*Kidney International, Vol. 49 (1996), pp. 127–134*

# Adhesion molecule interactions in human glomerulonephritis: Importance of the tubulointerstitium

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**Adhesion molecule interactions in human glomerulonephritis: Importance of the tubulointerstitium.** Infiltration of leukocytes into glomerular and interstitial regions of the kidney is a key event in the pathogenesis of human glomerulonephritis. This process is mediated by specific adhesion molecules, some of which are expressed in a coordinated fashion following endothelial cell activation. We have assessed the pattern of expression of the selectins (E, P and L), and the counter-receptors LFA-1 and ICAM-1, and VLA-4 and VCAM-1 in 119 renal biopsies using sequential sections, and have correlated this with the degree of histological damage (tubular atrophy and interstitial fibrosis) and the intensity of the macrophage infiltrate. Sections were stained with monoclonal antibodies using a standard alkaline phosphatase anti-alkaline phosphatase (APAAP) technique. There were strong correlations between the following: (1) expression of LFA-1, VLA-4, and L-selectin in the periglomerular region, interstitium and in focal interstitial infiltrates and the presence of macrophages in these regions; (2) *de novo* tubular expression of ICAM-1 and VCAM-1; (3) staining for ICAM-1 and VCAM-1 on focal cellular infiltrates within the interstitium; and (4) staining for E- and P- selectin on extraglomerular endothelium. These also strongly correlated with the degree of chronic histological damage. There was, however, no correlation between glomerular expression of adhesion molecules or glomerular macrophage infiltration and chronic histological damage. Although expression of VCAM-1 by the glomerular mesangium was strongly correlated with the presence of cells staining for VLA-4 within the glomerulus, glomerular expression of adhesion molecules correlated poorly with their expression in other sites. These results show that coordinated up-regulation of adhesion molecule expression in the tubulointerstitium is associated with interstitial fibrosis and tubular atrophy and may contribute, therefore, to the progression of renal disease.

Accumulation of leukocytes within the glomerulus and interstitium of the kidney is now accepted as one of the key pathogenetic mechanisms in both experimental and human glomerulonephritis. *In vitro* studies suggest that this involves a series of specific interactions between adhesion molecules expressed on circulating leukocytes and their ligands on endothelial and other cell types [1]. The three main groups of adhesion molecules involved in these interactions are the selectins, the integrins and certain members of the immunoglobulin supergene family.

To date, three selectins have been characterized. E-selectin is expressed predominantly by cytokine activated endothelium, L-selectin by circulating leukocytes and P-selectin by activated endothelial cells and platelets [1]. The selectins bind to a variety of carbohydrate/glycopeptide ligands. These include Sialyl Lewis$^x$ (binds to E-selectin, P-selectin and L-selectin), E-selectin ligand [ESL-1] which binds to E-selectin, P-selectin glycoprotein ligand [PSGL-1] which binds to P-selectin and GlyCAM-1 and CD34, both of which bind to L-selectin. [2, 3] The integrins are transmembrane, heterodimeric glycoproteins composed of non-covalently associated $\alpha$ and $\beta$ subunits. Two of the integrins involved in leukocyte-endothelial interactions come from the $\beta_2$ integrin family [CD11a/CD18 (LFA-1) and CD11b/CD18 (CR3, Mac-1)] while the other is the $\beta_1$ integrin VLA-4. These molecules are expressed predominantly on leukocytes. LFA-1 and Mac-1 (CR3) bind to ICAM-1, while VLA-4 binds to VCAM-1. Both ICAM-1 and VCAM-1 are members of the immunoglobulin supergene family and are expressed mainly on activated endothelium. More recently two further ICAM molecules have been identified, ICAM-2, which is constitutively expressed and is the counter-receptor for LFA-1 and ICAM-3 which also binds to LFA-1 [1, 4, 5].

*In vitro* studies suggest that the first step in leukocyte adhesion is 'rolling' of the leukocyte along the endothelium mediated by selectin-carbohydrate interactions [1, 4, 5]. The resultant decrease in cellular velocity brings leukocytes into close contact with chemoattractants (TNF-$\alpha$ and IL-8) released from the endothelial cell or inflammatory focus. These chemoattractants can activate leukocyte integrins by inducing a conformational change which permits the development of stronger interactions between these molecules and their respective counter-receptors ICAM-1 or VCAM-1 expressed on endothelial or other cell types [5].

