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Takahiko Ogawa · Noriaki Yorioka · Takafumi Ito
Yoshihiko Taniguchi · Junko Kumagai
Yukikazu Awaya · Michio Yamakido

Ultrastructural localization of vascular cell adhesion molecule-1 in proliferative and crescentic glomerulonephritis

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Abstract Recent studies have demonstrated an important role of vascular cell adhesion molecule-1 (VCAM-1) in the pathogenesis of nephritis. In the present study, renal biopsy specimens from patients with proliferative and crescentic glomerulonephritis were subjected to immunoelectron microscopy using an anti-VCAM-1 monoclonal antibody. In control normal kidney tissue, VCAM-1 expression was restricted to the free surface of parietal epithelial cells. In diseased glomeruli, VCAM-1 was expressed on the free surface of parietal and visceral epithelial cells, on the luminal surface of capillary endothelial cells, on infiltrating monocyte/macrophage-like cells, on mesangial cells, and in the matrix of the expanded mesangium. There was also VCAM-1 expression on almost all cell types in the crescents, including macrophage-like cells, fibroblast-like cells, and epithelial cells. Some cells also showed VCAM-1 positivity in the rough endoplasmic reticulum and the perinuclear space. Both the glomerular capillary lumen and urinary spaces of Bowman's capsule contained positive reaction products, which were often associated with exocytosis by the surrounding cells. VCAM-1 was predominantly expressed on the basal and lateral surfaces of a few proximal tubules, but it could not be localized ultrastructurally. These findings suggest that production and secretion of VCAM-1 by both infiltrating monocyte/macrophages and resident glomerular cells may be related to the pathogenesis of proliferative and crescentic glomerulonephritis.

Key words VCAM-1 · Glomerulonephritis · Ultrastructural study

T. Ogawa (✉) · N. Yorioka (✉) · T. Ito · Y. Taniguchi
J. Kumagai · Y. Awaya · M. Yamakido
2nd Department of Internal Medicine,
Hiroshima University School of Medicine,
1-2-3 Kasumi, Minami-ward, Hiroshima 734, Japan
Tel: (81)82-257-5196; Fax: (81)82-255-7360

Introduction

Many reviews have demonstrated an important role for adhesion molecules in the pathogenesis of various kinds of nephritis [2, 3, 5, 8, 10, 20].Vascular cell adhesion molecule-1 (VCAM-1), a cell surface glycoprotein belonging to the same immunoglobulin superfamily as intercellular adhesion molecule-1 (ICAM-1), is expressed by many types of cells, including endothelial cells, and plays a major part in the endothelial adhesion of activated monocyte/macrophages (but not neutrophils), which express its receptor, VLA-4 [9]. A number of light microscopic studies have detected increased VCAM-1 expression in the proximal tubules of patients with interstitial nephritis [6, 7, 19]. In addition, several groups of investigators [1, 6, 17, 19] have reported that the glomeruli and the proximal tubular cells from patients with proliferative glomerulonephritis or transplant rejection show up-regulation of VCAM-1 expression in association with an increase of VCAM-1 mRNA. These findings suggest the de novo synthesis of VCAM-1 in such conditions, although its exact intraglomerular localization at the electron microscopic level remains uncertain. In order to define the role of VCAM-1 in glomerulonephritis, it is important to localize its expression precisely within the glomerulus. In the present study, we used a monoclonal antibody to detect VCAM-1 in proliferative and crescentic glomerulonephritis, diseases which often feature a marked increase of glomerular VCAM-1 expression. In addition, to determine which VCAM-1-positive cells were monocyte/macrophages, CD68 immunoreactivity was used as a marker for cells of the monocytic series.

Materials and methods

Renal biopsies were originally taken for diagnostic purposes, and all specimens were subjected to light microscopic, immunofluorescent, and ultrastructural examination by standard methods. All 22 biopsy specimens investigated showed features of proliferative or crescentic glomerulonephritis (16 specimens were from patients with IgA nephropathy, 2 were from patients with Henoch-

Schönlein purpura nephritis, 2 were from patients with non-IgA mesangial proliferative glomerulonephritis, and 2 were from patients with anti-neutrophil cytoplasmic antibody-positive glomerulonephritis). Nontumour tissue from four kidneys that were resected because of localized carcinoma served as a normal control.

VCAM-1 and CD68 were detected using a mouse IgG1*k* antibody for recombinant soluble VCAM-1 [9] (4B9: Genzyme, Cambridge, Mass.) and a mouse IgG1*k* antibody for CD68 (KP1: Dakopatts, Glostrup, Denmark). The secondary antibody was horseradish peroxidase-labelled goat antimouse IgG/Fab′ (MBL, Nagoya, Japan). For all samples, negative controls for immunohistochemical procedures consisted of substitution of the specific antibodies with specific pathogen-free mouse serum (Dakopatts) or phosphate-buffered saline (PBS). In order to rule out the possibili-

