Expression of cell adhesion molecules in primary renal disease and renal allograft rejection

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Abstract

Background. In vitro studies have demonstrated that inflammatory mediators such as the cytokines TNFα and IL-1 upregulate or induce de novo expression of cell adhesion molecules on endothelial and epithelial cells. In the present study the expression of the cell adhesion molecules ICAM-1, VCAM-1, E-selectin and PECAM-1 was investigated in renal biopsies from patients with primary renal diseases (n = 66) and from renal allograft recipients (n = 42).

Methods. Expression of the cell adhesion molecules was determined by immunohistochemistry of frozen sections using monoclonal antibodies directed against PECAM-1, ICAM-1, VCAM-1, E-selectin and MHC class II molecules (APAAP method).

Results and Conclusions. PECAM-1 and ICAM-1 were expressed in the renal vasculature and disappeared in obliterated glomeruli with endothelial cell destruction. ICAM-1 but not PECAM-1 was upregulated in renal endothelia in acute allograft rejection and inflammatory primary renal diseases. Tubular de novo expression of ICAM-1 and VCAM-1 correlated with severe structural damage of the renal parenchyma including interstitial fibrosis. Vascular and/or glomerular VCAM-1 and E-selectin expression was pronounced in severe acute allograft rejection and also reflected the intensity of inflammatory reactions in primary renal diseases with or without autoimmune disorders. De novo expression of VCAM-1 and E-selectin in renal vessels and/or glomeruli and overexpression of ICAM-1 are markers of acute and severe inflammatory processes in biopsies from allograft recipients and patients with primary renal diseases.

Key words: E-selectin; ICAM-1; inflammatory kidney disease; PECAM-1; transplantation; VCAM-1; IL-1: interleukin 1; MHC: major histocompatibility complex; PECAM-1: platelet endothelial cell adhesion molecule-1; TNFα: tumour necrosis factor α; VCAM-1: vascular cell adhesion molecule-1.

Introduction

Infiltration of the renal parenchyma with inflammatory cells, including lymphocytes, monocytes, and granulocytes characterizes the histological picture of inflammatory kidney diseases, autoimmune disorders and renal allograft rejection [1,2].

In the earliest stages of inflammatory processes, leukocytes interact with the endothelial cell lining of the vasculature [3]. Endothelial cell injury promotes endothelial cell—leukocyte interaction that is mediated by cell surface adhesion molecules to support leukocyte adhesion and transmigration [4]. E-selectin, which belongs to the selectin family, turns up very early in the inflammatory process. It binds to sialylated glycoproteins on leukocytes to promote the weak attraction and rolling of leukocytes on endothelial cells [5]. The immunoglobulin-like molecules ICAM-1 and VCAM-1 are responsible for the firm secondary endothelial cell—leukocyte adhesion followed by transmigration of leukocytes through the endothelial cell lining [6]. ICAM-1, which binds to the β2-integrins LFA-1 and Mac-1 on leukocytes is expressed constitutively on endothelial cells and leukocytes and upregulated by cytokines, such as IL-1 and TNFα [7]. VCAM-1 expression is induced by cytokines on activated endothelial cells and some epithelial cells and binds to the β1-integrin VLA-4 [8]. Furthermore, ICAM-1 and VCAM-1 support T-cell activation by acting as costimulatory signals on target cells [9]. The role of PECAM-1 in leukocyte—endothelial cell interaction is not yet completely understood. It binds to itself or to the αvβ3-integris and is expressed on many endothelial cell types as well as on monocytes and platelets [10].

The aim of the present study was to determine the expression of the cell adhesion molecules ICAM-1,
VCAM-1, E-selectin and PECAM-1 in renal endothelial and epithelial cells in inflammatory kidney diseases and different forms of transplant rejection. To that end, the histopathological picture of inflammatory and non-inflammatory renal diseases and transplant rejection was correlated with the expression patterns of adhesion molecules.