A number of *in vivo* studies have demonstrated the importance of adhesion molecule interactions in the pathogenesis of experimental glomerulonephritis. Mulligan et al [6] have shown that antibodies to CR3 (Mac-1) and VLA-4 can decrease proteinuria in a rat model of nephrotoxic nephritis. More recently, Hill et al [7, 8] have used immunoelectron microscopy in the same model to demonstrate interactions between ICAM-1 and LFA-1 within the glomerulus and in the interstitium, while Tipping et al [9] have shown a reduction in glomerular neutrophil infiltration and proteinuria in nephrotoxic nephritis following administration of an antibody against P-selectin. Wuthrich et al [10, 11] have

Received for publication April 5, 1995
and in revised form August 3, 1995
Accepted for publication August 3, 1995

**Table 1.** Renal biopsy diagnoses

| No. | Diagnosis | | *N* |
|---|---|---|---|
| 1. | IgA nephropathy | | 27 |
| 2. | Other proliferative | | 9 |
| | Membranoproliferative glomerulonephritis | 3 | |
| | Mesangial proliferative glomerulonephritis | 3 | |
| | Post-infectious glomerulonephritis | 3 | |
| 3. | Vasculitis | | 7 |
| | Wegener's granulomatosus | 2 | |
| | Microscopic polyarteritis nodosa | 5 | |
| 4. | Crescentic glomerulonephritis | | 6 |
| 5. | Focal and segmental glomerulosclerosis | | 12 |
| 6. | Minimal change disease | | 13 |
| 7. | Membranous glomerulonephritis | | 10 |
| 8. | Amyloidosis | | 4 |
| 9. | Interstitial nephritis/acute tubular necrosis | | 6 |
| 10. | SLE | | 6 |
| 11. | Controls | | 9 |
| | Tumor nephrectomy specimens | 5 | |
| | Thin basement membrane nephropathy | 4 | |
| 12. | Others | | 9 |
| | Diabetes | 1 | |
| | Hypertension | 1 | |
| | Cryoglobulinemia | 1 | |
| | Chronic glomerulonephritis | 2 | |
| | IgA + acute tubular necrosis | 1 | |
| | IgA + membranous | 1 | |
| | Membranous + interstitial nephritis | 1 | |
| | No definite diagnosis | 1 | |

demonstrated glomerular and tubular upregulation of ICAM-1 and VCAM-1 in a mouse model of lupus nephritis.

Immunohistochemical studies have also described the pattern of expression of ICAM-1 and VCAM-1 in normal and diseased human kidneys [12–14]. However, no attempt has been made so far to analyze the expression of different adhesion molecules involved in interactions between the leukocyte and other cell types in the same kidney biopsy and to correlate this with the extent of histological damage. The aims of this study, therefore, were: (1.) To analyze and correlate the pattern of expression of the selectins (E, P and L), LFA-1, ICAM-1, VLA-4 and VCAM-1 in the same renal biopsy using sequential histological sections; and (2.) to correlate expression of the above molecules with the degree of histological damage and the extent of macrophage infiltration.

## Methods

### *Renal biopsies*

A total of 119 tissue blocks were used in this study. These comprised 114 renal biopsies and 5 specimens obtained from the non-involved pole of tumor nephrectomy specimens. The biopsies were obtained from two Glasgow hospitals (Glasgow Royal Infirmary and the Western General Hospital) and the Aberdeen Royal Infirmary. All the nephrectomy specimens were obtained from the Aberdeen Royal Infirmary. Specimens were snap frozen in liquid nitrogen and stored at −70°C until required. Histological diagnoses were supplied by the contributing Pathology Department and are listed in Table 1.

### *Immunohistochemical staining*

The antibodies used in this study were anti-LFA-1 (BB 16, Serotec, Kidlington, Oxford, UK), anti-VLA-4 (P4G9, Chemicon Int. Inc., Temecula, CA, USA), anti-ICAM-1 (B4H10, Serotec), anti-VCAM-1 (1G11B1, Serotec), anti-macrophage (FMC-33, Serotec), anti-E-selectin (E6, British Biotechnology Ltd., Abingdon, Oxford, UK), anti-P-selectin (AK 6, Serotec), and anti-L-selectin (DREG-56, Pharmingen, San Diego, CA, USA). Optimal dilutions for each antibody were obtained by titration experiments using normal and diseased human kidney. Six micron thick sections were cut on a cryostat at −20°C and then stored at −70°C until required. Sections were fixed in acetone for 20 minutes at room temperature, air dried for five minutes and incubated with the appropriate primary antibody for one hour. This was followed by 30 minute incubations with rabbit anti-mouse immunoglobulin (Dakopatts) diluted 1:20 in Tris-buffered saline and then with mouse alkaline phosphatase anti-alkaline phosphatase (APAAP) complexes (Dakopatts) diluted 1:40 in Tris-buffered saline, with the appropriate washes in between. Sections were then rinsed in tap water and developed using a substrate solution [Naphthol As Mx Phosphate (Sigma-Aldrich Co. Ltd., Poole, Dorset, UK)], Levamisole [Sigma] and Fast Red TRS salt [Sigma] in veronal acetate buffer) for 20 minutes. The slides were counterstained with hematoxylin and Scot's tap water solution and mounted using Apathy's aqueous mounting medium. For some antibodies (such as anti-VLA-4) an enhanced APAAP system was used in order to increase the sensitivity of the system. This entailed re-incubating the slides for 10 minutes each with secondary antibody and APAAP complexes prior to the addition of substrate. Negative controls comprised both normal and diseased kidneys in which the primary antibody was omitted.

