IJMS
Vol 29, No 4, December 2004

Original Article

Correlation of Corneal Allograft Rejection with Tumor Necrosis Factors-Alpha Gene Polymorphism

S.H. Jahadi-Hossieni[1], E. Kamali,[2]
M. Samani, A. Katbab, H. Khoshniat,
H. Movahhedan, M. nejabat, R. Salouti,
J. Sarouri[2]

Abstract

Background: Correlations between bone marrow, heart, kidney, liver, skin and lung transplant rejection or survival with human cytokine gene polymorphisms have been described. There are also reports about the role of cytokines and Tumor Necrosis Factors-Alpha (TNF-α) on corneal transplant in animal models. Further studies are needed to clarify the role of cytokines in corneal allograft rejection in humans.

Objective: To study whether corneal allograft rejection is associated with TNF-α gene polymorphism**.**

Methods: A total of 105 cases of corneal transplant were followed for a mean period of 25.9 months and the episodes of rejections recorded. We determined allele-specific PCR (ASPCR) TNF-α gene polymorphism of the patients and evaluated their association with rejection.

Results: The overall incidence of corneal graft rejection and its subsequent recovery were 21% and 63.6% respectively. Rejection was more common in the vascularized corneas (5.4 folds; $P<0.001$), and in eyes with anterior synechia (3.9 fold; $P<0.05$). There was no correlation between TNF-α gene polymorphism and the chance of allograft rejection.

Conclusion: No association was found between human TNF-α-308 G/A promoter gene polymorphism and corneal allograft rejection in our cases of uncomplicated penetrating keratoplasty.
Iran J Med Sci 2004; 29(4): 180-184.

Keywords ● TNF-α ● cytokine ● Cornea transplant ● Rejection

Departments of Ophthalmology &
[2]Immunology School of Medicine
and [1]Organ Transplant Rearch Center,
Namazee Hospital
Shiraz University of Medical Sciences
Shiraz Iran.

Correspondence:
SH. Jahadi-Hossieni MD
Organ Transplant Rearch Center,
Namazee Hospital
Shiraz University of Medical Sciences
Shiraz Iran.

Tel: +98 711 6263751
Fax: +98 711 6263752
E-mail: iahadih@sums.ac.ir

Introduction

The immune system recognizes and destroys a transplant organ when it is of a non-self nature.[1] The major human leukocyte antigen (HLA) system is a well known component of the immune system which determines the outcome in allogenic transplants.[2] The significance of the non-HLA components of the immune system is evidenced by the fact that allograft reactions can occur despite HLA compatibility between donors and recipients.[3,4]

Where as cornea is avascular and hidden from the immune system, most of the previous studies on the role of TNF-α in iorgan transplantation were carried out in organs having blood supplies and hence easily exposed to the immune system.[5,6,7-9]

There are some reports regarding TNF-α in corneal graft in animal models.[10,11] However, there is little information about the effects of human TNF-α gene polymorphisms on corneal allotransplants. In such cases the donor tissue was transplanted into an immunologically privileged site. Therefore, the present study investigates if TNF-α -308 G/A gene polymorphism has any effect on the outcome of corneal allograft and transplant rejection.

Patients and methods

The present study comprised 105 patients who underwent elective optical penetrating keratoplasties (full thickness corneal transplant) in non-inflamed eyes. The patients consisted of 58 males (55.2%) and 47 (44.8%) females and were in the range of 12-78 years with a mean age of 37.37 years. The underlying diseases included keratoconus (61%), corneal scar and opacity (23.8%), pseudophakic bullous keratopathy (10.5%), macular corneal dystrophy (2.9%) and Fuchs' endothelial dystrophy (1.9%).

Patients were considered as rejecters if showed one episode of clinically evident acute corneal allograft rejection during the follow up course of 2.5-60 months with a mean of 17.84 months. On the other hand, they were regarded as non-rejecters if no allograft rejection occurred until the end of the follow-up period of 4-100 months with a mean of 25.9 months.
Patients received postoperative systemic steroid therapy for one week followed by a steroid eye drop, which was tapered to four times a day during the first month and finally discontinued during the next 4 to 6 months.

Patients were visited regularly and episodes of corneal allograft rejections were observed by biomicroscopic examination of the eye. Graft failure, due to allograft rejection, was defined as persistence of the corneal edema with no improvement, despite intensive treatment for acute corneal allograft rejection. Recovered corneal allograft rejection was defined as relieved signs of corneal rejection, which resulted in a non-edematous clear cornea without anterior segment inflammation and/or keratic precipitates. Anterior synechia and recipient bed vascularization were considered as possible risk factors for rejection and according to their extent were graded from 0 to 4.

