Transplant Immunology 21 (2009) 13–17

Contents lists available at ScienceDirect

Transplant Immunology

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

Interleukin 8 −251T>A and *Interferon gamma* +874A>T polymorphism: Potential predictors of allograft outcome in renal transplant recipients from north India ☆

Ranjana Singh, Pravin Kesarwani, Dinesh K. Ahirwar, Rakesh Kapoor, Rama D. Mittal *

Department of Urology and Renal Transplantation, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Raebareli Road, Lucknow 226014, Uttar Pradesh, India

ARTICLE INFO

Article history:
Received 12 August 2008
Received in revised form 26 December 2008
Accepted 9 January 2009

Keywords:
IL8
IFNG
ARMS-PCR
Renal transplantation
Immunosuppressants
Allograft rejection

ABSTRACT

Background: Episodes of acute rejection represent an important risk factor for the development of chronic allograft nephropathy. We explored whether certain cytokine gene polymorphisms in renal transplant recipients may be useful markers for susceptibility to allograft rejection. *Interleukin 8* (*IL8*) −251 T>A and *interferon gamma* (*IFNG*) +874A>T gene polymorphisms were correlated with allograft outcome in renal transplant recipients.

Methods: Genotyping was done by amplification refractory mutational system-polymerase chain reaction (ARMS-PCR) in 264 healthy controls and 296 renal transplant recipients categorized into 235 non-rejecters and 61 rejecters.

Results: IL8 −251AA genotype was associated with 2.7-folds increased risk for allograft rejection in recipients experiencing rejection episodes as compared to non-rejecters (OR = 2.70, $P = 0.032$). Cox proportional analysis revealed >2-folds increased susceptibility for allograft rejection (HR = 2.38, $P = 0.010$) in *IL8* −251AA recipients. Kaplan–Meier analysis also demonstrated lower mean time to first rejection episode for *IL8* −251AA recipients (23 months) as compared to TT recipients (30 months) (log rank $P = 0.022$). No association of *IFNG* +874A>T was observed with allograft rejection, however, an increasing trend towards immunosuppressant toxicity was observed in patients with +874TT genotype at one month post transplantation.

Conclusion: Thus, *IL8* −251AA genotype may serve as potential predictor of allograft outcome in our North Indian cohort of renal transplant recipients.

1. Introduction

Renal transplantation is the preferred treatment of choice for most patients with end stage renal failure. The recent advent of novel immunosuppressive agents and new formulations has significantly improved the clinical outcome of renal transplant recipients. However, acute rejection episodes still occur in ~20–40% of organ transplant recipients, while chronic rejection and allograft dysfunction remain the most common causes of long-term graft loss [1]. Thus, identification of markers associated with or predictive of chronic allograft nephropathy should prove clinically useful.

Chemokines and cytokines are potent inflammatory mediators directly involved in immunologic mechanisms mediating alloimmune responses. Interleukin 8 (IL8; CXCL8) belongs to the superfamily of CXC chemokines attracting neutrophils and macrophages and manifests a wide range of proinflammatory effects [2,3]. It is reported to be associated with T helper 1 (Th1) type of immune response in renal allograft rejection [4]. The *IL8* gene is located on chromosome 4q13–21 and consists of four exons, three introns, and a proximal promoter region [5]. The T/A polymorphism located at −251 nucleotides relative to the transcriptional start site of the gene is known to affect its expression [6]. Hull et al. suggested mutant A allele of the (−251) T>A polymorphism to be associated with increased IL8 production by lipo-polysaccharide stimulated whole blood [7].

Interferon gamma (IFNG), another pro-inflammatory cytokine, produced by activated CD4+ T cells, defines the development of Th1 response and promotes cell mediated immunity [8]. Several groups have presented data that link Th1 cytokine pattern to allograft rejection [9,10]. Amirzargar et al. reported significantly higher IFNG serum concentrations at 2 weeks post transplantation in group with acute rejection versus those without acute rejection [11].

The human *IFNG* gene is located on chromosome 12q24.1. A single nucleotide polymorphism (SNP) in the first intron of *IFNG* gene (+874T>A) can putatively influence the secretion of IFNG [12]. Analysis of the biological role of this SNP suggested that +874A carriers are low IFNG producers [13]. In renal transplantation, the *IFNG* +874 TT genotype was

☆ Grant support: The study was supported by grant from the Council of Scientific and Industrial Research (CSIR) New Delhi, India (No. 27(0185)/08/EMR-II).

