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The interleukin-6 $^{-174}$promoter polymorphism is associated with long-term kidney allograft survival

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The interleukin-6 $^{-174}$promoter polymorphism is associated with long-term kidney allograft survival.

Background. Th1-dependent effector mechanisms may be responsible for allograft rejection. Recently, interleukin-6 (IL-6) has been shown to antagonize CD4$^+$ T cells to effector Th2 cells and, in the process, differentiate them into Th1 cells.

Methods. To assess the role of IL-6 in long-term allograft survival, 158 patients after first cadaveric kidney transplantation were analyzed for the biallelic $^{-174}$G→C promoter polymorphism of the IL-6 gene.

Results. Carriers of the $^{-174}$C-allele (genotype GC/CC) had an inferior three-year graft survival (71/104 = 68.3%; P = 0.0059) with a 3.7-fold increased relative risk of graft loss compared to carriers of the $^{-174}$GG-genotype (48/54 = 88.9%). The $^{-174}$GC/CC-genotype retained its negative impact on graft survival when other established prognostic factors and further cytokine polymorphisms ($^{-308}$TNF-α, TGF-β1 codon 10 & 25, $^{-592/-819/-1082}$IL-10 and $^{+874}$IFN-γ) were considered simultaneously.

Conclusions. Since the clinical impact on transplant outcome seems as important as matching for histocompatibility antigens, genotyping of the IL-6 $^{-174}$polymorphism may offer a new method for identifying patients at increased risk of allograft loss.

Interleukin-6 (IL-6) is a pleiotropic cytokine with a central role in host defense. It has diverse functions including stimulation of the hepatic acute phase response and differentiation or activation of macrophages, B- and T-cells. IL-6 is produced by many different cell types and, although initially thought to be a pro-inflammatory cytokine, it has recently been recognized as having additional anti-inflammatory and immunosuppressive properties [1–3]. It has been shown that IL-6 derived from antigen-presenting cells is able to initiate the polarization of naive CD4$^+$ T cells to effector Th2 cells, thereby antagonizing differentiation into Th1 cells [4]. Since Th1-dependent effector mechanisms are held responsible for allograft rejection, IL-6 may be a factor that decisively determines the success of allogenic organ transplantation.

Recently, a biallelic polymorphism within the promoter region of the IL-6 gene at position $^{-174}$G→C was detected, and the C-allele was found to be associated with lower in vitro and in vivo production of IL-6 [5]. As $^{-174}$CC homozygosity was found to be underrepresented in patients with systemic-onset juvenile chronic arthritis, a substantial clinical impact of this polymorphism was hypothesized [5]. In clinical transplantation, the $^{-174}$CC-genotype of the kidney donor was identified as a major risk factor for the occurrence of acute rejection episodes [6]. Even though the relevance of the recipient's genotype for acute rejections is still controversial, the $^{-174}$G-allele has recently been associated with renoprotection [7]. However, no data exist regarding long-term graft survival after allogenic kidney transplantation.

METHODS

Study design

We investigated the impact of the kidney recipients' IL-6 $^{-174}$promoter genotype on the three-year allograft survival and on the occurrence of biopsy-proven acute rejection episodes during the clinical course. The following cytokine promoter polymorphisms were investigated simultaneously, since they already have been linked to the occurrence of acute rejection episodes after kidney transplantation: $^{-308}$tumor necrosis factor-α ($^{-308}$TNF-α), transforming growth factor-β1 (TGF-β1) codon 10 & 25, $^{-592/-819/-1082}$IL-10, and $^{+874}$interferon-γ ($^{+874}$IFN-γ). Genotyping was performed in DNA samples of 158 Caucasian patients who received a first cadaveric kidney transplanta-

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Key words: pleiotropic cytokine, host defense, anti-inflammatory, immunosuppression, genotyping, T helper cells, kidney graft survival.

