Cytokine gene polymorphisms predict acute graft rejection following renal transplantation

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Cytokine gene polymorphisms predict acute graft rejection following renal transplantation.

Background. The proinflammatory cytokine tumor necrosis factor-α (TNF-α) has been implicated in the pathogenesis of acute rejection, while animal models suggest a role for interleukin-10 (IL-10) in promoting graft survival. It has also been shown that polymorphisms in the TNFA gene promoter (position -308) and in the IL-10 gene promoter (position -1082) correlate with differential production of these cytokines in vitro. The aim of this study was to determine whether TNF-α and IL-10 gene polymorphisms influence the incidence and severity of acute rejection in the first six months following renal transplantation.

Methods. The cytokine genotypes of 115 consecutive first cadaveric kidney allograft recipients and their donors were screened. The rejection episodes (REs) were defined clinically and confirmed histologically where possible and further classified according to severity (RS), namely steroid-resistant or responsive REs. The genotypes were then correlated with the REs and RS.

Results. The recipient TNF-α high producer genotype and IL-10 high producer genotype were significantly associated with multiple REs (≥2) in human leukocyte antigen (HLA)-DR mismatched transplants (P = 0.0047 and P = 0.045, respectively), whereas only the TNF-α high producer genotype was associated with steroid-resistant REs (P = 0.025). When recipient cytokines were analyzed together, the TNF-α high/IL-10 high producer genotype had the worst prognosis, whereas TNF-α low/IL-10 low producer genotype was protective.

Conclusions. We conclude that recipient TNF-α and IL-10 gene polymorphisms are determinants of REs and RS following kidney transplantation. Routine screening of these gene polymorphisms may have a clinical role in identifying patients at risk of multiple REs and severe rejections.

Although the one-year survival rate for cadaveric renal transplants has increased with greater understanding of histocompatibility matching and improved immunosuppression, the failure of allografts still poses a major clinical problem [1]. There is evidence that both the frequency and severity of acute rejection episodes (REs) may be a predisposing factor in reduced renal function and subsequent graft loss [1–3].

Acute rejections occur as a result of allocompatibility between donor and recipient [4], with the human leukocyte antigen (HLA) mismatch being a major contributor. This results in CD4+ T helper (Th) cell activation via their initial interaction with the HLA-DR and -DQ antigens, which are expressed on donor antigen-presenting cells (APC; direct recognition) or with processed donor antigens presented on recipient APCs by the major histocompatibility (MHC) class II antigens (indirect recognition). The effector cells then release an array of cytokines that promote various mechanisms involved in allograft rejection. Cytokines can be categorized into two broad groups [5]. The so-called Th1-type cytokines, including interleukin-2 (IL-2), tumor necrosis factor-α (TNF-α), TNF-β, and interferon-γ (IFN-γ), mediate cellular immune responses and are proinflammatory. Second, cytokines described as the Th2 type, which include IL-4, IL-5, and IL-10, have been shown to inhibit the development and function of Th1 cells, suppress inflammation, and enhance humoral pathways of the immune response.

The proinflammatory cytokine TNF-α, which stimulates macrophage function and increases MHC class II antigen expression, has been implicated in acute rejection [6] 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 into the graft [8]. The expression of ICAM-1 on the vascular endothelium has been associated with acute rejection in kidney transplants [9]. Conversely, IL-10 has the ability to inhibit APC function, TNF-α production...
Furthermore, it has been demonstrated that this polymorphism is associated with a sixfold to sevenfold increase or decrease in the production of mRNA, thus regulating cytokine production. It has been shown that a G to A polymorphism at position −308 on the TNFA gene promoter, giving alleles TNF1 and TNF2, respectively, is associated with a six-fold to sevenfold increase in transcription of the TNF-α gene in vitro [18, 19]. Furthermore, it has been demonstrated that this polymorphism determines TNF-α production in vitro [20]. We have previously shown that a G to A polymorphism (alleles IL-10 1*G and IL-10 1*A, respectively) at position −1082 in the IL-10 gene promoter correlates with lower IL-10 production in stimulated lymphocytes in vitro [17].

