SHORT REPORT

Influence of recipient and donor IL-1α, IL-4, and TNFα genotypes on the incidence of acute renal allograft rejection

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Aims: To determine whether polymorphisms of the genes encoding donor or recipient interleukin 1α (IL-1α), tumour necrosis factor α (TNFα), or IL-4 have any impact on the incidence of acute rejection after renal transplantation.
Methods: All donors and recipients were genotyped for three polymorphisms in the three cytokine genes: IL1A −889, TNFA −308, and IL4 −590.
Results: Statistical analysis of the data obtained revealed no association between the cytokine gene polymorphisms tested and the incidence of post-transplant acute rejection. After stratification for human leucocyte antigen (HLA) matching, it was found that kidneys from donors positive for the TNFA-A allele had a significantly increased incidence of acute rejection in HLA-DR mismatched transplants.
Conclusions: This finding argues for prospective TNFA genotyping of renal donors, with avoidance of allocation of kidneys from donors positive for the TNFA-A allele to HLA-DR mismatched recipients.

Transplantation of renal grafts is an established treatment for renal failure in a variety of medical conditions. It is now standard practice in the UK that pretransplant histocompatibility matching between the donor and recipient should be performed. This matching process is based on human leucocyte antigen (HLA) A/B/DR matching. It is clear that although HLA matching improves graft outcome, acute and chronic rejection of the allograft is still a problem with both cadaveric and living donors. However, at present there are no laboratory assays that are capable of predicting the incidence of acute rejection.

The aim of our study was to identify non-HLA risk factors for acute rejection, which could be used in pretransplant patient assessment. Candidates for these ''risk factors'' are the proteins known as cytokines, which act as communicators between cells, can alter the behaviour of cells, and operate as a network. Because of the profound influence of cytokines on such processes as wound healing, inflammation, and antibody production, there has been an explosion of interest recently in all aspects of cytokines related to solid organ allograft rejection, in the hope that it may provide a better understanding of the rejection process.

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Cytokines present in the renal graft may have originated from either the donor or the recipient. Mutations in cytokine polymorphism sequences may alter transcription factor sites, which could alter transcription itself and subsequent cytokine production. However, the exact role of cytokine gene polymorphisms in transplant outcome remains controversial, with some groups showing a correlation,[1–3] but others not.[4 5]

Tumour necrosis factor (TNF) is a proinflammatory cytokine. TNFα release has been correlated with the subsequent development of early graft failure. A G to A base change at position −308 of the TNFα promoter region has been described, resulting in two alleles TNF1 and TNF2.[6] The TNF2 allele is in linkage disequilibrium with the major histocompatibility complex (MHC) haplotype A1-B8-DR3. Wilson *et al* have provided evidence that the TNF2 allele is a much stronger transcriptional activator than the TNF1 allele.[7] The molecular mechanism to explain this has not been elucidated; there was no difference in affinity of the DNA binding proteins between the two alleles in the Raji cells tested.

The net effect of the three members of the interleukin 1 (IL-1) gene family (IL-1α, IL-1β, and IL-1 receptor antagonist) is to control inflammatory and host defence responses. IL-1 causes vasorelaxation, increases adherence of lymphocytes and neutrophils to endothelial cells, and might be implicated in the immunobiology of both acute and chronic graft rejection. The IL1A gene has a polymorphism present at position −889 of the promoter region, a C to T base change, which has been associated with juvenile rheumatoid arthritis.[8] This polymorphism lies upstream of a sequence that suppresses transcription of the IL1A gene.

IL-4 is a T helper type 2 cytokine, and is a growth costimulator for B and T cells, mast cells, erythroid progenitors, and myeloid progenitors. IL-4 inhibits the release of inflammatory mediators such as TNFα, IL-6, and IL-1α from activated monocytes, thus counteracting their damaging effects in the graft. IL-4 can also upregulate HLA class II MHC molecules on B cells. A C to T base change at position −590 in the 5′ region of the IL4 gene has been identified and linked with asthma.[9] The functional importance of this has yet to be understood.