Subjects and methods

Patients

One hundred and eight renal biopsies were investigated; 66 were collected from patients with unclear primary renal disease and 42 from allograft recipients. The biopsies of five patients with unclear primary renal disease had only minimal glomerular changes and were therefore selected as 'normal' renal tissue. In addition, five tissue samples from the disease-free part of tumour nephrectomies were investigated. The group of patients biopsied because of unclear primary renal disease comprised 27 women and 39 men with a mean age of 49 years. The distribution of histopathological diagnoses of this group is listed in Table 1. The group of patients with renal allografts consisted of 22 female and 20 male patients with a mean age of 46 years with different forms of allograft rejection (11 with minimally aggressive rejection, 16 with moderately and severely aggressive rejection, and six with chronic rejection), acute tubular necrosis (n = 5), and de novo or recurrent glomerulonephritis in their transplants (n = 4). Allograft recipients were treated with a standard immuno-suppressive therapy of steroids and cyclosporin A with or without azathioprine. Antilymphocyte antibody therapy was not given before biopsy. The patients with primary renal disease did not have any immunosuppressive treatment at the time of biopsy, with the exception of two patients with systemic lupus erythematosus (SLE) and two patients with severe nephrotic syndrome, who were already under therapy with steroids, cyclophosphamide or cyclosporin A.

Monoclonal antibodies

The following mouse monoclonal antibodies were used for the detection of adhesion molecules in frozen sections of kidney biopsies: anti-human ICAM-1 (Clone BBIG-I1), anti-human VCAM-1 (BBIG-V1) and anti-human E-selectin (BBIG-E4), were obtained from H. Biermann AG, Bad Nauheim, Germany; anti-human PECAM-1 (JC/70A) was from DAKO Diagnostika, Hamburg, Germany and anti-human MHC class II (B8.12.2) from Immunotech, Hamburg, Germany. Mouse IgG was used as a negative control (Sigma, Deisenhofen, Germany). Alkaline phosphatase-labelled goat anti-mouse secondary antibody, the APAAP detection system and the New Fuchs substrate system were obtained from DAKO Diagnostika (Hamburg, Germany). All other reagents were of the highest analytical grade available.

Immunohistochemistry

Tissue from renal biopsies was divided into two parts. One portion was fixed with 4% paraformaldehyde in PBS (phosphate-buffered saline) and paraffin-embedded for diagnostic evaluation. The other portion was snap-frozen in liquid-nitrogen-cooled isopentane and stored at −70°C until use. Immunohistochemistry was carried out on 5μm cryostat sections which were dried overnight at 37°C and stored at −20°C. Sections were fixed with acetone for 20 min at room temperature. After rinsing, sections were incubated for 2h with mouse monoclonal antibodies directed against ICAM-1 (1:8,000), VCAM-1 (1:500), E-selectin (1:100), PECAM-1 (1:200), MHC class II (1:25), and mouse IgG (1:100) at room temperature. After rinsing, sections were incubated with an alkaline-phosphatase-labelled secondary goat anti-mouse antibody (1:40) for 30 min and with the

Table 1. De novo expression of ICAM-1, VCAM-1 and E-selectin in primary renal diseases. The Table summarizes the results of 71 renal biopsies. Tissue specimens, which were defined as 'normal kidney', were derived from five patients with minimal glomerular changes and from the disease-free part of five tumour nephrectomies. E-selectin was never expressed in tubules and ICAM-1 was always present in the vasculature of normal and diseased kidneys, but was overexpressed in inflammatory diseases (see Results). In the patient with HSP, E-selectin was expressed in the glomerular tuft. Abbreviations: GN, glomerulonephritis; SLE, systemic lupus erythematosus; n, number of biopsies studied.

<table>
<thead>
<tr>
<th>Histopathological diagnosis</th>
<th>Tubules</th>
<th>Vasculature</th>
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<tbody>
<tr>
<td></td>
<td>ICAM-1</td>
<td>VCAM-1</td>
<td>E-selectin</td>
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<tr>
<td>Normal kidney</td>
<td>–</td>
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<td>–</td>
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<tr>
<td>Acute tubular necrosis (ATN)</td>
<td>3/6</td>
<td>3/6</td>
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<tr>
<td>Interstitial nephritis</td>
<td>3/4</td>
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<td>Amyloidosis</td>
<td>1/3</td>
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<tr>
<td>Diabetic glomerulonephrosis</td>
<td>–</td>
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<tr>
<td>Nephrosclerosis</td>
<td>–</td>
<td>1/3</td>
<td>–</td>
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<tr>
<td>Focal segmental glomerulonephrosis</td>
<td>–</td>
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<td>(FSG)</td>
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<tr>
<td>Membranous GN</td>
<td>2/9</td>
<td>4/9</td>
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<tr>
<td>Mesangial proliferative GN (IgA nephropathy)</td>
<td>4/12</td>
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<tr>
<td>Postinfectious GN</td>
<td>1/1</td>
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<tr>
<td>Focal necrotizing GN</td>
<td>3/9</td>
<td>9/9</td>
<td>7/9</td>
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<td>Lupus nephritis (SLE)</td>
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<td>Henoch–Schoenlein purpura (HSP)</td>
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<td>1/1</td>
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<tr>
<td>Haemolytic uraemic syndrome (HUS)</td>
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APAAP-detection system for another 30 min. After repeating the last two steps of the staining protocol (each for 10 min) sections were developed with New Fuchsin substrate system, weakly counterstained with Mayer’s haematoxylin solution and subsequently mounted on glycerol gelatin. Primary and secondary antibodies were diluted in 1% bovine serum albumin/PBS. Detection of MHC class II antigens was only performed on biopsies of 38 patients with unclear primary renal disease and not on specimens from renal allografts.

