Adhesion Molecules Expression in Noncrescentic Acute Post-Streptococcal Glomerulonephritis

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(J. Am. Soc. Nephrol. 1996; 7:2419-2427)

ABSTRACT
Clinicomorphological features of 11 cases of noncrescentic acute post-streptococcal glomerulonephritis (APSGN) were reviewed. Intraglomerular and interstitial leukocytes and their possible correlation with the adhesion molecules intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial-leukocyte adhesion molecule-1 (ELAM-1/E-selectin) were investigated by an immunohistochemical method. Intraglomerular leukocytes were primarily granulocytes (11.4 ± 10 cells/glomerular cross-section) and monocytes-macrophages (13.4 ± 19.4 cells/glomerular cross-section). The granulocytes outnumbered monocytes-macrophages in 7 of 11 specimens. The number of intraglomerular leukocytes correlated with proteinuria at the time of renal biopsy. Intraglomerular ICAM-1 staining was strongly positive in all biopsies, especially when intraglomerular monocytes-macrophages prevailed. Expression of Intraglomerular VCAM-1 and E-selectin in diseased kidneys did not differ from that in normal kidneys. Interstitial leukocytes were primarily monocytes-macrophages (158.9 ± 96.8 cells/mm²) and T lymphocytes (102.2 ± 63.9 cells/mm²). The number of interstitial leukocytes, especially monocytes-macrophages, correlated with serum creatinine level at the time of biopsy. Interstitial ICAM-1 staining was strongly positive on tubules, peritubular capillaries, and small vessels. The tubular positivity for ICAM-1 correlated with the number of interstitial monocytes-macrophages. Interstitial VCAM-1 and E-selectin were expressed as in normal kidney tissues. The data from this study demonstrate that APSGN is characterized by the presence of both intraglomerular and interstitial leukocyte infiltration, cor-

Acute post-streptococcal glomerulonephritis (APSGN) is histologically characterized by the presence of enlarged glomeruli containing an increased number of cells. The hypercellularity results from the proliferation of resident cells and from leukocyte infiltration (1,2). Polymorphonuclear leukocytes are the most easily identified cells and may be present in large numbers, but monocytes-macrophages have also been detected in both human and experimental models of the disease (1-3). In human studies using histochemical methods and electron microscopy, a high number of intraglomerular monocytes was found, especially in the early acute stage of the disease (4,5). In experimental serum sickness models, Sano (6) and Striker et al. (7) showed that much of the glomerular hypercellularity resulted from monocytes. In recent immunohistochemical studies, a few intraglomerular T lymphocytes were also found (8,9). Besides the acute glomerular involvement, an interstitial leukocyte infiltration, ranging from mild to severe, was also frequently described (1,2).

In recent years, it has become evident from many experimental and in vitro studies that adhesion molecules play a key role in inflammatory and immune responses (10-13). Intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) are constitutionally expressed on many different cell types, where they can be upregulated by cytokines and other mediators of inflammation (14,15). It is well known that the major ligand of ICAM-1, the β₂-integrin LFA-1, is present on all leukocytes, whereas the ligand of VCAM-1, the β₁-integrin VLA-4, is absent on neutrophils and present on lymphocytes and monocytes (16-20). The binding between VCAM-1 and VLA-4 is considered a preferential way of monocyte adhesion (21-23). Recent experimental studies (24-26) have shown that E-selectin (ELAM-1) expression is restricted to cytokine-stimulated endothelium, is prominent in acute inflammatory lesions, and correlates with the large influx of neutrophils.

We reviewed the clinicopathological features in 11 patients with APSGN to study (using an immunohistochemical method) intraglomerular and interstitial
infiltrating cells and their possible correlation with the expression of the adhesion molecules ICAM-1, VCAM-1, and E-selectin.

METHODS

Patients

Eleven patients (6 men and 5 women) with a mean age of 30.9 yr (range, 10 to 67), showing clinical and histological evidence of noncrescentic APSGN, were studied.

