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The Duffy antigen receptor for chemokines is up-regulated during acute renal transplant rejection and crescentic glomerulonephritis[1]

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The Duffy antigen receptor for chemokines is up-regulated during acute renal transplant rejection and crescentic glomerulonephritis.

Background. Recruitment of leukocytes during immune responses requires the coordinate expression of adhesion molecules in concert with chemokines and their receptors. The Duffy antigen receptor for chemokines (DARC) binds multiple chemokines and is expressed on postcapillary venules in the normal kidney. The chemokine receptor CCR5, which shares the ligand regulated upon activation, normal T-cell expressed and secreted (RANTES) with DARC, is expressed by infiltrating T cells in the renal interstitium. As DARC might be involved in the attraction of CCR5-positive cells, we studied the distribution of DARC and CCR5 in two forms of cell-mediated renal injury: renal allograft rejection and crescentic glomerulonephritis (cGN).

Methods. A total of 87 renal specimens, including 12 pretransplant biopsies, 47 transplant biopsies (Banff 1, $N = 10$; Banff 2, $N = 19$; and various other lesions $N = 18$), and 28 biopsies from patients with cGN, was analyzed. Immunohistochemistry for CCR5 and DARC was performed on serial sections of formalin-fixed and paraffin-embedded tissue.

Results. Compared with pretransplant biopsies, the mean number of DARC-positive interstitial venules was significantly increased during both transplant rejection and cGN. This was accompanied by an infiltration of CCR5-positive leukocytes. During transplant rejection, the number and distribution of CCR5-positive cells correlated with DARC-positive venules. Infiltrating CCR5-positive leukocytes were found mainly in the interstitium, often clustering around Bowman's capsules in biopsies from cGN. The number of glomerular CCR5 positive cells is low, but they are common in a subset of crescents.

Conclusions. We hypothesize that the increased number of DARC-positive venules in areas of interstitial injury and the colocalization with CCR5-positive infiltrating leukocytes may indicate a role for endothelial DARC expression during leukocyte adhesion and interstitial infiltration.

[1] **See Editorial by Lappin and Brady, p. 1816**

Key words: immune response, cell-mediated renal injury, allograft rejection, venules, endothelial DARC expression, leukocyte adhesion, kidney rejection.

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During acute transplant rejection and crescentic glomerulonephritis (cGN), a cell-mediated immune response leads to an influx of mononuclear leukocytes, mainly T cells and macrophages, into different compartments of the kidney [1–3]. This requires the concerted action of chemokines and adhesion molecules on infiltrating cells and the local endothelium [4]. Chemokines are small chemotactic proteins that are presently divided into four subgroups (CC, CXC, C, and CX_3C chemokines) according to their primary structure [reviewed in 5–7]. The interaction of chemokines with their respective receptors is essential for the local accumulation of specific inflammatory cells. The Duffy blood group antigen was identified as a chemokine receptor and was therefore renamed Duffy antigen receptor for chemokines (DARC) [8]. DARC can bind several chemokines of different subgroups, like regulated upon activation, normal T-cell expressed and secreted (RANTES), monocyte chemoattractant protein-1 (MCP-1), and interleukin-8 (IL-8) [8–10]. In addition to red blood cells, DARC is expressed on the endothelium of postcapillary venules of lymph nodes, spleen, and the kidney [11, 12]. The DARC expression on endothelium is present even in individuals who are Duffy negative on erythrocytes [13]. The function of DARC is still unknown, and binding of chemokines does not lead to the classic G-protein–coupled signal transduction of chemokine receptors. Darbonne et al proposed a function of DARC on red blood cells as a systemic "sink" for chemokines, but the role of DARC on endothelial cells remains to be defined [9, 12].

A recent study demonstrated an up-regulation of DARC on glomerular endothelium, collecting duct epithelium, and interstitial cells in children with HIV-associated renal diseases [14].

The chemokines macrophage inflammatory protein-1α (MIP-1α), MIP-1β, and RANTES are expressed in the kidney during transplant rejection and cGN. Inhibition of RANTES in experimental transplant rejection and cGN shows beneficial effects [15–28]. The mentioned chemokines bind to the chemokine receptor 5 (CCR5) and are able to induce transendothelial chemotaxis of T-cell subsets in vitro [29, 30]. T cells, macrophages, natural killer cells, and dendritic cells express CCR5 [31]. We recently demonstrated that a major part of the interstitial infiltrating cells in various forms of renal disease, including transplant rejection, are CCR5-positive T cells [32]. In the present study, we examined the distribution of CCR5-positive infiltrating leukocytes in relationship to DARC-positive venules. In areas of renal injury, we describe an up-regulation of DARC on the endothelium and a colocalization with CCR5-positive leukocyte infiltrates. We propose that DARC expressed on endothelial cells might act as a presenter of chemokines, and thus contribute to the local adhesion and infiltration of CCR5-positive cells at the site of renal injury.

METHODS

We studied a total of 87 renal biopsies, including 12 pretransplant biopsies, 47 transplant biopsies, and 28 biopsies from patients with cGN.