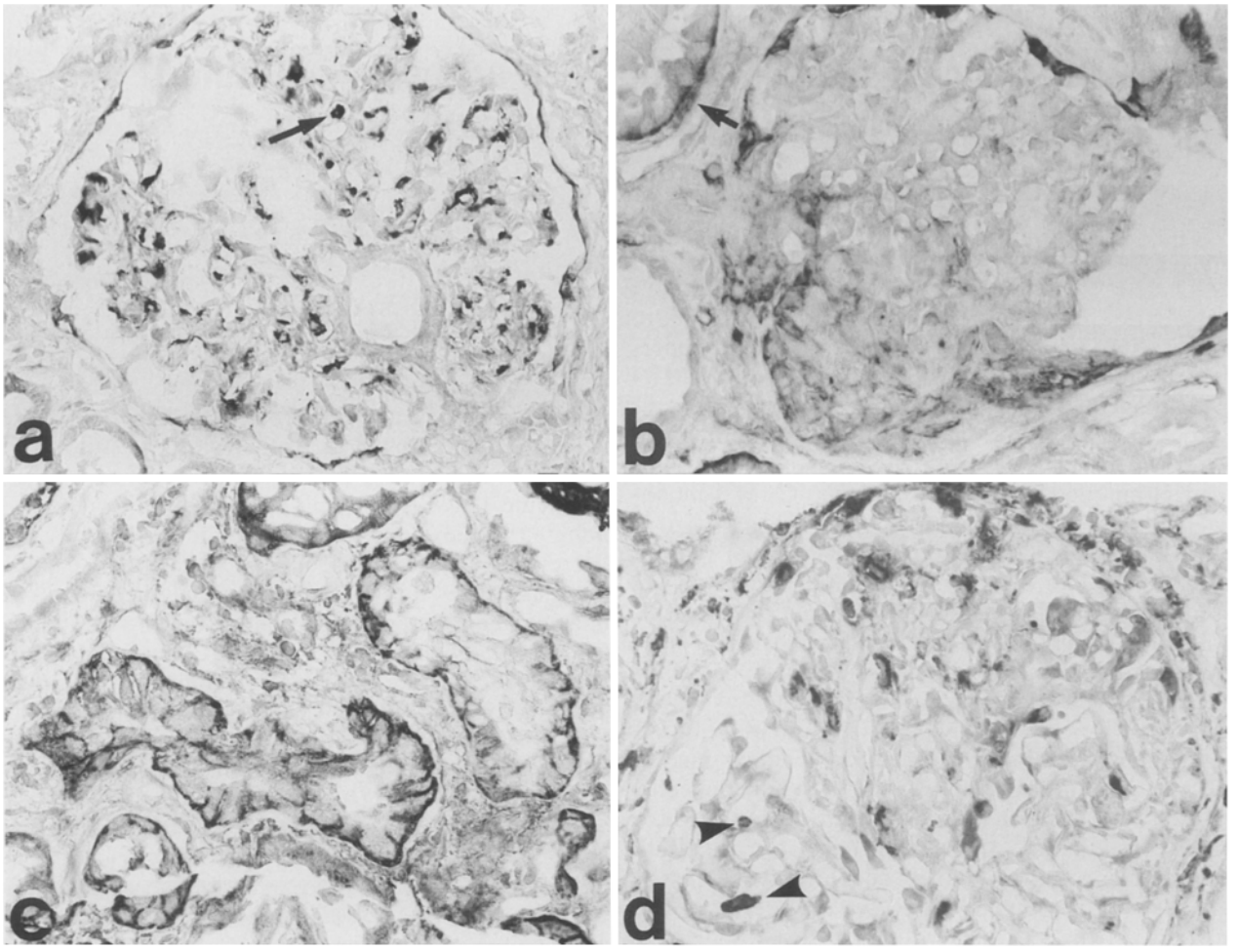

Fig. 1a–e Immunohistochemistry. **a** A glomerulus showing mild proliferative change. VCAM-1 expression is seen focally in the mesangium, on endothelial cells, and throughout the glomerular parietal epithelium. Also note VCAM-1 expression by a leucocyte in the capillary (*arrow*). × 340. **b** A glomerulus showing moderate proliferative change with a focal cellular crescent. VCAM-1 expression is prominent both in the crescent and throughout the glomerular parietal epithelium. Also note VCAM-1 expression in the mesangium (predominantly glomerular hilar region) and on the basal surface of a few tubular cells (*arrow*). × 340. **c** VCAM-1 expression in the proximal tubules. VCAM-1 is expressed predominantly on the basal and lateral surfaces of all the cells within some proximal tubular cross sections. × 340. **d** A glomerulus showing focal proliferative change. CD68-positive cells are localized within the upper half of the mesangial area of the glomerulus, where cell proliferation and/or matrix expansion is prominent and capillary lumina are collapsed, and are also seen in the periglomerular spaces. In the lower right and left portions of the same glomerulus, both proliferative changes and positive cells are largely absent except for intracapillary leucocytes (*arrowheads*). × 340. **e** In a single-stained serial section, a glomerulus showing severe proliferative change with a circumferential cellular crescent, and periglomerular inflammatory cell infiltration. A few CD68-positive cells are present in the glomerulus. More cells are concentrated in both the crescent and the periglomerular spaces. × 340

ty of cross-reaction between 4B9 and immunoglobulins in glomerular immune complexes, a potential problem because of the immunoglobulin-like domains in the VCAM-1 molecule, all specimens were incubated with an isotype-matched irrelevant antibody, a mouse IgG1*k* antibody for *Aspergillus niger* glucose oxidase (DAK-GO1: Dakopatts) that recognized neither VCAM-1 nor CD68.

All specimens were assessed immunohistochemically in single-stained serial sections. In brief, biopsy specimens were fixed in periodate-lysine-paraformaldehyde for 6 h at 4°C and washed successively in PBS containing 10%, 15%, and 20% sucrose. The specimens were then embedded in O.C.T. compound (Miles, Elkhart, Ind.) and rapidly frozen in liquid nitrogen. Serial cryostat sections (6 µm thick) were cut using a microtome, mounted on poly-L-lysine-coated glass slides, and air-dried. Sections were incubated in a moist chamber with the primary antibodies at 4°C for 48 h. After inactivation of endogenous peroxidase activity by incubation for 15 min with 0.3% hydrogen peroxide in methanol, the sections were incubated with the secondary antibody for 24 h at 4°C. The slides were washed thoroughly in PBS between each step. Reaction products were developed with 0.03% 3,3′-diaminobenzidine tetrahydrochloride (Dojin, Kumamoto, Japan) and 0.02% hydrogen peroxide, after which the sections were counterstained with methyl green and mounted for light microscopy.