Having established the relationship between cytokine production and cytokine gene polymorphism, a simple polymerase chain reaction (PCR)-based screening technique was developed and used to genotype renal transplant recipients and their donors. This was performed to determine the influence of these gene polymorphisms on renal allograft REs and severity (RS) in the first six months following renal transplantation and on chronic transplant nephropathy (CTN) and subsequent graft survival. Polymorphisms of the TNF-α and IL-10 gene promoters were analyzed in 115 consecutive first cadaveric renal patients transplanted between February 1990 and January 1991 at our center and who have a minimum follow-up of five years.

METHODS

Patients and donors

One hundred and fifteen consecutive recipients of first cadaveric renal transplants engrafted at a single unit between February 1990 and January 1991 were selected for the study. Recipients were allocated kidneys as previously described [21]. Selection for transplantation was based on ABO blood group compatibility, a negative complement-dependent cytotoxicity cross-match using all historic positive and current sera, and the best HLA match with no more than three HLA-A, -B and -DR antigen mismatches. All patients with primary function were given cyclosporine A (CsA) monotherapy at an initial dose of 15 mg/kg/day. The dosage was gradually reduced depending on blood levels. Transplant patients with delayed graft function were commenced on a triple therapy regimen of CsA, prednisolone, and azathioprine. Out of the 115 patients considered initially, 15 were subsequently excluded from the study: 4 with allograft vessel thrombosis or other surgical problems resulting in early loss of the graft, 1 because of nonadherence with the drug regimen, 4 because they lost their grafts to causes other than rejection in the first three months, and in 6 because either records were missing or they had been transferred to other centers for follow-up. Matching data for the remaining 100 patients showed that 74 out of 100, 75 out of 100, and 40 out of 100 had mismatches at the HLA-A, -B, and -DR loci, respectively. Only 1 of the 40 HLA-DR-mismatched transplants had two mismatches. Female recipients made up 29% of all recipients. Post-transplant patients were followed up regularly in the transplant clinic, and relevant data were collected. We were able to analyze only 88 corresponding donors because of the availability of donor DNA samples.

Control DNA samples

Frequencies for TNF-α and IL-10 alleles in nontransplant recipients were determined from laboratory staff and cadaveric kidney transplant donors from a different cohort.

Rejection episodes and severity

Rejection episodes were defined by clinical diagnosis (elevated serum creatinine in the absence of other pathology, including infection, urinary tract obstruction, allograft artery stenosis, or CsA toxicity) and were confirmed by a positive biopsy where possible. All biopsies were reviewed by a single renal pathologist (I.S.D.R.), and the Banff 93 working classification criteria were used in the histological classification of the biopsies [22]. A clinically suspected episode of acute rejection was usually confirmed with a biopsy of the allograft and was treated with three consecutive intravenous daily doses of 1 g methylprednisolone and was further classified according to severity, namely steroid-responsive or steroid-resistant acute rejection. Steroid-resistant rejection or a third episode of rejection was usually treated with anti-thymocyte globulin (ATG). Steroid-responsive acute rejections were rejections with creatinine levels returning to within 10% of prerejection levels after a pulse of steroid. Conversely, REs were considered steroid resistant when creatinine levels remained 10% above that of prerejection levels after a pulse of steroid and/or required ATG/OKT3 treatment. Biopsy confirmation of rejection was obtained in 23 out of 69 (33%) steroid-responsive rejections and 7 out of 9 (78%) of steroid-resistant rejections. Similarly, 10 out of 33 (30%) of steroid responsive rejections and 4 out of 5 (80%) of steroid-resistant rejections were histologically confirmed in patients with HLA-DR
mismatched transplants. The RE and the RS values were then correlated with patients’ cytokine genotypes (high or low producers). The influence of the cytokine genotype on the REs and the RS was assessed for the first six-months post-transplant. To study the effect of cytokine gene polymorphism on RS, only the most severe episode for each individual was used. Patients who died with functioning grafts or underwent transplant nephrectomy because of nonimmunological complications within this period were therefore excluded from these analyses (N = 5).