At the time of admission to our hospital, all patients showed increased antistreptolysin O (normal value < 200 IU/mL) titers (ranging from 250 to 800 IU/mL) and decreased C3 serum levels. In seven patients, a decreased serum C4 level was also present.

The interval time between streptococcal infection (a pharyngitis or tonsillitis in all patients) and the onset of nephropathy ranged from 7 to 22 days (mean value ± SD, 14.18 ± 5.34 days). The renal onset was an acute nephritic syndrome in seven patients, a nephrotic syndrome in one patient, and macroscopic hematuria in three patients. The mean prebiopsy follow-up period was 28.2 days (range, 7 to 75 days).

Serum creatinine concentration at the time of renal biopsy (RB) ranged from 61.8 to 866.3 μmol/L (m.v., 274.0 ± 290.5 μmol/L) and proteinuria from 0.1 to 11.7 g per day (m.v., 3.16 ± 3.49).

Kidney Tissue

Kidney tissue was obtained from all patients and, for comparison, from five cadaveric kidneys that could not be grafted because of vascular abnormalities. Tissue samples for light microscopy were fixed in Bouin’s fluid, embedded in paraffin, and stained according to standard techniques. For immunofluorescence and immunoperoxidase staining, the unfixed renal tissue was embedded in OCT compound (Miles Scientific, Naperville, IL), snap-frozen in a mixture of isopentane and dry ice, and stored at -80°C. Subsequently, 5-μm sections were placed on slides and stored at -20°C until they were immunostained.

Immunoperoxidase Labeling

We used an avidin-biotin technique, in which a biotinylated secondary antibody reacts with several peroxidase-conjugated streptavidin molecules. In brief, after incubation with 0.5% avidin (Sigma Chimica, Gallarate, Milan, Italy) and 0.01% biotin (Sigma) to suppress endogenous avidin-binding activity, tissue sections were incubated with the primary antibody. We used the following monoclonal antibodies: CD45 (monoclonal mouse anti-human leukocyte common antigen; Immunotech, Marseille Cedex, France), CD3 (monoclonal rabbit anti-human T-lymphocyte; Dako, Glostrup, Denmark), CD68 (monoclonal mouse anti-human monocyte-macrophage; Dako), CD15 (monoclonal mouse anti-human granulocyte; Dako), CD19 (monoclonal mouse anti-human B-lymphocyte; Dako), CD54 (monoclonal mouse anti-human ICAM-1; Serotec, Kidlington, Oxford, England), CD106 (monoclonal mouse anti-human VCAM-1; Serotec), CD62E (monoclonal mouse anti-human E-selectin; Serotec).

After being washed, the sections were sequentially fixed in a methanol·H₂O₂ solution (to block endogenous peroxidase) and incubated with the secondary biotinylated antibody (Dako) and with the peroxidase-labeled streptavidin (Dako). Peroxidase activity was detected with 3,5-diaminobenzidine (DAB, Dako), then sections were counterstained with Harry’s hematoxylin (BDH, Poole, England), dehydrated, and mounted in Entellan (Merck, Darmstadt, Germany).

Specificity of labeling was demonstrated by the lack of staining after substitution of phosphate-buffered saline for the primary antibody.

Quantitative Evaluation

All peroxidase-stained sections had five or more glomeruli and were evaluated by two independent observers who were blinded to any histological or clinical information. Minor differences were subsequently resolved by conference.

Immunohistological reaction intensity and/or quantity was evaluated separately for glomeruli, tubuli, vessels, and interstitium. Intraglomerular infiltrating cells were expressed as number of cells per glomerular cross section (gcs).

The interstitial labeled cells were counted using an eyepiece graticule (Leitz, Periplan 6F 12.5× MF mounted in a Leitz Dialux 20 microscope; (Leitz, Wetzlar, Germany) in ten consecutive fields, avoiding glomeruli and large vessels; the results were expressed as number of positive cells per square millimeter.

Results were evaluated using the mean value and the standard deviation of the mean.

