CC chemokine receptor 5 and renal-transplant survival


Summary

Background About 1% of white populations are homozygous carriers of an allele of the gene for the CC chemokine receptor 5 (CCR5) with a 32 bp deletion (CCR5Δ32), which leads to an inactive receptor. During acute and chronic transplant rejection, ligands for CCR5 are upregulated, and the graft is infiltrated by CCR5-positive mononuclear cells. We therefore investigated the influence of CCR5Δ32 on renal-transplant survival.

Methods Genomic DNA from peripheral-blood leucocytes of 1227 renal-transplant recipients was screened by PCR for the presence of CCR5Δ32. Demographic and clinical data were extracted from hospital records. Complete follow-up data were available for 576 recipients of first renal transplants. Graft survival was analysed by Fisher’s exact test and Kaplan-Meier plots compared with a log-rank test.

Findings PCR identified 21 patients homozygous for CCR5Δ32 (frequency 1.7%). One patient died with a functioning graft. Only one of the remaining patients lost transplant function during follow-up (median 7.2 years) compared with 78 of the 555 patients with a homozygous wild-type or heterozygous CCR5Δ32 genotype. Graft survival was significantly longer in the homozygous CCR5Δ32 group than in the control group (log-rank \( p=0.033 \); hazard ratio 0.367 [95% CI 0.157–0.859]).

Interpretation Patients homozygous for CCR5Δ32 show longer survival of renal transplants than those with other genotypes, suggesting a pathophysiological role for CCR5 in transplant loss. This receptor may be a useful target for the prevention of transplant loss.

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*Both investigators contributed equally to the work presented in this report.

Introduction

Progress in human molecular genetics offers the possibility of unravelling the complex genetic basis of many common diseases.1 Powerful molecular methods can identify single genes influencing predisposition to particular diseases. These developments have improved understanding of the pathophysiology of many disorders, and more specific therapeutic strategies can therefore be used.

Renal-transplant rejection is characterised by an interaction of donor kidney cells with recipient mononuclear cells. In this process, there is increased renal expression of chemokines such as RANTES and MIP-1α.2,3 By use of a RANTES antagonist, adhesion of monocytes to microvascular endothelium can be blocked, resulting in a substantial decrease in vascular injury and tubular damage in experimental renal transplantation in rats.3 The upregulation of proinflammatory chemokines such as RANTES and MIP-1α is accompanied by infiltration of the graft by mononuclear cells, consisting predominantly of T lymphocytes, monocytes/macrophages, and occasional eosinophils.2,4 During both acute rejection and chronic transplant dysfunction, many of the infiltrating mononuclear cells express the CC chemokine receptor 5 (CCR5), which mediates the effects of the chemokines RANTES, MIP-1α, MIP-1β, and others.5–11 CCR5 is one of a subfamily of G-protein-coupled receptors with seven transmembrane domains. Chemokine receptors are differentially expressed on leucocytes and various other cell types, and in combination with their ligands they have a central role in leucocyte trafficking.12–14 The available data suggest that the interaction of RANTES with its receptors, and especially CCR5, is important in transplant rejection.

A non-functional mutant allele of CCR5 with an internal deletion of 32 bp (CCR5Δ32) is found with high frequency in Europe and North America.15–17 Heterozygosity for this allele is found in 10–15% and homozygosity in about 1% of white populations.18–20 Because CCR5 is the major coreceptor for M-tropic HIV1 strains, individuals homozygous for CCR5Δ32 are highly resistant to HIV infection.15,17,21 CCR5Δ32 homozygotes appear healthy, and apart from their resistance to HIV infection, they show no obvious phenotype. CCR5Δ32 has been associated with a low risk of asthma.22

The observations of CCR5-positive T-cell infiltration and RANTES upregulation in transplant dysfunction led to our hypothesis that RANTES-mediated recruitment of mononuclear cells during rejection is impaired in individuals lacking a functional CCR5. Such individuals might therefore show less inflammatory response and improved graft survival after renal transplantation, a hypothesis explored by this observational pilot study.

Methods

Patients

We screened 1227 patients presenting to transplant clinics or dialysis units at six European centres between January, 1998, and March, 2000. The patients gave informed consent and were screened for the presence of CCR5Δ32. Demographic and clinical data were extracted from hospital records. Complete follow-up data were available for 576 recipients of first renal transplants. Graft survival was analysed by Fisher’s exact test and Kaplan-Meier plots compared with a log-rank test.