**Results**

**Expression of cell adhesion molecules in normal kidneys**

In normal kidneys a strong and homogenous expression of PECAM-1 was found exclusively on endothelial cells of glomerular and peritubular capillaries (Figure 1A) and of larger vessels (data not shown). ICAM-1 showed a similar distribution as PECAM-1, but was less strongly expressed and found predominantly in glomerular capillaries (Figure 1B) and large vessels (Figure 1C). VCAM-1 was always detected on epithelial cells of Bowman’s capsule (Figure 1D). E-selectin was not expressed in normal kidneys. MHC class II antigens were moderately expressed in peritubular capillaries, weakly in glomerular endothelial cells, but not in larger vessels (data not shown).

**Expression of cell adhesion molecules in primary renal diseases**

Histopathologically inflammatory primary kidney diseases can be divided into diseases with a mainly glomerular, tubulointerstitial, or vascular involvement of the renal parenchyma.

**Focal de novo expression of ICAM-1 and VCAM-1**

In various primary renal diseases and correlated with the severity of tissue damage or the intensity of acute inflammatory reactions (Figure 2A and 2B). ICAM-1 was particularly localized in the brush-border of tubular cells, whereas VCAM-1 showed a cytoplasmic staining pattern or a predominant basolateral localization in tubules [11]. Details of the frequency of distribution in different renal diseases are described in Table 1. De novo tubular expression of VCAM-1 was more frequent and pronounced than that of ICAM-1. Apart from acute tubular necrosis and chronic renal disease with tubular damage, de novo expression of VCAM-1 was detected in acute interstitial nephritis with tubular infiltration and acute glomerulonephritis, especially focal necrotizing glomerulonephritis and autoimmune diseases (e.g. P-ANCA positive vasculitis, Wegener’s granulomatosis, or SLE).

De novo expression of VCAM-1 in the renal vasculature (small vessels and occasionally peritubular capillaries) was associated with early stages of acute inflammatory glomerular disease with or without autoimmune disorders. Vascular expression of VCAM-1 was not detected in acute tubular necrosis, interstitial nephritis, diabetic glomerulosclerosis, nephrosclerosis, amyloidosis, focal segmental glomerulosclerosis and mesangial proliferative glomerulonephritis (IgA-nephritis), but was detected in seven of nine cases of focal necrotizing glomerulonephritis (6 of 9 patients were P- or C-ANCA positive), in the two patients with SLE (Figure 2C), in the patient with Henoch-Schoenlein purpura (HSP), in one of three patients with haemolytic uraemic syndrome (HUS), and in two of nine patients with membranous nephropathy. In addition to VCAM-1, E-selectin expression was weakly present in one biopsy from a patient with focal necrotizing glomerulonephritis and in one biopsy with a diagnosis of SLE in a small vessel (Figure 4C). Vascular VCAM-1 expression correlated with the intensity of the inflammatory process (Table 1). In five patients MHC class II molecules were coexpressed with VCAM-1 in endothelia of large vessels (Figure 2D).

**Glomerular expression of VCAM-1**

Glomerular expression of VCAM-1 was localized in cellular crescents in rapid progressive glomerulonephritis and in segmental lesions of the capillary tuft (Figures 3A–C). Cellular crescents were observed in one of twelve biopsies with a diagnosis of mesangial proliferative glomerulonephritis and in five of nine biopsies from patients with focal necrotizing glomerulonephritis, where VCAM-1 was always expressed in a homogenous and strong pattern in the crescents, and in one case in the mesangial cells of the glomerulus. Moreover VCAM-1 was localized in segmental lesions of destroyed capillary tufts (Figure 3C). The only glomerular expression of E-selectin occurred in the patient with HSP, who died a few days after acute onset of disease from multiorgan failure (Figure 3D).

