

The Role of Cytokines and Cytokine Gene Polymorphism in T-cell Activation and Allograft Rejection

S Bunnapradist,*_{MD}, S C Jordan,**_{MD}

Abstract

Introduction: Cytokines are largely responsible for the regulation of immunoregulatory events and therefore play an important role in transplantation immunology. Recent advances in genetics have made it possible to study the role of cytokine gene polymorphisms in allograft rejection. **Methods:** This review describes studies that have analysed genetic variants in relation to renal disease and transplantation. **Results and Conclusion:** Polymorphism of cytokine gene promoters can significantly influence the level of cytokine production, which in turn affects the amount of immunosuppression needed. It is possible that in future, genotyping of transplant recipients with respect to their cytokine gene polymorphisms may help determine their risk of allograft rejection, and thus, provide a guide to individualised immunosuppression in the post-transplant period.

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Introduction

Cytokines are peptide molecules that are responsible for intercellular signalling during immune activation events. Cytokines are responsible, in large part, for the regulation of all local and systemic immune inflammatory and immunoregulatory events. The existence of peptides that regulate immune events was known for more than 40 years, but only recently has the full extent of the cytokine networks been appreciated. Recent advances in gene cloning and expression, as well as the development of cytokine gene knockout mice, have allowed investigators to develop a clearer picture of the functions of specific cytokines. This review looks at how the new discoveries of cytokine gene polymorphism enhance our understanding of how the overexpression of specific cytokine genes leads to allograft rejection.

Cytokine Gene Polymorphism: Susceptibility Factor for Allograft Rejection?

a) Gene Polymorphism

Polymorphism of the blood group and human leukocyte antigen (HLA) genes result in allogenicity and hence, allogenic immune response after allotransplantation. The

variant in promoter and regulatory genes often result in different rates of RNA transcription and protein synthesis. This results in marked variation in protein production among individuals. With the advancement in polymerase chain reaction (PCR) technology, investigators are able to identify these polymorphisms and explain the difference in protein synthesis rate or protein structure which in turn leads to phenotype diversity such as blood grouping and HLA phenotypes in population.¹⁻⁵

b) Cytokine Gene Polymorphisms

Polymorphism in the regulatory regions of specific cytokine genes is associated with low level or excessive secretion of cytokines in response to a particular stimuli.¹⁻⁵ Recently, specific allelic types were shown to be associated with both acute rejection (AR) and chronic rejection (CR). Cytokine genes of interest included TNF- α , IL-10 and TGF- β where allelic variations appeared to predict AR and CR. These will be discussed in more detail below.

Tumor Necrosis Factor-alpha (TNF- α)

TNF- α and T-cell function: TNF- α is a cytokine produced primarily by lipopolysaccharide (LPS)-stimulated macrophages which mediates necrosis of transplanted

* Medical Director
Kidney-Pancreas Transplant Program

** Professor
Departments of Medicine, Pediatrics and Transplant Immunology
Cedars-Sinai Medical Center, Los Angeles, California, USA

Address for Reprints: Dr Suphamai Bunnapradist, Medical Director, Kidney-Pancreas Transplant Program, Cedars-Sinai Medical Center, 8635 W. 3rd St. Suite 590 W, Los Angeles, CA 90048, USA.

tumors.⁶ TNF- α also has immunoregulatory functions in human and experimental animal systems.⁶ Bonavida et al⁶ showed that the addition of TNF- α (1 to 10 μ g/mL) resulted in a dose-dependent inhibition of pokeweed mitogen (PWM)-induced antibody production by peripheral blood mononuclear cells (PBMCs). Satoh et al⁷ showed that recombinant TNF- α is a potent inhibitor of the development of autoimmune diabetes mellitus in the non-obese diabetic mice (NOD strain). These investigators felt that the ability to produce TNF in various mouse strains correlated with their propensity to develop autoimmune diseases. NOD mice were poor *in vitro* producers of TNF- α ; thus, it was felt that the lack of TNF production allowed autoimmune antibody production to proceed and autoimmunity to develop. Exogenous administration of TNF- α inhibited the dysfunctional T-cell activation and prevented the development of autoimmunity by induction of suppressor T cells.⁸ Other investigators have shown that exogenous administration of TNF- α to (NZB x NZW) F1 hybrid mice suppressed the progression of murine systemic lupus erythematosus (SLE). This was again postulated to be due to the induction of suppressor cell networks by TNF- α .⁹ It is unclear whether these observations will have clinical relevance.