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consent for CCR5-genotype analysis. The study protocol was approved by the local ethics committees. CCR5 genotyping was done on genomic DNA isolated from peripheral blood. Demographic data for recipients and donors (cold ischaemia time, HLA mismatches, donor’s age, panel reactive antibodies, recipient’s age and sex, and transplant survival) were extracted from hospital records. During routine visits, blood was collected into edetic acid tubes and stored at −20°C until use. 200 μL volumes of freshly thawed blood samples were used to isolate genomic DNA with a commercial kit (Qiagen Tissue Kit, Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

Genotype analysis

Samples of genomic DNA preparations (1 μL, 100–300 ng DNA) were used as templates to identify the CCR5 genotype of all individuals by PCR. Reaction mixtures consisted of 2·5 μL 10× AmpliTaq buffer, 4·0 μL deoxynucleotide triphosphates (1·25 mmol/L), 2·0 μL forward primer (20 pmol), 2·0 μL reverse primer (20 pmol), 1·0 μL genomic DNA, 13·3 μL water, and 0·2 μL Ampli Taq (1 U). PCR primers were designed with the computer program ‘Primer’ of the HUSAR software package (version 5), which is based on programs from the Genetics Computer group at the University of Wisconsin, Madison, USA. The following primers were used: forward primer 5′-TCAAAAGAAGGTCATT CATTACACC-3′; reverse primer 5′-AGCCAGAGG AGAAATACAAATC-3′. The size of the wild-type PCR product was 241 bp, whereas the CCR5Δ32 allele yielded a product of 209 bp (figure 1). The identity of the 209 bp fragment was confirmed by DNA sequencing. PCR reactions were carried out in a Robocycler Gradient 96 (Stratagene, Amsterdam, Netherlands) under the following conditions: 3 min 94°C (one cycle); 1 min 15 s 94°C, 1 min 15 s 58°C, 1 min 72°C (35 cycles); 7 min 72°C (one cycle). PCR products were analysed on 2% agarose gels.

Statistical analysis

Cumulative transplant survival was analysed by use of Kaplan-Meier plots. Statistical difference was examined with a log-rank test. Rates of graft loss 5 and 10 years after transplantation were compared by Fisher’s exact test.

Results

Of 1227 patients tested, 21 (1·7%) were homozygous for the CCR5Δ32 allele, 248 (20·2%) were heterozygous, and 958 (78·0%) were homozygous for the wild-type. Complete follow-up data were available for 576 recipients of first renal transplants.

Demographic and clinical data are given for these 576 patients in table 1. The proportion who received transplants from living donors was similar among the group homozygous for CCR5Δ32 and for the control group (table 1). The ethnic composition did not differ significantly between these groups; 98% of patients were white.

Biopsy-proven rejection episodes occurred in at least nine of the 21 patients homozygous for CCR5Δ32 (table 2). Despite these episodes, patients with this genotype showed good renal-transplant survival. During median follow-up of 7·2 years, only one of 21 patients with a defective CCR5 receptor lost graft function (patient 2, table 2). In this patient, the slowly progressive deterioration was presumed to be secondary to chronic allograft nephropathy, but further elucidation is not possible because no graft biopsy sample was obtained. Patient 11 died with a functioning graft. The other 19 patients were alive with functioning grafts in April, 2000.

In the control group of heterozygous and wild-type homozygous patients there were 78 graft failures. Thus, 5 and 10 years after transplantation the proportion with graft loss was significantly lower in CCR5Δ32 homozygous patients than in the control group (p=0·0108 after 5 years and p=0·0062 after 10 years, Fisher’s exact test). Comparison of transplant survival by a log-rank test showed significantly less graft loss for patients homozygous for CCR5Δ32 (figure 2), resulting in a hazard ratio of 0·367 (95% CI 0·157–0·859).

Discussion

In our survey of renal-transplant recipients, we found a good outcome for patients homozygous for CCR5Δ32, with no differences between the participating centres. No beneficial effect was observed for heterozygous transplant recipients. Homozygosity for the CCR5Δ32 allele shows an association with extended graft survival and may represent a significant advantage in renal transplantation. CCR5 may be a target for prevention of renal-transplant loss. Graft survival in our control patients was better than published data, perhaps because patients were recruited through transplant centres, favouring inclusion of those with functioning grafts.