The transplant biopsies were from patients with acute interstitial transplant rejection (Banff 1, $N = 10$) and acute vascular rejection (Banff 2, $N = 19$) according to the Banff classification [33]. To evaluate the course of CCR5 and DARC expression, we studied repeated biopsies. In addition to acute transplant rejections, this group included 18 biopsies with various other lesions like no rejection, borderline lesions, thrombotic microangiopathy, and chronic transplant rejection (as well as cases in which the material was insufficient for a definitive diagnosis). The cGN population included cytoplasmic IF antineutrophil cytoplasmic antibodies (c-ANCA) ($N = 8$) and perinuclear (p-ANCA) positive ($N = 4$), crescentic lupus nephritis ($N = 1$), Goodpasture's syndrome ($N = 1$), and unclassified patients ($N = 3$).

The monoclonal anti-DARC antibody of Fy6 specificity (clone 2C3) was raised in Balb/C mice by immunization with Chinese hamster ovary (CHO) cells expressing the Duffy glycoprotein. The monoclonal antibody 2C3 agglutinated DARC-positive [Fy(a+) and Fy(b+)] but not DARC-negative [Fy(a−b−)] red blood cells. The specificity was shown by flow cytometry and immunoblotting [abstract; Blanchard et al, *Vox Sang* 74(Suppl 1): 71, 1998]. The monoclonal antibody MC5 against human CCR5 and the applicability on paraffin-embedded material have previously been described in detail [32]. CD3-positive T cells (Dako, Hamburg, Germany) and CD68-positive macrophages (Dako) were evaluated in patients with cGN when appropriate biopsy material was available. A monoclonal rat antibody (MECA-79, ATCC number HB-9479) against high endothelial venules in lymphoid tissues was used on human tonsils and renal transplant biopsies [34].

Immunohistochemistry was performed on 2 μm thick serial sections of formalin-fixed, paraffin-embedded material as previously described [32]. In brief, slides were deparaffinized in xylene and rehydrated in graded ethanols. Endogenous peroxidase was blocked by 3% H_2O_2 in methanol for 15 minutes. Antigen retrieval was performed by microwave treatment in a citrate-based buffer. First antibody was applied for one hour. For signal amplification and color development, a commercial biotin-streptavidin kit was used according to the instruction of the manufacturer (Super Sensitive Ready to Use Kit; Biogenex, San Ramon, CA, USA). After counterstaining with hemalaun, slides were embedded in Aquatex (Merck, Darmstadt, Germany).

For the quantitation of the immunohistochemistry, positive cells or positive vessels were counted in 10 high-power fields (×400) for each biopsy by one observer without knowledge of the diagnosis of the transplant biopsies. CCR5-positive cells were separately counted in the interstitium and in glomeruli. Mean cell counts were compared by Wilcoxon test. Correlations were evaluated with the Spearman-Rho correlation coefficient, and $P < 0.05$ was considered statistically significant. Bars give the SEM.

RESULTS

DARC expression on two different types of venules in the tonsil, including the sites of leukocyte recirculation

The monoclonal anti-DARC antibody (2C3) with specificity for the Fy6 antigen was used on human tonsils to optimize the immunostaining conditions. To identify the distribution of DARC-positive venules, we used the monoclonal rat antibody MECA-79. This antibody binds to addressins on high endothelial venules of lymph nodes, which serve as adhesion molecules involved in lymphocyte homing [35, 36]. DARC is expressed on two types of venules in human tonsils, as shown in Figure 1. The first and most common type of DARC-positive endothelium has the same distribution and morphology as high endothelium venules stained with MECA-79 (Fig. 1B, C). The second are venules with a flat endothelium, which display no reactivity with MECA-79 (data not shown). Omitting the first antibody completely abolished the signal (Fig. 1A).

Fig. 1. Immunohistochemistry for the Duffy antigen receptor for chemokines (DARC) and rat monoclonal antibody (MECA-79) on human tonsils and for DARC on pretransplant control biopsies. (*A*) Negative control (first antibody replaced by diluent, original ×400). (*B*) Parallel section stained with a monoclonal antibody against DARC (2C3, original ×400). Note the two different types of venules stained for DARC with high endothelium (arrow) on the left and flat endothelium on the right (arrowhead). (*C*) High endothelial venules stained with MECA-79 (arrow, original ×200). In pretransplant renal biopsies (*D* and *E*), only a few DARC-positive small postcapillary venules are seen (arrow, D, original ×100) and a single DARC-positive venule in the lower half of E (arrowhead, original ×200).

The number of DARC-positive venules in normal renal tissue was low

In contrast to the recent description of the expression of MECA-79 in heart transplantation, we did not find any MECA-79 reactivity in over 20 renal specimens, ranging from normal renal tissue to severe transplant rejection (data not shown) [37]. In contrast, DARC staining was present on some peritubular venules in pretransplant biopsies (Fig. 1D, E). Vessels that stained positive for DARC were on average larger in diameter compared with the size of the vessel wall than the majority of interstitial capillaries. Although they did not stain with MECA-79, endothelial cells were larger and higher, similar to those seen in high endothelial venules (as compared with other peritubular vessels). Taking these morphological findings together, we assumed that these vessels represent postcapillary venules. For simplicity, they are referred to as venules for the remainder of this article. No DARC expression was found on the endothelium of glomerular capillaries or on endothelium of arteries. In pretransplant biopsies, the mean number of DARC positive venules was low (0.3 positive vessels per high-power field). The distribution of DARC in the normal kidney is consistent with previous publications [11, 14].