The proinflammatory cytokine TNF- α is released at the site of inflammation by macrophages, causing endothelial cell activation, up-regulating major histocompatibility complex (MHC) and adhesion molecule expression. It also causes vasodilatation and increases vascular permeability. By these mechanisms, it maintains the inflammatory response against the allograft by facilitating the recruitment and activation of leukocytes. Plasma levels of TNF- α are known to be associated with allograft rejection. Transcription of TNF- α is also under control of calcineurin and nuclear factor of activated T cells (NF-AT). Thus, cyclosporin and tacrolimus can partially inhibit TNF- α . Different susceptibility may render different individuals more or less sensitive to the immunosuppressive effects of these agents.^{1,3,4}

Guanine (G) to adenosine (A) substitution at position -308 on the TNF- α gene promoter gives alleles TNF1 and TNF2 respectively. Expression of the TNF2 allele is associated with a 6- to 7-fold increase in transcription of TNF- α gene *in vitro*. Several studies in heart, kidney and lungs transplants revealed correlation between serum TNF- α levels and transplant rejection, where high levels are associated with more frequent rejection. One study did not show the direct effect of high TNF- α cytokine production and more frequent episodes of rejection. However, excessive TNF- α production was shown to be associated with a higher incidence of steroid-resistant rejection in kidney transplant recipients.^{1,3,4} Data from our lab have shown that anti-TNF- α antibodies can favourably modify parameters of severe AR in a highly histoincompatible model of lung

allograft rejection.¹⁰

Transforming Growth Factor-Beta (TGF- β)

TGF- β is a multifunctional peptide with pleiotropic effects on a number of cell types. Receptors for TGF- β are present on most cell types and T cells are known to produce TGF- β . TGF- β is a known modulator of immune cell growth and differentiation. TGF- β is known to have regulatory effects on T cells, B cells, NK cells, LAK cells and monocytes/macrophages.^{11,12} TGF- β is a potent inhibitor of T- and B-cell activation, and when antigen is presented in the context of high concentrations of TGF- β , a deviation to a Th2 type (tolerogenic) immune response is seen.¹³ In conjunction with IL-2 and IL-5, TGF- β acts as a stimulus for IgA production by activated lymphocytes.¹⁴ TGF- β 1 knockout mice were born without obvious deformities, but died at 3 to 4 weeks of age due to massive mononuclear infiltration of all organs.¹⁵ TGF- β 's potent immunoregulatory capabilities suggest that it may find a use in regulation of dysfunctional immune responses as are seen in autoimmune diseases of the kidney and other organs. In addition, TGF- β may find a role in the prevention and possibly treatment of acute allograft rejection, since recent experiments in rat heart transplant models have shown that allografts transfected with the human TGF- β gene showed absence of AR.¹⁶ Despite this, these allografts develop scarring and what appears to be a form of accelerated chronic rejection. It is also of interest that the potent immunosuppressive drug cyclosporine A is known to induce TGF- β gene expression in tissues while suppressing the expression of IL-2.¹⁷ The calcineurin inhibitor, tacrolimus (FK-506), appears to be a less potent inducer of TGF- β .⁵ Unfortunately, TGF- β is now known to play a very important role in promoting fibrosis of the kidney and lung in chronic transplant rejection and in autoimmune diseases.¹⁸ This finding would obviously limit the use of TGF- β transfection in human transplantation.

