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The cytokine gene polymorphisms in patients with chronic kidney graft rejection

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Abstract

Chronic allograft rejection remains an important cause of morbidity after kidney transplantation. The aim of the study was to examine the association between IL-2, IL-6 and TNF-α promoter polymorphisms and chronic kidney allograft rejection. The study included 64 patients with long-term stable graft function and 62 with chronic allograft nephropathy. Among patients with chronic allograft nephropathy a statistically significant prevalence of the IL-6 CC genotype associated with low IL-6 expression was observed ($p<0.01$, OR 3.18; 95% CI 1.27–8.15).

There were no statistically significant differences in distribution of IL-2 and TNF-α genotypes between patients with stable graft function and chronic allograft rejection. The results of present study suggest that the genetically determined low IL-6 production may be the risk factor of chronic allograft nephropathy development.

Keywords: Kidney allograft; Chronic rejection; Cytokine; Polymorphism

1. Introduction

Chronic rejection is characterized by progressive functional deterioration of allogenic grafts beginning months or years after transplantation. Although the precise nature of molecular and cellular events that initiate and maintain chronic rejection is speculative, multiple immunological and nonimmunological factors, such as cold ischemia time, reperfusion injury, and lipid abnormalities, may contribute to this process [1]. Alloantigen-induced immune activation, however, plays the most important role in the induction of chronic rejection. Evidence for this notion was provided by studies demonstrating that organs disparate for minor and/or major histocompatibility antigens can undergo chronic rejection whereas syngenic organs remain free of chronic lesions [2]. Some of the so-called "nonimmunological factors" implicated in chronic rejection may also be mediated by immune activation. Consistent with this notion are a series of recent findings demonstrating that CD4+T cells play an essential role in the development of graft vasculopathy induced by ischemia reperfusion injury in syngenic grafts and the blockade of T cell activation prevents this process [3].

Cytokine genotypes have previously been studied in patients undergoing solid organ transplantation, and certain polymorphisms have been implicated in the development of complications such as acute rejection and chronic allograft failure [4], although the role of individual gene variants is controversial. The immune system is regulated by an array of cytokines, which influence cellular activation, differentiation, and function.

IL-2 acts as a growth and differentiation factor for natural killer (NK) cells, some B lymphocytes, and lymphokine-

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activated killer (LAK) cells. In addition, IL-2 activates monocytes to produce cytokines, particularly TNF-α. Stimulation of IL-2 production in T cells is observed upon antigen-induced activation. In a natural setting of antigenic stimulation, IL-2 synthesis is initiated following engagement of the T cell receptor. Activation of NK cells by IL-2 results in increased INFγ production and increased cytotoxic effects [5].

IL-6 is a pleotropic cytokine involved in many different aspects of the response of inflammation. It is produced by a wide range of cells of both hematopoetic and nonhematopoetic origin, including macrophages, lymphocytes, endothelial cells, mesangial cells, and vascular smooth muscle. IL-6 is an integral mediator of acute phase response and modulates both local and systemic immunity [6].

TNF-α, is a powerful inducer of the inflammatory response and is central regulator of innate immunity. Inflammatory responses to TNF-α are mediated both directly and through stimulation of other proinflammatory cytokines. The proinflammatory cytokine TNF-α, which stimulates macrophages function and increases MHC class II antigen expression, has been implicated both in acute rejection and chronic rejection [7]. The activation of endothelial cells and the subsequent expression of the intercellular adhesion molecule-1 (ICAM-1) induced by TNF-α can enhance vascular permeability and therefore augment the infiltration of proinflammatory granulocytes in to the graft [8]. The level of production of these cytokines at the site of the allograft could be important in accelerating rejection [9,10].

The level of cytokine production has been associated with polymorphisms in cytokine gene promoters [11]. Promoter region polymorphisms, it is postulated, may directly or indirectly affect the binding of transcription factors and might consequently increase or decrease the production of mRNA thus regulating cytokine production. It has been shown that a G to A polymorphism at position −308 on the TNF gene promoter, giving alleles TNF1 and TNF2, respectively, is associated with six- to sevenfold increase in transcription of the TNF-α gene in vitro [12]. Recent reports suggest that −330 IL-2 and −174 IL-6 promoter polymorphisms may influence these cytokines expression. The homozygous IL-2 GG and IL-6 GG genotypes are associated with increased cytokines production, homozygous IL-2 TT and IL-6 CC with decreased [11].

2. Material

One hundred twenty six recipients of first cadaveric kidney transplants were selected for the study. Selection criteria included patients with long-term stable graft function and gradual progressive loss of graft function that are not explained by causes such as chronic calcineurin inhibitors nephrotoxicity, acute rejection episodes, recurrence of underlying disease, infections, urinary obstruction, allograft artery stenosis. Patients with graft loss were excluded from the study. The study included 64 patients with long-term stable graft function without episodes of acute graft rejection and history of CMV-infection (35 males, 29 females, aged 21–60 years, mean 41.7 years, duration of allograft 2–11 years, mean 5.8 years, donor age 18–60 years, mean 38.7 years, serum creatinine in range of normal values, absence of proteinuria, absence of abnormalities in biopsy as well as in ultrasound and nuclear scans) and 62 with chronic allograft rejection (34 males, 28 females aged 20–65 years, mean 42.2 years, duration of allograft 2–10 years, mean 5.6 years, donor age 19–62 years, mean 39.5 years). In any of these patients the CMV-infection and acute rejection episodes were diagnosed. Among patients with long-term stable graft function the underlying renal diseases were as follows: glomerular nephropathy (19 subjects), diabetes nephropathy (17 subjects), hypertension nephropathy (10 subjects), polycystic kidney disease (4 subjects), other diseases (7 subjects), not known (7 subjects). Among patients with chronic allograft nephropathy the underlying renal diseases were as follows: glomerular nephropathy (18 subjects), diabetes nephropathy (16 subjects), hypertension nephropathy (11 subjects), other diseases (9 subjects), not known (8 subjects). Chronic allograft nephropathy was diagnosed clinically in patients having a continuously rise in serum creatinine at least 30% above baseline, usually accompanied by new or worsening hypertension and proteinuria (above 500 mg/24 h). Anatomical problems were excluded by ultrasound and nuclear scans. Biopsy criteria included the presence of intestinal fibrosis, tubular atrophy, and particularly the characteristic vascular changes such as hypertrophy of the arterial intima and smooth muscle (intimal thickening) and glomerular sclerosis [13]. All biopsies were reviewed by a renal pathologist, and the Banff 93 working classification criteria were used in the histological classification of the biopsies [14].

Immunosuppressive therapy consisted of cyclosporin A, azathioprine and prednisone.

The study was approved by the local ethics committee and written informed consent was obtained from all subjects.

3. Methods

3.1. Isolation of genomic DNA

DNA was isolated from 300 μL EDTA blood by using a DNA isolation kit (Puregene, Biozym, Hessich Oldendorf, Germany).

The genotyping was done using PCR-RFLP method as previously described [8,9].