A Monocyte Chemoattractant Protein-1 (MCP-1) Polymorphism And Outcome After Renal Transplantation

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Abstract. Among the factors modulating transplant rejection and cardiovascular disease, chemokines and their respective receptors deserve special attention. In this respect, increased expression of MCP-1 and the corresponding receptor CCR2 have been demonstrated in renal transplant rejection and coronary artery disease. The impact of the MCP-1–2518G and CCR2–64I genotypes on renal allograft function was investigated in 232 patients who underwent transplantation over an 11-yr period. Genomic DNA was genotyped using PCR with sequence-specific primers followed by restriction fragment length polymorphism analysis. Eighteen (7.8%) patients were homozygous for the MCP-1–2518G mutation. The G/G allele of MCP-1-2518 behaved as a determinant for long-term allograft survival and resulted in reduction of the mean graft survival, as compared with the heterozygous (A/G) or wild-type (A/A) allele (67 ± 14 versus 95 ± 4 mo; Log rank P = 0.0052). The 64I mutation of CCR2 had no effect on kidney graft failure (93 ± 6 and 91 ± 5 mo, respectively; P = 0.81). None of the investigated polymorphisms showed a significant shift in gene frequency in acute rejection and rejection-free groups. In conjunction with these findings, peripheral blood mononuclear cells from kidney transplant recipients carrying the G-allele were characterized by a 2.5-fold higher MCP-1 secretion (P < 0.05). In conclusion, recipients of renal transplants homozygous for the –2518 G mutation of the MCP-1 gene are at risk for premature kidney graft failure. This variant of MCP-1 may be a future predictor for long-term kidney graft failure.

During acute allograft rejection, monocytes and T effector cells are directed into the transplant and produce a characteristic tubular or vascular infiltrate (1). The complex process of extravasation and influx of leukocyte subsets into the site of tissue injury appears to be mediated to a significant extent by the expression of specific chemokines and chemokine receptors (2). Specifically, the expression of the CC-chemokine MCP-1 together with the corresponding chemokine receptor CCR2 could be detected on the mononuclear cells infiltrating the kidney graft (3–8). Furthermore, elevated levels of MCP-1 were also detected in the serum and urine of patients at the time of acute and chronic rejection (9,10).

Currently, in experimental transplantation targeted deletion of CCR1, CCR5, CX3CR1, or CXCR3 or specific blockade of these receptors, have resulted in improved allograft survival (1,11). Furthermore, we have recently shown that human transplant recipients without a functional CCR5 due to a 32-bp deletion in the CCR5 gene (Δ32 mutation) have significantly better allograft survival (12). The interaction of MCP-1 and CCR2 has not been blocked experimentally in a transplant model to date. Preliminary data, however, suggests a prolonged vascularized cardiac allograft survival in CCR2 knockout mice compared with wild-type (11).

A substantial number of allografts are lost due to death of the recipient from cardiovascular disease (13,14). Detailed studies of atherosclerotic vascular disease in a rodent model were able to demonstrate the involvement of MCP-1/CCR2 in the complex process of atherogenesis (15,16,17). Interestingly, the MCP-1–2518G variant in homozygous patients appears to be a genetic risk factor for CAD (18). Thus, chemokines and chemokine receptors may affect allograft function not only through acute and chronic allograft rejection but perhaps indirectly through the accelerated cardiovascular comorbidity of the recipients.

Common mutations are described for CCR2 and MCP-1. Although there is to date no evidence that the CCR2–64I polymorphism alters CCR2 expression or function on leukocytes, this variant is associated with delayed progression of HIV infection and a lower risk for the development of sarcoidosis (19,20,21). The MCP-1 polymorphism at position –2518 (G or A) relative to the major transcriptional start site was shown to influence the level of MCP-1 production in response to interleukin1β (IL-1β) in vitro (22). In this study, we ana-
lyzed whether MCP-1–2518 and CCR2–64I variants are associated with altered kidney allograft outcome.

**Materials and Methods**

**Patients Demographics**

A total of 232 first renal allograft recipients transplanted at two centers from 1990 through 2001 were analyzed. Demographic data for donor and recipient age and gender, HLA-mismatch, panel reactive antibodies, cold ischemia time, immunosuppressive therapy, presence of rejection episodes, and graft survival were extracted from the hospital record. Acute rejection was determined by allograft biopsy in 84% of renal transplant recipients. In the remaining, rejection was defined by an increase in creatinine level by 30% from baseline that was not attributable to other causes with subsequent return to baseline after treatment with pulse steroids or antilymphocytic antibodies. Graft survival was defined as recipient survival with a functioning renal transplant. The Internal Review Board approved the study, and written consent was obtained at the time of enrollment.

**Identification of Genotypes**

Genotype analysis for CCR2-64I and MCP-1-2518 was performed on genomic DNA isolated from peripheral blood with PCR with sequence-specific primers followed by restriction fragment length polymorphism analysis as described previously (18,20). Briefly, after PCR amplification, PCR products were digested with PvuII (recognizes the MCP-1-2518 A/G transition) or BsaB I (CCR2-64I mutation) (New England Biolabs, Waltham, MA). MCP-1-2518 G/A and CCR2–64I variants were detected by electrophoresis on 4% NuSieve (3:1) gel.

