Antibodies to Intercellular Adhesion Molecule 1/Lymphocyte Function-associated Antigen 1 Prevent Crescent Formation in Rat Autoimmune Glomerulonephritis

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Summary

In patients with glomerulonephritis widespread crescents are associated with a poor prognosis. Crescent formation appears to depend on the migration of mononuclear cells into Bowman's space, and therefore the interaction between leukocytes and glomerular endothelium may be a critical event in the genesis of crescents. We performed the present study to determine the effects of mouse monoclonal antibodies to the adhesion molecules intercellular adhesion molecule 1 (ICAM-1) and lymphocyte function-associated antigen 1 (LFA-1) in a model of crescentic glomerulonephritis in Wistar-Kyoto rats, induced by immunization with bovine glomerular basement membrane (GBM). By 10–14 d after immunization, the rats had developed circulating anti-GBM antibodies, reactive with the $\alpha 3$ chain of type IV collagen (the Goodpasture antigen), accompanied by proteinuria, accumulation of rat immunoglobulin (Ig)G in the GBM, increased expression of ICAM-1 by glomerular endothelial cells, infiltration of glomerular tufts with LFA-1^+ T cells and monocyte/macrophages, and early crescents. At 5 wk all rats had diffuse fibrocellular crescents, glomerular sclerosis, and tubulointerstitial damage. All rats developed severe renal insufficiency and died by 5 or 6 wk. The administration of monoclonal antibodies to rat ICAM-1 and LFA-1 markedly decreased the severity of the renal disease. In a group of rats injected three times a week with the monoclonal antibodies, from 2 d before immunization with GBM to day 14, glomerular abnormalities and proteinuria were virtually absent at day 14; even at 5 wk glomerular disease was quite mild, with only slight crescent formation and with only a mild decrease in renal function. When treatment was continued until 5 wk, the beneficial effects were even more marked, with virtual absence of crescents and with preservation of normal renal function. In a group of rats in which treatment was initiated on day 14, shortly after the appearance of glomerular abnormalities, progression of the disease was appreciably retarded, and the decrease in renal function was inhibited. The kidneys of rats treated from days −2 to 14 with antibodies to ICAM-1 and LFA-1 showed bright linear staining for rat IgG along the GBM, which did not differ in intensity from that seen in untreated rats. Furthermore, the titers of anti-GBM antibodies at 2 wk in treated rats were not lower than that seen in most of the untreated rats. There was, however, moderate reduction of anti-GBM antibodies at 5 wk in the treated rats. In addition, in rats in which treatment was started after onset of the disease, the titers of anti-GBM antibodies did not decrease, although the progression of disease was inhibited. We conclude that the preventive or therapeutic effects of antibodies to ICAM-1 and LFA-1 in rat anti-GBM glomerulonephritis probably resulted mainly from interference with interaction between leukocytes and activated glomerular endothelium, although reduction in the autoimmune response may have contributed to the beneficial effects. The results raise the possibility that similar treatment might be used to limit the progression of glomerular damage in human crescentic glomerulonephritis.

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In patients with glomerulonephritis the formation of crescents is usually associated with an ominous prognosis (1). Crescents are thought to be induced by an influx of circulating monocytes/macrophages (2–4) and plasma proteins (5, 6) into Bowman's space, mainly through gaps in the glomerular basement membrane (GBM)[1] (7, 8). Gaps are apparently induced by proteolytic enzymes released by leukocytes that have migrated across the endothelium (9). Thus, the interaction between endothelium and leukocytes may be a critical event in the formation of crescents.

The migration of leukocytes through inflamed blood vessels requires the adhesion of leukocytes to the endothelium, a phenomenon mediated by endothelial and leukocyte surface molecules functioning as receptors and ligands (10). One of the most important interactions is between intercellular adhesion molecule 1 (ICAM-1) and LFA-1. ICAM-1 (CD54) is a member of the Ig superfamily, which is expressed on a variety of hematopoietic cells, as well as on endothelial cells, fibroblasts, and certain epithelial cells. LFA-1 (CD11a/CD18) is a leukocyte cell surface glycoprotein widely expressed on cells of the hematopoietic lineage (11).