The staining procedure for immunoelectron microscopy was the same as for light microscopic immunohistochemistry. After visualization of the reaction products, the sections were fixed in 2% osmium tetroxide for 1 h at room temperature, dehydrated in a graded ethanol series, and embedded in Epon 812. Ultrathin sections were then cut straight from the surface of the block, lightly stained with lead citrate, and examined with an electron microscope (H-7100, Hitachi, Tokyo, Japan).

Results

In the normal control kidneys, VCAM-1 expression was restricted to a few of the parietal epithelial cells and proximal tubules. In addition, a few CD68-positive cells were seen within the glomeruli and the interstitium.

In the diseased glomeruli with mild proliferative changes, VCAM-1 expression was seen focally in the mesangium, on endothelial cells, throughout the glomerular parietal epithelium, and occasionally on intracapillary leucocytes (Fig. 1a). With the progression of glomerular damage, VCAM-1 expression became more intense at all sites, and increased markedly in the cellular/fibrocellular crescents (Fig. 1b). In the tubules, VCAM-1 expression was confined to the basal and lateral surfaces of a few proximal tubules (Fig. 1c). A few CD68-positive cells were scattered in mesangium, periglomerular spaces, tubules, and perivascular sites (Fig. 1d). In serial sections, CD68-positive cells were

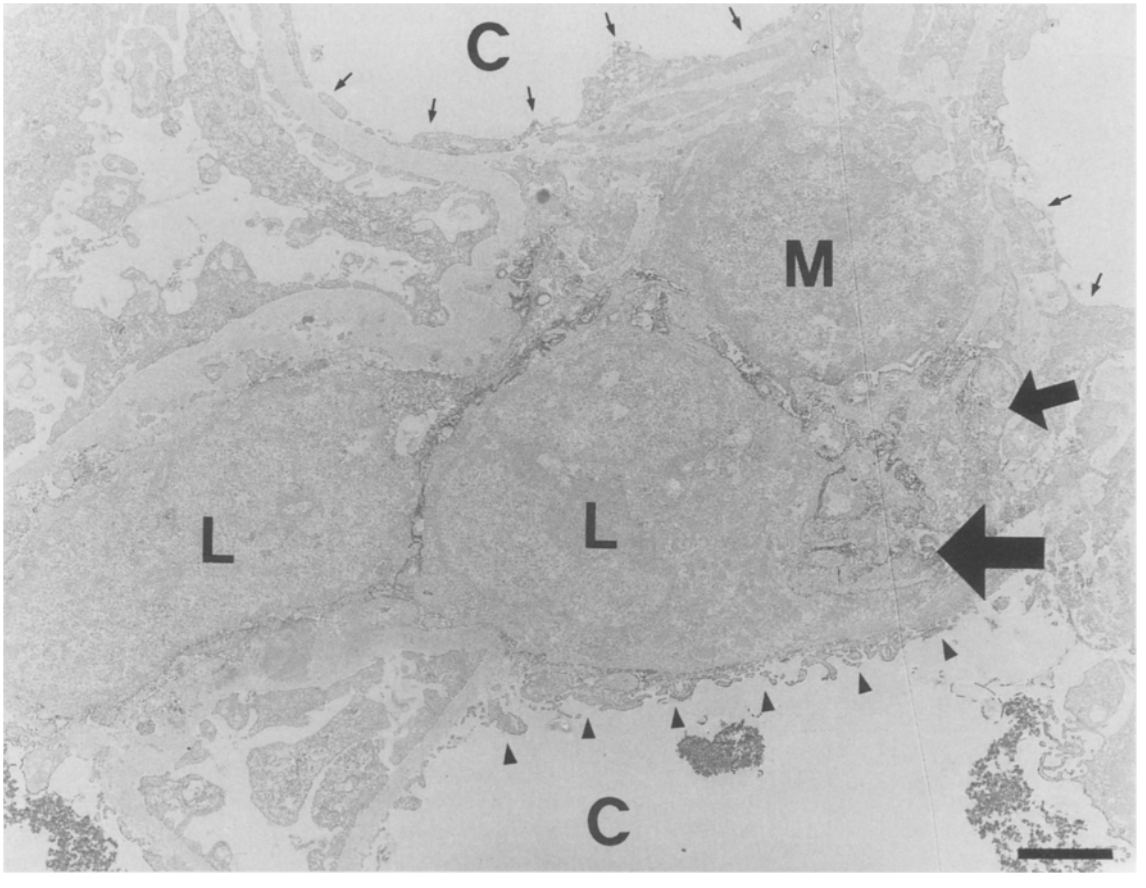

Fig. 2 Immunoelectron micrograph showing three mononuclear cells with a large nucleocytoplasmic ratio. VCAM-1 is expressed at the interfaces between these cells in the mesangial area. The ultrastructural morphology suggests that two of the cells are of the monocyte/macrophage lineage (*L*) and the other is a mesangial cell (*M*). VCAM-1 is also expressed at the interface between a monocyte/macrophage-like cells and an endothelial cell. The former cell has extended many processes into the VCAM-1-positive matrix (*large arrows*). VCAM-1 expression is seen along the luminal surface of capillary endothelial cells adjacent to the infiltrating macrophage-like cell (*arrowheads*), while it is sparse on the other endothelial cells (*arrows*). Also note amorphous immunoreactive granules in the capillary lumina (*C*). × 6,700, *bar* 2 µm

