Expression of the C-C chemokine receptor 5 in human kidney diseases

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Background. Chemokines are proteins that contribute to the migration of leukocytes to sites of tissue injury. CCR5 is a receptor for the C-C chemokine RANTES, which is expressed in inflammatory kidney diseases and transplant rejection.

Methods. In order to study the distribution of CCR5, we developed a series of monoclonal antibodies against human CCR5. These antibodies were then evaluated by flow cytometry, Western blot, and immunohistochemistry on formalin-fixed, paraffin-embedded tonsils. Eighty biopsies from patients with membranous glomerulonephritis (N = 9), IgA nephropathy (N = 10), lupus nephritis (N = 10), membranoproliferative glomerulonephritis (N = 10), acute interstitial nephritis (N = 13), chronic interstitial nephritis (N = 10), acute transplant rejection (N = 9), and chronic transplant rejection (N = 9) were stained for CCR5 and CD3 expression in parallel sections.

Results. One monoclonal antibody (MC-5) showed a single protein band of approximately 38 kD corresponding to CCR5 in Western blot. By indirect immunohistochemistry, a cell membrane signal was detected exclusively on monocellular inflammatory cells. All control stainings with an isotype-matched mouse IgG2a were negative. CCR5-positive cells were identified in areas of interstitial infiltration in biopsies of chronic glomerulonephritis, interstitial nephritis, and transplant rejection. The staining of CCR5 showed the same distribution as CD3-positive T cells. In patients with impaired renal function, a significantly higher number of CCR5-positive cells was found as compared with patients with normal renal function. In contrast to the prominence of CCR5-positive cells in the interstitial infiltrate, the number of CCR5-positive cells within the glomeruli was low, even in cases with proliferative glomerulonephritis. No CCR5 expression could be detected on intrinsic cells of glomerular, tubular, or vascular structures.

Conclusions. The pattern of CCR5 and CD3 cell infiltration suggests that CCR5-positive T cells may play a role in interstitial processes leading to fibrosis. Further studies are required to define the pathophysiological relevance of these cells in progressive renal diseases.

Chemokines are members of a large and rapidly growing family of small chemotactic proteins [1]. According to the position of the first two of four conserved cysteine residues, these cytokines are divided into subgroups [2]. The C-C chemokine RANTES (regulated upon activation of normal T cell expressed and secreted), a 68-amino acid protein, can be expressed by stimulated fibroblasts [3], mesangial cells [4], and tubular epithelial cells [5]. The role of chemokines in the cellular infiltrate of inflammatory renal diseases has been studied in vitro [4] and in vivo [6]. In the mouse model of anti-glomerular basement membrane nephritis, an up-regulation of RANTES mRNA was observed [6]. Blocking the RANTES effect by MetRANTES led to a decrease of proteinuria and a decreased number of T cells [7]. Elevated RANTES levels have been described in patients with human immunodeficiency virus (HIV) nephropathy (Kimmel PL et al, abstract; J Am Soc Nephrol 5:279, 1994) and during human transplant rejection [8]. The production of RANTES by cultured tubular cells can be stimulated not only by proinflammatory cytokines [5], but also by exposure to albumin [9].