The number of DARC-positive venules increased during transplant rejection

In allograft biopsies from patients with acute rejection, the mean number of DARC-positive venules is significantly higher than in pretransplant biopsies (Figs. 2B, D; 3; and 4A). DARC-positive venules were especially prominent at sites of strong interstitial leukocyte infiltration (Fig. 2A). There was no difference regarding the expression pattern and number of DARC-positive venules between patients with interstitial and vascular rejection (Fig. 3). Even in vascular rejection, no DARC expression was detected on endothelium of glomerular capillaries and arteries. Attachment of leukocytes to DARC-positive endothelia and transmigration was apparent in transplant rejection, as illustrated in Figure 4F.

The up-regulation of DARC was not specific for allograft rejection but was a feature of cGN

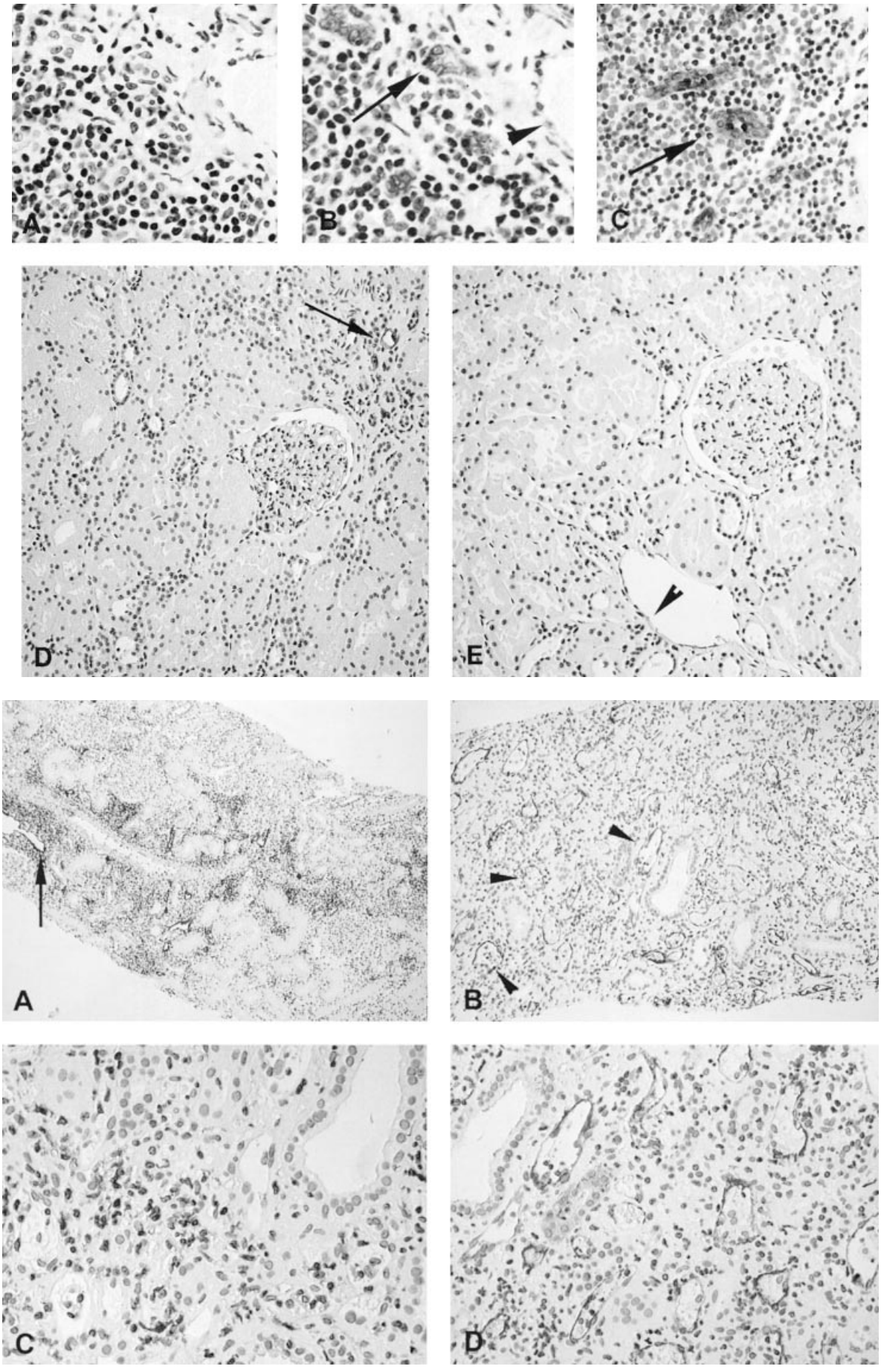
In biopsies with cGN, the distribution of DARC at sites of interstitial infiltration closely resembled the staining pattern in biopsies undergoing transplant rejection (Figs. 3 and 5C, D). Attachment of leukocytes to DARC-positive endothelia of interstitial venules was a common feature (Fig. 5C). In contrast to the prominent increment of DARC-positive interstitial venules, we could not detect DARC staining on glomerular endothelium. Liu et al described DARC-positive cells outside the endothelium in interstitial infiltrates [14]. We found only a very small number of DARC-positive cells outside the endothelium. These cells did not show a distinct pattern and could not be further characterized.