The present report describes the effect of mAbs against rat ICAM-1 and LFA-1 in Wistar-Kyoto rats with a lethal autoimmune anti-GBM nephritis (12). This disease is characterized by development of antibodies reactive with the Goodpasture's antigen (13), linear deposition of rat IgG in the GBM (12), infiltration of glomeruli by T lymphocytes and monocyte/macrophages, widespread crescent formation, and glomerular sclerosis. We found that injections of anti-ICAM-1 and LFA-1 antibodies prevented the formation of crescents, markedly reduced the severity of other glomerular and tubulointerstitial lesions, and prolonged the survival of the animals.

Materials and Methods

Animals. Wistar-Kyoto rats weighing 150–180 g were purchased from Charles River Breeding Laboratories (Wilmington, MA), and allowed free access to food and water.

Antigen Preparation and Rat Immunization. GBM was isolated from fresh bovine kidneys using a differential sieving method (14). GBM was sonicated, digested with collagenase (type VII; Sigma Chemical Co., St. Louis, MO) at 37°C for 16 h in 0.05 M Hepes buffer (pH 7.4) with 0.01 M $CaCl_2$, and centrifuged at 14,500 *g* for 30 min. The pellets were resuspended in 0.1 M citrate buffer, pH 3.0. The rats were immunized (on day 0) by a single subcutaneous injection of 50 mg (wet weight) of the GBM homogenate emulsified with an equal volume of CFA (Sigma Chemical Co.), and an injection at a different subcutaneous site with 5×10^9 cells of pertussis vaccine (Michigan Public Health, Lansing, MI).

mAbs. A mouse IgG1 anti-rat ICAM-1 mAb (1A29) and a mouse IgG2 anti-rat LFA-1 mAb (WT.1) were generated and characterized as previously described (15, 16). An IgG1 mouse anti-diethyl thiamine pentaacetic acid (DTPA) antibody and an IgG2a mouse anti-human CD44 antibody, which did not react with rat tissues, were used as isotype controls. The ascitic supernatants were purified on protein A-Sepharose CL-4B columns.

Histologic Methods. Samples of renal tissue were obtained at 7, 10–14, and 35 d, and 10–20 wk after immunization. Tissue was fixed in 10% buffered formalin, and embedded in paraffin. Sections of 4 μm were stained with hematoxylin-eosin (H&E) and by the periodic acid-Schiff method. The number of nuclei was counted in cross-sections of 10 glomeruli randomly selected in a histologic section from six rats in each group. Crescents were counted in 50 glomeruli randomly selected in sections from six rats of each group. Crescents were roughly divided into three groups: (*a*) circumferential; (*b*) those involving about one-half of the glomerulus; and (*c*) those involving a quarter or less of the glomerulus.

Immunofluorescence Methods. Fluorescein-conjugated (FITC)-IgG fractions of goat or rabbit antisera to rat IgG, C3, and fibrinogen were purchased from Cappel Laboratories (Cochranville, PA). ICAM-1 and LFA-1 were revealed by successive incubations of tissue sections with normal goat serum, relevant mouse mAbs, and affinity-purified FITC-goat anti-mouse IgG (Cappel Laboratories) that had been extensively absorbed with rat serum. As controls, we used the irrelevant isotype-matched antibodies against DTPA and human CD44. The immunofluorescence methods, including controls, were performed as previously described (17). The intensity of staining for rat IgG along the GBM was semiquantitatively graded on a scale from 0 to +++ (0, absent; +, minimal; ++, moderate; +++, marked). The interpretations were performed without knowledge of the group in at least 15–20 glomeruli from three sections from each group. When extensive formation of crescents and glomerulosclerosis were present (groups II and III) evaluation of glomerular staining was based on residual, relatively undamaged, capillary loops. The sections were mounted in a medium containing *p*-phenyllenediamine to reduce fading (18) and examined with a Nikon microscope equipped with epifluorescence optics and appropriate filters.

