and approved the final manuscript.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.molimm.2011.11.009.

References