Typing of TNF-α polymorphism by ASPCR
Genomic DNA was extracted from peripheral blood leukocytes by a salting out procedure.[12] PCR-based DNA analysis was carried out with some modification under conditions previously described.[14] Based on this method, an ASPCR was used to detect the G→A transition polymorphism at position -308 of TNF-α gene. Three primers were used for the ASPCR: the forward primer (position -144/-164: 5'-TCTCG GTTTCTTCTCCATCG-3') was used in combination with either the reverse primer (position 328/-308 G: 5'-ATAGGTTTTGAGGGGCATGG -3'), complementary to the TNFA1 allele, or the reverse primer (position -320/-308 A: 5'-ATAG GTTTTTG AGGGGCATGA-3'), which is complementary to the TNFA2 allele. As an internal control the β-globin specific primers (forward primer: 5'-ACACAACTGTGTTCACTAGC-3', and reverse primer: 5'-CAACTTCATCCACGTT C ACC-3') were included to the reactions. Fifty micro-liters of PCR reaction mixture were comprised of genomic DNA samples (500 ng), 200 μmol/L dNTPs, 2 mM MgCl2, 1X Taq DNA polymerase buffer, 2 unit of Taq DNA polymerase (Bohringer Manheim, Germany) and 10 pmol of each test primers. Reaction conditions used with the thermal cycler (master cycler, Ependerof) were as follows: 95°C for 5 minutes; 31 cycles of 95°C for 90 seconds, 61°C for 150 seconds, and 72°C for 60 seconds; and 72°C for 10 minutes.
Reaction products were separated on a 2% agarose gel and stained with ethidium bromide.

Statistical analysis:
Experimental data for the distribution of TNF-α alleles and clinical data were compared by Chi-square or Fisher exact tests, using EPI6 statistical software package.

Results

The number of cases with their genders and underlying diseases are shown in Table 1. The overall incidences of rejection and recovery following the rejection were 21% (22 eyes), and 63.6% (14 eyes) respectively. From 22 eyes with allotransplant reaction, 21 eyes experienced endothelial cell rejection and one eye epithelial cell rejection. In 12 eyes with recipient bed vascularization there was a 5.4-folds more common corneal allograft rejection (9 eyes) compared to 93 nonvascularized eyes, in which only 13 cases were rejected ($P<0.0001$). Recovery following rejection in eyes without recipient bed vascularization was 1.8-fold more common (9 cases) than eyes with similar problems (5 cases), although the difference was not statistically significant. The presence of anterior synechia was also associated with a 3.9-folds increase in corneal allograft rejection (4 out of 5 cases) compared to its absence (21 out of 100 cases) ($P<0.05$). Recovery following rejection was 4.3-folds higher in eyes without anterior synechia. How-

Table 1: Frequency of the underlying diseases in corneal transplant patients

		Indication					Total
		KCN n (%)	OP n (%)	MD n (%)	PBK n (%)	FD n (%)	
gender	Female	24 (51)	12 (25.5)	2 (4.3)	7 (14.9)	2(4.3)	47
	Male	40 (69)	13 (22.4)	1 (1.7)	4 (6.9)	0	58
Total		64	25	3	11	2	105

KCN = Keratoconus, OP= Opacity, MD = Macular Dystrophy, PBK = Pseudophakic Bullous Keratopathy, FD = Fuchs' Endothelial Dystrophy

ever, the difference was not statistically significant.

In the present study no difference was found between TNF-α gene polymorphism and the chance of allograft rejection. However, no apparent correlation was found between the chance of recovery of rejected allograft and different inherited TNF-α alleles or genotypes (genotype A_1A_1=GG and genotype A_1A_2= AG) Table 2 shows the number and the types of rejections with various TNF-α polymorphism.

Discussion

The major risk in allogenic organ transplantation is transplant rejection by T cell-mediated and humoral immune reaction.[14] It has been postulated that cytokines, including TNF-α, are involved in the entire process of transplant rejection.[15] In addition to the classical HLA system, cytokine gene polymorphisms are among the most important factors in transplant outcome.[7] Previous studies have suggested some associations between TNF-α gene polymorphism and the outcome of hematopoietic stem cell,[17] heart,[8,21] liver[19,20], skin,[5] and kidney[7,21] transplantations.

Most of those studies were done on organs with blood supplies and hence were exposed to the immune system. Considering the avascularity of cornea, the aim of this study was to clarify whether there was any correlation between human TNF-α-308G/A polymorphism with corneal allograft rejection.

In our study, TNF-α gene polymorphism was not found to be associated with either corneal allograft rejection, or recovery following rejection in patients who underwent penetrating keratoplasty for diverse etiologies. This was in contrast to forgoing organ transplant.