**Chronic transplant nephropathy**

Chronic transplant nephropathy was defined clinically as a gradual deterioration of allograft function with a mean rise in yearly serum creatinine levels of over 15% (in the absence of infection, urinary tract obstruction, allograft artery stenosis, or CsA toxicity). Nine recipients in this cohort had CTN, of which 6 (66%) were also confirmed with biopsy.

**Long-term graft survival**

The effect of the polymorphisms on five-year graft survival was also assessed. Patients who died with functioning grafts or who lost their grafts because of nonimmunological reasons were excluded from this analysis.

**DNA extraction**

Genomic DNA from whole blood or frozen cells was obtained by phenol extraction and ethanol precipitation following proteinase K (Boehringer Mannheim, Mannheim, Germany) digestion.

**Cytokine promoter region polymorphism analysis**

The TNFA promoter polymorphism at position –308 was analyzed by PCR-restriction fragment length polymorphism (PCR-RFLP) as previously described (abstract; Wilson et al, *Human Mol Genet* 1:353, 1992). Individuals with the rarer allele TNF2 (including both homozygotes and heterozygotes) were considered TNF-α high producers. IL-10 promoter gene polymorphism was analyzed using sequence-specific oligonucleotide probes (SSOP) as previously described by Turner et al [17]. The biotinylated oligonucleotide probes were designed to detect polymorphism in a dot blot technique. Individuals who were IL-10 1*A positive (including both homozygotes and heterozygotes) were classified as IL-10 low producers according to previously defined criteria [17]. The technical aspects of genotyping have been described in detail [23].

**Statistical analysis**

Patients were grouped into the predicted high or low producer phenotypes according to their genotypes as defined previously [16, 17, 23]. Differences in TNF-α and IL-10 high/low production frequencies between patient and control groups were analyzed by the Fisher’s exact test for 2 × 2 tables (Epi Info 6; CDC, Atlanta, GA, USA). Results are presented as relative risk (RR), with 95% confidence intervals (CI). Patients with the high or low cytokine producer genotypes were further divided into two groups in terms of REs: those who had less than 1 or no RE (≤1) and those who had multiple RE (≥2). The influence of HLA-A, -B, and -DR matching and the influence of cytokine genotype on the REs and RS were analyzed using the Fisher’s exact test for 2 × 2 tables unless otherwise stated. Similarly, the effect of cytokine gene polymorphism on CTN and graft survival was analyzed. A two-tailed P value of less than 0.05 was taken to be statistically significant, as well as a P of less than 0.10 as a trend.

**RESULTS**

**Cytokine production genotype frequencies**

In the renal transplant group, 80 out of 100 (80%) of patients had the low IL-10 producer genotypes (IL-10 1*G/IL-10 1*A and IL10 1*A/IL-10 1*A) compared with 75 out of 119 (63%) of the same genotypes in controls (P = 0.08). The TNF-α high producer genotype (TNF2/TNF2, TNF2/TNF1) and the TNF-α low producer genotype (TNF1/TNF1) frequencies were similar in both groups. We also assessed the influence of cytokine gene polymorphisms on original disease but found no significant association, perhaps because of the low numbers in each category.

**Effect of human leukocyte antigen-A, -B, and -DR mismatch on rejection episodes**

Mismatch in both HLA-A, -B, and -DR did not influence REs and RS in the first six-months post-transplant. However, there was a statistically greater proportion of recipients with HLA-DR–mismatched transplants with multiple REs as compared with those with zero mismatches when only early REs up to three-months post-transplantation were considered (12 out of 40 with HLA-DR–mismatched transplants had multiple RE as compared with only 7 out of 60 with matched transplants, P = 0.042, Yates analysis).