Chemokine receptors are classified according to their ligands into the CC, CXC, and CX3C receptor families (CCR, CXCR, and CX3CR) [10]. These receptors are seven-transmembrane–spanning G-protein–coupled proteins [11]. CCR5 is a receptor for the C-C chemokines RANTES, macrophage inflammatory protein-1α (MIP-1α), macrophage inflammatory protein-1β (MIP-1β), and monocyte chemoattractant protein-2 (MCP-2) [12, 13]. The identification of CCR5 as the major coreceptor for macrophage-tropic HIV-1 strains has led to a rapid progression of knowledge about the physiological and pathophysiological role of CCR5 [14-19].
Approximately 20 to 30% of peripheral T cells and 10% of monocytes are CCR5 positive [19]. To date, little is known about the expression and distribution of chemokine receptors on different cells during renal diseases. Schadde et al (abstract; Schadde E et al, J Am Soc Nephrol 8:485, 1997) described the expression of the chemokine receptors 1 through 5 in a mouse model of anti-glomerular basement membrane nephritis. An up-regulation of CCR1, -2, -3 and -5 was observed by reverse transcription-polymerase chain reaction (RT-PCR) in kidney cortex, in the tubular fraction, and was most prominent in the glomerular fraction. The RT-PCR technique, however, did not permit an assignment of the CCR expression to specific cells. By in situ hybridization, CCR5 expression was detected in infiltrating cells in allograft rejection but not in intrinsic renal cells of the normal kidney [20]. In a study on cryosections, Rottman et al described CCR5 by immunohistochemistry. In one case of interstitial nephritis, CCR5 was found on interstitial infiltrating cells and endothelium and vascular smooth muscle cells [21]. As RANTES seems to play an important role in different animal models of inflammatory kidney diseases, as well as in human renal diseases and transplant rejection, we aimed to characterize the cellular distribution of the RANTES receptor CCR5 in kidney diseases. The identification of CCR5-positive cells is important in the characterization of infiltrating cells and their potential role in the inflammatory process. This information is a prerequisite for eventual therapeutic studies to block CCR5. Therefore, we examined the CCR5 expression pattern in a series of inflammatory kidney diseases and during renal transplant rejection on formalin-fixed and paraffin-embedded renal biopsies by immunohistochemistry. We found that the CCR5-positive cells were primarily CD3-positive cells, and in all disease, entities were present almost exclusively in the interstitium. The mean number of CCR5-positive cells was found to be significantly higher in patients with glomerular diseases and impaired renal function than in patients with normal renal function.

METHODS

Generation of the CHO/CCR5 cell line

Dihydrofolate reductase (DHFR)-deficient Chinese hamster ovary (CHO) cells were stably transfected with the cDNA sequence of CCR5 as previously described [22]. One clone growing in the presence of 20 nm methotrexate expressed high levels of CCR5 and was chosen for immunization of BALB/c mice. Full functional activity of the overexpressed CCR5 protein was demonstrated by receptor down-modulation and calcium influx induced by RANTES (data not shown).

Generation and characterization of the monoclonal anti-CCR5 antibody MC-5

BALB/c mice were immunized intraperitoneally for seven times with 10⁷ CHO/CCR5 cells as described earlier in this article. Four days after the last injection, the spleens were removed, splenocytes fused with the P3 × 63-Ag8 myeloma cell line, and the cells plated on six 96-well, flat-bottomed microtiter plates. Supernatant from each well, containing approximately 10 hybridomas, was tested by flow cytometry analysis on CHO/CCR5 versus CHO/CXCR4 cells. To ensure homogeneity, hybridoma cells from a well with specific binding activity against CCR5 were cloned twice by limited dilution. The specificity of the resulting monoclonal anti-CCR5 antibody MC-5 was tested on CHO cells overexpressing the receptors CCR1-3 and CXCR4 (not shown), as well as on peripheral blood mononuclear cells (PBMCs) from two donors with a homozygous Δ32 deletion in the CCR5 gene. In all cases, no binding of the antibody MC-5 was detected.

Detection of cell surface CCR5 by flow cytometry analysis

Five × 10⁶ CHO/CCR5 cells or PBMCs were incubated with supernatant or purified antibody from the MC-5 hybridoma for 30 minutes on ice. After three washing steps with phosphate-buffered saline (PBS), fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse F(ab)₂ fragments were added for 30 minutes at a dilution of 1:50 (F313; Dako, Carpinteria, CA, USA). Cells were washed three times, incubated for 10 minutes in 10% mouse serum, and finally stained with PE- or Tricolor-conjugated anti-CD4 or anti-CD8 antibodies (CALTAG). After a final washing step, cells were analyzed on a flow cytometer (FACSCalibur; Becton-Dickinson, Mountain View, CA, USA).