**MCP-1 Production**

MCP-1 released from unstimulated and stimulated peripheral blood mononuclear cells (PBMC) was determined by enzyme-linked immunosorbent assay (ELISA) as described (22). Briefly, PBMC from kidney transplant recipients were isolated with density gradient centrifugation. PBMC were cultured for 24 h with and without addition of 10 ng/ml IL-1β (Endogen, Woburn, MA). MCP-1 concentrations were assayed in cell-free supernatants by specific ELISA according to the manufacturer’s instructions (BioSource International, Inc., Camarillo, CA).

**Statistical Analyses**

Patient and graft survival was analyzed with Kaplan-Meier estimates. Groups were compared with log rank test. Multivariate analysis for the respective risk factors was performed with Cox regression. P values were adjusted for multiple testing according to the method described by Bonferroni. P < 0.05 was considered significant.

**Results**

The overall gene frequencies of MCP-1-2518 G (28.3%) and CCR2-64I (11.8%) were similar to those previously reported (18,20,22). Eighteen patients (7.8%) were homozygous for the MCP-1-2518G mutation, and 94 patients (40.5%) were heterozygous. Three patients (1.3%) were homozygous for the CCR2-64I mutation; 45 patients (19.4%) were heterozygous. Individuals with or without the MCP-1-2518 G/G genotype did not differ with respect to other risk factors for allograft failure such as donor age, cold ischemia time, ethnic background, living donor, HLA-mismatch, panel of reactive antibodies, immunosuppressive therapy, recipient age and gender, and occurrence of acute rejections (Table 1).

**Association of the Polymorphism with Acute Rejection**

The overall incidence of acute rejection within the first year was 36.4%. Neither of the investigated polymorphisms showed a significant shift of gene frequency in acute rejectors versus nonrejectors. The gene frequency for the MCP-1-2518 G allele

<table>
<thead>
<tr>
<th>Table 1. Demographic and clinical data for 232 recipients of first kidney transplants according to the MCP-1 –2518 genotype</th>
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<tr>
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<tr>
<td>Mean recipient age (± SD)</td>
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<tr>
<td>Gender</td>
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<tr>
<td>Male</td>
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<tr>
<td>Ethnicity</td>
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<td>White</td>
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<td>African American</td>
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<td>Hispanic</td>
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<td>Asian</td>
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<td>Cadaveric donors</td>
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<td>Donor age (± SD)</td>
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<tr>
<td>Cold ischemia time</td>
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<tr>
<td>HLA-mismatch (total)</td>
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<tr>
<td>Panel reactive antibody (%)</td>
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<tr>
<td>Calcineurin inhibitors</td>
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<tr>
<td>Patients with acute rejection</td>
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</table>
was 28.4% for rejectors and 28.2% for nonrejectors (P = 0.67). CCR2-64I gene frequency was 11.4% in the rejection group versus 12.0% in the rejection free group (P = 0.37). Similarly, no difference was found by analyzing by genotypic distribution (A/A & A/G versus G/G) of MCP-1-2518 (Table 1).

Effect of Graft Survival on Genotypes
A total of 39 grafts (17%) lost function, and mean graft survival was 91 mo. Patient and graft survival with respect to the MCP-1 genotype is shown in Figure 1. In accordance with a previous study, the G/G genotype was compared with the combined A/A and A/G genotypes (18). The G/G allele of MCP-1-2518 behaves as a determinant for long-term allograft survival and results in significant reduction of the mean graft survival, as compared with the heterozygous (A/G) or wild-type (A/A) allele (67 ± 14 versus 95 ± 4 mo; log rank P = 0.0052; Table 2 and Figure 1).

After multivariate correction for the risk factors listed in Table 1 the Cox proportional hazard analysis revealed that only the presence of the G/G genotype was associated with graft loss. The G/G genotype represented a 4.5-fold risk for graft loss (95% CI, 2.3 to 8.8; P = 0.027).

The reason for three graft losses within 12 mo after transplantation for the G/G MCP-1 genotype were transplant vein thrombosis in one case, and two patients lost their graft because of severe rejection.

The 64I mutation of CCR2 had no effect on kidney graft survival (Table 2). Death with a functioning graft occurred in eight patients (3.4%). Censoring these patients’ graft survival data did not change the significantly poorer outcome for patients with the MCP-1 G/G genotype (data not shown).

**MCP-1 Production**
The functional effect of this MCP-1 mutation in immunosuppressed transplant recipients is not known. We therefore examined the effect of the -2518 G-allele on MCP-1 production after stimulation with the proinflammatory cytokine IL-1β. Baseline MCP-1 production was comparable in PBMC with or without the G-allele (543 ± 88 versus 305 ± 131; P = 0.10). However, after stimulation the G-allele was associated with a significant 2.5-fold higher MCP-1 production compared with PBMC without the G-allele (Figure 2). At the time of blood collection, the two groups studied for MCP-1 production did not differ in the immunosuppressive therapy (P = 1.0 by Fisher’s exact test).