**Cytokine gene polymorphisms and rejection episodes**

Recipients and donors were grouped into high or low cytokine producer genotypes, and these data were correlated with REs (Table 1). When all patients were analyzed independently of HLA-DR matching, no association was found between cytokine gene polymorphism and REs. However, the recipient TNF-α high producer genotype (TNF2 positive) showed an association with increased REs only when the HLA-DR–mismatched transplants were analyzed separately. A greater propor-
of the recipients with TNF-α high producer genotype had multiple REs (7 out of 10, 70%) compared with recipients with the TNF-α low producer (TNF2 negative) genotype (5 out of 28, 18%, $P = 0.0047$, RR = 3.92, 95% CI, 1.61 to 9.57). Similarly, a greater proportion of recipients with IL-10 high producer genotype (IL-10 1*A negative) had multiple REs (6 out of 10, 60%) as compared with only 6 out of 28 (21%) recipients with the IL-10 low producer genotype (IL-10 1*A positive; $P = 0.045$, RR = 2.80, 95% CI, 1.17 to 6.69). Furthermore, when the recipients were classified according to the presence or absence of REs, the recipient TNF-α high producer genotype, but not the IL-10 high producer genotype, was associated with an increase in the incidence of REs in HLA-DR±mismatched transplants. Nine out of 10 (90%) of the recipients with the TNF-α high producer genotype (TNF2 positive) had one or more REs compared with 13 out of 28 (46%) of recipients with the TNF-α low producer genotype (TNF2 negative, $P = 0.06$). No associations with multiple REs were found when donor TNF-α and IL-10 genotypes were analyzed (Table 2).

Cytokine gene polymorphism profile and rejection episodes

To assess the combined effect of cytokine polymorphism combinations in relation to REs, we analyzed data for both TNF-α and IL-10 together for individual recipients. Only HLA-DR±mismatched transplants were analyzed because we have shown that only mismatching at this locus increases REs in recipients with TNF-α and IL-10 in this cohort (Table 1). A greater proportion of recipients with the TNF-α high/IL-10 high producer genotype (TNF2 positive/IL-10 1*A negative) had multiple REs (3 out of 3, 100%) as compared with 9 out of 35 (26%) with all other genotypes ($P = 0.026$, RR = 3.89; 95% CI, 2.21 to 6.83; Table 2). Conversely, significantly fewer patients with the TNF-α low/IL-10 low producer genotype (TNF2 negative/IL-10 1*A positive) had multiple REs (2 out of 21, 10%) as compared with all other genotypes (10 out of 17, 59%, $P = 0.003$, RR = 0.16, 95% CI, 0.04 to 0.64, Yates analysis). However, no associations were found with the presence or absence of REs and the recipient TNF-α high/IL-10 high producer genotype (TNF2 positive/IL-10 1*A negative).

### Table 1. Association between recipient and donor cytokine genotype and frequency of rejection episodes (RE) in the first six months following renal transplantation

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Predicted phenotype</th>
<th>Number of individuals and RE</th>
<th>All transplants</th>
<th>HLA-DR mismatched transplants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>≤1 RE</td>
<td>≥2 RE</td>
</tr>
<tr>
<td>Recipient TNF-α</td>
<td>TNF2 positive</td>
<td>high</td>
<td>24</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>TNF2 negative</td>
<td>low</td>
<td>50</td>
<td>9</td>
</tr>
<tr>
<td>Recipient IL-10</td>
<td>IL-10 1*A negative</td>
<td>high</td>
<td>11</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>IL-10 1*A positive</td>
<td>low</td>
<td>63</td>
<td>14</td>
</tr>
<tr>
<td>Donor TNF-α</td>
<td>TNF2 positive</td>
<td>high</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>TNF2 negative</td>
<td>low</td>
<td>39</td>
<td>9</td>
</tr>
<tr>
<td>Donor IL-10</td>
<td>IL-10 1*A positive</td>
<td>high</td>
<td>23</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>IL-10 1*A negative</td>
<td>low</td>
<td>40</td>
<td>14</td>
</tr>
</tbody>
</table>