Immunoblotting

For immunoblotting, confluent CHO cells were washed twice with PBS and lysed with hot Laemli buffer. The cell lysate was boiled immediately for five minutes and separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; 10% acrylamide gel). Gels and polyvinylidene fluoride (PVDF) membrane (Immobilon-P, Millipore Corp., Germany) were equilibrated with transfer buffer (Tris 25 mM; glycine 192 mM; methanol 20%; pH 8.3) for 10 minutes. Electrophoretic transfer was performed with the Trans-Blot SD Semi-Dry Transfer Cell (Bio-Rad, Richmond, CA, USA) using a constant current of 3 mA/cm² for 45 minutes. The membranes were stained in Ponceau solution (Ponceau Red 0.5%, glacial acetic acid 1%) for five minutes to control the protein transfer. Blocking was done with 10% nonfat dry milk in TBST (Tris-HCl 20 mM, sodium chloride...
137 mm, Tween 20; 0.5%; pH 7.6) for one hour. Blocking solution was washed away in TBST for five minutes. MC-5 was diluted 1:100 in TBS with 10% nonfat dry milk. A first antibody was incubated overnight at 4°C. Membranes were rinsed in TBST twice and washed in TBST (1 × 15 min, 2 × 5 min). A secondary antibody [horseradish peroxidase (HRP) conjugated rabbit anti-mouse; Dako] was used in a dilution of 1:2000 for 30 minutes. Afterward, membranes were washed three times in TBST and once in TBS for 15 minutes each.

For detection, we used the ECL solution (Amersham Life Science, Arlington Heights, IL, USA) following the manufacturer’s protocol.

**Immunohistochemistry**

Four monoclonal antibodies and a polyclonal rabbit serum against CCR5 were tested on a formalin-fixed, paraffin-embedded tissue of human tonsils. Isotype-matched mouse IgG was used on parallel sections as a negative control. By enzyme-linked immunosorbent assay (ELISA), IgG concentration of the diluted supernatant was approximately 3 μg/ml, and an excess concentration of 10 μg/ml IgG2a was used as a negative control. One antibody (MC-5) turned out to be suitable on paraffin-embedded material with the following protocol. Slides were deparaffinized and rehydrated (10 min in 100% ethanol; 96, 70, 50% ethanol and aqua dest. for a few seconds each). Endogenous peroxidase activity was blocked by H2O2 in methanol (5 ml of 30% H2O2 in 70 ml methanol) for 15 minutes. Slides were rinsed in Tris buffer (Tris 0.05 M, pH 7.5). In preliminary experiments, we established that autoclaving of the formalin-fixed and paraffin-embedded tissue slides provided better results than microwave treatment or protease digestion. Therefore, all stainings were performed after autoclaving for 10 minutes at one bar in a citrate buffer [Stock-Solution 10X; citrate-monohydrate 0.01 M (244; Merck, Darmstadt, Germany); pH 6; final concentration diluted in aqua dest.]. After 10 minutes of autoclaving and 40 minutes of cooling, slides were again rinsed in Tris buffer for 15 minutes. The first antibody (MC-5, 1:10 in PBS with 10% human serum) was incubated for one hour. After rinsing with Tris buffer, one drop of Link (biotinylated goat anti-mouse immunoglobulins; Super Sensitive Ready to Use Kit; BioGenex, San Ramon, CA, USA) was added and incubated for 30 minutes. The same was done with Label (peroxidase-conjugated streptavidin; Super Sensitive Ready to Use Kit; BioGenex). Slides were briefly rinsed in PBS. Staining was done with AEC according to the manufacturer’s protocol (Super Sensitive Ready to Use Kit; BioGenex). Staining was followed under the microscope. Slides were counterstained with hemalaun for approximately one minute and embedded in Aquatex (Merck). To compare the expression pattern, we used markers for T cells (CD3, 1:400; Dako), macrophages (CD68, 1:100; Dako) and B cells (1:25, CD79a, Dako).

**Patient characteristics and quantitative evaluation of biopsies**

We evaluated a total of 80 human kidney biopsies; the clinical data are summarized in Table 1. All biopsies were stained with anti-CCR5 and anti-CD3 in parallel.