The tubulointerstitium was the main site of CCR5-positive leukocyte infiltration

The number of CCR5-positive infiltrating leukocytes in pretransplant biopsies was low (Fig. 3). The up-regulation of DARC was accompanied by an infiltration with CCR5-positive cells during acute transplant rejection (Fig. 3). A major part of the leukocytes infiltrating the renal allograft interstitium expressed CCR5 during rejection episodes (Fig. 4B, C, E), and CCR5-positive leukocytes were present at all sites of allograft rejection, that is, tubules, endothelia, and occasionally glomeruli (Fig. 4C–E). A single biopsy with acute vascular transplant rejection and acute transplant glomerulopathy showed a high number of CCR5-positive cells within the glomeruli.

In cGN, as in other glomerular diseases, the main site of CCR5-positive cell infiltration was the tubulointerstitium (Fig. 5B, E) [32]. A prominent clustering of CCR5-positive cells was apparent in the interstitium surrounding the glomeruli in early cell-rich stages of cGN resembling cellular cuffing of glomeruli (Fig. 5E). Forty percent of cellular crescents contained CCR5-positive cells (Fig. 5A, B), while CCR5-positive cells were rarely found in intact glomerular capillary loops, consistent with our previous observation in other forms of glomerulonephritis [32].

Fig. 2. Immunohistochemistry of renal transplant biopsies from patients with acute vascular rejection stained with a monoclonal anti-DARC antibody (*A*, original ×100; *B*, original ×200; and *D*, original ×400) or with a monoclonal anti-CCR5 antibody (*C*, original ×400). Note the prominent DARC expression on venules located in areas of interstitial infiltration in A (arrow). (B) Note the high number of DARC-positive venules (arrowhead, compare Figure 1D and E). (C) Note the number of CCR5-positive cells surrounding the DARC-positive venules on a parallel section of D.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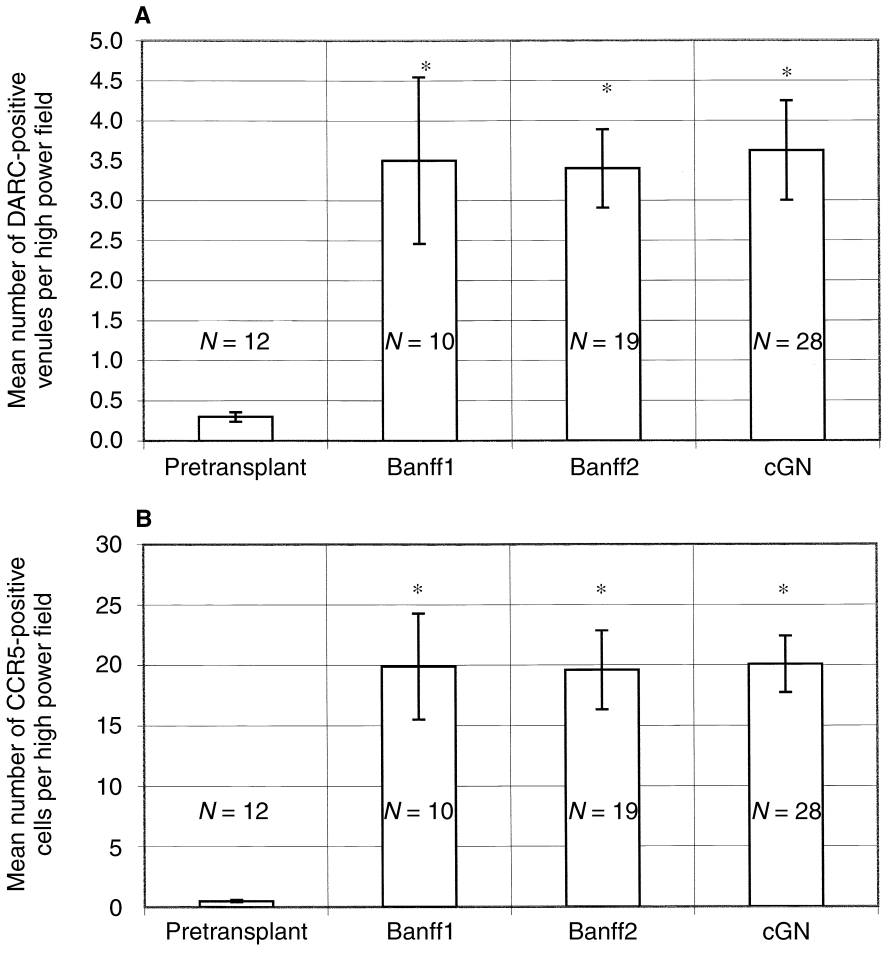

Fig. 3. Mean numbers of DARC-positive venules (*A*) and chemokine receptor 5 (CCR5)-positive cells (*B*) per high-power field in pretransplant biopsies, in biopsies with acute interstitial rejection (Banff 1, $P < 0.01$ vs. pretransplant), in biopsies with acute vascular rejection (Banff 2, $P < 0.01$ vs. pretransplant), and in biopsies with cGN ($P < 0.01$ vs. pretransplant).