### *Scoring of stained sections*

All sections were examined by a pathologist (JGS) who was blinded to their identity. Anatomical areas were chosen for scoring each antibody based upon a preliminary survey of staining patterns obtained with normal and abnormal tissues. We made a special attempt to score those regions in which there appeared to be *de novo* expression of a specific molecule. A semiquantitative scoring scale from 0 to 3 was used as follows: 0 = no specific staining, 0.5 = possibly positive, 1+ = weakly positive, 2+ = moderately positive, 3+ = strongly positive. Scoring was generally influenced by the extent rather than the intensity of staining.

Histological regions scored for staining with individual monoclonal antibodies were:

LFA-1: glomerulus, periglomerular region, interstitium, focal interstitial infiltrates

ICAM-1: glomerular endothelium, extraglomerular vascular endothelium, tubules, focal interstitial infiltrates

VLA-4: glomerulus, periglomerular region, interstitium, focal interstitial infiltrates

VCAM-1: glomerular mesangium, extraglomerular vascular endothelium, tubules, focal interstitial infiltrates

Macrophages: glomerulus, periglomerular region, interstitium, focal interstitial infiltrates

E-selectin: extraglomerular vascular endothelium

P-selectin: extraglomerular vascular endothelium

L-selectin: periglomerular region, interstitium, focal infiltrates

### *Chronic histological damage*

One hundred and seven of the 119 sections were also assessed for the degree of tubular atrophy and interstitial fibrosis. The following scoring scale was used: 0 = no damage, 0.5 = possible damage, 1+ = mild damage, 2+ = moderate damage, 3+ =

severe damage. Biopsies with a histological damage score of 0 to 1+ (no to mild damage) were compared against biopsies with a histological score of 2 to 3+ (moderate to severe damage) in some analyses (see below).

### *Statistics*

Spearman's correlation coefficient was used to identify correlations between (a) expression of different adhesion molecules in the same biopsy and (b) the degree of chronic histological damage and expression of a specific adhesion molecule in the same biopsy. The Kruskall-Wallis test with multiple comparisons was used to compare adhesion molecule expression between different groups (Groups 1 to 10 in Table 1) and against controls (Group 11). The Mann-Whitney *U*-test was used for comparing adhesion molecule expression in biopsies classified as showing severe histological damage as compared to biopsies with mild histological damage. All statistics were performed using the BMDP Statistical Software Package (BMDP Statistical Software, Inc., Los Angeles, CA, USA). A $P$ value of $< 0.05$ was considered to be significant.

## Results

### *Distribution of adhesion molecules in normal kidney*

*1. LFA-1 and ICAM-1.* Small numbers of LFA-1 positive cells were present within the glomerulus and in the periglomerular and interstitial areas. ICAM-1 was strongly expressed on the glomerular and extraglomerular endothelium (including peritubular capillaries), but was absent from the tubules and interstitium in normal kidney (Fig. 1).

*2. VCAM-1 and VLA-4.* Small numbers of VLA-4 positive cells were found in the glomerular, periglomerular and interstitial areas. There was weak (0.5 to 1+) expression of VCAM-1 within the glomerulus in a mesangial distribution and on the endothelium of extraglomerular vessels but not peritubular capillaries. Bowman's capsule was strongly positive for VCAM-1 in all kidneys. In contrast to ICAM-1 there was some tubular expression of VCAM-1 even in normal kidneys. There was strong staining of isolated cells in both proximal and distal tubules and weaker, diffuse staining of tubules, mainly on the basolateral side (Fig. 2).

*3. Selectins.* There was minimal staining for E-selectin and P-selectin on extraglomerular endothelial cells. Glomeruli were negative. L-selectin was restricted to occasional interstitial cells in normal kidneys.

### *Distribution of adhesion molecules in diseased kidney*

*1. LFA-1 and ICAM-1.* Large numbers of LFA-1 positive cells were present in the glomerular, periglomerular and interstitial regions in biopsies with an inflammatory infiltrate (Fig. 3). A number of diseased kidneys had *de novo* tubular expression of ICAM-1 and ICAM-1 positive cells were also present within focal interstitial infiltrates in diseased kidneys (Fig. 4). There were no major changes in the expression of ICAM-1 in glomerular and extraglomerular endothelium in disease, except for the specific instances discussed below. Staining for ICAM-1 on glomerular endothelium, however, was so strong that it may have been difficult to detect an increase in the sections.

*2. VCAM-1 and VLA-4.* Large numbers of VLA-4 positive cells were present in inflammatory infiltrates in the glomerular, periglomerular and interstitial regions. There was no change in the pattern of mesangial, Bowman's capsule or vascular expression of VCAM-1 in diseased kidneys. VCAM-1 positive cells were present in focal interstitial infiltrates and on all the cells of a tubular cross section in some diseased kidneys in contrast to expression by single tubular cells in normal kidneys (Fig. 5).

*3. Selectins.* There was a marked increase in E-selectin and P-selectin expression on the extraglomerular vascular endothelium in some biopsies with prominent interstitial infiltrates (Fig. 6). A large number of L-selectin positive cells were present within glomerular and interstitial infiltrates in diseased kidneys. In one biopsy from a patient with crescentic glomerulonephritis due to Wegener's granulomatosus, there was *de novo* expression of E-selectin and P-selectin on glomerular endothelium (Fig. 7).

