AN ANALYSIS OF CYTOKINE GENE POLYMORPHISMS ON ACUTE REJECTION IN RENAL RECIPIENTS

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ABSTRACT

Introduction*: 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.*

Materials and methods*: TNF-β (+252A/G), IL-10 (-592A/C), IL-1β (-511C/T) and IL-1ra (86bp VNTR) gene polymorphisms were investigated in 157 renal recipients 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% CI = 1.884-18.456, P = 0.002).*

Conclusion*: Increased PRA levels have more significant impacts than cytokine gene polymorphisms on the likelyhood of developing acute renal graft rejection. The necessary pre- and/or post-transplant measures to lower the PRA levels should be taken.*

Keywords*: Gene polymorphism, panel reactive antibody, acute rejection, renal transplant.*

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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 investigated the association of IL-1β, IL-1 receptor antagonist (IL-1ra) or TNF-β gene polymorphism with acute renal graft rejection[1-2]. 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[3].

Although it has been established that elevated PRA can induce severe AR in renal transplantation[4,5], pre-transplant PRA levels were only involved in limited studies[6-8] 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.

Materials and methods

Study population

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. Thirty-four 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, demographic and clinicopathological characteristics of the recipients, and transplant characteristics were collected. AR group and non-AR group were compared with regarding genotyping and other variables. The study was approved by the ethics committee of the Third Xiangya Hospital. AR was confirmed based on clinical or biopsy findings according to Banff criteria[9]. 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[10,11]. A volume of 25μl PCR reactions consisted of approximately 100ng genomic DNA, 12.5μl 2×HSTM Mix (Dongsheng Biological Technology Co., Ltd, Guangzhou, China), 10μM 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 (8μL) 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-1β gene 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-1 ra gene containing variable numbers of a tandem repeat 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

Thirty-four AR recipients (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 $P < 0.3$, a PRA level >10% remained significant risk factor in a multivariate logistic regression analysis (OR = 5.897, 95% 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[1,4,12,13]. In view of the pivotal role that cytokines play in the immune response, 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 fac-

tors, on renal transplantation was evaluated in the present study. Only limited studies investigated the association of both gene polymorphism of cytokines and pre-transplant PRA level with acute renal graft rejection[6-8].

Variables	AR groups (n=34)	Non-AR groups (n=123)	*P*
Univariate analysis			
Donor characteristics			
Age (yr mean ± SD)	34.30±6.52	35.17±9.31	0.752
Gender no. (%)			0.64
Male	27(79.4)	93(75.6)	
Female	7(20.6)	30(24.4)	
Donor type no. (%)			0.39
Deceased	16(47.1)	73(59.3)	0.211
Living	17(50.0)	47(38.2)	
DCD	1(2.9)	3(2.4)	
Recipient characteristics			
Age (yr mean ± SD)	36.50±8.84	38.20±10.58	0.337
Gender no. (%)			0.893
male	23(67.6)	65(52.8)	
female	11(32.4)	58(47.2)	
Primary kidney disease no. (%)			0.704
Glomerulonephritis	29 (85.4)	110 (89.4)	
Adult polycystic disease	3 (8.8)	5 (4.1)	
Diabetes	1 (2.9)	3 (2.4)	
Other	1 (2.9)	5 (4.1)	
PRA level >10%	9 (26.5)	6 (4.9)	0.001
Initial immunosuppression no. (%)			0.68
Cyclosporin	16(22.5)	55(77.5)	
Tacrolimus	18(20.9)	68(79.1)	
Use of antilymphocytic agents before AR	3 (8.8)	19 (15.4)	0.317
Transplant characteristics			
Cold ischemia time	3.53±1.65	3.12±1.79	0.653
HLA no. of 0 mismatches (%)	14(60.9)	70(56.9)	0.102

Table 1: Donor, recipient and transplant characteristics of all recipients with and without AR.
Abbreviations: AR; acute rejection, SD; standard deviation, DCD; donation after cardiac death, PRA; panel-reactive antibodies, HLA; human leukocyte antigen.

Genotype	AR groups (n=34)	Non-AR groups (n=123)	*P*
TNF-β (+252A/G)			0.515
A/A	4(11.8)	29(23.6)	
A/G	19(55.9)	58(47.2)	
G/G	11(32.3)	36(29.2)	
IL-10 (-592A/C)			0.238
A/A	14(41.2)	67(54.5)	
A/C	15(44.1)	44(35.8)	
C/C	5(14.7)	12(9.7)	
IL-1β (-511C/T)			0.182
C/C	5(14.7)	32(26.0)	
C/T	20(58.8)	62(50.4)	
T/T	9(26.5)	29(23.6)	
IL-1ra (86bp VNTR)			0.449
1/1	25(73.5)	98(79.7)	
1/2	6(17.6)	23(18.7)	
1/4	3(8.9)	2(1.6)	

Table 2: TNF-β, IL-10, IL-1β and IL-1ra genotypic frequencies in recipients with and without acute rejection.
Abbreviations: TNF; tumor necrosis factor, IL; interleukin, ra; receptor antagonist; AR; acute rejection, VNTR; variable numbers of a tandem repeat.

	OR (CI: 5–95)	*P*
IL-1β (-511C/T)	0.438(0.144-1.335)	0.147
IL-10 (-592A/C)	1.113(0.745-2.331)	0.258
Donor type	1.137(0.606-2.862)	0.487
HLA no. of 0	1.436(0.813-3.156)	0.151
PRA level (>10% vs.≤10%)	5.897(1.884-18.456)	0.002

Table 3: Logistic regression of potential risk factors for acute rejection
Abbreviations: OR; odds ratio, CI; confident interval, HLA; human leukocyte antigen, PRA; panel-reactive antibodies.

However, none of these authors established a significant association between PRA levels and AR.

Our results are consistent with other studies regarding no influence of IL-10, TNF-β, IL-1β or IL-1ra gene polymorphism on acute renal graft rejection[1,12,14]. However, the present study revealed that a PRA level >10% associated with acute renal graft rejection. The possible reasons to explain this was our subjects who mainly underwent humoral (12/18) or antithymocyte globulin-requiring (9/16) rejection, implicating that AR in our present study

mostly comprised acute humoral rejection. Theoretically, cytokine or its receptor antagonist are mainly related to acute cellular rejection while PRA facilitate to acute humoral rejection.

Our findings indicated that increased PRA levels had more significant impacts on the risk for acute renal graft rejection. Therefore, it is crucial for clinicians to focus more on pre- and post-transplant PRA levels. We should take necessary pre- and/or post-transplant measures such as plasma exchange, immunoadsorption, or intravenous immunoglobulin, to eliminate pathogenic antibodies or make their inactivation[15-17].

Acute renal graft rejection is a complicated phenomenon involving numerous factors, such as cell- and/or antibody-dependent components, HLA matching, and immunosuppressive agents and the underlying mechanism is not yet fully understood. Considering the relatively small sample sizes in the present study which may lead to have insufficient power to detect slight effect of cytokine or its receptor antagonist gene polymorphisms on AR, the conclusion from the present study should be interpreted with caution. 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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