There are multiple variations in genes encoding TGF- β production. Two polymorphisms in the region of the gene encoding the lead sequence of the protein located at +600 and +650 after the transcription start site result in a change in the amino acid sequences at codon 10 and 25. Codon 10 is part of an alpha helical structure which directs transport of the TGF- β protein through the cell. The substitution of proline for leucine results in a modification of the charge and distortion of the alpha helix. Also, the leader sequence of TGF- β gene must be cleaved by an enzyme in order for the protein to be released from the cell. Substitution of arginine for proline at codon 25 alters the charge and structure of the enzyme cleavage site. These differences result in high or low cytokine producer phenotypes. The highest TGF- β production is associated with the presence of leucine at codon 10 and arginine at codon 25. High TGF- β production

in vitro is associated with the codon 25 arginine variant, TGF- β production *in vivo* is also affected by the codon 10 leucine sequence.⁵

In one study of kidney transplant recipients, there was a strong association between high TGF- β producer and acute rejection. Many of these individuals were also high producers of TNF- α . If kidney transplant recipients are high producer of both genotypes, then the relative risk of suffering two or more acute rejection episodes increases more than 4-fold ($P < 0.007$), with a particular elevation in the risk of corticosteroid-resistant rejection ($P < 0.002$). If the graft is obtained from a donor who is also from the high TGF- β producer genotype, then the risk of acute rejection and steroid-resistant rejection increases even further.⁵ This seemingly ironic association between high TGF- β production and AR could be explained by a recently discovered effect of TGF- β on activated cytotoxic T cells. TGF- β induces CD103, an E-cadherin and epithelial cell adhesion molecule. This effect may explain why cytotoxic T cells would redirect to renal tubular epithelial cells and induce tubulocytolysis.^{5,19}

Since TGF- β is involved in the development of two cardinal features of chronic allograft dysfunction, fibrosis and arteriosclerosis, multiple investigations have been done to link the polymorphisms and rate of chronic allograft loss. The most compelling evidence came from lung transplant recipients. The higher levels of TGF- β were associated with the presence of lung fibrosis by 6 to 18 months. The high levels strongly correlated with the high producer genotype. Patients' deaths were also associated with TGF- β genotypes. In this study, the highest TGF- β producers all died within 7 years while 65% of the low producer genotypes still survived at 7 years. In heart transplant recipients, the presence of a high producer genotype, whether derived from donor or recipient tissues correlated well with development of transplant coronary artery disease (TCAD). One study demonstrated that 90% of patients with the low TGF- β producer genotype were free of TCAD at 7 years, compared with only 39% of the high TGF- β group. Transplantation from donors that were high TGF- β producers resulted in 40% graft survival compared to 75% survival for grafts from donors that were low TGF- β producers at 7 years.⁵

Interleukin-10 (IL-10)

IL-10 was originally described as cytokine synthesis inhibitory factor because it is a Th2 cytokine which inhibits the production of Th1 cytokines.²⁰ Human and mouse IL-10 are highly homologous to the Epstein-Barr virus (EBV) gene BCRF-1 (B-cell replacement factor-1), now designated viral-IL-10 or vIL-10.²¹ In humans, IL-10 is produced by Th0, Th1 and Th2 CD4⁺ T cells. IL-10 is also produced by

B cells and numerous other cell types.

IL-10 is a pleiotropic cytokine that exerts both immunosuppressive and immunostimulatory effects on various cell types. The predominant effect of IL-10 production is suppression of Th1 (γ -IFN and IL-2) cytokine production. In rat models of lung transplantation, we have shown that overexpression of IL-10 is seen in a model that develops spontaneous tolerance of the allograft. This is also associated with suppressed expression of Th1 cytokines. Conversely, in rat lung allograft models where rejection is seen, Th1 cytokines predominate and IL-10 expression is reduced.²²