*4. Macrophages.* A large number of infiltrating cells within the glomerulus and in the periglomerular and interstitial areas were found to be macrophages as assessed by staining with the monoclonal antibody FMC-33.

Negative controls which were routinely performed in all cases showed no positive staining (Fig. 8).

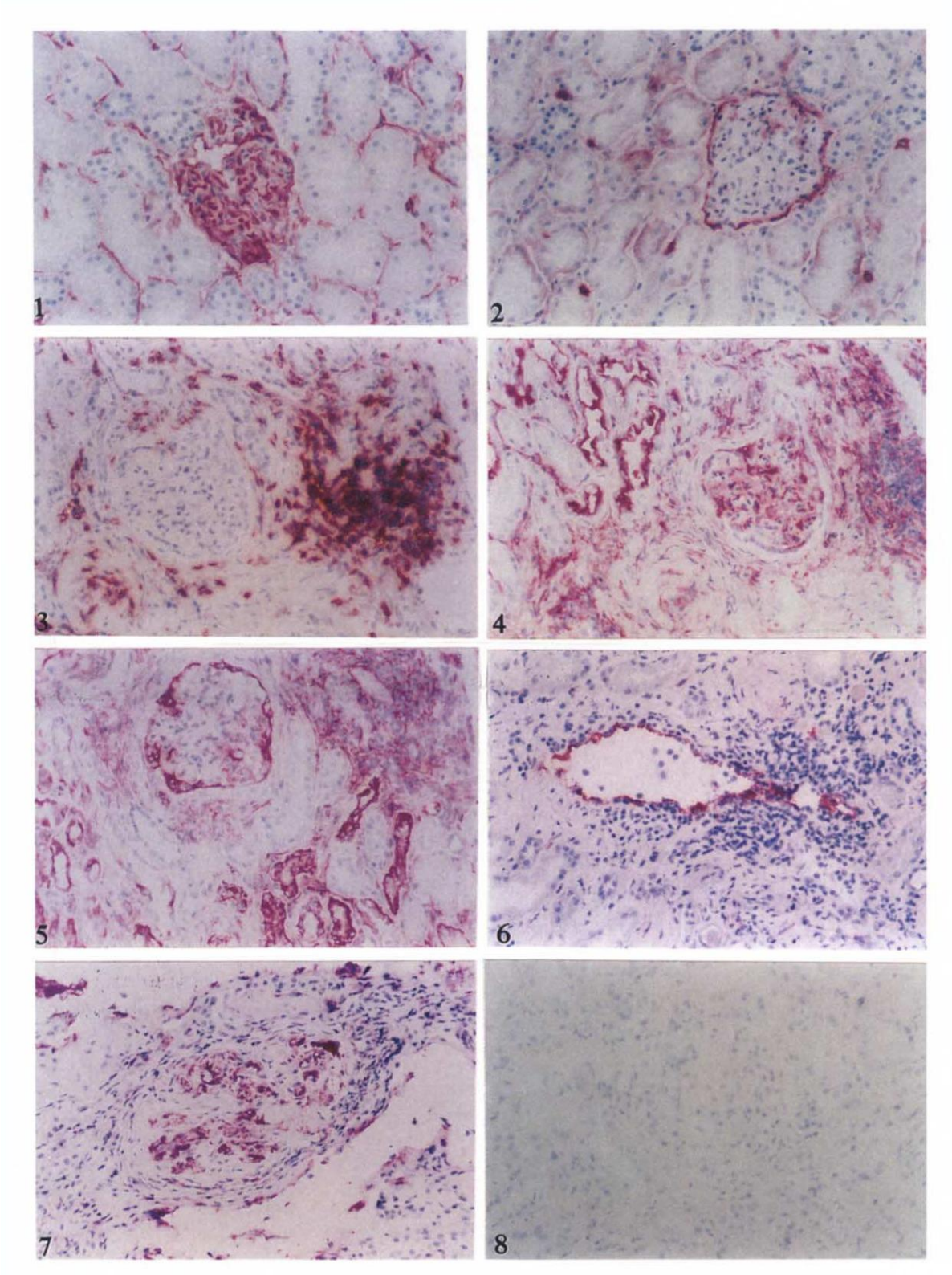
### *Comparison of adhesion molecule expression in different diagnostic groups*

There were relatively few differences when adhesion molecule expression in specific diseases was compared with their staining in controls. The Kruskall-Wallis test with multiple comparisons was used to compare groups 1 to 10 (Table 1) against group 11 (controls) for this analysis. Group 12 was not used because of the heterogeneity of diagnoses in this group. Glomerular endothelial expression of ICAM-1 was significantly greater in the control group than in minimal change disease ($P < 0.05$) and systemic lupus erythematosus ($P < 0.05$). Expression of ICAM-1 on extraglomerular vascular endothelium was also significantly greater in control biopsies than in crescentic glomerulonephritis ($P < 0.05$).

The individual groups (1 to 11) were then compared with each other. The only statistically significant differences in adhesion molecule expression was increased staining for LFA-1 and VLA-4 by extraglomerular inflammatory cells in acute tubular necrosis and acute interstitial nephritis, reflecting the intense interstitial infiltrate seen in these biopsies; this also occurred when these biopsies were compared with controls ($P < 0.05$).