MATERIALS AND METHODS

Selection of patient group, and determination of rejection episodes and severity

The study cohort comprised 277 renal allograft recipients and donor pairs. Ethical approval was obtained. Patients were transplanted between 21 January 1991 and 10 December 1996 at St James's University Hospital, Leeds, UK. HLA matching was determined by both serological and molecular techniques. There were 103 favourably matched and 174 non-favourably matched donor–recipient pairs in this patient cohort. The favourably matched cohort comprised 19 ''000'',

Abbreviations: IL, interleukin; HLA, human leucocyte antigen; MHC, major histocompatibility complex; PCR, polymerase chain reaction; TNF, tumour necrosis factor

Table 1 The genotype, phenotype, and allele frequencies of IL1A, IL4, and TNFA of 277 renal allograft recipients and 233 cadaveric donors

	IL1A −889				IL4 −590				TNFA −308			
Recipients												
Genotype	c/c	c/t	t/t		c/c	c/t	t/t		g/g	g/a	a/a	
Acute rejection	70	56	14		79	52	9		85	50	5	
Frequency	0.50	0.40	0.10		0.56	0.38	0.06		0.60	0.36	0.04	
No rejection	74	49	14		82	52	3		83	48	6	
Frequency	0.54	0.35	0.11		0.60	0.38	0.02		0.61	0.35	0.04	
p Value	0.6728				0.2226				0.9104			
Phenotype	c	Not c	t	Not t	c	Not c	t	Not t	g	Not g	a	Not a
Acute rejection	126	14	70	70	131	9	61	79	135	5	55	85
Frequency	0.90	0.10	0.50	0.50	0.94	0.06	0.44	0.56	0.96	0.04	0.40	0.60
No rejection	123	14	63	74	134	3	55	82	131	6	54	83
Frequency	0.90	0.10	0.46	0.54	0.98	0.02	0.40	0.60	0.96	0.04	0.39	0.61
p Value	0.9215		0.5006		0.1580		0.5501		0.9512		0.9968	
Allele frequency	c		t		c		t		g		a	
Acute rejection	196		84		210		70		220		60	
Frequency	0.70		0.30		0.75		0.25		0.78		0.22	
No rejection	197		77		216		58		214		60	
Frequency	0.72		0.28		0.79		0.21		0.78		0.22	
p Value	0.6336				0.2812				0.9314			
Donors												
Genotype	c/c	c/t	t/t		c/c	c/t	t/t		g/g	g/a	a/a	
Acute rejection	63	48	11		66	53	3		79	36	7	
Frequency	0.52	0.39	0.09		0.54	0.43	0.03		0.65	0.30	0.05	
No rejection	63	37	11		73	35	3		73	34	4	
Frequency	0.58	0.33	0.09		0.60	0.38	0.02		0.66	0.31	0.03	
p Value	0.6356				0.1718				0.7430			
Phenotype	c	Not c	t	Not t	c	Not c	t	Not t	g	Not g	a	Not a
Acute rejection	111	11	59	63	119	3	56	66	115	7	43	79
Frequency	0.90	0.10	0.50	0.50	0.94	0.06	0.44	0.56	0.96	0.04	0.40	0.60
No rejection	100	11	48	63	108	3	38	73	107	4	38	73
Frequency	0.90	0.10	0.46	0.54	0.98	0.02	0.40	0.60	0.96	0.04	0.39	0.61
p Value	0.9931		0.5149		0.7666		0.0931		0.6471		0.9807	
Allele frequency	C		T		C		t		g		a	
Acute rejection	174		70		185		59		194		50	
Frequency	0.71		0.29		0.76		0.24		0.80		0.20	
No rejection	163		59		181		41		180		42	
Frequency	0.73		0.27		0.82		0.18		0.81		0.19	
p Value	0.6853				0.1654				0.7570			

15 ''100'', 40 ''010'', and 29 ''110'' individuals. All patients had a negative complement dependent cytotoxicity crossmatch result before transplantation. Patients received cyclosporine A, prednisolone, and azathioprine as immunosuppressive treatment post-transplant. DNA samples from patients and donors were prepared from EDTA buffy coat cells, according to published methods.[10] Forty four donors donated two kidneys to patients within the group.