Positive cells were counted in 10 high-power fields (×400) to a maximum of 50 cells. Cell count per interstitium and glomerulus were separately evaluated by an observer without the knowledge of either the underlying disease or the serum creatinine. The overall positive cell number reflected the positive cells within the glomerulus plus the cells in the tubulointerstitium. Cell counts were compared by Wilcoxon test, and a P < 0.05 was considered as statistically significant. Bars give the standard error of the mean (SEM).

**RESULTS**

**Characterization of the monoclonal antibody MC-5 against CCR5**

By flow cytometry analysis, MC-5 detected CCR5 on overexpressing CHO cells (data not shown) and on PBMCs isolated from peripheral blood of normal donors (Fig. 1A). As the negative control, isotype-matched mouse IgG2a, untransfected CHO cells, and PBMCs from individuals homozygous for the Δ32-CCR5 allele (lacking CCR5 expression) were used (Fig. 1A). Immunoblotting showed a single band of approximately 38 kD in cells overexpressing CCR5 (Fig. 1B), but not in cells overexpressing CXCR4. These results confirm the specificity of MC-5 for CCR5.

On sections of formalin-fixed and paraffin-embedded tonsils (Fig. 1C), we found CCR5-positive cells mainly in the interfollicular region with a cell membrane-staining pattern. In the follicles, only a few cells were positive. The pattern of CCR5-positive cells correlated well with the CD3-positive T cells, but not with the distribution of CD68-positive macrophages and CD79-positive B cells. These results demonstrate the feasibility of staining for CCR5 with MC-5 in paraffin-embedded tissue, and show that mostly CD3-positive T cells stain for CCR5.

**Staining for CCR5 in human kidney biopsies**

Biopsies from 80 patients (43 men and 37 women) with various renal diseases were included in this study. Irrespective of the type of kidney disease, CCR5 expression was only found on infiltrating mononuclear cells. No expression of CCR5 could be detected on intrinsic cells of the glomerulus, tubuli, or vasculature. The pattern of distribution between CCR5- and CD3-positive cells in parallel tissue sections was closely comparable. The correlation between CD3- and MC-5–positive cells

**Segerer and Mack et al:** CCR5 in human kidney diseases
Table 1. Characteristics of the patients at the time of biopsy (median and range)

<table>
<thead>
<tr>
<th>Renal disease</th>
<th>N</th>
<th>Age (years)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Sex ratio female:male</th>
</tr>
</thead>
<tbody>
<tr>
<td>Membranous glomerulonephritis</td>
<td>9</td>
<td>70 (39)</td>
<td>0.9 (4.7)</td>
<td>3:6</td>
</tr>
<tr>
<td>IgA nephropathy</td>
<td>10</td>
<td>41 (55)</td>
<td>1.0 (5.6)</td>
<td>6:3</td>
</tr>
<tr>
<td>Lupus nephritis</td>
<td>10</td>
<td>27 (45)</td>
<td>1.1 (1.7)</td>
<td>9:1</td>
</tr>
<tr>
<td>Membranoproliferative glomerulonephritis</td>
<td>10</td>
<td>51 (54)</td>
<td>1.8 (2.5)</td>
<td>6:4</td>
</tr>
<tr>
<td>Acute interstitial nephritis</td>
<td>13</td>
<td>46 (59)</td>
<td>8.2 (17.6)</td>
<td>5:8</td>
</tr>
<tr>
<td>Chronic interstitial nephritis</td>
<td>10</td>
<td>43 (60)</td>
<td>2.6 (11.7)</td>
<td>5:5</td>
</tr>
<tr>
<td>Acute transplant rejection</td>
<td>9</td>
<td>35 (36)</td>
<td>1.3 (3.1)</td>
<td>4:5</td>
</tr>
<tr>
<td>Chronic transplant rejection</td>
<td>9</td>
<td>37 (52)</td>
<td>2.5 (3.5)</td>
<td>3:6</td>
</tr>
</tbody>
</table>

also supports the interpretation that the CCR5-positive cells are mainly CD3-positive T cells (Fig. 2).