### *Spearman's correlations between expression of different adhesion molecules in the same renal biopsy*

There were strong correlations between the expression of LFA-1, VLA-4 and L-selectin and the presence of macrophages in periglomerular and interstitial areas and in focal infiltrates within the interstitium (Table 2). There was a weaker correlation between staining for LFA-1 and VLA-4 in the glomerular and extraglomerular areas of the same renal biopsy. ICAM-1 and VCAM-1 were expressed on focal infiltrates in some biopsies and this generally correlated with extraglomerular (that is, periglomerular, interstitial and focal interstitial infiltrates) but not glomerular expression of LFA-1, VLA-4 and L-selectin and the presence of macrophages. The expression of L-selectin by cellular infiltrates in the interstitium correlated strongly with interstitial and periglomerular, but not glomerular, expression of LFA-1, VLA-4, the presence of macrophages and staining for ICAM-1 and VCAM-1 within focal interstitial infiltrates.




Staining for ICAM-1 and VCAM-1 on renal tubules correlated strongly with cellular infiltration, determined by staining for LFA-1, VLA-4, L-selectin, and macrophages, in extraglomerular areas (periglomerular region, interstitium and focal infiltrates) (Table 3). Although correlations with glomerular infiltration were weaker, tubular expression of ICAM-1 was positively associated with glomerular cells expressing VLA-4 and the presence of macrophages, while the latter was weakly associated with tubular

**Fig. 1.** *ICAM-1 expression in normal kidney.* Strong endothelial expression of ICAM-1 in the glomerulus and in the peritubular capillaries. There is no tubular expression of ICAM-1 and no ICAM-1 positive cells are present in the interstitium. APAAP ×200.

**Fig. 2.** *VCAM-1 expression in normal kidney.* Strong expression of VCAM-1 by Bowman's capsule cells. There is weak mesangial positivity within the glomerulus. Note the strong VCAM-1 expression in individual tubular cells (generally only one cell was strongly positive per tubular cross section). APAAP ×200.

**Fig. 3.** *LFA-1 expression in focal and segmental glomerulosclerosis.* Note the large number of LFA-1 positive cells within a focal interstitial infiltrate. A similar, although not identical pattern of staining was obtained for VLA-4, L-selectin and the macrophage marker FMC-33. APAAP ×200. (Figs. 3, 4 and 5 are sequential sections from the same renal biopsy).

**Fig. 4.** *ICAM-1 expression in focal and segmental glomerulosclerosis.* Note ICAM-1 positive cells within the interstitial infiltrate as well as *de novo* tubular expression of ICAM-1. There is no significant change in the expression of ICAM-1 on glomerular endothelium or peritubular capillaries as compared to controls. APAAP ×200. (Figs. 3, 4 and 5 are sequential sections from the same renal biopsy).

**Fig. 5.** *VCAM-1 expression in focal and segmental glomerulosclerosis.* Note VCAM-1 positive cells within the interstitial infiltrate. All the cells within some tubular cross section are positive in this biopsy. There is no change in the expression of VCAM-1 by Bowman's capsule cells or by the glomerular mesangium as compared to normal kidney. APAAP ×200. (Figs. 3, 4 and 5 are sequential sections from the same renal biopsy).

**Fig. 6.** *E-selectin expression in essential mixed cryoglobulinemia.* Strong expression of E-selectin on extraglomerular vascular endothelium in close proximity to a cellular infiltrate. E-selectin and P-selectin expression was usually linked to the presence of an inflammatory infiltrate. APAAP ×200.

**Fig. 7.** *E-selectin expression in Wegener's granulomatosus.* Strong expression of E-selectin by the glomerular endothelium in a patient with rapidly progressive glomerulonephritis due to Wegener's granulomatosus biopsied soon after admission. Staining with P-selectin was identical (not shown). APAAP ×200.

**Fig. 8.** *Negative control, IgA nephropathy.* Negative control staining in a patient with IgA nephropathy.

**Table 2.** Spearman's correlations between cellular infiltrates and adhesion molecule expression in human glomerulonephritis (representative data)

| | LFA-1 (g) | Mø (g) | LFA-1 (int) | Mø (int) | L-Sel (int) |
|---|---|---|---|---|---|
| LFA-1 (g) | 1 | | | | |
| Mø (g) | 0.32 (0.001) | 1 | | | |
| LFA-1 (int) | 0.31 (0.001) | 0.28 (0.008) | 1 | | |
| Mø (int) | 0.22 (0.028) | 0.33 (0.001) | 0.65 (0.0001) | 1 | |
| L-Sel (int) | 0.10 (0.369) | 0.18 (0.129) | 0.60 (0.0001) | 0.68 (0.0001) | 1 |
| ICAM-1 (inf) | 0.16 (0.139) | 0.23 (0.033) | 0.39 (0.0001) | 0.31 (0.002) | 0.45 (0.0001) |

Spearmans correlation coefficients between staining for adhesion molecules (LFA-1/ICAM-1/VCAM-1/L-selectin) and macrophages (Mø) within the glomerulus (g), in the interstitium (int) and in focal interstitial infiltrates (inf). Results (not shown) for VLA-4 (g and int) and for VCAM (inf) were similar to those for LFA-1 and ICAM-1, respectively. (Numbers in brackets indicate *P* values).

staining for VCAM-1. Expression of E-selectin and P-selectin on extraglomerular vascular endothelium also correlated strongly with extraglomerular cellular infiltration, again excepting the glomerulus, and with tubular expression of ICAM-1 and VCAM-1. ICAM-1 and VCAM-1 were expressed on focal infiltrates in some biopsies and this correlated with tubular staining for ICAM-1 and VCAM-1 and with E-selectin and P-selectin on extraglomerular vascular endothelium.