Table 2: Frequency of rejection and its subtypes in various TNF-α genotypes

TNF-α	Rejection [n (%)]			Rejection Type n (%)	
	Yes*		No		
	Recovered	failed		END	EPI
GG	11(13.4)	7(8.6)	64(78)	17(94)	1(6)
AG	3(18.7)	1(6.3)	12(75)	4(100)	0

*Genotype distribution is also not different between cases recovered from Rejection or failed after rejection. END= Endothelial, EPI= Epithelial. For more detail see text.

Corneal transplants have the best prognosis and the lowest rate of rejection compared with other organ.[23] This accounts for the presence of blood-aqueous barrier, recipient bed avascularity, lack of classic antigen-presenting cells (APCs) in the avascular corneal tissue and the so called "anterior chamber-associated immune deviation" (ACAID).[23,24]

TNF–a exerts a crucial effect on ACAID,[25] its introduction into the anterior chamber of the eye disrupts the its immune privilege and suppresses ACAID.[26] It has been claimed that recipient's bed vascularization of at least three quadrants is amongst the predisposing factors of corneal allograft rejection.[27] It has also been reported that the risk of corneal graft rejection, in donor and / or recipient ABO incompatible penetrating keratoplasties, is higher in vascularized than in non-vascularized corneas.[28] In fact disruption of the eye's immune privilege can occur with interruption of the blood-ocular barrier, corneal neovascularization and the access of classic APCs to the center of the graft.[24]

The majority of our patients were cases of uncomplicated penetrating keratoplasty, without high risk factors such as recipient bed vascularization, active ocular inflammation, disrupted blood-aqueous barrier, and anterior synechia. The present study showed that corneal allograft rejection was increased by 5.4-folds in eyes with recipient bed vascularization and by 3.9-folds in anterior synechia. However, the analysis of our data did not show any statistically significant correlation between TNF-α gene polymorphism and either occurrence of rejection and/or its subsequent recovery. This was probably due to small number of eyes having these two risk factors in our series.

It seems reasonable to assume that the corneal donor tissue is more readily exposed to the immune system in eyes with recipient bed vascularization, anterior synechia, vascular congestion, and ocular inflammatory diseases.[28] All of these situations erode corneal privilege and increase the chance of exposure of the transplanted tissue to TNF-α secreting immune cells which increase the chance of corneal allograft rejection. The present study

did not contain sufficient number of cases with simultaneous rejection accompanied by vascularization and/or anterior synechia. This, however, would provide clinically reliable data in regard to the extent of the aforementioned two complications in terms of rejection and/or recovery following the rejection.

A full understanding of gene function requires more detailed knowledge about the interactions among different cytokines in corneal transplants more readily exposed to the immune system. Further studies are needed to clarify the probable roles of TNF-α and other cytokines in corneal allograft rejection in corneal transplants with bed vascularization, or anterior synechia, along with congested and inflammed eyes such as tectonic corneal grafts. In general, it seems unlikely that preoperative tests for TNF-α-308G/A gene polymorphism in nonvascularized cases of corneal transplantation, would help assess the risk of developing corneal allograft rejection.

Acknowledgement

This work has been financially supported by organ transplantation research center grant number 81-7, Shiraz University of Medical Sciences.

References

1 Vartdal F, Thorsby E. [Immunologic reactions in transplantation]. *Tidsskr Nor Laegeforen* 1999; **119**: 3167-70.

2 Harris PE, Cortesini R, Suciu-Foca N. Indirect allorecognition in solid organ transplantation. *Rev Immunogenet* 1999; **1**: 297-308.

3 Arancibia-Carcamo CV, Osawa H, Arnett HA, et al. A CIITA-independent pathway that promotes expression of endogenous rather than exogenous peptides in immune-privileged sites. *Eur J Immunol* 2004; **34**: 471-80.

4 Visentainer JE, Lieber SR, Persoli LB, et al. Serum cytokine levels and acute graft-versus-host disease after HLA-identical hematopoietic stem cell transplantation. *Exp Hematol* 2003; **31**: 1044-50.

5 Jiang DY, Chen B. Clinical study on the immunoregulation effects of cytokines on the acellular xenogenic dermal matrix]. *Zhonghua Shao Shang Za Zhi* 2003; **19**: 351-4.

6 Shaw BE, Maldonado H, Madrigal JA, et al. Polymorphisms in the TNFA gene promoter region show evidence of strong linkage disequilibrium with HLA and are associated with delayed neutrophil engraftment in unrelated donor hematopoietic stem cell transplantation. *Tissue Antigens* 2004; **63**: 401-11.

