Transplant Immunology 30 (2014) 34–39

Contents lists available at ScienceDirect

Transplant Immunology

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

Study of the cytokine polymorphisms in correlation to rejection and graft survival in renal allograft donors and recipients from a homogenous Saudi population

Ameera Gaafar [a,*], Alia Iqniebi [a], Atia Sheereen [a], Abdelmoneim Eldali [b], Hannu Turpeinen [d], Chaker Adra [a,e], Khalid Al Meshari [c], Khalid Al Hussein [a,**]

[a] Stem Cell & Tissue Re-engineering Program, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia
[b] Department of Biostatistics, Epidemiology and Scientific Computing, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia
[c] Renal Transplant Program, King Faisal Specialist Hospital & Research Centre, Riyadh, Saudi Arabia
[d] Institute of Biomedical Technology and BioMediTech, University of Tampere, Tampere, Finland
[e] Transplantation Research Center, Brigham and Women's Hospital and Children's Hospital Boston, Harvard Medical School, Boston, MA, USA.

ARTICLE INFO

Article history:
Received 24 June 2013
Received in revised form 28 November 2013
Accepted 28 November 2013

Keywords:
Cytokines
Polymorphism
Renal transplant
IL-10 genotype
HLA-DRB1
SNP

ABSTRACT

Objectives: Allograft outcome can be improved with the discovery of risk factors that influence adverse events and may allow individualization of patients' treatment. Rejection is the main hurdle to successful transplantation and the immune response is the key effecter to rejection development. Hence, the major objective of the present study was to assess the relationship between single nucleotide polymorphisms (SNPs) in 5 cytokine genes, HLA mismatch and graft outcome in a cohort of 100 Saudi kidney transplant recipients and 100 living related donors at a single transplant center.
Materials & methods: Genotyping of the following positions: TNFA (−308 G/A), TGFB1 (codon 10 T/C, codon 25 C/G), IL-10 (−1082 G/A, −819 C/T, −592 C/A), IL-6 (−174 C/G), and IFNG (+874 T/A) were performed.
Results: The majority of the donors whose recipients presented with either cellular or antibody mediated graft rejection (90% and 100%) respectively were found to be significantly ($p = 0.0351$) associated with intermediate or high IL-10 producing haplotypes, compared to those with stable grafts (58.66%). Haplotypes linked with lower IL-10 production were not detected in the donors or their recipients with antibody mediated graft rejection compared to donors with stable graft (41.33%). The distribution of donor IL-10-1082 haplotypes (GG, GA, AA) showed a statistically significant association of IL-10-1082 GA genotype ($p = 0.0351$) with rejection, when grouped according to patients' rejection status. No other statistically significant deviations were observed in the donors' genotypes. Analyses of cytokine polymorphisms in the recipients revealed no significant association. Multivariate logistic regression analyses showed that only HLA-DRB1 mismatch significantly influenced graft loss ($p = 0.0135$).
Conclusion: This study demonstrates that the donor IL-10 genotypes and HLA-DRB1 mismatch are key determinants in graft outcome after renal transplantation.

1. Introduction

Kidney transplantation is often the best medical treatment for patients with end stage renal disease. Despite recent improvement in immunosuppressive medication and HLA-matching, allograft rejection continues to be problematic and remains the leading cause of graft loss after renal transplantation. Enhanced HLA matching between donors and recipients, optimized immunosuppression, and/or decline of cold ischemia time have improved short-term graft survival rates and decreased early mortality in kidney transplantation [1]. Recent observations signify the importance of the innate immune system in both graft rejection and induction of tolerance following organ transplantation [2]. Tissue injuries during transplantation have a profound effect on the immunological responses, leading to production of inflammatory mediators such as cytokines [3,4]. Cytokines are immunoregulatory proteins produced and secreted by activated immune cells that interact with

* Correspondence to: Ameera Gaafar, PhD Scientist, Stem Cell & Tissue Re-engineering Program, King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, 11211 Riyadh, Saudi Arabia. Tel.: +966 1 4647272 32537x32537; pager: 6281; fax: +966 1 4427858.
** Correspondence to: Khalid Al Hussein Senior Scientist, Stem Cell & Tissue Re-engineering Program, King Faisal Specialist Hospital and Research Centre, P.O. Box 3354, 11211 Riyadh, Saudi Arabia. Tel.: +966 1 4647272 27864x32537; pager: 6281; fax: +966 1 4427858.
E-mail addresses: agaafar@kfshrc.edu.sa (A. Gaafar), Khussein@kfshrc.edu.sa (K. Al Hussein).

0966-3274/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.trim.2013.11.006

specialized receptors on target cells to stimulate certain cellular responses. They display a high degree of pleiotropism and do not operate in segregation but rather as a complex network of interactions [5,6]. It has been reported that the production of cytokines is under genetic control [7–13]. Polymorphisms within the coding, non-coding intron, or promoter regions have been recognized in a large number of these genes. Therefore, it is important to investigate the association of cytokine polymorphisms with kidney allograft rejection. Improving transplantation outcomes necessitates a better understanding of the genetic basis of the immune responses to organ, tissue allografts and identification of risk factors that predispose patients for delayed graft function, acute rejection episodes, chronic allograft function, or graft survival [14,15]. A major genetic determinant of acute rejection after kidney transplantation is donor–recipient mismatching at HLA-DR [16]. In recent years, investigators have also demonstrated intriguing associations between transplant outcomes and polymorphisms in genes encoding a variety of cytokines (TNFA, TGFB1, IFNG, lymphotoxin, IL-1, IL-4, IL-6 and IL-10), adhesion molecules (ICAM-1, L-selectin, and E-selectin), and co-stimulatory/regulatory molecules (B7 and CTLA-4) [17–20]. Cytokine gene expression, or protein levels, that lead to up or down regulation of perforin and granzyme B (e.g. IFN-γ and IL-10), have been measured in PBMC and plasma of kidney transplanted donors and recipients [21–23]. Pro-inflammatory cytokines correlated with the development of renal injury and thus could be used as diagnostic tools for graft rejection. However, in the literature, the impact of cytokine SNPs in renal transplants is contradictory. In addition, most of the data available were based on graft outcomes from cadaver donors, or donors of diverse ethnic backgrounds. To date to our knowledge, there is no single report investigating the association of cytokine gene polymorphism in a kidney transplant cohort in a highly consanguineous society such as the Saudi population [24,25]. Therefore, it is plausible to predict that such a community with less genetic variability may provide an excellent model for studying the genes underlying the immune responses that influence kidney transplant results. In particular, our study was strengthened by the fact that most of the kidney transplants were offered by related donors and subjected to comparable treatment regimes in a single center. Therefore, the present study was initiated to examine the impact of gene polymorphisms of pro- and anti-inflammatory cytokines on the development of graft rejection in a cohort of renal transplant recipients and their living related donors.