Morphologically, two types of CCR5-positive cells were distinguishably clustered around and within cellular crescents. The majority of the cells consisted of small, round cells with prominent expression of CCR5 similar to most of the CCR5-positive cells within the interstitium (Fig. 5A). These cells were identified as CD3-positive lymphocytes on consecutive sections. The other cell type represented by larger cells exhibited a slightly weaker CCR5 staining (Fig. 5B). Morphologically and by immunohistochemistry on serial sections, these cells could be identified as CD68-positive macrophages. Interestingly, although we found CD68-positive cells within glomerular tufts, these cells did not express CCR5. In concordance with our previous data, we could not demonstrate CCR5 expression on intrinsic renal cells [32].

In transplant rejection, CCR5-positive cells and DARC-positive venules show a morphological and numerical correlation

As described previously in this article, DARC-positive venules were mainly localized in areas of interstitial leukocyte infiltration. A main part of these infiltrating cells expressed CCR5, and these cells could be located in and around DARC-positive venules (Figs. 2C and 4F, G). Besides the colocalization in areas of interstitial infiltration of CCR5-positive leukocytes and DARC-positive venules, the mean number of DARC-positive venules correlated significantly with the mean number of CCR5-positive cells ($R^2 = 0.35$, Spearman-Rho correlation coefficient, 0.659, $P < 0.01$).

To evaluate the time course of DARC expression and CCR5-positive cells, we obtained data from patients with repeated biopsies. Two patients with pretransplant biopsies and later acute vascular rejection showed a strong increase in both the number of interstitial DARC-positive venules and CCR5-positive cells. Figure 6 illustrates the course of the mean number of DARC-positive venules and CCR5-positive cells in a patient with seven biopsies of the same transplant. The numbers were low in two biopsies without signs of acute rejection. The first episode of interstitial rejection led to the strongest increment of DARC expression and CCR5-positive cells. One later episode of vascular rejection again led to another increase of both DARC-positive venules and