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Table 1. Genotype and allele frequencies and corresponding graft survival

	Caucasian controls $N = 383$ [5]		Patients $N = 158$		
	Number	%	Number	%	3-year graft survival %
Genotype frequency					
GC	169	44.1	72	45.6	69.4
CC	70	18.3	32	20.2	65.6
GC/CC	239	62.4	104	65.8	68.3
GG	144	37.6	54	34.2	88.9
Allele frequency					
G-174	457	59.7	180	57.0	
C-174	309	40.3	136	43.0	

tion at the Medical University of Lübeck between 1982 and 1998. Only patients with a cyclosporine (CsA)-based immunosuppression (CsA/Neoral™ and methylprednisolone or CsA/Neoral™ and azathioprine and methylprednisolone) and a complete follow-up of at least 36 months were included. Patients who died with functioning grafts within the observation period of 36 months after kidney transplantation were excluded from analysis. We analyzed all promoter polymorphisms using polymerase chain reaction-single strand polymorphism (PCR-SSP; Cytokine Genotyping Tray; One-Lambda Technologies, Canoga Park, CA, USA) on a Perkin-Elmer 9600 Thermocycler (Perkin-Elmer, Norwalk, CT, USA). The PCR conditions were: 96°C for 130 seconds; 63°C for 60 seconds; 9 cycles of 96°C for 10 seconds; 63°C for 60 seconds; 20 cycles of 96°C for 10 seconds; 59°C for 50 seconds; and 72°C for 30 seconds.

Statistics

Allograft survival was calculated according to the method of Kaplan and Meier. The comparison of the survival curves was analyzed with the long-rank test. Multifactorial Cox regression analysis was performed to investigate the impact of the other prognostic factors on the observed association of the $^{-174}$genotype with graft survival. Since several polymorphisms were tested in parallel, a Bonferroni correction was applied to exclude that our findings resulted by chance.

RESULTS

Both IL-6 $^{-174}$alleles were common in our Caucasian kidney graft recipients and the genotype/allele frequencies were comparable to recently published healthy controls ($P = 0.73$, global chi-squared test; Table 1) [5]. While the overall three-year graft survival rate was 75.3% (119/158) in our cohort, Kaplan-Meier analysis revealed inferior graft survival for the $^{-174}$GC/CC (71/104, 68.3%) compared to the $^{-174}$GG-genotype (48/54, 88.9%; $P = 0.0059$, log rank test; Fig. 1). A multifactorial Cox regression analysis established that adjustment for other prognostic factors such as HLA-matching, sex and age of the donor and recipient, year of transplantation, presence of preformed lymphocytotoxic antibodies, and the occurrence of acute rejection episodes during the early post-transplant period had no significant influence on this result. When all factors were considered simultaneously, the CC/CG-genotype retained its negative impact on graft survival ($P = 0.014$). Carriers of the $^{-174}$C-allele were identified as having a 3.7-fold increased relative risk for graft loss within three years (95% confidence interval 1.3 to 10.5), resulting in a 20.6% difference in three-year graft survival compared with $^{-174}$C-allele negative patients (genotype GG). Since further cytokine polymorphisms were tested simultaneously, a Bonferroni correction was applied to exclude that the observed association resulted by chance ($P = 0.047$). Except for the IL-6 $^{-174}$polymorphism, none of the other cytokine polymorphisms tested were found to impact long-term graft survival significantly. Furthermore, acute rejection episodes during the clinical course of the patients were not associated with the investigated cytokine polymorphisms.

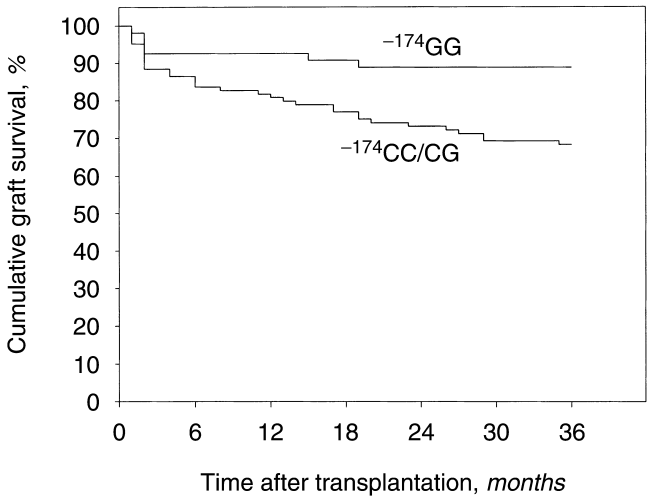

Fig. 1. Kaplan-Meier analysis of kidney allograft survival rates according to the interleukin-6 (IL-6) promoter region polymorphism $^{-174}$genotype (GG versus CC/GC). $P = 0.0059$ by the log rank test.