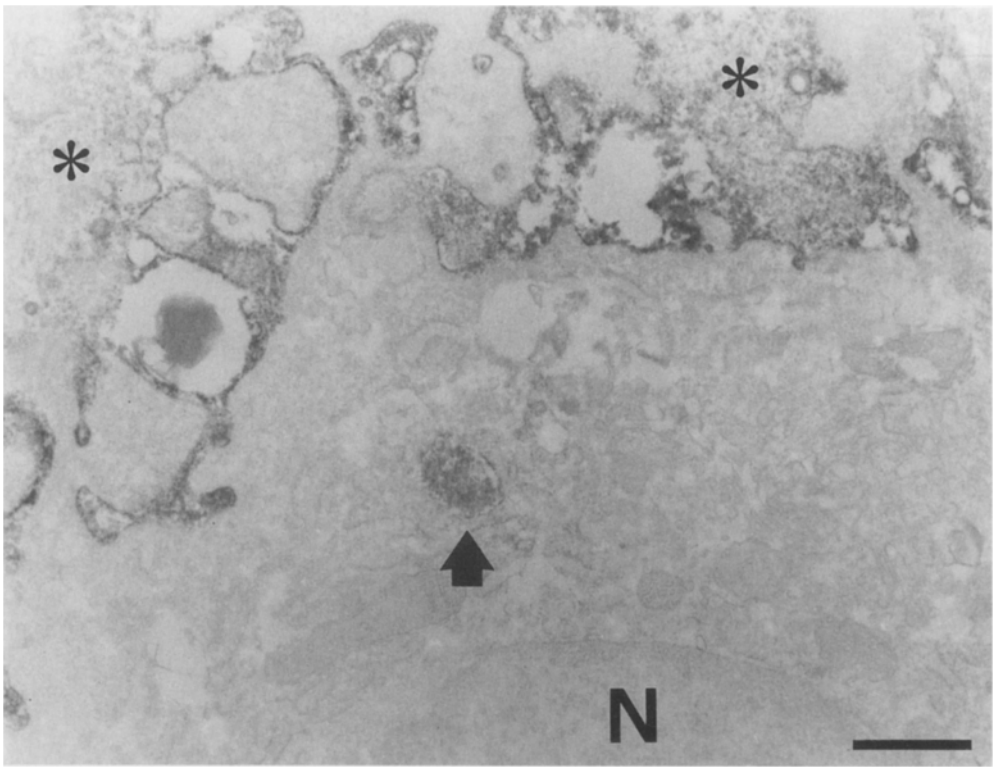

Fig. 3 At a higher magnification, an infiltrating cell (probably a monocyte/macrophage) shows many cell surface processes. VCAM-1 is evenly distributed on the outer surface of the plasma membrane, and there is also immunoreactivity in the mesangial matrix (*asterisks*). The *arrow* indicates an immunoreactive multivesicular body (*N* nucleus). × 34,000, *bar* 0.5 μm

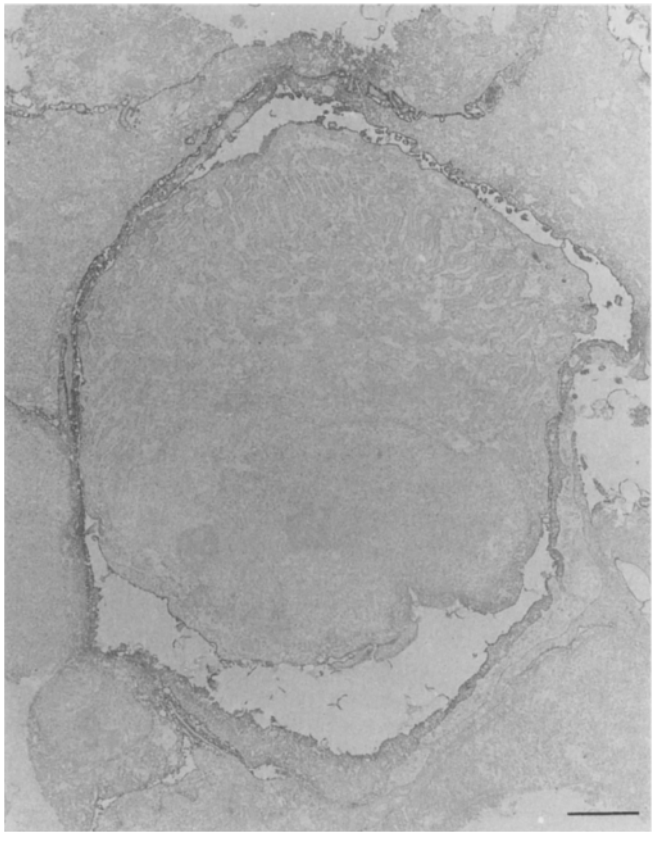

Fig. 4 Immunoelectron micrograph of VCAM-1 expression in a cellular crescent. The infiltrating cells (morphologically monocyte/macrophages) are clustered together. Immunoreactivity is present along the surface of the cell membrane. × 5,500, *bar* 2 μm

seen in a VCAM-1-positive glomerulus, and CD68-positive cells were often present within the VCAM-1-positive cellular crescents and on the surrounding infiltrating inflammatory cells (Fig. 1e).

In the negative control sections, there was no relevant staining of either the glomeruli or the tubulointerstitium.