Cytokine gene polymorphism and rejection severity

When the effect of cytokine gene polymorphisms on RS was analyzed, only the recipient TNF-α high producer genotype (TNF2 positive) was associated with increased RS (Table 3). Seven out of 21 (33%) recipients with the TNF-α high producer genotype had steroid-resistant rejections compared with 2 out of 29 (7%) recipients with the TNF-α low producer genotype (TNF2 negative, $P = 0.025$, RR = 4.83, 95% CI, 1.11 to 20.97). Five out of the seven steroid-resistant rejections in the recipients with the TNF-α high producer genotype were biopsy confirmed rejections: one was Banff grade 4 (III), two were grade 4 (IIb), and the remaining two were grade 4 (Ia) and 4 (I) rejections. Although the IL-10 high producer genotype (IL-10 1*A negative) was not significantly correlated to steroid-resistant rejections, there was a positive trend linking the IL-10 high producer genotype to steroid-resistant rejections ($P = 0.065$, RR = 3.20, 95% CI, 1.05 to 9.78). Out of the four steroid-resistant rejections in the IL-10 high producer group, only two had biopsy confirmation, and both were graded 4 (I). No associations with RS were found when donor TNF-α and IL-10 genotypes were analyzed (Table 3).
Table 2. Association between recipient tumor necrosis factor-α (TNF-α) and interleukin-10 (IL-10) genotype profile and rejection episodes (RE) in the first six months following renal transplantation in HLA-DR mismatched transplants

<table>
<thead>
<tr>
<th>Cytokine genotype</th>
<th>Predicted phenotype</th>
<th>Number of patients and cytokine producer genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF2 positive</td>
<td>IL-10 1*A negative</td>
</tr>
<tr>
<td></td>
<td>TNF-α high</td>
<td>IL-10 high</td>
</tr>
<tr>
<td>≤1 RE</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>≥2 RE</td>
<td>3</td>
<td>4</td>
</tr>
</tbody>
</table>

*Significantly more patients with the TNF-α high/IL-10 high producer genotype had multiple RE (3/3, 100%) as compared to 9/35 (26%) with all other genotypes (P = 0.026, RR = 3.89, 95% CI 2.21–6.83)

*Significantly fewer patients with the TNF-α low/IL-10 low producer genotype had multiple RE (2/21, 8%) as compared to 10/17 (59%) with all other genotypes, (P < 0.003, RR = 0.16, 95% CI 0.04–0.064)

Table 3. Association between recipient and donor cytokine genotype and rejection severity (RS) in the first six months following renal transplantation

<table>
<thead>
<tr>
<th>Cytokine genotype</th>
<th>Predicted phenotype</th>
<th>Steroid responsive RE</th>
<th>Steroid resistant RE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recipient TNF-α</td>
<td>TNF2 positive</td>
<td>high</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>TNF2 negative</td>
<td>low</td>
<td>27</td>
<td>2</td>
</tr>
<tr>
<td>Recipient IL-10</td>
<td>IL-10 1*A negative</td>
<td>high</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>IL-10 1*A positive</td>
<td>low</td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td>Donor TNF-α</td>
<td>TNF2 positive</td>
<td>high</td>
<td>13</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>TNF2 negative</td>
<td>low</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>Donor IL-10</td>
<td>IL-10 1*A negative</td>
<td>high</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>IL-10 1*A positive</td>
<td>low</td>
<td>25</td>
<td>6</td>
</tr>
</tbody>
</table>

Table 4. Association between recipient TNF-α and IL-10 genotype profile and RS in the first six months following renal transplantation in HLA-DR mismatched transplants

<table>
<thead>
<tr>
<th>Cytokine genotype</th>
<th>Predicted phenotype</th>
<th>Steroid responsive RE</th>
<th>Steroid resistant RE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TNF2 positive</td>
<td>IL-10 high</td>
<td>1</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>TNF2 positive</td>
<td>IL-10 low</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>TNF2 negative</td>
<td>IL-10 high</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>TNF2 negative</td>
<td>IL-10 low</td>
<td>22</td>
<td></td>
</tr>
</tbody>
</table>

95% CI, 2.25 to 14.67). Only one third of the steroid-resistant rejections in the recipients with the TNF-α high/IL-10 high producer genotype were biopsy proven and were graded as Banff grade 4 (I). Conversely, significantly fewer patients with the TNF-α low/IL-10 low producer genotype (TNF1 negative/IL-10 1*A positive) had steroid-resistant rejections (1 out of 23, 4%) as compared with all other genotypes (8 out of 27, 30%, P = 0.028, RR = 0.15, 95% CI, 0.02 to 1.09).