Membranous glomerulonephritis

We studied nine cases of membranous glomerulonephritis. This was the disease group with the highest median age (Table 1). Eight patients suffered from early membranous glomerulonephritis (stages I and II), and one had stage III disease.

No CCR5-positive cells were found within the glomerulus (Fig. 3A; note the different scale for glomerular and interstitial infiltrates; Fig. 4 A, B). The mean CCR5-positive cell number per high-power field of cortical tissue was approximately one. In only one case with chronic stage III disease, renal insufficiency and severe interstitial fibrosis was a mean of 12 positive cells per high-power field observed in the cortical tissue.

IgA nephropathy

Ten patients had the typical findings of IgA nephropathy. As in membranous glomerulonephritis, no CCR5-positive cells were found within the glomerulus (Fig. 3A). Three patients with impaired renal function showed an interstitial infiltrate with prominent CCR5 expression (Fig. 3B and Fig. 4 C, D).

Lupus nephritis

Biopsies from 10 cases of diffuse proliferative lupus nephritis (as defined by WHO IV) were examined. This was the group with the lowest median age and 9 of the 10 patients were women. In spite of the proliferative nephritis, the number of CCR5-positive cells in the glomerulus was low (Fig. 3A). In contrast, there was a considerable CCR5-positive mononuclear interstitial infiltrate (Fig. 4E). The mean overall CCR5-positive cell number was 11.5 per high-power field (Fig. 3B). In some cases, the CCR5-positive infiltrate was pronounced in the periglomerular region (Fig. 4E). Nine biopsies were evaluated for the number of CD68-positive macrophages. A mean of 7.5 ± 0.9 CD68-positive cells was found in the interstitium and 9.2 ± 1.7 within the glomerulus per high-power field. Therefore, the number of CD68-positive macrophages within the glomerulus (9.2) exceeded the negligible number of CCR5 with 0.2 and CD3 with 0.1 positive cells by far. In contrast, the number of CD68-positive macrophages (7.5 ± 0.9) within the interstitium was lower than the number CCR5- (11.3 ± 2.4) and the CD3- (18.2 ± 3.9) positive cells. Although there were very few CD3- and CCR5-positive cells in the glomerulus of systemic lupus erythematosus (SLE) patients, a strong infiltrate with CD68-positive macrophages was apparent (Fig. 4F).

Membranoproliferative glomerulonephritis

A total of 10 biopsies with the typical features of membranoproliferative glomerulonephritis was studied. The number of CCR5-positive cells within the glomerulus appeared to be higher than in other glomerulopathies, whereas the mean CCR5-positive cell infiltrate in the cortical tissue and the percentage of CCR5-positive cells were similar to the other forms of glomerulonephritis (Fig. 3A and Fig. 4 G, H, and Fig. 5).

Acute interstitial nephritis

We studied 13 biopsies of patients with acute interstitial nephritis. This was the group with the highest median serum creatinine value at the time of biopsy (Table 1). The histological picture ranged from mild signs of tubular damage with a minimal interstitial infiltrate to a prominent mononuclear infiltrate with tubulitis (Fig. 6 A, B) and interstitial edema. There was usually no or only mild interstitial fibrosis. In four patients, eosinophils were detected in the infiltrate. Very few, if any, CCR5-positive cells were found in the glomerulus (Fig. 3A).

In contrast, in the interstitium, a mean of 20 CCR5-positive cells (Fig. 3B) was found with an accentuation in the peritubular region (Fig. 6 A, B). At a higher magnification, CCR5-positive cells could be detected that infiltrate the tubular epithelium in areas with tubulitis (Fig. 6 C, D).