Immunoelectron microscopic examination of the normal control kidneys, revealed no expression of VCAM-1 by any cells within the glomeruli, although it was expressed on the free surface of the parietal epithelial cells of Bowman's capsule.

In diseased kidneys, VCAM-1 was expressed on the free surface of the parietal and visceral epithelial cells, on the luminal surface of the capillary endothelial cells, on and around infiltrating monocyte/macrophage-like cells, on parts of the surface of the mesangial cells (Fig. 2), and in the matrix of the expanded mesangium (Fig. 3). However, it was not expressed in the parts of the mesangium without proliferative change, even in the same glomerulus. In the cellular and fibrocellular crescents, VCAM-1 expression was found on the surface of almost all cells, including monocyte/macrophage-like cells, fibroblast-like cells, and epithelial cells (Fig. 4). Some cells also showed VCAM-1 positivity within the rough endoplasmic reticulum and the perinuclear space as well as on the surface of the plasma membrane (Fig. 5). The infiltrating monocyte/macrophage-like cells and fibroblast-like cells contained small vesicles that featured tiny immunoreactive granules on their inner surfaces. Exocytosis of VCAM-1-positive materials was also often seen (Fig. 6). Additionally, both the glomerular capillary lumen and the urinary spaces in Bowman's cap-

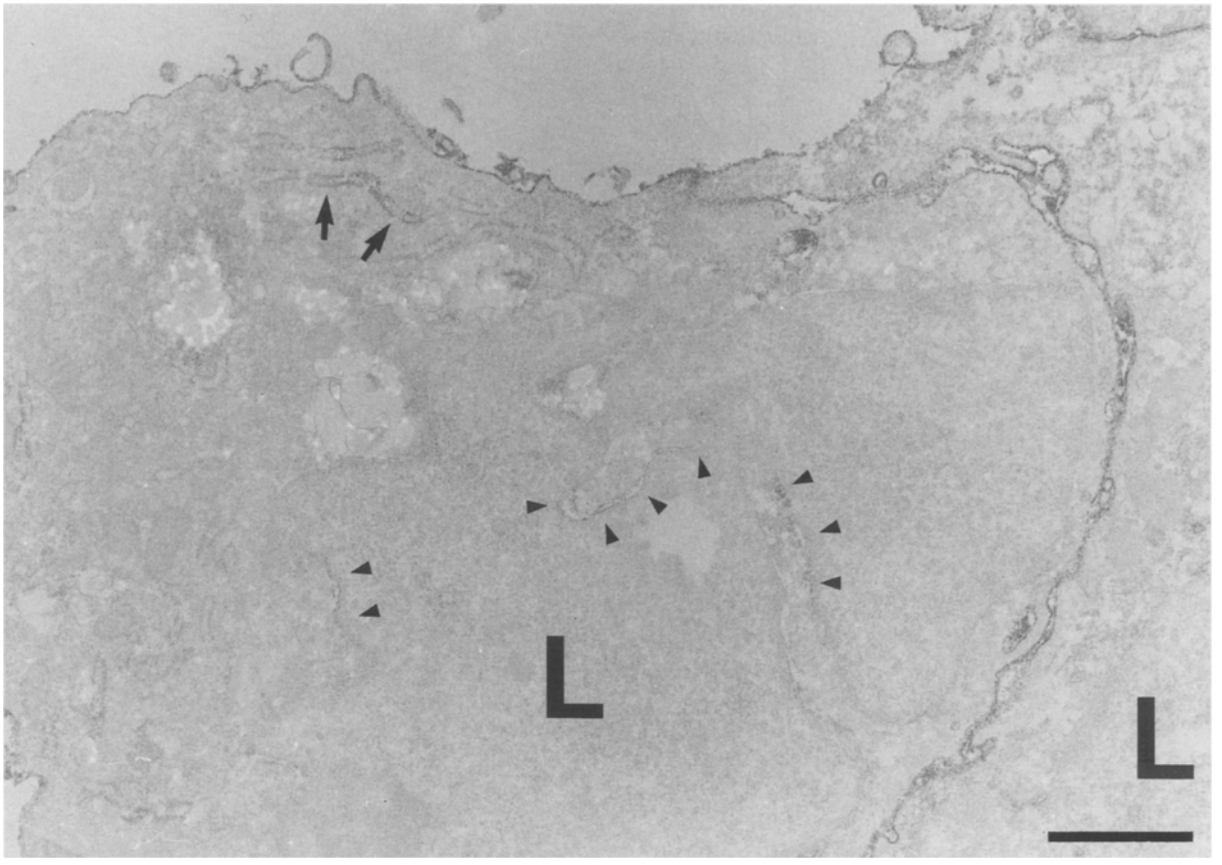

Fig. 5 Another part of the glomerulus shown in Fig. 4. VCAM-1 is evenly distributed on the outer surface of the plasma membrane of the infiltrating cells, which are probably monocyte/macrophages (*L*). Immunoreactivity is present in well-developed rough endoplasmic reticulum (*arrows*) and in the perinuclear space (*arrowheads*). × 20,000, *bar* 1 μm