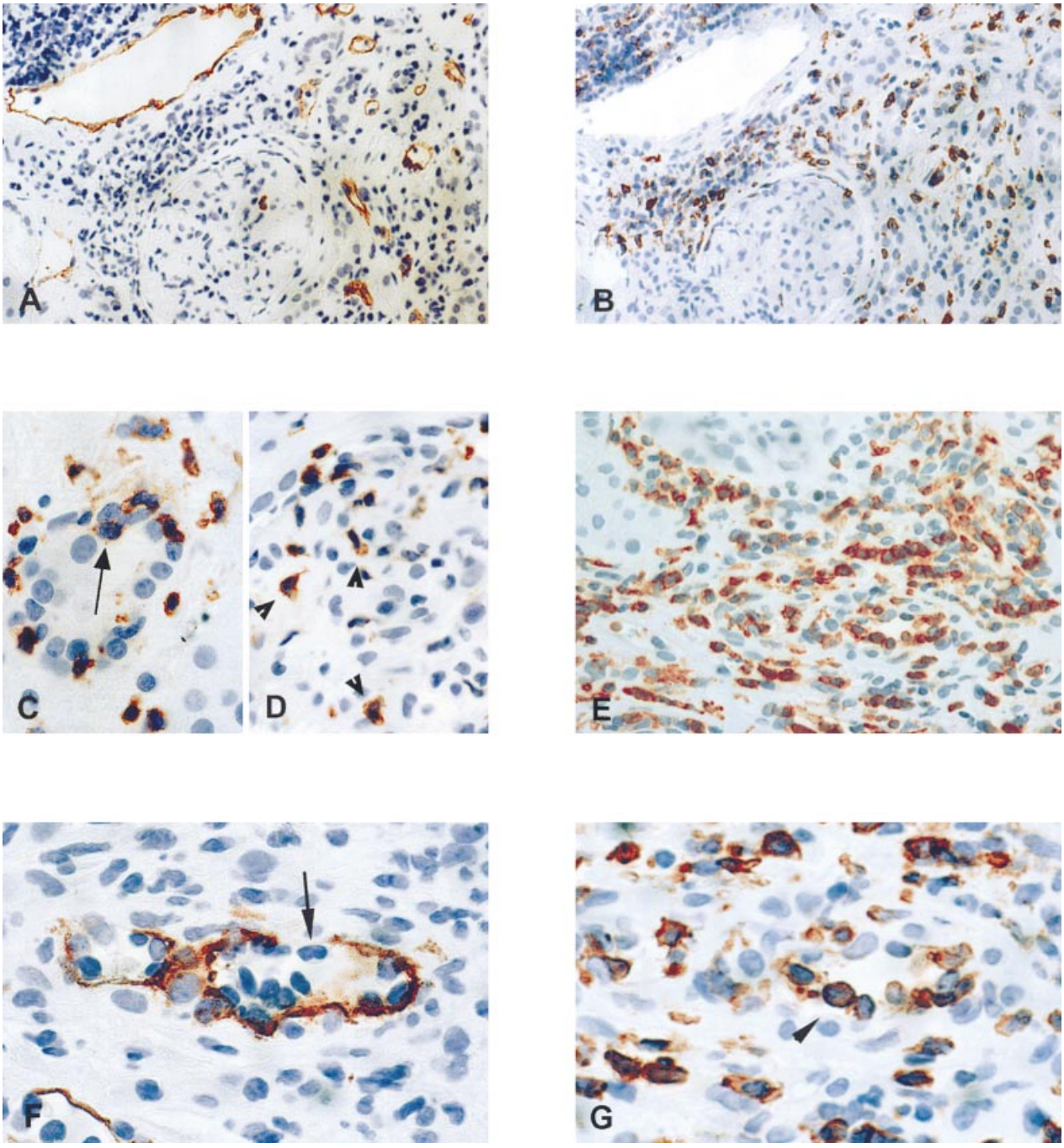

Fig. 4. Immunohistochemistry of renal transplant biopsies from patients with acute interstitial rejection (Banff 1, in *A, B*, and *C*) or with acute vascular rejection (Banff 2, in *D, E, F*, and *G*) stained with a monoclonal anti-DARC antibody (A, original ×200; F, original ×1000) and with a monoclonal anti-CCR5 antibody (B, original ×200; C, D, and E, original ×400; G, original ×1000). Note the high number of DARC-positive interstitial venules in A, but the absence of DARC on glomerular endothelium. (C and D) The infiltration of CCR5-positive cells in tubulitis (C) and acute allograft glomerulopathy (D). (E) The massive infiltration of the interstitium by CCR5-positive cells. (F and G) Parallel sections and colocalized CCR5-positive cells with a DARC-positive capillary. One leukocyte is just transmigrating the endothelium in F (arrow). Most of the cells within the DARC-positive vessel are CCR5 positive (arrowhead in G; G, original ×1000 is a higher magnification of E). Reproduction of this figure in color was sponsored by Fresenius Medical Care Deutschland GmbH.