2. Materials & methods

2.1. Study population

One hundred recipients and 100 donors of living related renal transplants engrafted between May 2006 and April 2008 were selected for this study. All renal transplants were carried out at a single center (Kidney Transplant Unit, King Faisal Specialist Hospital & Research Center, Riyadh, Saudi Arabia). Living kidney donors were at least 18 years of age. Selection for transplants were made following ABO blood group compatibility, a negative complement-dependent cytotoxicity cross-match using all historic positive, current antibody and HLA matching (HLA-A, -B, and DRB1). Donor specific antibodies were determined by solid phase immunoassay (SPI of Luminex platform), utilizing single antigen testing for HLA class I & II antigens. To determine the donor relevance of HLA antibodies detected by SPI, molecular typing techniques (SSO and SSP) were utilized to type live donors for all HLA class I and II loci. C4d staining was determined by immunohistochemistry on paraffin-embedded tissues. Immunosuppressive medication included Prednisone, Cyclosporine A or FK 506, Mycophenolate Mofetil and Rapamycin. Out of 100 considered initially, one was later disqualified from the study as he died soon after the transplantation. Seven patients presented with clinical and pathological complications other than rejection, so they were excluded from the analysis. Table 1 summarizes the demographic data of 92 recipients with and without rejection. Rejection was identified using clinical, biochemical, and histological criteria. All biopsies were assessed by a renal pathologist [26]. The diagnosis of acute humoral rejection was determined, as per the Banff Criteria, by the triad of histological changes (peri tubular capillaritis or glomerulitis, plus ATN), positive C4d staining, and the demonstration of donor relevant HLA antibodies (positive DSA and/or positive cross match) [26]. Finally, 17 patients suffered from acute rejection within the first 3–6 postoperative months, as proven by clinical symptoms, rise of serum creatinine (>0.3 mg/dL) and histological examination of graft tissue after biopsy. Patients with acute rejection were further subdivided based on the nature of their pathology into cellular (10) and antibody mediated rejection (7). Patients with stable renal function at the time of the analysis (n = 75) were compared with patients with acute rejection.

Renal allograft function was monitored and checked by measuring serum creatinine levels and the incidence of acute renal rejection was established by needle core biopsy. The average follow-up time data of either clinical or antibody production was 5 years after transplantation.

This study was approved by the Institution's Research Advisory Council (RAC), and Ethics Committee, and in accordance with the Ethics Committee of KACST, Riyadh, Saudi Arabia. Written informed consents were obtained from all participants.

2.2. DNA extraction

Venous blood was collected from all kidney transplant recipient/donor pairs and used for the isolation of genomic DNA as follows: genomic DNA was extracted from 5 mL of peripheral blood using Puregene DNA purification kit (Gentra Systems). Finally the DNA was dissolved in 1 mL of DNA Hydration solution, properly labeled and stored at −80 °C. Based on our committee rules and study approval protocols, confidentiality was strictly observed for all the samples analyzed.

Table 1
Characteristics of the studied 92 patients and 92 donors.

Group	Non-rejected (75)	Rejected (17)	p-Value
Age, mean (min–max), year			
Recipients	40.5 (2–76)[a]	34 (17–66)	0.15
Donors	30.4 (20–59)	28.4 (13–66)	0.4
Recipient gender M/F	48/27	8/9	0.27
Donor gender M/F	54/21	12/5	1.0
Antibody rejection (N)	0	7	–
Cellular rejection (N)	0	10	–
Chronic allograft nephropathy			
No/early/late, %	73 (97.33)/0/2 (2.67)	17 (100)/0/0	1.0
HLA-A/B/DRB1 mismatch, %			
0	6 (8%)	1 (5.9%)	0.64
1–2	30 (40%)	7 (41.2)	
3–4	33 (44%)	6 (35.3)	
5–6	6 (8%)	3 (17.6)	
PRA I[b], % >5%	8.2 (0–91)	22.0 (0–91)	0.14
PRA II[c], % >5%	12.2 (0–99)	16.3 (0–99)	0.53
Creatinine before transplant	838.6 (93–1917)	723.5 (328–1399)	0.16
Creatinine after transplant	88.9 (43–136)	105.9 (70–204)	0.09
CIT	185.5 (51–267)	199.1 (77–315)	0.34
DGF: yes/no	74/1	15/2	0.087
Status at last follow-up:			
Alive	71	12	–
Dead	2	0	–
Lost graft	2	5	<0.001

CIT: cold ischemia time. DGF: delayed graft function.
[a] Data shown as means (range) or frequencies.
[b] Panel reactive antibody class I.
[c] Panel reactive antibody class II.