Renal transplantation is a highly effective therapy for chronic renal failure, but the decline in renal function in the graft is accelerated and exceeds the expected loss of glomerular function in kidneys with advancing age. Thus, chronic allograft nephropathy is a persistent problem, which may result from both immunological and non-immunological insults. The increasing number of molecular analyses in medicine allows the identification of different factors contributing to renal-transplant failure. For example, the finding that thrombophilia is a risk factor for renal-transplant failure emphasised the importance of genetic factors for non-immunological injury and forms the basis for specific preventive interventions. 2, 24 Our study, identifying the

<table>
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<th>Characteristic</th>
<th>Wild-type and heterozygous CCR5Δ32 (n=555)</th>
<th>Homozygous CCR5Δ32 (n=21)</th>
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<tr>
<td>Mean (SD) age at transplantation in years</td>
<td>44 (3) (14-3)</td>
<td>39-4 (10-1)</td>
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<tr>
<td>Mean (SD) donor’s age in years</td>
<td>41-4 (15-5)</td>
<td>39-3 (12-5)</td>
</tr>
<tr>
<td>Mean (SD) cold ischaemia time in h</td>
<td>16 (8)</td>
<td>17 (7)</td>
</tr>
<tr>
<td>Mean (SD) % panel reactive antibodies</td>
<td>13 (8)</td>
<td>5 (11-2)</td>
</tr>
<tr>
<td>Mean (SD) number of HLA mismatches</td>
<td>2-8 (1-6)</td>
<td>2-6 (1-5)</td>
</tr>
<tr>
<td>Number with transplants from living donors</td>
<td>51</td>
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Table 1: Demographic and clinical data for 576 recipients of renal transplants
chemokine receptor CCR5 as an important factor for transplant loss, illustrates this point. Experimental and clinical research implicates cytokines and chemokines in the process of transplant rejection. Evidence has accumulated that the interaction of chemokines such as RANTES and MIP-1α with their respective receptors has a role in the inflammatory infiltration associated with transplant rejection. According to their chemical structure, RANTES and MIP-1α are classified as CC chemokines, involved in the attraction of T lymphocytes and macrophages to sites of injury. They both bind to CC chemokine receptors 1 and 5 (CCR1, CCR5), expressed on human lymphocytes and macrophages, and induce chemotaxis. The persistent intrarenal generation of chemokines and the CCR5-positive cell infiltrate have been postulated to mediate initiation and maintenance of the inflammatory response in transplant rejection. Indeed, interference with the interaction between chemokine receptor and ligand significantly decreased rates of rejection of experimentally transplanted organs. Passive immunisation of rats with antibodies to RANTES attenuated acute lung-allograft rejection. Targeted deletion of the chemokine receptor CCR1, another receptor for RANTES and MIP-1α, mitigated heart-allograft rejection in mice. Interestingly, there was also less intragraft expression of CCR5 in these animals, which decreased even further after administration of cyclosporin.

For the human CCR5 gene, a mutant allele with a 32 bp deletion (CCR5Δ32) exists, which encodes a functionally defective receptor. Individuals homozygous for CCR5Δ32 are highly resistant to HIV1 infection, appear healthy, and show no other phenotype. These characteristics imply that the loss of CCR5 can be compensated for or that the receptor has no function under the conditions analysed so far. However, individuals carrying the mutation are at lower than normal risk of asthma. Furthermore, the CCR5Δ32 allele may have a positive effect on the course of autoimmune diseases such as rheumatoid arthritis and multiple sclerosis. The significantly prolonged renal-graft survival that we observed in renal-transplant recipients homozygous for CCR5Δ32 adds another advantage for this mutation. These results also identify CCR5 as an important determinant of graft survival. Since patients homozygous for CCR5Δ32 show no obvious deleterious effects, CCR5 should be an ideal therapeutic target for future interventions intended to prevent renal-transplant loss. Such interventions could involve chemokine antagonists, CCR5 blockers, or cytotoxic antibodies directed against CCR5-positive cells.

Our study shows the power of molecular medicine in identifying important and specific targets for therapeutic interventions.

Contributors
Michael Fischereder contributed to collection of blood samples and patients’ data, data analysis, and preparation of the report. Bruno Luckow contributed to the idea of the study, genotype analysis, and preparation of the report. Berthold Hocher provided blood samples and patients’ data, data analysis, and preparation of the report. Rudolf Wüthrich contributed to the idea and.
provided all data for the Zürich patients (including genotype information). Uwe Rothenpieler provided blood samples and information on patients. Helmut Schneebberger did the statistical analyses. Ulf Panzer provided complete data for the Hamburg patients (including genotype analysis). Rolf Stahl contributed to the idea and provided complete data for the Hamburg patients (including genotype analysis). Ingeborg Hauser, Klemens Budde, Hans Neumann, Bernhard Kramer, and Walter Land provided blood samples and information on patients. Detlef Schröndorf contributed to the idea, management of the project, and preparation of the report.

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References