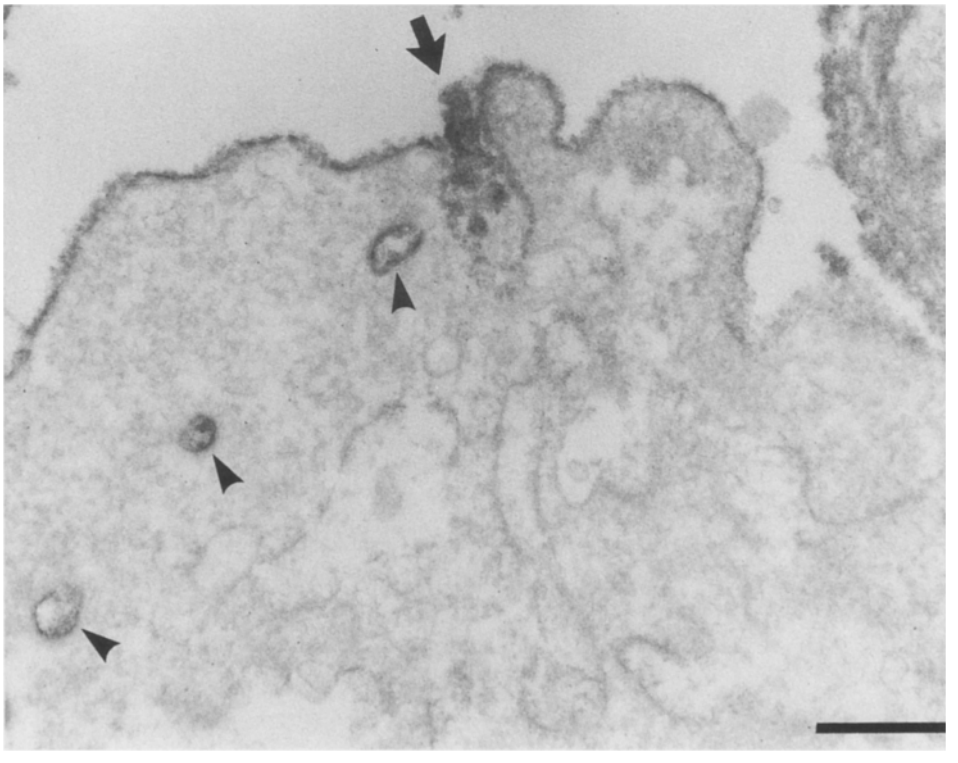

Fig. 6 A high magnification of a cell (probably a macrophage) in a cellular crescent. VCAM-1 is evenly distributed on the plasma membrane. Small vesicles (*arrowheads*) contain immunoreactive granules on their inner surface. The *arrow* indicates exocytosis of VCAM-1. × 60,000, *bar* 0.3 μm

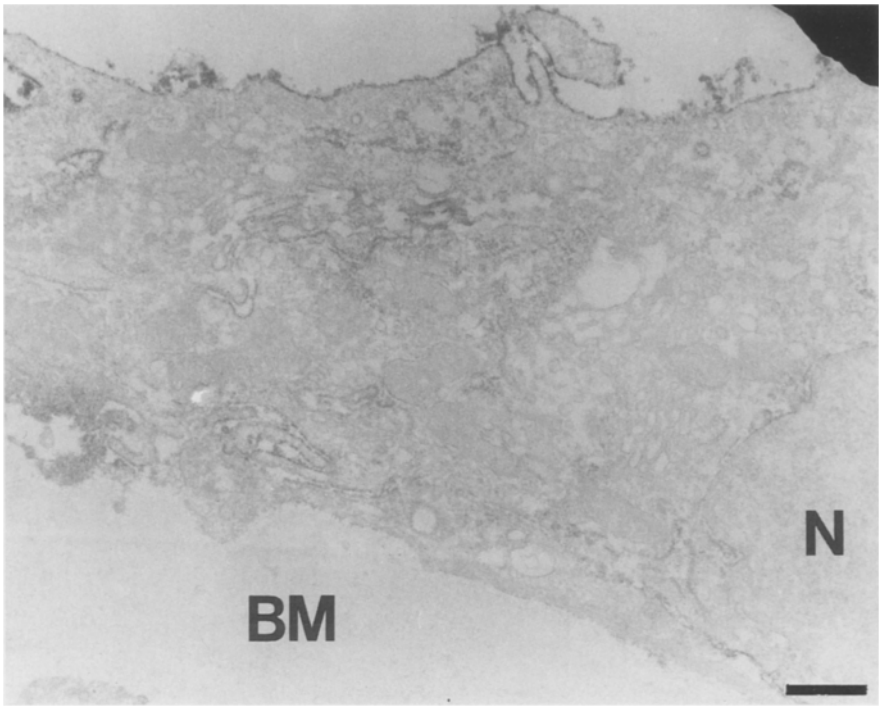

Fig. 7 VCAM-1 expression on the exposed surface of a parietal epithelial cell. Expression is also seen in the rough endoplasmic reticulum, the perinuclear space, the Golgi complex, and the basal surface of the plasma membrane (*N* nucleus, *BM* Bowman's basement membrane). × 22,000, *bar* 0.5 μm