The following variables were interrelated: (1) staining for LFA-1, VLA-4, L-selectin and macrophages in the periglomerular region, interstitium and in focal cellular infiltrates; (2) tubular expression of ICAM-1 and VCAM-1; (3) staining for ICAM-1 and VCAM-1 on focal cellular infiltrates within the interstitium of the kidney; and (4) expression of E-selectin and P-selectin on extraglomerular vascular endothelium. The coordinate expression of the above molecules occurred regardless of the primary diagnosis.

In contrast, staining for ICAM-1 on the glomerular endothelium showed no strong correlations with any of the variables discussed. However, a very weak negative correlation with interstitial staining for VLA-4 was noted. There was a relatively weak correlation between mesangial expression of VCAM-1 and extraglomerular expression of VLA-4, LFA-1, L-selectin and the presence of macrophages. Within the glomerulus, there was a strong correlation between staining for VLA-4 and mesangial VCAM-1 (Table 3). There was, however, no significant relationship between expression of ICAM-1 on glomerular endothelium and glomerular staining for LFA-1. Glomerular macrophage infiltration was also not related to glomerular expression of ICAM-1 or VCAM-1.

### *Spearman's correlations between the degree of histological damage and the expression of leukocyte, tubular and vascular adhesion molecules*

The degree of interstitial fibrosis and tubular atrophy was assessed. This was then correlated with adhesion molecule expression. There was a strong and significant positive correlation between the degree of chronic histological damage and: (1) interstitial staining for LFA-1 ($r = 0.56$, $P < 0.0001$), VLA-4 (0.56, $P < 0.0001$), L-selectin (0.69, $P < 0.0001$) and macrophages (0.62, $P < 0.0001$); (2) ICAM-1 (0.61, $P < 0.0001$) and VCAM-1 (0.45, $P < 0.0001$) expression in tubules and within focal interstitial infiltrates (respectively; 0.36, $P < 0.001$, and 0.52, $P < 0.0001$); (3) E-selectin (0.71, $P < 0.0001$) and P-selectin (0.72, $P < 0.0001$) expression on the extraglomerular vascular endothelium.

This occurred regardless of the primary diagnosis. Interestingly, these were the same molecules that had earlier been found to be expressed in these locations in a coordinate fashion. There were no significant correlations between the glomerular expression of LFA-1, VLA-4, ICAM-1, VCAM-1 or glomerular macrophage infiltration and the degree of tubulointerstitial damage.

**Table 3.** Spearman's correlations between adhesion molecule expression in different sites in human renal biopsies (representative data)

| | ICAM-1 (tub) | VCAM-1 (tub) | E-Sel (ve) | ICAM-1 (ge) | VCAM-1 (mes) |
|---|---|---|---|---|---|
| LFA-1 (g) | 0.15 (0.131) | 0.19 (0.056) | 0.28 (0.01) | 0.17 (0.095) | 0.19 (0.056) |
| VLA-4 (g) | 0.26 (0.009) | 0.18 (0.069) | 0.18 (0.101) | 0.20 (0.046) | 0.42 (0.0001) |
| Mø (g) | 0.30 (0.004) | 0.25 (0.018) | 0.25 (0.035) | 0.16 (0.141) | 0.10 (0.357) |
| LFA-1 (int) | 0.62 (0.0001) | 0.56 (0.0001) | 0.62 (0.0001) | 0.07 (0.478) | 0.21 (0.043) |
| VLA-4 (int) | 0.63 (0.0001) | 0.53 (0.0001) | 0.51 (0.0001) | −0.23 (0.021) | 0.25 (0.018) |
| Mø (int) | 0.67 (0.0001) | 0.56 (0.0001) | 0.62 (0.0001) | 0.04 (0.690) | 0.33 (0.002) |
| L-Sel (int) | 0.55 (0.0001) | 0.62 (0.0001) | 0.66 (0.0001) | 0.05 (0.662) | 0.13 (0.260) |
| ICAM-1 (inf) | 0.30 (0.002) | 0.52 (0.0001) | 0.25 (0.024) | 0.03 (0.762) | −0.09 (0.390) |
| VCAM-1 (inf) | 0.51 (0.0001) | 0.36 (0.0001) | 0.39 (0.0001) | −0.11 (0.261) | −0.03 (0.791) |
| VCAM-1 (tub) | 0.54 (0.0001) | 1 | | | |
| E-Sel (ve) | 0.61 (0.0001) | 0.56 (0.0001) | 1 | | |
| ICAM-1 (ge) | −0.56 (0.571) | 0.05 (0.636) | 0.12 (0.302) | 1 | |
| VCAM-1 (mes) | 0.23 (0.031) | 0.22 (0.031) | 0.29 (0.014) | 0.15 (0.155) | 1 |

