Analysis of TNF-β, IL-10, and IL-1 Cluster Gene Polymorphisms and Clinical Risk Factors on Acute Renal Graft Rejection

Jie Zhao¹, Qifa Ye¹, Qiquan Wan*² and Jiandang Zhou²

¹Department of Transplant Surgery, the Third Xiangya Hospital, Central South University, Changsha 410013, Hunan, China
²Department of Clinical Laboratory of Microbiology, the Third Xiangya Hospital, Central South University, Changsha 410013, Hunan, China

Abstract

Introduction: There is growing evidence of the genetic association between certain cytokine and acute renal graft rejection. We sought to investigate the role of recipients’ TNF-β, IL-10, IL-1β and IL-1 receptor antagonist (ra) gene polymorphism as well as other variables such as PRA levels and HLA mismatches in acute renal graft rejection.

Methods: TNF-β (+252A/G), IL-10(-592A/C), IL-1β (-511C/T) and IL-1ra (86bp VNTR) gene polymorphisms were determined in 157 renal allograft recipients with and without acute rejection using PCR, TNF-β, IL-10, IL-1β and IL-1ra genotypic variants were investigated for correlation with acute rejection within the first year after renal transplantation.

Results: Patients with increased panel-reactive antibody (PRA) levels were predisposed to acute renal graft rejection (P=0.001). After adjusting for all variables of P<0.3, a PRA level >10% remained significant risk factor in a multivariate logistic regression analysis (OR=5.897, 95% confidence intervals=1.884-18.456, P=0.002). No significant difference was found between recipients with and without acute rejection regarding TNF β, IL-10, IL-1β and IL-1ra gene polymorphisms.

Conclusion: Increased PRA levels have more significant impacts than cytokine gene polymorphisms on the likelihood of developing acute renal graft rejection. We should take necessary pre-transplant and/or post-transplant measures such as plasma exchange or immune adsorption to lower the PRA levels. To identify the actual role of both PRA levels and gene polymorphisms in acute renal graft rejection additional studies with larger sample sizes will be necessary.

Keywords: Cytokine; Receptor antagonist; Gene polymorphism; Panel reactive antibody; Acute rejection; Renal transplantation

Introduction

Acute rejection (AR) still represents a major clinical problem accounting for most renal graft failures and hinders the success of renal transplantation. There is growing evidence of the genetic association between certain cytokine or its receptor antagonist and AR after renal transplantation. Some studies [1-3] investigated the association of IL-1β, IL-1 receptor antagonist (IL-1ra) or TNF-β gene polymorphism with acute renal graft rejection. However, the effects of these polymorphisms on AR after renal transplantation are still controversial. Panel reactive antibodies (PRA) are pre-existing antibodies targeting the human leukocyte antigen (HLA) and the complement system has an essential role in PRA related kidney rejection by the classical C1q-dependent pathway [4]. Although it has been established that elevated PRA can induce severe AR in renal transplantation [5,6], pre-transplant PRA levels were only involved in limited studies [7-11] which investigated the association of gene polymorphism with acute renal graft rejection. We sought to ascertain whether polymorphisms of the genes encoding recipients TNF β, IL-10 and IL-1β and IL-1ra as well as other variables such as PRA levels and HLA mismatches had impacts on the incidence of acute renal graft rejection and among these variables, which the most important risk factor for AR was.

Recipients and Methods

Between January 2008 and December 2009, 178 recipients underwent kidney transplantation at the Third Xiangya Hospital of Central South University (Changsha, China). From this original group, 21 recipients were excluded because of two or more kidney transplantations, simultaneous transplantations, lost to follow-up or technical problems. This left 157 patients available to be included in the present study with a follow-up period of 1 year. Out of the 157 available transplants, 34 recipients suffered at least one rejection episode, and 18 of these were biopsy proven, with the remaining 16 clinically proven. Twelve of those 18 biopsy proven AR were present of humoral rejection. Nine of those 16 clinically proven AR were antithymocyte globulin-requiring AR. All subjects were divided into AR group (n=34) and non-AR group (n=123). Donor information (age, gender and donor type), demographic and clinicopathological characteristics of the recipients (age, gender, primary disease, pre-transplant PRA level, initial immunosuppression (Cyclosporin/Tacrolimus) and use of antilymphocytic agents before AR), and transplant characteristics (cold ischemia time, HLA no. of 0 mismatches) were collected. AR group and non-AR group were compared with regarding genotyping and other variables. This study was performed with written informed consent and approved by the ethics committee of our hospital. AR was confirmed based on clinical or biopsy findings according to Banff criteria [12]. Clinical rejection was identified by increased creatinine levels in the lack of the evidence of infection, obstruction, or drug toxicity. Patients included in the “non-AR” group were defined as having no rejection episodes within the first year after transplantation.
Genotyping of TNF β (+252A/G), IL-10 (-592A/C), IL-1β (-511C/T) and IL-1ra (86bp VNTR) polymorphism