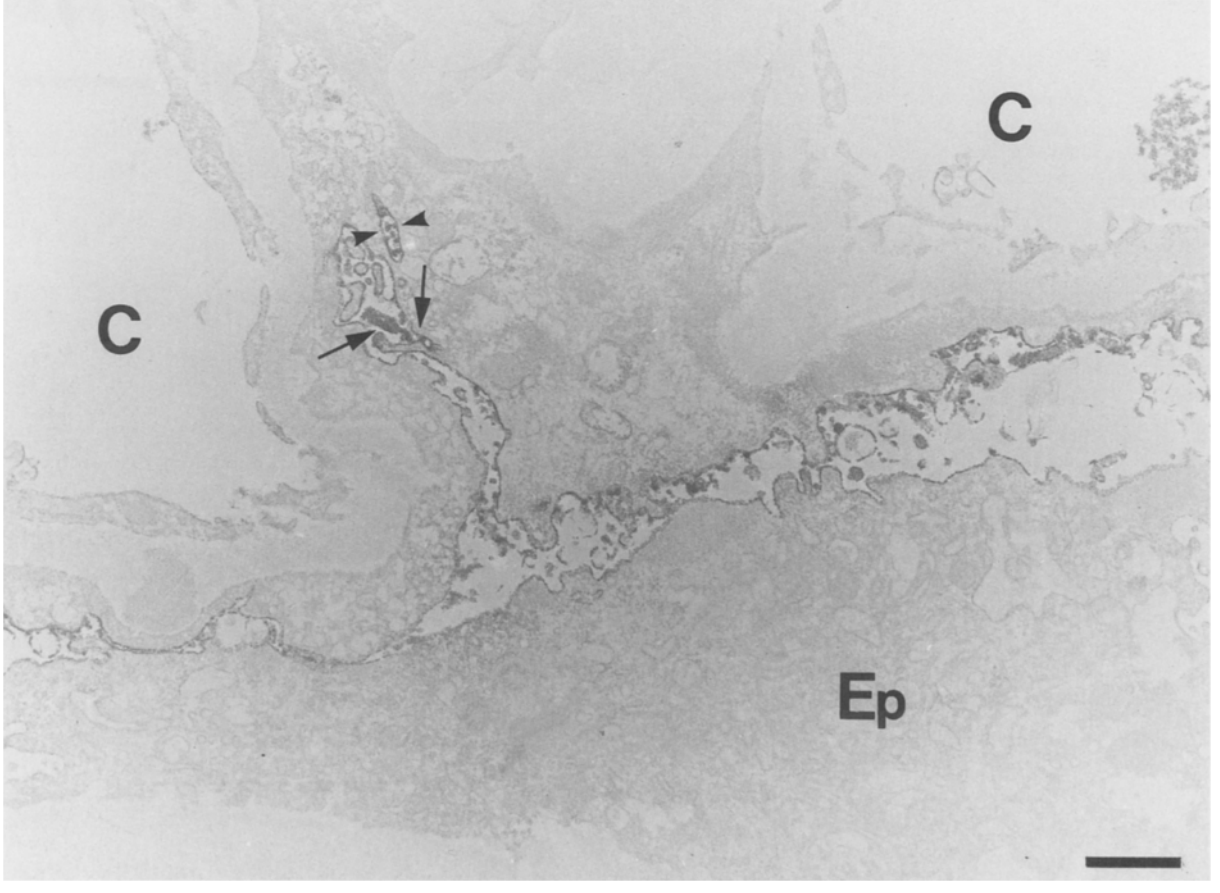

Fig. 8 VCAM-1 expression on the luminal plasma membrane of visceral and parietal epithelial cells. Exocytosis of VCAM-1 by a visceral epithelial cell is seen (*arrows*). A membrane-limited small structure contains tiny immunoreactive granules on its inner surface (*arrowheads*). Also note that there is no VCAM-1 expression by epithelial cells on the surface adjacent to the basement membrane or on the luminal surface of the capillary endothelial cells. Immunoreactive amorphous granules are seen in both the capillary lumen and the urinary space (*Ep* parietal epithelial cell, *C* capillary lumen). × 12,600, *bar* 1 μm

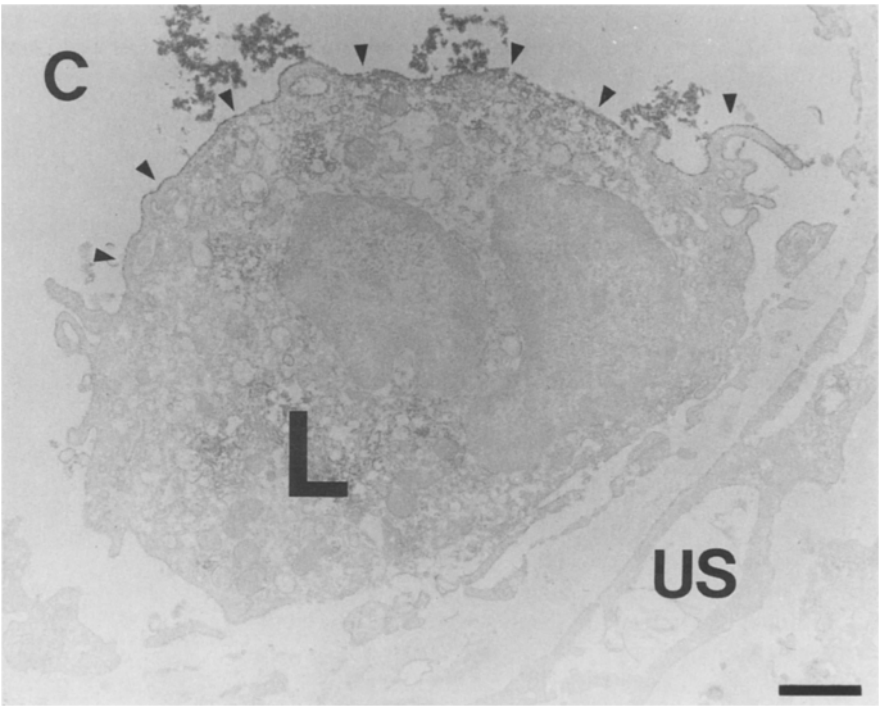

Fig. 9 A leucocyte (probably a monocyte; *L*) is seen adjacent, but not adherent, to the glomerular capillary endothelium. There is VCAM-1 expression on the luminal surface (*arrowheads*), while there is no expression on the surface close to the endothelial cells or on the luminal surface of the endothelial cells themselves. Also note the amorphous immunoreactive granules in the capillary lumen (*C* capillary lumen, *US* urinary space). × 10,000 *bar* 1 μm

sule contained amorphous positive reaction products, which were often associated with exocytosis by the surrounding capillary endothelial cells and by the parietal and visceral epithelial cells (Figs. 7, 8). Nonadherent circulating monocytic cells also occasionally showed VCAM-1 expression on the surface of the plasma membrane (Fig. 9). In the tubules, VCAM-1 was expressed predominantly on the basal and lateral surfaces of a few proximal tubules at the light microscopic level, but we could not determine its ultrastructural localization owing to the limitations of the frozen sections examined.