Spearman's correlation coefficients between adhesion molecules (LFA-1/ICAM-1/VCAM-1/VLA-4/L-selectin/E-selectin/P-selectin) and macrophages (Mø) within the glomerulus (g), glomerular endothelium (ge), mesangium (mes), in the interstitium (int) and tubules (tub), in focal interstitial infiltrates (inf) and extraglomerular vascular endothelium (ve). (Numbers in brackets indicate *P* values).

*Comparison of adhesion molecule expression in biopsies with mild or severe chronic histological damage*

It was possible to divide 107 of the biopsies into 2 groups; 64 were classified as having mild damage (0 to 1+) and 43 as having moderate to severe damage (2 to 3+) based on an assessment of the extent of tubulointerstitial injury (interstitial fibrosis and tubular atrophy). Twelve biopsies could not be classified. Using the Mann-Whitney *U*-test, the group with moderate to severe damage had a significantly greater expression of the adhesion molecules ICAM-1 ($P < 0.0001$) and VCAM-1 ($P < 0.0001$) on tubular cells and in focal infiltrates. VCAM-1 expression within the mesangium was not different. Endothelial staining for E-selectin ($P < 0.0001$) and P-selectin ($P < 0.0001$) was increased in the extraglomerular vessels. A more intense cellular infiltrate in the biopsies classed as severe was reflected by increased interstitial staining for LFA-1 ($P < 0.0001$), VLA-4 ($P < 0.0001$), L-selectin ($P < 0.0001$), and macrophages ($P < 0.0001$). There was no significant change in glomerular expression of LFA-1 and VLA-4 or in the number of glomerular macrophages between biopsies with mild and moderate/severe damage.

## Discussion

This is the most complete study of adhesion molecule expression in human glomerulonephritis reported so far. In contrast to other studies [12–14], there was generally no correlation between adhesion molecule expression and particular disease entities. Exceptions were the reduction in glomerular endothelial staining for ICAM-1 in minimal change disease and SLE and on extraglomerular vascular endothelium in crescentic glomerulonephritis; these findings are of uncertain significance. There was, however, a strong positive correlation between expression of different adhesion molecules in certain areas of the same renal biopsy: (1) expression of LFA-1, VLA-4, and L-selectin in the periglomerular region, interstitium and in focal interstitial infiltrates and the presence of macrophages in these regions; (2) *de novo* tubular expression of ICAM-1 and VCAM-1; (3) staining for ICAM-1 and VCAM-1 on focal cellular infiltrates within the interstitium; and (4) staining for E-selectin and P-selectin on extraglomerular endothelium.

Remarkably, almost all of these variables were strongly correlated with each other. The exception was ICAM-1 on focal cellular infiltrates with staining for E-selectin on extraglomerular endothelium. The correlations were positive and highly significant. Therefore, if a particular biopsy had an extraglomerular cellular infiltrate that expressed molecules such as LFA-1, VLA-4 and L-selectin, then that biopsy usually had strong expression of ICAM-1 and VCAM-1 on tubules and in focal cellular infiltrates, and up-regulation of E-selectin and P-selectin on the extraglomerular vascular endothelium. This is in keeping with the results of Fuggle et al [15], who showed a strong correlation between the number of infiltrating leukocytes and the magnitude of expression of the endothelial cell adhesion molecules ICAM-1, VCAM-1 and E-selectin in renal transplant biopsies. In addition, an interstitial infiltrate expressing LFA-1, VLA-4 and L-selectin generally occurred in close physical proximity to extraglomerular vessels expressing E and P-selectin.

Each of the positively correlated variables noted above were also independently correlated with more marked chronic interstitial changes, suggesting that the expression of adhesion molecules could be linked to tissue injury *in vivo*. This is in keeping with an accumulating body of evidence showing a close linkage between cellular adhesion and tissue injury. *In vitro* studies have demonstrated that cellular adhesion can promote renal injury by priming respiratory burst activity and degranulation in activated leukocytes [16]. Also, monoclonal antibodies against the common chain of the $\beta_2$ integrin CD18 not only reduce adhesion of macrophages to mesangial cells *in vitro*, but decrease superoxide anion production and mesangial cell injury in this system [17]. It is likely that the close contact between adherent cells increases tissue injury caused by degranulation and respiratory burst activity due to reduced effectiveness of free radical scavengers and protease inhibitors [1]. Juxtaposition of leukocytes and endothelial cells is also thought to promote production of toxic leukotriene mediators [18] due to a pooling of the enzymatic processes of the two cell types. Finally, *in vivo* studies in a rat model of nephrotoxic nepritis treated with the interleukin-1 receptor antagonist have demonstrated that interstitial ICAM-1 expression is closely linked to interstitial leukocyte infiltration and tubulointerstitial damage [19].