Chronic interstitial nephritis

Ten patients showed a histological pattern of chronic interstitial nephritis with an interstitial infiltrate, interstitial fibrosis, and sometimes signs of tubulitis. Tubuli showed signs of atrophy. The CCR5-positive infiltrate
Fig. 1. Flow cytometry analysis of peripheral blood mononuclear cells (PBMCs) from normal donors (A, left) and donors homozygous for the Δ32/Δ32 deletion of CCR5 (A, right), stained with MC-5 for CCR5 and a monoclonal antibody against CD4. (B) Immunoblot with protein from Chinese hamster ovary (CHO) cells overexpressing CCR5 or CXCR4. A single protein band is only detected with the monoclonal antibody MC-5 in cells overexpressing CCR5, but not in those overexpressing CXCR4. (C) CCR5-positive cells are found mainly in the interfollicular region in formalin-fixed, paraffin-embedded human tonsils. A CCR5-positive membrane staining pattern of small lymphocytes is observed. Negative control with isotype-matched IgG2a (magnification ×100, left panel). Positive staining with MC-5 (magnification ×100, center panel). Membrane staining pattern with MC-5 (magnification ×600, right panel).
was less prominent, and the percentage of CCR5-positive T cells was smaller than in acute interstitial nephritis (Fig. 3B and Fig. 6 E, F).

Transplant rejection

We studied a total of 18 transplant biopsies. Half of them showed signs of acute rejection. Biopsies were performed in the mean 32 days after transplantation. Seven of the nine acute rejections were classified as grade I and one as grade II according to the Banff classification. In two cases, vascular rejection with endotheliitis was present (Fig. 7 A, B), and the rest of the cases showed mild to severe tubulitis. In one case, prominent glomerulitis was present. The mean cell count of CCR5-positive cells and the percentage of CCR5-expressing T cells (Fig. 5) was the highest in this group. CCR5-positive cells were seen in all areas with mononuclear infiltrates. CCR5-positive cells invaded the epithelium of tubuli (Fig. 7C). In cases of vascular rejection, CCR5-positive cells were found in the subendothelial region (Fig. 7 A, B). Interestingly, in the one case with prominent transplant glomerulitis, a considerable number of CCR5 (1.4 per high-power field)- and CD3 (2.4 per high-power field)-positive cells were present within the glomerulus. Overall, however, the mean CCR5-positive cells within the glomerulus was comparable to the primary glomerulopathies.

Nine patients showed signs of chronic allograft nephropathy with mild to severe interstitial fibrosis (Fig. 7 D, E) and, in most cases, prominent intima-fibrosis. In three cases, focal-segmental glomerulosclerosis was present. The CD3- and CCR5-positive infiltrate was essentially interstitial with a mean of 15.6 CCR5-positive cells per high-power field (Fig. 3B).

Relationship between CCR5-positive cells and serum creatinine

When CCR5-positive cells were evaluated irrespective of diagnosis but according to the serum creatinine at the time of biopsy (normal serum creatinine; creatinine between 1.2 and 3; and creatinine ≥3 mg/dl), the mean cell count rose from 3.9 ± 1.3 in the normal to 14.4 ± 1.1 in the group with creatinines ≥1.2 to 3.0 to 19.8 ± 2.9 positive cells in the group with creatinine values over 3 mg/dl (Fig. 8). A similar increase was found for CD3-positive T cells with 7.8 ± 2.5 in the normal creatinine group, to 21.8 ± 1.3 and 28.4 ± 2.9 in the over 3 mg/dl creatinine group, respectively. Ten cases had to be excluded because of unknown serum creatinine values at the time of biopsy. As the interstitial infiltrate has been shown to be an important predictor of renal survival [32], we studied the mean cell count of CD3- and CCR5-positive cells in patients with glomerulonephritis with normal and impaired renal function. Figure 8 shows a significantly higher number of CCR5-positive cells in patients with glomerulonephritis with normal and impaired renal function. CD3-positive T cells were also found in a significantly higher number in patients with impaired renal function.
DISCUSSION

We studied the distribution of CCR5-positive cells in some of the most common renal diseases, including renal transplant rejection. The most important findings are: (a) a prominent CCR5-positive interstitial infiltrate consisting mainly of T cells, even in cases of primary glomerular disease; (b) the rare occurrence of CCR5-positive cells within the glomerulus, even in cases with considerable glomerular CD68-positive macrophage infiltration; (c) the absence of CCR5 expression by intrinsic renal cells in any of the kidney diseases examined; (d) a significant increase of CCR5-positive interstitial cells in patients with renal insufficiency. Unfortunately, the limited amount of material available from renal biopsies did not allow us to further analyze the cell infiltrate or the expression of adhesion molecules in parallel.