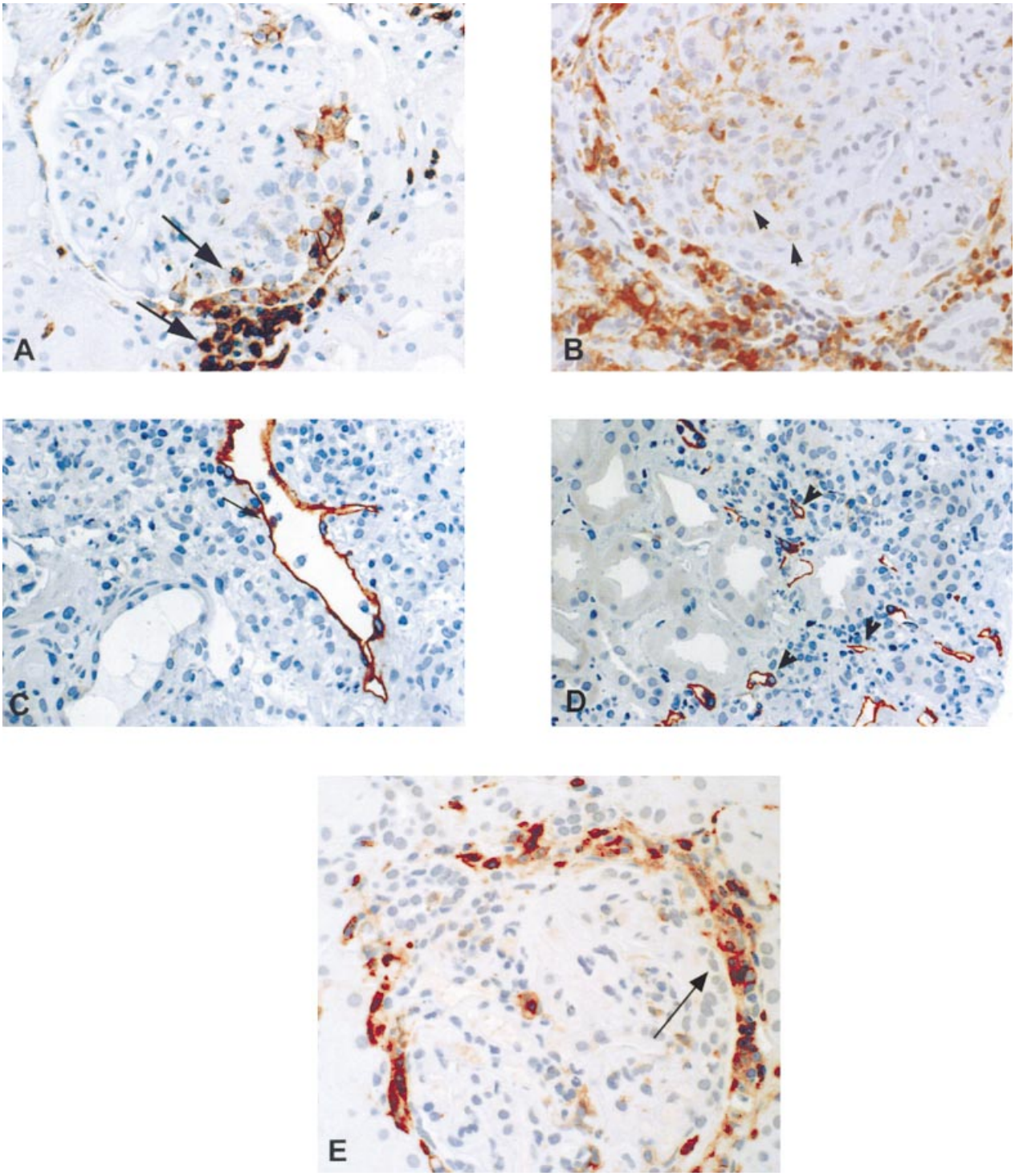

Fig. 5. Immunohistochemistry of renal biopsies from patients with cGN stained with a monoclonal antibody against CCR5 (MC5, *A*, *B*, and *E*, original ×400) and with a monoclonal antibody against DARC (2C3, *C* and *D*, ×400). Note the two different types of CCR5-positive cells in crescents: the small cells with strong CCR5 staining in A (arrow) and the large cell type with a weak membrane staining in B (arrowhead). A leukocyte sticks to the DARC-positive endothelium in C (arrow). DARC-positive venules are localized at sites of infiltration (arrowhead) in D. (E) Note the prominent clustering of CCR5-positive cells around Bowman's capsules. Reproduction of this figure in color was sponsored by Fresenius Medical Care Deutschland GmbH.

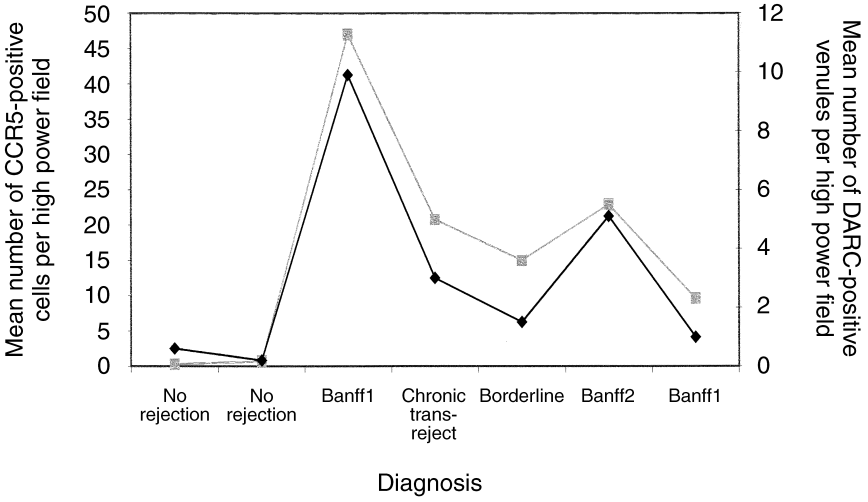

Fig. 6. The mean number of DARC-positive venules (right) and CCR5-positive cells (left) in seven biopsies of the same transplant. The diagnoses are given on the *x* axis (chr. tx: signs of chronic transplant rejection; borderline, borderline lesions). Note the parallel course of (◆) DARC-positive venules and (■) CCR5-positive infiltrating cells.

CCR5-positive cells. This case illustrates the up-regulation during an acute episode of transplant rejection and a decrease of the number of DARC-positive venules in a later biopsy without acute rejection. Furthermore, it shows a parallel course of DARC-positive venules and CCR5-positive cell infiltration.

DISCUSSION

The major findings of this study can be summarized as follows: (*1*) DARC is expressed on the endothelium of a low number of postcapillary venules in the normal human kidney. In contrast to a high expression in the endothelium of tonsils, DARC-positive endothelial cells in the kidney do not express the addressins labeled by MECA-79. (*2*) During cell-mediated injury of transplant rejection or cGN, the expression of DARC increases markedly on the endothelium of venules, but not on glomerular or arterial endothelium. In contrast to the description in heart transplants, we found no MECA-79 reactivity on renal endothelia during transplant rejection and in normal specimens. (*3*) DARC-positive peritubular venules are located in areas of interstitial infiltrates consisting mainly of CCR5-positive cells. During transplant rejection, this results in a positive correlation between the mean number of DARC-positive venules and CCR5-positive cells. In individual cases, a parallel rise and fall of DARC-positive venules and CCR5-positive interstitial infiltrates during rejection episodes could be documented. (*4*) Intrinsic renal cells do not express CCR5 in normal kidneys during transplant rejection and cGN. CCR5-positive leukocytes account for a major part of the interstitial infiltrates during transplant rejection and cGN. Crescents contain two types of CCR5-positive cells, which morphologically and by immunohistochemistry represent T cells and macrophages. In contrast, CCR5-positive cells were rare within the glomerular tuft in either transplant rejection or cGN.

Expression of DARC in the normal kidney has been described by immunohistochemistry and in situ hybridization with somewhat conflicting results. Consistent with our data, DARC expression was found on a subset of endothelial cells lining postcapillary venules and peritubular capillaries [11, 14, 38]. Chaudhuri et al demonstrated DARC expression on endothelium of glomeruli and principal cells of collecting ducts, in addition to peritubular capillaries in a detailed morphological study [38], whereas other groups found no DARC expression on tubular epithelium or glomerular capillaries in the normal kidney [11, 14]. In concordance with Liu and Hadley, we did not detect DARC expression on glomerular endothelial cells in control biopsies. The differences in DARC expression on tubular epithelia and glomerular endothelium between these studies may be due to different underlying diseases, different sensitivities and specificities of the applied antibodies, for example, monoclonal versus polyclonal, or to differences in tissue preparations, for example, frozen versus paraffin-embedded material. At present, clear evidence exists for DARC expression on endothelia of a restricted type of venules in the normal kidney, and we describe a markedly increased DARC expression on endothelia of peritubular venules during transplant rejection and cGN.