Effect of cytokine genotype on chronic transplant nephropathy and long-term graft survival

At five-years post-transplant, 9 out of 100 patients had lost their grafts through chronic rejection. There was no correlation between TNF-α and IL-10 genotypes, with CTN and the subsequent survival of graft at five-years post-transplant.

DISCUSSION

Cytokines have been implicated in the regulation of acute rejection. In this study, we investigated the role of recipient and donor cytokine gene polymorphism on REs and RS. These gene polymorphisms have been shown to influence interindividual production of the respective cytokines in vitro. The interindividual differences in cytokine production have the potential to influence the various immune and inflammatory responses...
within the graft. This difference in cytokine production between individuals is expected to be exacerbated in the presence of HLA-A, -B, or -DR mismatching. Although this study could not demonstrate that HLA-A, -B, and -DR matched transplants had significantly fewer REs or less severe rejections in the first six months following transplantation, we observed a trend indicating a protective effect of HLA-DR matching in REs in the first three months following renal transplantation compared with mismatched transplants. This is not unusual and supports other studies with similar observations [24]. The CD4+ Th cells are activated directly via donor alloantigens associated with the HLA molecules expressed on the graft. Therefore, matching at the HLA-DR locus would reduce Th-cell activation and alloantigen recognition. The smaller number of REs might be attributed to the down-regulation of Th-cell effector functions and cytokine release.

The analysis of the high/low cytokine producer genotype frequencies showed that the frequency of the IL-10 low producer genotype in renal recipients was higher when compared with normal controls. Even though this difference was only approaching statistical significance, it may suggest that the IL-10 high producer genotype could have a protective effect against certain of the original diseases progressing to end-stage renal failure. This finding fits in well with the observation that in vitro pretransplant B-cell responses in patients with end-stage renal disease were significantly lower compared with healthy controls [14] because IL-10 is essential for B-cell growth and development.

When recipient TNF-α gene polymorphisms were analyzed individually in all recipients, we did not find any relationship between the genotypes and the incidence of multiple REs (Table 1). This result could be explained by the effect of high-dose CsA immunosuppressive therapy during the early post-transplant period. The action of CsA in inhibiting the production and release of the proinflammatory Th1 cytokines is well documented [25]. The transcription of the TNF-α gene, which requires the translocation of the cytoplasmic nuclear factor of activation of T cells, may be inhibited by CsA [25]. Alternatively, the effect of the TNF-α high producer genotype might have been masked when we analyzed all of the patients together independently of HLA-DR matching status. This explanation is more plausible because 60% of our transplants were matched at the HLA-DR locus. Furthermore, in patients with highly allogeneic HLA-DR–mismatched transplants, the TNF-α high producer genotype was linked with the presence of REs and was strongly associated with an increase in the incidence of multiple REs (Table 1). This may be attributed to the fact that CsA does not totally block cytokine production [26]. From our results, we suggest that CsA monotherapy post-transplant may be insufficient for blocking TNF-α release in patients with the TNF2-positive genotype who also received a HLA-DR–mismatched transplant. Furthermore, the recipient TNF-α high producer genotype was associated with steroid-resistant REs (Table 3).