7 Gu XW, Zhao M, Li LY, et al. Cytokine gene polymorphism in sensitized kidney transplant recipients and its association with acute rejection episodes. *Di Yi Jun Yi Da Xue Xue Bao* 2003; **23**: 1211-3.

8 Abdallah AN, Cucchi-Mouillot P, Biteau N, et al. Analysis of the polymorphism of the tumour necrosis factor (TNF) gene and promoter and of circulating TNF-alpha levels in heart-transplant patients suffering or not suffering from severe rejection. *Eur J Immunogenet* 1999; **26**: 249-55.

9 Hahn AB, Kasten-Jolly JC, Constantino DM, et al. TNF-alpha, IL-6, IFN-gamma, and IL-10 gene expression polymorphisms and the IL-4 receptor alpha-chain variant Q576R: effects on renal allograft outcome. *Transplantation* 2001; **72**: 660-5.

10 Williams KA, Standfield SD, Mills RA, et al. A new model of orthotopic penetrating corneal transplantation in the sheep: graft survival, phenotypes of graft-infiltrating cells and local cytokine production. *Aust N Z J Ophthalmol* 1999; **27**: 127-35.

11 Sano Y, Osawa H, Sotozono C, Kinoshita S. Cytokine expression during orthotopic corneal allograft rejection in mice. *Invest Ophthalmol Vis Sci* 1998; **39**: 1953-7.

12 Bouma GB, Crusius JBA, Oudkerk Pool M. Secretion of tumor necrosis factor alpha and lymphotoxin alpha in relation to polymorphisms in TNF genes and HLA-DR alleles. Relevance for inflammatory bowel disease. *Scand J Immunol* 1996; **43**: 456-63

13 Wilson AG, de Vries N, Pociot F, et al: An allelic polymorphism within the human tumor necrosis factor α promoter region is strongly associated with HLA A1, B8, and DR3 alleles. *J Exp Med* 1993; **177**: 557-60.

14 Michaels PJ, Fishbein MC, Colvin RB. Humoral rejection of human organ transplants. *Springer Semin Immunopathol* 2003; **25**: 119-40.

15 Ferrara JL, Cooke KR, Teshima T. The pathophysiology of acute graft-versus-host disease. *Int J Hematol* 2003; **78**: 181-7.

16 Mullighan C, Heatley S, Doherty K, et al. Non-HLA immunogenetic polymorphisms and the risk of complications after allogeneic hemopoietic stem-cell transplantation. *Transplantation* 2004; **77**: 587-96.

17 Nagler A, Or R, Nisman B, et al. Elevated inflammatory cytokine levels in bone marrow graft rejection. *Transplantation* 1995: **60**: 943-8.

18 Turner D, Grant SC, Yonan N, et al. Cytokine gene polymorphism and heart transplant re-

jection. *Transplantation* 1997; **64**: 776-9.
19 Warle MC, Farhan A, Metselaar HJ, et al. Cytokine gene polymorphisms and acute human liver graft rejection. *Liver Transpl* 2002; **8**: 603-11.
20 Mas VR, Fisher RA, Maluf DG, et al. Polymorphisms in cytokines and growth factor genes and their association with acute rejection and recurrence of hepatitis C virus disease in liver transplantation. *Clin Genet* 2004; **65**: 191-201.
21 Sankaran D, Asderakis A, Ashraf S, et al. Cytokine gene polymorphisms predict acute graft rejection following renal transplantation. *Kidney Int* 1999; **56**: 281-8.
22 Niederkorn JY. The immune privilege of corneal allografts. *Transplantation* 1999; **67**:1503-8.
23 Rocha G, Deschenes J, Rowsey JJ. The immunology of corneal graft rejection. *Crit Rev Immunol* 1998; **18**: 305-25.
24 Ferguson TA, Herndon JM, Dube P. The mmune response and the eye: a role for TNF alpha in anterior chamber-associated immune deviation. *Invest Ophthalmol Vis Sci* 1994; **35**: 2643-51.
25 Okamoto S, Streilein JW. Role of inflammatory cytokines in induction of anterior chamber-associated immune deviation. *Ocul Immunol Inflamm* 1998; **6**: 1-11.
26 Hill JC: The relative importance of risk factors used to define high-risk keratoplasty. *Ger J Ophthalmol* 1996; **5**: 36-41.
27 Inoue K, Tsuru T. ABO antigen blood-group compatibility and allograft rejection in corneal transplantation. *Acta Ophthalmol Scand* 1999; **77**: 495-9.
28 Coster DJ, Williams KA. Management of high-risk corneal grafts. *Eye* 2003; **17**: 996-1000.