The monoclonal antibody MECA-79 binds to several L-selectin ligands, which are involved in lymphocyte homing to lymph nodes [39]. A differential expression of DARC and colocalization with MECA-79–positive high endothelial venules has recently been described [40]. The absence of MECA-79 staining during renal allograft rejection is in contrast with an up-regulation of MECA-79 reactivity during heart transplant rejection, and points toward differences in the counter-receptors

or the decoration of counter-receptors involved in the infiltration of renal allografts [37].

The up-regulation of DARC on endothelial cells at sites of inflammation leads to the question of DARC function. In lymphoid organs such as tonsils, DARC is constitutively expressed on high endothelial venules, the site of T-cell transmigration, and homing. In the kidney, DARC is expressed at sites where the extravasation of leukocytes into the interstitium is thought to take place. The cascade of interactions between adhesion molecules, chemokines, and chemokine receptors during adhesion and transmigration of leukocytes is most effective in vascular beds with low shear stress. In the kidney, these are postglomerular capillaries and venules, the site of DARC up-regulation. In fact, adherent and transmigrating leukocytes could be observed on DARC-positive endothelia of venules during transplant rejection and cGN (Figs. 4F and 5C). The parallel course of changes in DARC-positive venules and CCR5-positive leukocyte infiltrates observed in serial biopsies from one transplant strongly suggests the participation of DARC and CCR5 in a common process during transplant rejection (Fig. 6). As a hypothesis, we propose that chemokines may be presented by DARC on the surface of activated endothelia in postglomerular venules and thus participate in the local adhesion and transmigration of CCR5-positive cells. Besides our morphological data, experimental studies support this hypothesis. Middleton et al demonstrated the localization of the DARC ligand IL-8, but not MIP-1α on the luminal surface of endothelial cells after intradermal injection [41]. An alternative hypothesis of DARC acting as a "sink" for chemokines and perhaps functioning as an anti-inflammatory molecule cannot be ruled out by our morphological data. However, the recent description of abnormalities in the leukocyte recruitment in DARC-deficient mice would be more consistent with our hypothesis [42]. As these mice did not display a phenotype under normal conditions, the main role of DARC seems to be during pathological responses. In addition to the erythroid-specific mutation leading to the "bone marrow silent" in blacks, apparently healthy true Fy-null individuals have been described, which is consistent with this view [43–45].

An up-regulation of the CCR5 ligands RANTES, MIP-1α, and MIP-1β during transplant rejection and cGN, and the beneficial effect of chemokine blockade have been described in animal models [reviewed in 5]. In a previous study, we have shown that in various forms of glomerular diseases, CCR5-positive cells are rare within the glomerular tuft, but constitute a major part of the interstitial infiltrate and correlate with impaired renal function [32]. Our present results show that in cGN, a considerable number of CCR5-positive leukocytes are present within crescents, while CCR5-positive leukocytes are rare in the glomerular tuft. This is in concordance with the expression of the CCR5 ligands MIP-1α and MIP-1β [28]. We could distinguish two subsets of CCR5-positive cells, most likely representing macrophages and T cells in the crescents. The presence of CCR5-positive macrophages in crescents and their absence within the glomerular tuft of cGN or noncrescentic lupus nephritis imply differences in chemokine receptor expression between certain macrophage populations, as CCR5-negative macrophages are present in the glomerular tuft in these biopsies. Of interest is the marked periglomerular infiltrate with CCR5-positive cells. These might be involved in the rupture of Bowman's capsule, leading to the invasion of interstitial cells, that is, T cells and fibroblasts, and result in irreversible fibrosis of the glomerulus, as described in animal models [46].

Although crescent formation is a feature of severe glomerular injury, we did not detect DARC expression on endothelial cells of glomerular capillaries. Thus, the positive correlation of DARC-positive endothelia with CCR5-positive infiltrates also holds true in the negative sense; that is, DARC-negative endothelia rarely show CCR5-positive infiltrates.

In summary, our results show a marked up-regulation of DARC on postglomerular endothelia in areas of interstitial and periglomerular infiltrates in both transplant rejection and cGN. These areas are also those with a CCR5-positive leukocyte infiltrate. The concordance of these phenomena suggest that DARC on endothelia and chemokine receptors on leukocytes, together with their ligands such as RANTES, may work hand in hand during leukocyte adhesion and transmigration in cell-mediated renal injury.

NOTE ADDED IN PROOF

A new nomenclature for chemokine receptors has been published, in which Duffy was separated from chemokine receptors with known signal transduction and is referred to as a chemokine-binding protein.

Murphy PM, Baggiolini M, Charo IF, Hebert CA, Horuk R, Matsushima K, Miller LH, Oppenheim JJ, Power CA: International union of pharmacology. XXII. Nomenclature for chemokine receptors. *Pharmacol Rev* 52:145–176, 2000.

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