Intraglomerular, tubular, and vessel staining of adhesion molecules were scored semiquantitatively on a four-point scale: no staining, 0; focal staining, 1; diffuse staining, 2; massive staining, 3. Tubular staining was scored in ten consecutive fields per biopsy. Means and standard deviations of the scores were calculated.

Statistical significances (P < 0.05) were analyzed using t test and regression test.

RESULTS

Histological Features

By light microscopy, we found the presence of endocapillary proliferation and intraglomerular leukocyte infiltration of variable degrees, ranging from mild to intense. Extracapillary proliferation was absent in all cases. Subepithelial deposits (humps) were seen by light microscopy in ten biopsies. In eight cases, interstitial infiltration was also evident, ranging from mild to severe.

Immunofluorescence was typically positive for C3, with a granular "starry sky" pattern.

Electron microscopy was performed in five cases, confirming the intraglomerular leukocyte infiltration and the presence of subepithelial deposits (humps).

Immunohistochemical Features

Intraglomerular infiltration and adhesion molecule expression. The results are shown in Table 1.

Intraglomerular infiltration ranged from 7.9 to 70 cells/gcs (m.v., 23.8 ± 21 CD45+ cells/gcs). These cells were primarily monocytes-macrophages (m.v., 13.4 ± 19.4 CD68+ cells/gcs) and granulocytes (m.v., 11.4 ± 10 CD15+ cells/gcs) with less evidence of T lymphocytes (m.v., 1.9 ± 1.4 CD3+ cells/gcs) (Figure 1).

No B lymphocytes were found. In seven cases, the granulocytes were prevalent, whereas in four cases (Table 1: PT 8 through 11), monocytes-macrophages outnumbered granulocytes. The number of intraglo-
TABLE 1. Intraglomerular infiltration and adhesion molecule expression

<table>
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<th>PT</th>
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m.v. ± SD 23.8 ± 21 1.9 ± 1.4 13.4 ± 19.4 11.4 ± 10.7

a PT, patient; m.v., mean value; SD, standard deviation.

Intrarenal leukocytes correlated with the degree of urinary proteins at RB (Figure 2).

Although a positive trend between the number of intraglomerular leukocytes and the prebiopsy follow-up was present, we did not find a statistical significance between these parameters even when the interval between onset of streptococcal infection and biopsy was considered.

Glomerular ICAM-1 staining, which in normal kidneys was present only on the endothelium, appeared stronger and more diffuse in all diseased kidneys. The four cases with a prevalent monocyte-macrophage infiltration demonstrated the most diffuse and intense ICAM-1 positivity (Figure 3). Furthermore, ICAM-1 did not correlate with glomerular nephritis duration. Glomerular VCAM-1 only lined the Bowman's capsule, as it also appeared in the normal kidneys, and was occasionally positive on scattered cells. Glomerular E-selectin was always negative, as in normal tissues.

Intrarenal infiltration and adhesion molecule expression. The presence of a diffuse intrarenal infiltration (Table 2, Figure 4), ranging from mild to intense (m.v., 245.6 ± 137.9 CD45+ cells/mm²), was found with immunohistochemical evaluation in all biopses, characterized by monocytes-macrophages (m.v., 158.9 ± 96.8 CD68+ cells/mm²) and T lymphocytes (m.v., 102.2 ± 63.9 CD3+ cells/mm²). The number of interstitial granulocytes was low (m.v., 15.7 ± 14.6 CD15+ cells/mm²).

Although only two patients showed high serum creatinine levels, we found a significant correlation between the number of intraglomerular leukocytes, primarily monocytes-macrophages, and serum creatinine level at RB (Figure 5). Some infiltrating cells were also positive for ICAM-1 (m.v., 82.7 ± 59.1), VCAM-1 (m.v., 33.6 ± 25.4), and E-selectin (m.v., 4.6 ± 9.5) (Table 2).

Tubules and vessels. In normal kidneys, interstitial E-selectin was negative and tubular epithelial cells had no ICAM-1, although some tubules were positive for VCAM-1. Some capillaries expressed both ICAM-1 and VCAM-1, the latter also being positive on some small vessels.