When the high IL-10 production genotype was analyzed in all the patients, it was not associated with REs. However, in recipients with HLA-DR–mismatched transplants, the IL-10 high producer genotype was associated with an increase in the incidence of multiple REs (Table 1). This association was weaker than that of TNF-α. This correlation was surprising considering the anti-inflammatory functions of IL-10. We had expected that the high IL-10 production would act against the production of the proinflammatory Th1-type cytokines such as TNF-α and hence suppress acute graft rejection. However, recent reports have suggested that IL-10 may enhance antibody responses against the graft [13] and low IL-10 responses can be used to predict a low risk for acute graft rejection [14]. Furthermore, IL-10 is a potent stimulator, inducing differentiation and proliferation of B cells, thus driving the immune response toward the humoral pathway [27].

Previously, our laboratory has documented in heart transplant recipients that a certain combination of TNF-α and IL-10 gene polymorphisms (the TNF-α high/IL-10 low group) was associated with higher levels of rejection [28]. This underlines the complex nature of allograft rejection and emphasizes the importance of studying the role of a cytokine in the context of others. Therefore, to determine the combined effect of TNF-α and IL-10 genotypes on REs and RS in renal transplant patients, we categorized the patients into the four possible genotype combinations and correlated these with the REs (Tables 2 and 4). We found the TNF-α high/IL-10 high producer genotype to give a strong association with the incidence of multiple REs in HLA-DR–mismatched transplants and with steroid-resistant REs. Contrary to previous studies, which indicated a protective role of IL-10 in transplant rejection [12, 29], we found that the IL-10 high producer genotype did not protect against the influence of the TNF-α high producer genotype. A recipient TNF-α high/IL-10 high producer genotype may influence REs by the up-regulation of both the cellular and humoral responses. Why this genotype combination is associated with rejection in kidney but not in heart transplants is uncertain. One reason may be because of an organ to organ variation that may affect the microenvironment in which the cytokines exert their influence. Another reason may be the use of a different immunosuppressive regimen for patients receiving heart transplants (triple therapy of CsA, prednisolone, and azathioprine). We also note that when patients with delayed graft function were analyzed separately, two out of five (40%) with the TNF-α high/IL-10 low producer genotype had multiple REs compared with 0 out of 16 (0%)
with all other genotypes ($P = 0.047$). Interestingly, these patients received a triple therapy immunosuppressive regimen, too.

When biopsy grades were analyzed in association with RS and both TNF-α and IL-10 genotypes, the results were not conclusive because of the small numbers, although the TNF-α high producer genotype seemed to be linked with more severe rejections according to the Banff 93 criteria [22].

In this study, the donor genotype was not associated with REs and RS. This emphasizes the importance of recipient TNF-α and IL-10 production rather than the local production of cytokines by donor organs in the activation of T cells and the initiation of an immune response against the kidney.

Analysis of our data suggests that CTN and long-term graft survival is unaffected by TNF-α and IL-10 gene polymorphisms. This may be due to the small number of patients who had CTN and graft failure. Other cytokines, such as IL-2, IFN-γ, IL-4, and the fibrogenic TGF-β, have been implicated in rejection and should also be analyzed for the presence of genetic polymorphisms so as to assess its overall effect on acute and chronic rejection and subsequent graft loss. It is already known that TGF-β1 and IFN-γ gene polymorphisms are associated with the chronic rejection of lung allografts [30, 31].

In this study, we have demonstrated that recipient TNF-α and IL-10 gene polymorphisms that were previously associated with differential production of these cytokines in vitro can be linked to clinical outcome in HLA-DR–mismatched renal allografts. Seventy-eight percent (74 out of 95) of the transplant recipients in this study had a low level of initial immunosuppression (CsA monotherapy), and this may have allowed the effect of the cytokine gene polymorphism to be seen more clearly. With the advent of powerful immunosuppressive drugs, the incidence of acute REs has decreased in recent years, however, not without adverse effects of the drugs. For this reason, we propose that kidney recipients receiving HLA-DR–mismatched transplants be screened for their TNF-α and IL-10 genotypes to predict transplant outcome in the early phases of post-transplantation. The screening of genes of a larger range of cytokines that are supposedly important and implicated in both acute and chronic rejection may, in the future, help to predict risk of acute kidney graft rejection.

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