* Corresponding author. Department of Urology, SGPGIMS, Raebareli Road, Lucknow-226014, India. Tel.: +91 522 2668004 8x2116; fax: +91 522 2668 017.
E-mail addresses: ramamittal@yahoo.com, rmittal@sgpgi.ac.in (R.D. Mittal).

0966-3274/$ – see front matter © 2009 Published by Elsevier B.V.
doi:10.1016/j.trim.2009.01.001

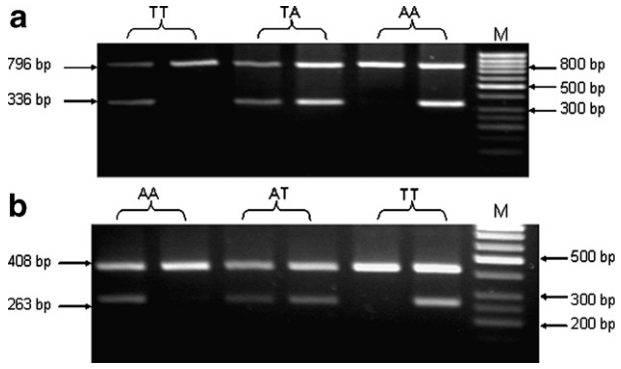

Fig. 1. ARMS PCR assay for analyzing *IL8* −251T>AA (a) and *IFNG* +874A>T (b) polymorphism showing wild type, heterozygous and homozygous variant genotypes. PCR products were separated on 2% agarose gel electrophoresis. Lane M was loaded with 50 bp molecular marker.

associated with elevated serum creatinine at 5 year post transplantation [14]. However, Hahn et al. observed no association of *IFNG* gene polymorphism with acute rejection episodes [15].

2. Objective

Despite the importance of the *IL8* gene in allograft rejection, no investigation of the role of *IL8* (−251T>A) polymorphism in relation to allograft rejection has been undertaken. Moreover, reports of *IFNG* (+874A>T) in several studies reported have been inconclusive which prompted us to undertake this study. In light of the above, our objective was to investigate the possible correlation and association of *IL8* (−251T>A) and *IFNG* (+874A>T) polymorphism for susceptibility to allograft rejection, in a cohort of renal transplant patients and healthy controls representing the general population of north India.

3. Materials and methods

3.1. Study population

A total of 264 unrelated healthy individuals and 296 renal transplant recipients of similar ethnicity from North India (particularly Lucknow and the adjoining cities of north India) were enrolled in the present study. The healthy individuals (mean age 35.17 ± 12.39 years; male/female 214/50) were randomly selected from the hospital staff, students and blood donors visiting the hospital. The transplant recipient's cohort (mean age 35.42 ± 10.67 years; male/female 243/53) included patients transplanted between July 2003 and March 2007 from live related donors at the tertiary care hospital of Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India. Selection for transplantation was based on ABO blood group compatibility, a negative complement-dependent cytotoxicity cross-match using all historic positive and current sera. Patients were molecular typed for human leukocyte antigen (HLA) class I — A, B, C antigens and class II — DR, DQ antigens. The demographic data regarding donor's age, recipient's age and sex, immunosuppression regimen (cyclosporine/MMF/prednisilone or tacrolimus/MMF/prednisilone), presence of rejection episodes and graft survival were referred from the hospital records with the assistance of a collaborating renal transplant surgeon. 236 recipients were on cyclosporine based immunosuppression therapy and 60 recipients were on tacrolimus based immunosuppression therapy. Occurrence of rejection episode was based on clinical diagnosis with

Table 1
Clinical and demographic characteristics of renal transplant recipients.