In APSGN biopsies, ICAM-1 showed an intense positivity involving all the interstitial components: tubules, peritubular capillaries, and small vessels (Figure 6).

Interstitial VCAM-1 did not significantly differ from normal kidneys and E-selectin was positive only rarely on interstitial capillaries.

We found a significant correlation between ICAM-1 tubular expression and the CD68 interstitial infiltration (Figure 7).

DISCUSSION

The primary histological feature in APSGN is the presence of a diffuse and global glomerular hypercellularity, resulting from proliferation of resident cells and leukocyte infiltration (1,2). In agreement with previous human immunohistochemical studies (8,9), our data showed that intraglomerular leukocytes were primarily granulocytes and monocytes-macrophages. In particular, monocytes-macrophages outnumbered granulocytes in four cases. There were few T lymphocytes, and no B lymphocytes were found.

In experimental serum sickness models, Sano (6) and Striker et al. (7) found that much of the glomerular hypercellularity resulted from monocytosis, and Hunsticker et al. (27) found that the peak proteinuria coincided with the time of intraglomerular monocyte accumulation. In our cases, also confirming previous results obtained by our group, the number of intraglomerular leukocytes correlated with urinary proteins at RB (4) and not with serum creatinine levels (28), suggesting that the acute glomerular involvement is responsible more for protein losses than for the impairment of renal function.

The mechanism of acute and transient intraglomerular leukocyte accumulation in APSGN is still not well known. In recent years, it has become more and more clear that adhesion molecules are essential for leukocyte entry to the tissue (10–13). Most authors (12,26,29–31) agree that leukocyte adhesion to the endothelium may be schematically divided into two
Figure 1. Intense intraglomerular leukocyte infiltration (a) primarily resulting from granulocytes (b) and macrophages (c). There are few intraglomerular T lymphocytes (d). (Immunoperoxidase stain; original magnification: a-c, ×10; d, ×20).

Figure 2. Correlation between intraglomerular leukocytes and urinary proteins at the time of renal biopsy. gcs, glomerular cross-section.

steps: first, the interaction between selectins (as E-selectin) and their carbohydrate ligands induces leukocytes to roll onto endothelium. The second step is characterized by the interaction between leukocyte integrins and endothelial immunoglobulin (Ig)-like molecules (as ICAM-1 and VCAM-1), and produces the immobilization that precedes diapedesis. ICAM-1 and VCAM-1 are constitutionally expressed on many different cell types, where they can be upregulated by cytokines and other mediators of inflammation (14,15). It is well known that the major ligand of ICAM-1, the β2-integrin LFA-1, is present on all leukocytes, whereas the ligand of VCAM-1, the β1-integrin VLA-4, is absent on neutrophils and present on lymphocytes and monocytes (16–20).

In normal glomeruli, we found, as have many other authors (32–34), a constitutive expression of ICAM-1 on endothelial cells, whereas VCAM-1 was present on Bowman's capsule and E-selectin was negative. All of our cases with noncrescentic APSGN showed an i-
Increased intraglomerular ICAM-1 expression, with a particular intensity in the four cases in which monocytes-macrophages outnumbered granulocytes. An increased expression of intraglomerular ICAM-1 that significantly correlated with the number of glomerular monocytes-macrophages was recently found in APSGN by Parra et al. (35), suggesting that in APSGN, ICAM-1 is primarily involved in the glomerular monocyte-macrophage infiltration.