Recognition of these associations, however, does not establish

which if any is the primary event, and it does not indicate that all actually contribute to the pathogenesis of renal injury. For example, tubular expression of ICAM-1 and VCAM-1 may be, simply, a by-product of extensive leukocyte infiltration. Alternatively, it may be important in localizing these cells to the interstitium of the kidney or in allowing tubular epithelial cells to function as antigen presenting cells, which could then play a role in immune-mediated renal injury [20, 21]. The large number of functional studies in different animal models of glomerulonephritis that have demonstrated improvements in both clinical and histological manifestations of renal disease following inhibition of adhesion molecules suggests, however, that adhesion molecule expression is important in the pathogenesis of glomerulonephritis [6, 22, 23]

While the significance of positive associations is difficult to interpret, the absence of strong associations between glomerular expression of LFA-1, VLA-4, ICAM-1, VCAM-1, and glomerular macrophage infiltration and chronic histological damage suggests that glomerular adhesion molecule expression does not play a major role in the pathogenesis of tubulointerstitial disease.

Unlike other authors [12–14], this study did not identify distinctive patterns of adhesion molecule expression in different histopathological types of glomerulonephritis. The few exceptions were of uncertain significance. The absence of such relationships is not unexpected, since this study suggests that tubulointerstitial adhesion molecule expression is related to the degree of chronic histological damage, rather than the etiology of the glomerular lesion. Thus biopsies with advanced IgA nephropathy with significant interstitial infiltration had strong expression of endothelial and cellular selectins, tubular and interstitial ICAM-1 and VCAM-1 and interstitial LFA-1 and VLA-4. On the other hand biopsies from patients with early IgA disease without an interstitial infiltrate had negligible expression of the above molecules. A similar association was seen in groups with other primary diagnoses. As a result, comparisons of adhesion molecule expression between different diagnostic groups generally showed no difference as most groups contained a mixture of biopsies with mild and severe damage. However, when the biopsies were separated into two groups based on the degree of chronic histological damage and not primary diagnosis, significant differences in adhesion molecule expression between these two groups were identified. These findings once again support the hypothesis that regardless of the nature and severity of the initial insult, there may be a common pathway that is responsible for progressive renal dysfunction. This could involve altered adhesion molecule expression.

It was anticipated that expression of ICAM-1 on glomerular and extraglomerular vascular endothelium would correlate with leukocyte infiltration as ICAM-1 is thought to play a major role in allowing circulating cells to migrate into extravascular locations within the kidney. However, staining for ICAM-1 within the glomerulus or in the extraglomerular vascular endothelium (including peritubular capillaries), did not correlate strongly with either glomerular or interstitial cell infiltration. Within the glomerulus, however, the explanation could be trivial; staining for ICAM-1 was so intense on normal glomerular endothelium that an increase would probably have remained undetected with our immunohistochemical technique.

There was a strong relationship between glomerular expression of VCAM-1, most of which appeared to be within the mesangium, and glomerular infiltration with cells staining for VLA-4. Since VLA-4 is the ligand for VCAM-1, this correlation was anticipated, although the mesangial location of VCAM-1 suggests that the interaction with VLA-4 may be important in retaining VLA-4 positive cells rather than in assisting their emigration from the circulation.

The absence of detectable staining for E-selectin and P-selectin within the glomerulus in all but one biopsy was surprising, since rolling of leukocytes mediated by the selectins and their carbohydrate ligands is the first step in the adhesion of leukocytes to vascular endothelium. In the single biopsy showing strong expression of both E-selectin and P-selectin on the glomerular endothelium, the patient was a young male with aggressive Wegener's granulomatosus who had a renal biopsy soon after admission. It is possible, therefore, that other patients may have had glomerular expression of E- and P-selectin during the early stages of glomerular inflammation, but did not undergo renal biopsy until expression had declined. This is consistent with the results of Tipping et al [9], who demonstrated strong expression of P-selectin within the glomerulus as early as one hour after induction of anti-GBM nephritis in mice.

In summary, this study has documented the pattern of expression of multiple adhesion molecules in the same renal biopsy and has shown coordinate expression in the tubulointerstitium, but not the glomerulus. Coordinate upregulation occurred irrespective of the primary diagnosis but was closely linked to the degree of chronic histological damage. These results suggest that there is a common pathway of tubulointerstitial injury irrespective of the primary diagnosis and that adhesion molecule expression within the tubulointerstitium may be an important mechanism in its pathogenesis.

## Acknowledgments

Dr. Roy-Chaudhury was supported by a grant from the Aberdeen Renal Research Fund. Research reported in this paper was supported by a grant from the Medical Research Council, U.K. and the Scottish Hospitals Endowment Research Trust (SHERT). We wish to acknowledge the generous support of clinicians and pathologists at the Aberdeen Royal Infirmary, Aberdeen, the Western General Hospital, Glasgow and the Glasgow Royal Infirmary, Glasgow who kindly made renal biopsy material available to us. This work was presented at the 27th Annual Meeting of the American Society of Nephrology and published in abstract form (*J Am Soc Nephrol* 5:793, 1994).

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