CCR5-positive cells in glomerular disease

The type of leukocyte infiltration in inflammatory diseases depends on the local expression of adhesion molecules in concert with the release of chemokines and the expression of their receptors on specific leukocyte subsets [4]. In renal inflammatory diseases, two types of leukocytes, that is, monocytes/macrophages and T-cell subsets, are implicated both in the generation of inflammatory diseases and in their progression to chronic renal insufficiency [23]. We found a prominent expression of CCR5 in the interstitial infiltrate. The distribution of cells indicated that these were mainly T cells. The highest number of CCR5-positive cells was found in acute transplant rejection and in cases with acute interstitial nephritis. The distribution of CCR5-positive cells that was observed by immunohistology in transplant rejection is comparable to the results reported by Eitner et al with in situ hybridization [20]. Among the primary glomerular diseases, lupus nephritis showed the highest number of CCR5-positive cells within the interstitium, followed by cases with membranoproliferative glomerulonephritis. The percentage of CCR5-positive T cells (43 to 63%) within the interstitial infiltrate was consistently higher than in the peripheral blood (20 to 30%), but differed little between the various types of glomerular diseases. Hooke, Gee, and Atkins described the interstitial leukocyte populations in biopsies of various forms of glomerulonephritis [24]. The percentage of T cells in cases with proliferative glomerulonephritis ranged between 39 and 77% [24]. A T-cell predominance in the interstitial infiltrate has been noted by different investigators in chronic glomerulonephritis, as well as in interstitial nephritis, and has been related to progressive disease [25].

CD3-positive, CCR5-positive cells in glomeruli

A striking result of our study is the rare appearance of CD3-positive, CCR5-positive cells within the glomerulus even in proliferative glomerular diseases. On the other hand, the interstitial infiltrate shows CCR5 expression on a prominent part of the infiltrating cells. The low number of T cells infiltrating the glomerulus is in agreement with the results of Hooke, Gee, and Atkins, who found a mean of 0.3 T cells per glomerular cross section in normal renal tissue, and no significant increase in cases with glomerulonephritis [24]. Even in cases of lupus nephritis showing a significant macrophage infiltration, we rarely detected CCR5-positive cells within the glomerulus (9.2 CD68-positive vs. 0.2 CCR-5-positive cells within the glomerulus per high-power field). This suggests that in immune-mediated glomerulonephritis, the infiltrating macrophages are either CCR5 negative or express low, undetectable amounts of immunostainable CCR-5. This is surprising, because in other inflammatory diseases activated monocytes and macrophages are fre-

Fig. 5. Percentage of CCR5-positive cells among the CD3-positive cells in the cortical tissue of biopsies according to the disease entity. Abbreviations are: ac.int., acute interstitial nephritis; ch.int., chronic interstitial nephritis; IgAN, IgA nephropathy; SLE, lupus nephritis; MN, membranous nephropathy; MPGN, membranoproliferative glomerulonephritis; ac.reject, acute transplant rejection; ch.reject, chronic transplant rejection.
**Fig. 6.** Parallel sections of a renal biopsy from cases with acute (A–D) and chronic (E, F) interstitial nephritis stained with anti-CD3 (A, E; magnification ×400) or MC-5 (B, D; magnification ×400; C, magnification ×100). Note the CCR5- and CD3-positive cells in the peritubular infiltrate with the same distribution pattern. (D) Note the massive tubulitis by CCR5-positive cells. A prominent CD3 (E)- and CCR5 (F)-positive interstitial infiltrate in a case of chronic interstitial nephritis.

