Molecular Immunology 50 (2012) 1–8

Contents lists available at SciVerse ScienceDirect

Molecular Immunology

journal homepage: www.elsevier.com/locate/molimm

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

Jeffrey Damman[a,*], Julian L. Kok[a,b], Harold Snieder[c], Henri G. Leuvenink[a], Harry van Goor[d], Jan-Luuk Hillebrands[d], Marcory C. van Dijk[d], Bouke G. Hepkema[e], Anna Reznichenko[b], Jaap van den Born[b], Martin H. de Borst[b], Stephan J Bakker[b], Gerjan J. Navis[b], Rutger J. Ploeg[a,f], Marc A. Seelen[b]

[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

ARTICLE INFO

Article history:
Received 19 October 2011
Received in revised form
16 November 2011
Accepted 19 November 2011
Available online 15 December 2011

Keywords:
Complement
Transplantation
Kidney
MBL
Ficolin
Polymorphism

ABSTRACT

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 mannan 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.

Multivariate 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.

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 cardic death.

* Corresponding author at: Department of Surgery, University Medical Center Groningen, University of Groningen, CMC V, Y2144, Hanzeplein 1, 9713 GZ Groningen, The Netherlands. Tel.: +31 624751035; fax: +31 848390782.
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

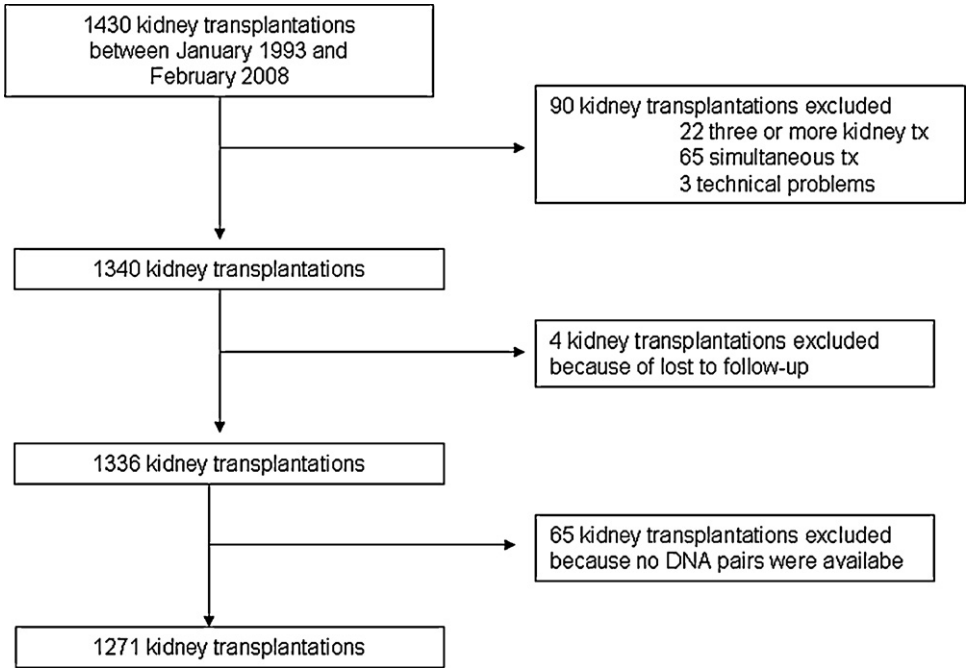

Fig. 1. Kidney transplantations that were included and excluded from the study.

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 (L-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 MASP2 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