DISCUSSION

This study reports, to our knowledge for the first time, a significant association of the IL-6 $^{-174}$promoter polymorphism with long-term kidney allograft survival. Its clinical impact may be as important as matching for histocompatibility antigens, since the difference in graft sur-

Table 2. Cox multifactorial regression analysis

Variable	Coefficient	95% CI
IL-6 $^{-}$174 genotype	3.7	1.3–10.5
HLA-matching	4.2	1.4–12.3
Sex of donor/recipient	1.1	0.4–2.8
Age of donor	1.2	0.5–2.8
Age of recipient	1.0	0.4–2.5
Years post-transplantation	2.7	1.2–6.1
Preformed antibodies	4.9	1.7–14.0
Acute rejections	1.1	0.5–2.6

vival between $^{-174}$C-allele negative and positive individuals is almost identical to the 18% difference in five-year graft survival of first cadaver kidney transplants with 0 and 6 HLA-mismatches [8]. Due to the high frequency of the $^{-174}$C-allele in our Caucasian population, the introduction of genotyping into clinical routine may offer a simple method for identifying a significant proportion of patients at risk of graft loss.

Several published studies already have addressed the potential impact of cytokine genotypes on solid organ transplantation [7, 9–13]. However, these investigations revealed variable results that may be attributed to the different study designs including: (*a*) patient group (ethnic origin), (*b*) transplanted organ (cadaveric kidney vs, heart, lung or liver), (*c*) immunosuppressive protocol (CsA based versus tacrolimus versus additional thymoglobulin), (*d*) definition of rejection (biopsy-proven), and (*e*) observation period (clinical course vs. several months). Thus, a comparison of our findings with the results from different centers is difficult [10].

The impact of cytokine polymorphisms on long-term graft survival in patients under CsA-based triple therapy has not been subjected to large clinical trials. Previous investigations concentrated on patient cohorts treated predominantly with CsA monotherapy [13]. These studies failed to detect an influence of the $^{-1082}$IL-10, $^{-308}$TNF-α and IFN-γ exon 1 CA repeat polymorphisms on kidney allograft survival. Likewise, there was no significant association of the TGF-β1 genotype with long-term kidney graft survival [9], even though it appears to be a major factor in lung transplantation [14] and has recently been proposed to be relevant for kidney transplantation [15].

As multiple and methylprednisolone-resistant acute rejections are closely linked to long-term graft outcome, a simultaneous impact of the IL-6 $^{-174}$genotype on acute rejections might have been expected. However, our results confirm the findings of Marshall et al, who failed to detect an association with various cytokine polymorphisms including IL-6 and acute rejection episodes within 30 days (CsA-based triple therapy) [10]. In contrast, another recent investigation described the IL-6 $^{-174}$G-allele as being protective against acute rejection when a period of several months after transplantation was analyzed [7]. In accordance with these observations, our data indicate that the impact of the kidney recipients' IL-6 $^{-174}$genotype on allograft outcome may be detectable only when the long-term course after transplantation is evaluated. One possible explanation could be that the higher dosage of immunosuppressive drugs, especially corticosteroids, might disguise the impact of the IL-6 $^{-174}$genotype in the early phase after transplantation. Moreover, analysis of 11 cytokine polymorphisms identified the IL-6 $^{-174}$CC-genotype of the kidney donor as being strongly predictive of acute rejection within 30 days [10], suggesting the donor genotype to prevail and to be much more relevant at this stage. However, replica retrospective studies of different cohorts and prospective clinical studies including both the recipient and the donor genotype will be required to confirm that observation. To explain the potential pathogenetic link between the $^{-174}$genotype and allograft outcome, we suggest that immunologic mechanisms be considered first, since the $^{-174}$polymorphism has been shown to be functionally relevant for the IL-6 gene expression [5]. Functional studies will have to focus on the three hypotheses: (*a*) the IL-6 $^{-174}$polymorphism itself is of functional relevance for graft survival; (*b*) it is in linkage disequilibrium with other flanking, functionally relevant polymorphisms [16]; and (*c*) it has an impact on the pharmacodynamic efficacy of the immunosuppressive drugs used in our cohort. The investigation of these hypotheses may provide new insights into the role of IL-6 in the allogenic immune response and tolerance induction.

In conclusion, we report a significant association of the IL-6 $^{-174}$polymorphism with long-term kidney allograft survival. Our results imply a protective effect of the $^{-174}$G-allele versus the $^{-174}$C-allele in the multifactorial pathogenesis of allograft rejection.

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