VCAM-1, through the interaction with its ligand VLA-4, is thought to be a preferential way of monocyte recruitment (21–23). Nevertheless, in our specimens, VCAM-1 was almost negative in the glomerular tufts, as it also appears in the normal kidney.
In a previous study on renal vasculitis (36) and in 18 cases of IgAGN showing necrotizing-extracapillary lesions (data not published), our group has found an intense positivity of VCAM-1, strictly corresponding to the areas of necrotizing-extracapillary damage with evident monocyte-macrophage accumulation. With a double-staining procedure, we identified the parietal epithelial cells positive for VCAM-1 in the crescents, whereas in the necrotic areas it was quite impossible to determine exactly what type of cell was positive. Furthermore, in our experience, VCAM-1 was negative in the tuft of 21 cases of cryoglobulinemic glomerular nephritis, characterized by a massive monocyte infiltration but the absence of extracapillary proliferation. Therefore, all of our data seem to confirm the hypothesis that intraglomerular VCAM-1 might be particularly involved in the monocyte recruitment in necrotizing-extracapillary damage. Obviously, when using only immunohistochemistry, we can hypothesize but not demonstrate that VCAM-1 glomerular positivity is linked to a different mechanism of monocyte recruitment.

Recent experimental studies have shown that E-selectin is absent on normal endothelium before cytokine stimulation (24,25). Its expression, although also involved in chronic inflammation (37,38), is prominent in acute inflammatory lesions and correlates with the large influx of neutrophils (25, 26). On the other hand, it has been found that the cytoplasmic domain of E-selectin contains tyrosin residues that have been suggested to mediate the internalization of
Many authors have described in APSGN the presence of diffuse interstitial infiltration ranging from mild to severe, and sometimes associated with focal accumulation of mononuclear leukocytes and polymorphonucleates (1,2). By light microscopy, we found interstitial infiltrations of varying degrees in eight patients. With immunohistochemistry, all cases showed a diffuse interstitial infiltration primarily composed of monocytes-macrophages and T lymphocytes. A few granulocytes were also present. The number of interstitial infiltrating cells significantly differed from those found in normal kidneys.

The greater sensitivity of immunohistochemistry to detect infiltrating leukocytes was also underlined by Alexopoulos et al. in membranous glomerulonephritis (41), and, in our opinion, confirms the benefits of routine use of monoclonal antibodies on renal biopsies to more precisely determine quantity and quality of interstitial leukocyte infiltration.

In 1977, Bohle et al. (42), using morphometric analysis, discovered for the first time a correlation between serum creatinine levels and the increase in interstitial volume in acute endocapillary glomerulo-
nephritis, and explained the correlation on the basis of a reduction of RBF brought about by compression of the postglomerular vasculature.

In our patients, we could not find any correlation between the presence of interstitial edema and the degree of renal failure. Moreover, it is well known that in both primary and secondary glomerular nephritis, the intensity of interstitial infiltration better correlates with the impairment of GFR than do glomerular lesions (43–45).

In our cases, taking into account that a very high serum creatinine level was present in only two patients, a significant correlation between interstitial infiltration, and in particular of monocytes-macrophages, and serum creatinine level at RB was found. Therefore, also considering the absence of correlation between intraglomerular infiltration and serum creatinine level, our data might suggest that the interstitial inflammation may also be responsible for the functional damage in acute processes.

In our specimens, we also found some cells expressing adhesion molecules, primarily ICAM-1 and VCAM-1. The presence of adhesion molecules on leukocyte surface was suggested by Brady et al. (46) to be important in cell-cell and cell-matrix interactions to promote transcellular biosynthesis of lipoxygenase products, therefore enhancing the inflammatory process.

In the interstitium of our patients, we found ICAM-1 to be strongly expressed, and to involve tubules, peritubular capillaries, and small vessels. Moreover, the tubular ICAM-1 expression significantly correlated with the interstitial monocyte-macrophage number, further suggesting that ICAM-1 in noncrescentic APSGN is especially involved in acute monocyte recruitment. In effect, interstitial VCAM-1 and E-selectin did not show substantial differences from normal kidneys.

In conclusion, our data demonstrate that APSGN is characterized by the presence of both intraglomerular and interstitial leukocyte infiltration that respectively correlate with proteinuria and serum creatinine at RB. Among the adhesion molecules studied, ICAM-1 seems to be the most involved in recruitment of both intraglomerular and interstitial leukocytes, especially of monocytes.

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