The expression of PECAM-1 in the renal vasculature was not affected by inflammatory kidney diseases. PECAM-1, however, was diminished in obliterated glomeruli with endothelial cell destruction, such as in amyloidosis, chronic glomerulonephritis or diabetic glomerulosclerosis (Figure 4A). ICAM-1 was overexpressed in glomeruli, peritubular capillaries and larger vessels in acute inflammatory processes, such as focal necrotizing or postinfectious glomerulonephritis or autoimmune disorders (Figure 4B).

**Expression of cell adhesion molecules in renal allografts**

During allograft rejection, the expression of PECAM-1 did not differ from normal kidneys (see Figure 1A). In kidneys with moderate and severe allograft rejection (n=16), characterized by tubulitis and/or vasculitis [12], ICAM-1 expression was diffusely increased in glomerular cells and particularly upregulated in peritubular capillaries (Figure 5A) and larger vessels (Figure 5B). Overexpression of ICAM-1 in renal endothelia was combined with a focal de novo expression of ICAM-1 on the apical surface of tubular cells in five of sixteen cases. These changes correlated with the severity of allograft rejection with respect to both, cellular tissue infiltration and clinical course of the rejection episode. In chronic rejection, ICAM-1 upregulation was less striking. In four cases of membranoproliferative glomerulonephritis of the renal allograft, ICAM-1 expression was not different from normal...
kidneys in the endothelium, but was expressed *de novo* in the tubular brush-border in two of four biopsies.

In moderate and severe allograft rejection, VCAM-1 was found to be expressed *de novo* on tubular cells \((n=8/16)\), peritubular capillaries \((n=3/16)\) and small vessels \((n=3/16)\) in a focal pattern (Figure 5C). In contrast, glomerular endothelial cells never expressed VCAM-1. In some cases of chronic transplant rejection with arteriosclerosis, VCAM-1 was expressed in non-glomerular endothelial cells of small vessels \((n=3/6)\) and single tubules. Occasionally, infiltrating leukocytes were VCAM-1-positive. In two of four cases of glomerulonephritis of the allograft VCAM-1 was focally expressed on tubules and in one case in a small vessel.
E-selectin was found only as focal E-selectin staining in single endothelial cells of peritubular capillaries in severe allograft rejection \((n=4/16)\). One of these patients had a recurrent HUS. De novo expression of E-selectin and VCAM-1 was associated with the most severe forms of allograft rejection resulting in subsequent transplant loss in 50% of these patients.

During acute tubular necrosis \((n=5)\) the expression pattern of adhesion molecules remained unchanged with the exception of weak focal tubular VCAM-1 expression in one case.

**Discussion**

The expression of the adhesion molecules ICAM-1, VCAM-1, E-selectin, and PECAM-1 was studied in...
normal renal tissue, renal allografts and biopsies from patients with primary renal disease by immunohistochemistry of frozen sections. The data indicate that immunohistochemical assessment of the vascular and/or glomerular expression of ICAM-1, VCAM-1 and E-selectin may serve as a diagnostic parameter of the severity of renal allograft rejection and reflect the intensity of inflammation in acute glomerulonephritis, particularly when associated with autoimmune diseases. In contrast, PECAM-1 expression was not affected by inflammatory processes, but reduced in obliterated glomeruli with endothelial cell destruction.
Tubular expression of cell adhesion molecules seems to be a marker of extensive structural damage, due to inflammatory processes, acute tubular necrosis, or chronic kidney disease.

The expression of VCAM-1 [11,13,14], or ICAM-1, VCAM-1 and E-selectin [15,16] in renal allograft rejection has been examined by immunohistochemistry [11,13–16], in situ hybridization [13], electron-microscopy [11] or in combination with cell culture studies of renal tubular cells [16]. In most of the studies, vascular and tubular expression of VCAM-1 and vascular expression of E-selectin, as well as upregulation of ICAM-1 was found. The intensity and localization of the expression of the cell adhesion
molecules, however, varied between the different investigators depending on the antibodies used, the sample size of the investigated biopsies, and the selection of tissue specimens with respect to the severity of the rejection or the time after transplantation. In our patient population de novo expression of VCAM-1 and E-selectin, which was detectable in <25% of the 42 transplant biopsies, correlated with the severity of the rejection episode, suggesting that the expression of adhesion molecules could serve as an additional diagnostic or prognostic marker for allograft rejection.