IL-10 also has potent effects on macrophages including down regulation of MHC class II expression which would reduce the ability of macrophages to interact with T cells and NK cells.²³ IL-10 also inhibits a number of potent cytokines involved in inflammatory responses including prostaglandin E₂, TNF- α , IL-1, IL-6, IL-8.^{24,25} In addition, IL-10 induces the release of soluble TNF receptors, and inhibits the induction of ICAM and B7 gene expression.^{24,25} IL-10 can also induce the expression of IL-1 receptor antagonist (IL-1RA) which has potent anti-inflammatory properties. IL-10 induces the Fc γ R1 which is important in mediating ADCC.²⁶ The potent anti-inflammatory actions of IL-10 may account for the EBV capturing this mammalian gene in order to suppress any Th1-mediated inflammation that would jeopardise EBV survival in the host.²² We have also seen vIL-10 expression in fine needle aspiration biopsies (FNAB) of renal allografts from patients with posttransplant lymphoproliferative disorder (PTLD) associated with EBV infection. After cessation of immunosuppressive medication, vIL-10 expression continued and was associated with good allograft survival without medications. This suggests that overexpression of vIL-10 may suppress Th1 responses to the allograft as well.

IL-10 has potent effects on B cells. IL-10 induces IgA synthesis in CD40/CD40L-activated B cells.²⁷ IL-10 also has a number of other activating effects on tonsillar and splenic B cells.^{25,26} IL-10 knockout mice appear to have normal B-cell and T-cell development and function, but suffer from chronic bowel inflammation due to uncontrolled immune reactivity to bowel antigens.²⁸

Studies investigating IL-10 production reflect the different properties of IL-10. An increase in intragraft IL-10 expression has been associated with long-term survival in rodent models. However, high numbers of intragraft IL-10 secreting T cells are associated with accelerated vascular rejection in patients with high antibody sensitisation to HLA class II.¹⁻⁴

Three single base substitutions have been described in the IL-10 promoter at positions -1082, -819 and -592. The presence of arginine at position -1082 (IL-10 1*A) is associated with low IL-10 production after stimulation of

T cells *in vitro*.

Assuming that IL-10 has only an inhibitory effect on the immune processes involved in rejection, patients who have high IL-10 and low TNF- α producing genotypes should have a better prognosis than other patients. However, Turner et al³ showed that the different IL-10 genotypes did not correlate with rejection outcomes. This may reflect the diverse roles of IL-10 described above and, both high and low production may be associated with different harmful routes of allograft rejection. The beneficial effects of over-expression of vIL-10 in animal models and in patients with PTLTD as described above stand in contrast to the data for IL-10 which relate to the inability of vIL-10 to stimulate NK and cytotoxic T-cell activation.

Conclusion

Cytokines are critical to initiation and perpetuation of allograft injury. Most cytokines are proinflammatory and increase antigen expression on the allograft which may have implications for acute and chronic rejection. Other cytokines, such as IL-10 and TGF- β , are immunoregulatory and may promote graft survival, although this view is too simplistic. Polymorphism of cytokine gene promoters can significantly influence the level of cytokine production. Immunosuppressive agents are known to modulate cytokine activity by interfering with or enhancing (i.e. TGF- β induction by CsA) cytokine production, competitive binding at the receptor sites or suppressing cytokine signalling networks. Thus, one can postulate that the overall state of immunosuppression is the net effects of HLA mismatch, systemic and intra-graft cytokine gene expression and effects of immunosuppressive medications. By determining the cytokine polymorphism of donors and recipients prior to transplantation, the transplant physician may be able to categorise the transplant recipients into high, intermediate and low risk for rejection. This would apply to both acute and chronic rejection. Since donor cytokine promoter gene polymorphism may also play a role independent and additive to the recipients' cytokine profile, measurement of donors' cytokine production profile should be a novel method to categorise organs as "marginal" versus good. Further studies are needed in both animals and clinical transplantation to confirm the effects of cytokine gene polymorphism. Early data which linked cytokine gene promoter polymorphism to delayed graft function also needs to be explored. Given that different immunosuppressive agents may regulate specific cytokine networks but not others, genotyping individuals for cytokine gene polymorphism may eventually become part of standard pretransplant work up. This data could be very useful in determining the level of immunosuppression needed by each patient. Overall, this information could help reduce graft loss in those at highest risk for AR/CR as determined by cytokine gene profiling

and conversely allow for reduction of immunosuppressive agents in those at low risk.

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