One hundred and forty patients experienced at least one rejection episode in the first year, whereas 137 patients remained rejection free. Patients included in the ''no acute rejection'' group were defined as having no rejection episodes in the first year after transplantation. Patient files were reviewed to provide clinical outcome data. All laboratory techniques were performed without knowledge of the rejection status of each individual.

PCR restriction fragment length polymorphism genotyping

All donors and recipients were genotyped for three polymorphisms in three cytokine genes: IL1A −889, TNFA −308, and IL4 −590. All assays have been described previously.[6 8 9]

IL-1

The primer sequences used were: forward, 27 mer 5′-AAGCTTGTTCTACCACCTGAACTAGGC-3′; reverse, 20 mer 5′-TTACATATGAGCCTTCCATG-3′,[8] which amplify a 99 bp sequence. Polymerase chain reaction (PCR) products with a C at position –889 are digested by NcoI to give two fragments of 83 bp and 16 bp. PCR products with a T at position −889 are not digested by NcoI.

TNF

The primer sequences used were: forward, 20 mer 5′-AGGCAATAGGTTTTGAGGGCCAT-3′; reverse, 20 mer 5′-TCCTCCCTGCTCCGATTCCG-3′,[6] which amplify a 107 bp sequence. PCR products with a G at position −308 are digested by NcoI to give two fragments of 87 bp and 20 bp. Those with an A at position −308 are not digested by NcoI.

IL-4

The primer sequences used were: forward, 20 mer 5′-ACTAGGCCTCACCTGATACG-3′; reverse, 20 mer 5′-GTTGTAATGCAGTCCTCCTG-3′,[9] which amplify a 252 bp sequence. PCR products with a C at position −590 generate a BsmFI recognition sequence; digestion produces two fragments of 60 bp and 192 bp. PCR products with a T at position −590 are not digested by BsmFI.

Take home messages

- Kidneys from donors positive for the TNFA-A allele had a significantly increased incidence of acute rejection in HLA-DR mismatched transplants
- Prospective TNFA genotyping of renal donors should be carried out and kidneys from donors positive for the TNFA-A allele should not be given to HLA-DR mismatched recipients

Table 2 Analysis of donor IL1A −889, IL4 −590, and TNFA −308 polymorphisms in rejectors and non-rejectors

	Genotype	DR M rejectors	DR M non-rejectors	p Value	DR MM rejectors	DR MM non-rejectors	p Value
IL1A	IL1A T* positive	21	25	0.8953	37	24	0.8136
	IL1A T* negative	26	41		38	21	
IL4	IL4 T* positive	28	26	0.06	28	14	0.6212
	IL4 T* negative	19	40		47	31	
TNFA	TNFA-A* positive	14	25	0.4896	26	7	0.0395
	TNFA-A* negative	33	41		49	38	

The donors were divided into rejectors and non-rejectors, and HLA-DR matched (DR M) transplants versus HLA-DR mismatched (DR MM) transplants. The p values shown represent the combined results from the two randomly selected groups

Statistical analysis

The genotype, allele, and phenotype frequencies of IL1A, IL4, and TNFA gene polymorphisms were calculated in recipients and donors. Allele frequencies and phenotype frequencies were obtained by counting the number of individuals positive for a particular allele. Associations were analysed using the statistical package Epi Info 6 to generate a χ^2 value from a $2 \times n$ contingency table. Yates corrected p values were used where appropriate. To correct probability values for multiple comparisons, the patient–donor pairs were randomly divided into two groups, and genotyping was performed in each group. Associations with rejection were deemed significant if found in both cohorts.

RESULTS AND DISCUSSION

Table 1 provides an analysis of cytokine gene polymorphism data in recipient and donor groups. This revealed no association between the polymorphisms tested and the incidence of post-transplant acute rejection. These findings are in accord with some previously published studies,[4 5] but not with others.[1–3]

When results were stratified on the basis of HLA-DR matching, analysis of the donor TNFα high producer genotype (TNF2 positive) was found to be significantly associated with increased incidence of acute rejection in HLA-DR mismatched transplants ($p = 0.0395$; relative risk, 1.4). No such relation was established for IL-4 or IL-1α (table 2).

These data argue for the prospective typing of renal donors for TNFA gene polymorphisms with avoidance of allocation of kidneys from donors positive for the TNFA-A allele to HLA-DR mismatched recipients.

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