Genomic DNA was extracted from peripheral blood leukocytes. TNF β, IL-10, IL-1β and IL-1ra gene polymorphisms were detected by polymerase chain reaction (PCR) using previously reported primers [13-15]. A volume of 25 μl PCR reactions consisted of approximately 100 ng genomic DNA, 12.5 μl 2xHSTM Mix (Dongsheng Biological Technology Co., Ltd, Guangzhou, China), 10 μM of each primer (Huada gene science and technology Co., Ltd, Wuhan, China), and double-distilled water. The cycling conditions were: initial denaturation at 94°C for 4 minutes; 35 denaturation cycles at 94°C for 30 seconds, annealing at 56°C for 30 seconds, extension at 72°C for 30 seconds; final extension at 72°C for 5 minutes. The amplified products (8 μL) containing the NcoI polymorphic site at position +252 of TNF β gene, the RsaI polymorphic site at position -592 of IL-10 gene or the AvaI polymorphic site at position -511 of IL-1βgene were digested with 10 units of NcoI, RsaI or AvaI restriction enzyme (Fermentas) at 37°C for 3 hrs, respectively. Amplification of polymorphic regions of IL-1ra gene containing variable numbers of a tandem repeat (VNTR) of 86 base pairs was also performed using PCR. The PCR fragments and restriction fragments were eventually analyzed on 2% agarose gel electrophoresis.

Statistical analysis

SPSS (version 17.0, SPSS, Inc., Chicago, IL) was used to perform statistical analyses with a P values under 0.05 considered statistically significant. Continuous and categorical variables were analyzed using the Student’s t-test, Fisher exact test and Chi-square test, respectively. For multivariate logistic regression models, all variables with P<0.30 in the univariate analysis were included to identify independent risk factors for AR.

Results

A total of 157 recipients including 34 AR recipients (age range 23-58; mean age 36.50 ± 8.84, male-23 and female-11) and 123 non-AR recipients were finally analyzed. Table 1 showed the traditional risk factors for AR including demographic and clinical characteristics of both donors and recipients, and transplant characteristics. Table 2 showed the frequencies of TNF β, IL-10, IL-1β and IL-1ra variants in all recipients. Table 1 and Table 2 also showed that in univariate analysis, higher AR incidence was found to be statistically significant to PRA (P=0.001).

After adjusting for all variables of P<0.3 such as initial donor type, IL-10 genotypes, IL-1β genotypes, and HLA no. of 0 mismatches, a PRA level >10% remained significant risk factor in a multivariate logistic regression analysis (OR=5.897, 95% confidence intervals (CI)=1.884-18.456, P=0.002) (Table 3). No any significant difference was found between AR group and non-AR group regarding TNF β, IL-10, IL-1β and IL-1ra gene polymorphisms as well as other variables.

Discussion

AR represents a major clinical problem after renal transplantation and hinders the success of renal transplantation. There are different results in different studies regarding the exact role of cytokine gene polymorphisms in acute renal graft rejection [1,3,5,16,17]. Reasons for controversy among the results of different studies may be due to the variable clinical diagnosis processes, different PRA, HLA match status, immunosuppressive protocols, small sample size and ethnicity and so on [18-20].

In view of the pivotal role that cytokines play in the immune response, we evaluated the influence of genetic variants of TNF-β, IL-1β and IL-10 and IL-1ra genes, two important pro-inflammatory cytokines and two important anti-inflammatory factors, on renal transplantation. Only limited studies [7-11] investigated the association
of both gene polymorphism of cytokines and pre-transplant PRA level with acute renal graft rejection. However, it was so surprising that none of these authors established a significant association between PRA levels and AR. The reason for these results may be due to the relatively small amount of PRA-positive subjects it these studies. Hence, we also investigated whether other variables such as PRA levels had significant effects on acute renal graft rejection.

Our results are consistent with other studies regarding no influence of IL-10 [1,3,16,21,22] TNF-β [23], IL-1β [1,3], or IL-1ra [1,3] gene polymorphism on the presence of acute renal graft rejection. However, the present study revealed that a PRA level >10% associated with susceptibility to acute renal graft rejection. The possible reasons to explain this was our subjects who mainly underwent humoral (12/18) or T-cell-mediated (9/16) rejection, implicating that AR in our present study mostly comprised acute humoral rejection. Theoretically, cytokine or its receptor antagonist is mainly related to acute cellular rejection while PRA facilitate to acute humoral rejection. It is crucial for clinicians to focus more on pre- and post-transplant PRA levels, with the aim to suppress acute renal humoral rejection. It is necessary to analyze a very large sample to have sufficient statistical power to demonstrate or discard an acute cellular rejection. The next step in this study will be identifying the actual mechanism is not yet fully understood. Considering the relatively small sample sizes in the present study which may lead to have insufficient statistical power to detect slight effect of cytokine or its receptor antagonist gene polymorphisms on AR, our conclusion should be interpreted with caution. Additional studies with large sample size and better study designs including adding donor genotype studies can add the statistical power to find any association of different cytokine polymorphisms and acute cellular rejection. It is necessary to analyze a very large sample size to have sufficient statistical power to demonstrate or discard an association between cytokine polymorphisms and acute renal graft rejection. The next step in this study will be identifying the actual role of both PRA levels and cytokine or its receptor antagonist gene polymorphisms in acute cellular and humoral rejection, respectively after renal transplantation by extensive studies of larger sample sizes and PRA-positive subjects, or of additional polymorphisms and combinations of polymorphisms, and better study designs.

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