In vitro experiments have demonstrated that upregulation or induction of adhesion molecules on endothelial or epithelial cells is dependent on the stimulation with cytokines such as TNFα, IL-1 and IFNγ [17]. Local overexpression of TNFα has been demonstrated...
in kidney biopsies during allograft rejection [18]. Therefore upregulation of adhesion molecules in the tissue of rejecting kidneys seems to be a logical step in the cascade of inflammatory tissue reaction. The patho-
genetic importance of the expression of adhesion mole-
cules, however, is not clearly understood. Adhesion molecules may mediate the infiltration process but may also serve as a costimulatory signal for T-cell-receptor activation by antigen presenting cells. Current immunosuppressive drugs do not seem to downregulate or to prevent cytokine-induced expression of adhesion molecules. Further clues to the definite role of adhesion molecules for transplant rejection will be obtained from interventional studies with anti-adhesion mole-
cules or oligonucleotides, such as ongoing clinical trials with anti-ICAM-1 or anti-LFA-1 in transplant patients [19,20].

The expression of adhesion molecules in inflammat-
ory primary renal diseases compared with non-
inflammatory renal diseases or biopsies without histopathological changes has been studied by only a few investigators, but with controversial results. Roy-Chaudhury et al. [21] examined a large number of biopsies and described a coordinate upregulation of adhesion molecules closely linked to chronic histo-
logical damage, irrespective of the primary diagnosis. In contrast to these data we and other groups [22–25] found a strong correlation between the expression of VCAM-1 in tubuli, glomeruli and vasculature and the diagnosis of acute necrotizing glomerulonephritis and autoimmune disease. Moreover we observed differences in the tubular versus vascular or glomerular expression of cell adhesion molecules in different renal diseases. Whereas tubular expression of ICAM-1 and VCAM-1 was correlated with structural tubular damage and interstitial fibrosis, vascular and glomerular expression of VCAM-1 and occasionally of E-selectin was strongly linked to acute severe inflammatory renal diseases, such as focal necrotizing glomerulonephritis with cres-
cent formation and glomerulonephritis in autoimmune disorders.

An increased cytokine production has also been described in experimental glomerulonephritis with autoimmune disorders, which would explain the upregu-
lation of the adhesion molecules ICAM-1, VCAM-1 and E-selectin in the diseased kidney [26]. Our observa-
tion of a concomitant induction of VCAM-1 and MHC class II molecules (see Figures 2C and 2D) in renal vessels agrees with the hypothesis of endothelial cell activation in those illnesses. Elevated levels of circulating ICAM-1 and VCAM-1 have been measured in the serum from patients with Wegener’s disease and SLE [27]. The glomerular expression of VCAM-1 was strik-
ing in the cellular crescents and in the segmental lesions of necrotizing glomerulonephritis. Glomerular epithelial cells are involved in extracapillary inflammation, but the reason for the accumulation of macrophages, epithelial cells and lymphocytes are unknown. Since VLA-4 positive leukocytes have been detected in glom-
erular crescents, it is possible that Bowman’s capsular cells with a strong expression of VCAM-1 attract

VLA-4 positive macrophages and lymphocytes which in turn could increase local cytokine production [21]. Whether VCAM-1 expression in segmental necrosis of the capillary tuft derived from endothelial cells or adjacent podocytes cannot be answered by immuno-
histochemistry, but could be investigated by electron-
microscopy.

The increased tubular expression of ICAM-1 and VCAM-1 in all forms of chronic renal disease might be the reason for or the consequence of chronic tubulo-intestinal infiltration in primary glomerular lesions, causing interstitial fibrosis and progression of renal disease [28].

Immunosuppression is the therapy of choice for autoimmune diseases and rapid progressive glomerulo-
nephritis [29]. In other forms of glomerulonephritis a significant therapeutical efficacy of immuno-
suppressants has not been proven [30]. In acute inflammatory renal diseases overexpression of cell adhesion molecules in renal vasculature and glomeruli could be an additional indicator for the probability of success of immunosuppressive therapy.

Whether therapies with anti-adhesion molecules are useful for the treatment of autoimmune disorders or crescentic glomerulonephritis has to be tested in experi-
mental models and, if successful, in clinical trials [31]. Sequential histological evaluation during the course of the disease is not possible in patients, but would be useful in animal models. Another target of immuno-
suppressive therapy could imply the regulation of transcription factors for genes encoding both cell adhe-
sion molecules and cytokines, such as NF-κB, that is upregulated in nephrotoxic nephritis, an animal model for autoimmune disease [32].

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