**Discussion**
Despite advances in immunosuppression over recent years, long-term graft function remains adversely affected by a disproportionately high rate of first-year graft failure and accelerated decline due to allograft nephropathy or recipient mortality. Multiple associations have been reported for gene polymorphisms on of HLA, cytokine, and costimulatory molecules on clinical outcomes of kidney transplantation (23). Few

**Table 2. Survival time of the first kidney graft according to the MCP-1 –2518 and CCR2-64I genotype**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Graft Survival Time mo; mean ± SEM</th>
<th>95% CI</th>
<th>Log-rank P</th>
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</thead>
<tbody>
<tr>
<td>MCP-1 –2518</td>
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<tr>
<td>A/A &amp; A/G</td>
<td>214</td>
<td>95 ± 4</td>
<td>(87 to 103)</td>
<td></td>
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<tr>
<td>G/G</td>
<td>18</td>
<td>67 ± 14</td>
<td>(40 to 94)</td>
<td>0.0052</td>
</tr>
<tr>
<td>CCR2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCR2/CCR2</td>
<td>184</td>
<td>91 ± 5</td>
<td>(82 to 101)</td>
<td></td>
</tr>
<tr>
<td>CCR2/64I &amp; 64I/64I</td>
<td>48</td>
<td>93 ± 6</td>
<td>(81 to 104)</td>
<td>0.81</td>
</tr>
</tbody>
</table>
studies however have explored consequences of genetically determined alterations of receptor-ligand interactions in the chemokine system.

Our group was the first to report a significant association between an allelic variation of a chemokine gene with kidney graft survival (12). Whereas the CCR5Δ32 mutation demonstrated a beneficial effect on kidney graft survival, our present study demonstrates a significant association of allograft failure with G allele of MCP-1-2518 polymorphism. Renal allograft recipients with the homozygous for the G allele experience premature graft loss with a significantly shortened mean graft survival of 28 mo. Our functional data has also shown that PBMC isolated from kidney transplant recipients who are carriers of this allele show an increased production of MCP-1.

The presence or absence of heterozygous CCR2-64I variant did not influence the kidney graft outcome. This is in accordance with our previous observation that CCR2-64I was not associated with acute rejection or graft survival in 207 liver transplant recipients (24). Our data contrast however with a recent study where the heterozygous CCR2-64I variant was associated with decreased incidence of acute rejection (P = 0.014) in 163 kidney transplant recipients. Long-term graft survival was not addressed in this study (25).

The increased levels of MCP-1 expressed by carrier of the G allele of the -2518 polymorphism has two potentially negative effects on kidney graft outcome. An increased mononuclear cell infiltration attracted by increased levels of MCP-1 can enhance both rejection and the development of transplant-associated atherosclerosis (26). Although we cannot exclude subclinical rejections, we found no influence of the MCP-1 variant on acute rejection in our population. This is in accordance with data obtained from a fully MHC-disparate mouse cardiac rejection model where the MCP-1/CCR2 had a primary role in the development of transplant vasculopathy while playing only a secondary role in acute rejection (WW Hancock, personal communication).

The role of MCP-1 and CCR2 in the pathogenesis of atherosclerosis may be through increasing mononuclear cell recruitment into the intima, thereby contributing to both antigen specific and nonspecific effects. This role is supported by the fact that CCR2 as well as MCP-1 knockout mice, when backcrossed with apo E-deficient or LDL receptor–deficient mice, have an inhibitory effect on the formation and development of atherosclerotic lesions (15,16). The findings in animal models are consistent with human cardiovascular studies. The MCP-1-2518 (G/G) so-called high MCP-1 producer genotype was at increased risk for CAD. The fact that this effect was not seen in heterozygotes -2518 (A/G) suggests a recessive mode of action of this chemokine on CAD (18). The same study found that individuals homozygous for CCR2-64I were at reduced risk for severe CAD, tested by coronary angiography (18). Due to the low number of CCR2-64I homozygotes, we were unable to assess whether this variant is associated with reduced risk of premature kidney graft loss. Our data indicate however that the heterozygous genotypes for MCP-1-2518 as well as CCR2-64I do not have an apparent effect on graft survival.

Identification of MCP-1-2518 (G/G) genotype as a risk factor for premature allograft failure expands our knowledge of the complex genetic factors contributing to successful transplantation (23). Beyond an improvement in understanding the pathophysiology of allograft failure, this interaction also offers an interesting potential for future specific therapy. Just as with CCR1, CCR5, and CXCR3, specific blockade of MCP-1/CCR2 interaction appears possible. In experimental atherosclerosis, another disease model that is modulated by MCP-1/ CCR2, overexpression of inactive MCP-1 resulted in significant inhibition of atherosclerotic lesion formation (27,28). Similar interventions might improve allograft survival. Future studies with experimental transplantation in animals with pharmacologic blockade of MCP-1 will possibly determine the effects of interventions specifically directed against this chemokine.

Acknowledgments
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References
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