Characteristics	Non-rejecters $n=235$	Rejecters $n=61$	P value[a]
Age at time of transplantation[b] (years)	35.31 ± 10.85	35.85 ± 10.06	0.725
Donors age[b]	37.12 ± 11.81	38.00 ± 10.79	0.553
Gender (male/female)	194/41	49/12	0.686[c]
Body weight (kg)[b]	52.50 ± 9.85	54.35 ± 7.69	0.206
Baseline serum creatinine (mg/dl)[b]	1.14 ± 0.28	1.29 ± 0.42	0.029
HLA mismatch (total number)	3.94 ± 2.16	4.31 ± 2.25	0.357
Recipient diagnosis			
Chronic glomerulonephritis	124 (52.7)	34 (55.7)	
Chronic intestinal nephritis	47 (20.0)	12 (19.8)	
Diabetic nephropathy	30 (12.8)	6 (9.8)	
Others	34 (14.5)	9 (14.7)	0.935[c]

Figures in parenthesis represent the percentage.
[a] — P value calculated by ANOVA test.
[b] Data represented as mean ± SD.
[c] — χ^2 P value.

Table 2
Genotype and allele frequencies of *IL8* −251T>A and *IFNG* +874A>T in 264 healthy controls and 296 transplant recipients.

Polymorphism	Genotype/ allele	Phenotype	Controls n (%)	Patients n (%)	OR[a] (95% CI)	P_{trend}
IL8 −251A>T rs 4073	TT	Low	109 (41.3)	121 (40.9)	1 (Ref)	–
	TA	Medium	108 (40.9)	133 (44.9)	1.17 (0.81–1.69)	0.404
	AA	High	47 (17.8)	42 (14.2)	0.81 (0.49–1.34)	0.412
	T allele	Low	326 (61.7)	375 (63.3)	1 (Ref)	–
	A allele	High	202 (38.3)	217 (36.7)	0.93 (0.73–1.19)	0.580
IFNG +874A>T rs 2430561	AA	Low	95 (36.0)	84 (28.4)	1 (Ref)	–
	AT	Medium	126 (47.7)	145 (49.0)	1.37 (0.93–2.02)	0.113
	TT	High	43 (16.3)	67 (22.6)	1.77 (1.09–2.89)	**0.022**
	A allele	Low	316 (59.8)	313 (52.9)	1 (Ref)	–
	T allele	High	212 (40.2)	279 (47.1)	1.33 (1.05–1.69)	**0.019**

The homozygote for the low producer allele was taken as reference category.
OR, odds ratio; CI, confidence interval.
P values in boldface are significant.
[a] Data were calculated by logistic regression analysis and adjusted by age, gender and body weight status of the study subjects.

Table 3
Influence of *IL8* −251T>A and *IFNG* +874A>T polymorphism on allograft outcome in renal transplant recipients categorized into 235 non-rejecters and 61 rejecters.

Genotype/ allele	Non-rejecters n (%)	Rejecters n (%)	OR (95% CI)[a]	P_{trend}	HR (95% CI)[b]	P value
IL8 −251T>A						
TT	100 (42.6)	21 (34.4)	1 (Ref)	–	1 (Ref)	–
TA	108 (46.0)	25 (41.0)	1.64 (0.66–4.09)	0.287	1.08 (0.61–1.94)	0.784
AA	27 (11.5)	15 (24.6)*	2.70 (1.09–6.69)	**0.032**	2.38 (1.23–4.64)	**0.010**
T allele	308 (65.5)	67 (54.9)	1 (Ref)	–		
A allele	162 (34.5)	55 (45.1)**	1.56 (1.04–2.34)	**0.031**		
IFNG +874A>T						
AA	63 (26.8)	21 (34.4)	1 (Ref)	–	1 (Ref)	–
AT	121 (51.5)	24 (39.3)	0.58 (0.30–1.14)	0.114	0.65 (0.36–1.17)	0.152
TT	51 (21.7)	16 (26.2)	0.92 (0.43–1.96)	0.823	0.95 (0.49–1.84)	0.878
A allele	247 (52.6)	66 (54.1)	1 (Ref)	–		
T allele	223 (47.4)	56 (45.9)	0.94 (0.63–1.40)	0.761		

*χ^2 P value 0.030; **χ^2 P value 0.032.
The homozygote for the low producer allele was taken as reference category.
OR, odds ratio; CI, confidence interval; HR, hazard ratio.
P values in boldface are significant.
[a] Data were calculated by logistic regression analysis and adjusted by age, gender and body weight status of transplant recipients.
[b] HR were calculated by Cox proportional survival model and adjusting age, gender and body weight status of transplant recipients as covariates.