Discussion

Although a marked increase of VCAM-1 expression has been detected in some of the proximal tubular cells in patients with interstitial nephritis [6, 7, 19], there have been few reports on the types of cells within the glomerulus that express this molecule [1].

In contrast to the widespread expression of ICAM-1 in glomerulonephritis [15], VCAM-1 staining was restricted to relatively small areas corresponding to the lesions of diseased glomeruli. However, more cell types showed VCAM-1 expression than were reported previously in experimental models and human nephritis [19, 21], including infiltrating cells of the monocyte/macrophages series as well as resident glomerular cells.

In mesangial areas showing cell proliferation and/or an increase of the matrix, VCAM-1 expression was markedly increased both at the interface between infiltrating macrophage-like cells and the mesangial or endothelial cells and on the luminal surface of adjacent capillary endothelial cells. In contrast, no VCAM-1 expression was seen in mesangial areas without proliferative changes. If VCAM-1 has an important role in leucocyte adhesion and migration into the interstitium, as was reported previously in allograft rejection [12], our findings suggest that it may also have a pathogenic role in the mesangial expansion associated with proliferative glomerulonephritis by promoting the migration of circulating monocytic cells and their adhesion to mesangial cells after initial adhesion to the endothelium. This hypothesis is in agreement with the in vitro findings of Brady et al. [4].

VCAM-1 expression on the surface of nonadherent circulating monocytic cells was an unexpected finding. It appeared to be inconsistent with previous reports that circulating lymphocytes and monocytes show surface expression of VLA-4, the ligand for VCAM-1 [11, 18]. We have no definite explanation for this phenomenon, but the following facts may suggest an explanation. First, increased soluble VCAM-1 levels have been found in the serum of patients with the glomerular disease [14], and some of our patients also had a high serum VCAM-1 level (data not shown). Second, the glomerular capillaries occasionally contained amorphous VCAM-1-positive reaction products, which might have represented soluble VCAM-1. Third, exocytosis of VCAM-1 by endothelial cells and by visceral or parietal epithelial cells was often detected. These findings suggest that soluble VCAM-1 secreted by local tissue cells may bind to receptors on the surface of monocytic cells rather than being actively produced by the monocytic cells themselves. Binding of VCAM-1 to other cells in the glomerulus may also have occurred. Thus, it was difficult to determine whether our antibody (4B9) detected expression of VCAM-1 by renal cells and infiltrating leucocytes or simply stained soluble

VCAM-1 trapped in the kidney, and this should be kept in mind when our results are interpreted.

Crescents consist of a variety of cells, such as monocyte/macrophages, polymorphonuclear leucocytes, lymphocytes, fibroblast-like cells, and visceral and parietal epithelial cells. During crescent formation, cells of the monocyte/macrophage lineage may be prominent [13]. In our patients, cells positive for CD68, a common monocyte antigen, were often concentrated in the cellular crescents and in the expanded mesangium. As with intracapillary monocytic cells, VCAM-1 expression was also detected on the surface of the macrophage-like cells in crescents. This may have simply been because soluble VCAM-1 secreted by the surrounding cells was bound to receptors on the macrophages. However, the following findings suggested that VCAM-1 was synthesized in the rough endoplasmic reticulum of macrophage-like cells, transported intracellularly, incorporated into the plasma membrane, and discharged by exocytosis into the urinary space: VCAM-1-positive reaction products were detected within the rough endoplasmic reticulum, the perinuclear space, and cytoplasmic multivesicular bodies, while exocytosis was noted at the plasma membrane. The factors that trigger the local synthesis of VCAM-1 are not known. However, activated cells of the monocyte/macrophage lineage can produce some cytokines with the ability to induce expression of adhesion molecules [16, 21], so cytokines secreted by infiltrating macrophages may have an autocrine effect. Mulligan et al. [16] have also suggested that in a certain phase of nephritis, the progression of monocytic cell accumulation, cytokine production, and adhesion molecule expression may all be coordinated. VCAM-1 may play an important part in crescent growth by facilitating the adhesion of macrophages to resident glomerular cells as well as to other macrophages.

We found that VCAM-1 was expressed predominantly on the basal and lateral surfaces of a few proximal tubules at the light microscopic level, as previously reported, but we failed to detect its precise ultrastructural localization because of the limitations of the frozen section material that was available for study. Despite this, the different pattern of expression from that of ICAM-1, which was confined to the brush border [15], suggests that VCAM-1 may have a different role in glomerulonephritis from this other ligand.

In conclusion, our findings suggest that the local production and secretion of VCAM-1 by both infiltrating monocyte/macrophages and resident glomerular cells may be related to the pathogenesis of proliferative and crescentic glomerulonephritis, both through promotion of the accumulation of macrophages in the mesangium and through facilitation of interactions between macrophages and resident glomerular cells or other macrophages in the crescents. However, since our data are purely morphological, we are unable to determine whether VCAM-1 expression has a key role in glomerular damage or is a consequence of such damage. Further studies in experimental models of glomerulonephritis are needed to determine the effect of blocking antibodies directed against VCAM-1.

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