**Fig. 7.** Parallel sections of a renal biopsy from cases with acute (A, B) and chronic (D, E) renal transplant rejection stained with anti-CD3 (A, D) or MC-5 (B, E; magnification ×400). A case of acute vascular transplant rejection with CD3-positive cells within the vessel wall and some subendothelial located CCR5-positive cells (↑) is illustrated in (A) and (B). (C) illustrates the infiltration of the tubular epithelium by a CCR5-positive cell in a renal biopsy from a patient with acute transplant rejection (↑, magnification ×1000). A chronic transplant rejection with marked interstitial fibrosis is shown in D and E. Publication of this figure in color was made possible by a grant from Fresenius Medical Care, Homburg, Germany.
cular structures. This is consistent with in situ hybridization results that also failed to find CCR5-positive intrinsic renal cells [20]. An immunohistological study on CCR5 distribution in different organs has been reported on frozen sections [21]. In a kidney with interstitial nephritis, Rottman et al described CCR5 on mononuclear, vascular smooth muscle, and endothelial cells. The CCR5 expression reported in this single case on cryosection is at variance with these immunohistochemical results in 80 biopsies and the in situ hybridization studies by Eitner et al [20].

**T cell infiltrate and CCR5-positive cells**

Many authors have described the important role of interstitial fibrosis during chronic renal failure [29–32]. We found a statistically significant increase in CCR5-positive cells and CD3-positive T cells in patients with impaired renal function in glomerular diseases. This relationship between the T cell infiltrate and renal insufficiency is in concordance with studies on IgA nephropathy [33] as well as in various other forms of glomerulonephritis [23]. The high degree (43 to 86%) of CD3-positive T cells that also express CCR5 is interesting as in peripheral blood only 20 to 30% of T cells are CCR5 positive [19]. This could relate to either a specific attraction of CCR5-positive cells into the interstitium and/or potential activation of the T cells in the interstitium by the cytokine milieu. As in different glomerular diseases, in which the relative portion of CCR5-positive cells compared with CD3-positive cells in the interstitium seems to be similar, a common pathway for their accumulation and the progression of disease might be hypothesized. It has been postulated that a dangerous cycle could be initiated by the proteinuria common to glomerular disease. The proteinuria would cause chemokine expression by tubulointerstitial cells with infiltration of chemokine receptor-bearing T cells and perpetuation of the inflammatory reaction, which in turn, would contribute to the progression of renal disease. This hypothesis is supported by up-regulation of RANTES in tubular cells exposed to albumin in vitro and RANTES expression by tubulointerstitial cells in experimental and human glomerular diseases and transplant rejection [6, 8]. Although our finding of a predominance of CCR5-positive T cells in nephritis and transplant rejection fits well to this model, we cannot establish a causal relationship that would require further experimental studies.

In conclusion, we identified CCR5-positive, CD3-positive cells as a prominent fraction of the interstitial infiltrate of various glomerular and interstitial diseases, as well as in transplant rejection. The relationship between the CCR5-positive T cells and the degree of renal insufficiency in glomerular diseases may indicate a possible role for these cells in the progression of renal disease. The prognostic value of CCR5-positive cells for the de-

![Fig. 8. Mean CCR5-positive cells in cortical tissue from biopsies irrespective of disease entity (A) according to normal serum creatinine, creatinine between 1.2 and 3, and creatinine over 3 mg/dl. (B) The results in patients with glomerulonephritis and normal (<1.2 mg/dl) or elevated (>1.2 mg/dl) serum creatinine. The numbers in the bars indicate number of biopsies examined.](image-url)
development of interstitial fibrosis and renal insufficiency will have to await a future longitudinal study. The absence of CCR5 on monocytes/macrophages in the glomerulus, on the other hand, argues in favor of other chemokine receptors as contributors to the glomerular macrophage infiltration in proliferative glomerulonephritis. The further identification of the specific chemokine receptors on the different leukocyte populations and their role in renal diseases may then allow therapeutic interventions directed at specific chemokine receptors.

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