Immunoperoxidase Methods. Mouse IgG bound to renal tissues of rats injected with the mAbs to ICAM-1 and LFA-1 was detected by an immunoperoxidase technique using biotin-labeled affinity-purified horse anti-mouse IgG (Vector Labs, Inc., Burlingame, CA), previously absorbed with rat serum, and avidin-biotinylated horseradish peroxidase complexes (Vector Labs, Inc.). The phenotype of inflammatory cells was determined by the use of mouse mAbs to rat T lymphocytes (W3/13) (Accurate Chem. & Sci. Corp., Westbury, NY) and to rat monocyte/macrophages (ED1) kindly provided by Dr. C. D. Dijkstra (19). Sections of frozen kidney were cut at 4 μm in a cryostat, fixed in acetone for 10 min at room temperature, air dried, and incubated with the primary mouse mAb followed by biotin-labeled affinity-purified horse anti-mouse IgG (Vector Labs, Inc.) (dilution, 1:200; 30 min), and avidin-biotinylated horseradish peroxidase complexes (Vector Labs, Inc.) (dilution, 1:100; 45 min). The sections were then treated for 1–5 min with 0.04% AEC (Sigma Chemical Co.) in 0.03% H_2O_2 in 0.1 M acetate buffer, pH 5.0, rinsed with distilled water, and counterstained with hematoxylin. Controls for the immunoperoxidase studies were performed as previously described (20). The number of positive cells was counted in 10 randomly selected glomerular cross-sections obtained from six rats of each group. The intensity of interstitial mononuclear cell infiltration was estimated by counting T lymphocytes and monocyte/macrophages in five randomly selected renal cortical areas of 0.25 mm^2, in sections from six rats in each group, using a 19-mm diameter micrometer disk (Olympus Corp., Lake Success, NY) inserted into an ocular lens of the microscope (21).

Urinary Output and Protein Excretion. 16-h urinary samples were collected in metabolic cages and their volumes were measured. Uri-

[1] *Abbreviations used in this paper:* DTPA, diethyl thiamine pentaacetic acid; GBM, glomerular basement membrane; ICAM-1, intercellular adhesion molecule 1.

nary protein excretion was measured by the quantitative biuret method.

Serum Creatinine. Serum creatinine was measured by a quantitative colorimetric assay (Sigma Chemical Co.).

Serum Antibody to the α3 Chain of Type IV Collagen. Serum samples from individual rats in groups I, II, IV, and VI were tested by an ELISA kit (Biocarb Diagnostics; SciMedx Corp., Denville, NJ). In group II, 16, of 20 randomly selected rats were tested at 2 wk, and seven at 5 wk. In group IV, all rats were tested at 2 wk, and seven of eight at 5 wk. In group VI, all rats were tested at 5 wk. Microtiter strips coated with the α3 chain of type IV collagen (the Goodpasture antigen [22]) were incubated with a 1:25 dilution of the rat sera as well as three reference-positive human sera, which had been arbitrarily defined by the manufacturer to contain 1,000, 100, and 10 ELISA units, respectively. In addition, normal human serum was analyzed. All samples were tested in duplicate. After a 1-h incubation and three washes, specific binding of rat or human IgG was detected using either an alkaline phosphatase–conjugated rabbit antibody to rat IgG or an alkaline phosphatase–conjugated antibody to human IgG. Finally, the substrate *p*-nitrophenyl-phosphate was added, and absorbances were read at 405 nm in a EL-310 (BioTek Instruments, Winooski, VT) ELISA auto reader at time 0 and at 60 min for each well. The net absorbance change was determined for each well by subtracting time 0 from 60 min. The optical densities of the three reference sera were plotted against the defined ELISA units on a log-linear scale in order to construct a standard curve. The value for each unknown rat serum was then determined from the standard curve.

PBL. Samples of peripheral blood were collected in heparinized tubes from normal rats, and from rats at 2 and 5 wk after immunization. Total leukocytes were counted using a hemocytometer (Fisher Scientific Co., Pittsburgh, PA). Differential leukocyte counts based on 100 cells were performed on blood smears stained with Wright-Giemsa (Sigma Chemical Co.).

Statistical Analysis. The data are expressed as means ± SD. An unpaired *t* test was used for comparison.

Experimental Design. The experimental design is shown in Table 1.

Results

The main results are summarized in Table 2; further details are given below.

Table 1. *Experimental Design*

Group	No. of rats	Immunization (day 0)	mAbs	Antibody injections* (d) −2 0 7 14 21 28 35
I	6/6	None/CFA	None	
II	20	GBM + CFA	None	
III	6	GBM + CFA	Anti-DTPA + anti-human CD44	◄——►
IV	8	GBM + CFA	Anti-rat ICAM-1 + anti-rat LFA-1	◄——►
V	6	GBM + CFA	Anti-rat ICAM-1 + anti-rat LFA-1	◄————►
VI	6	GBM + CFA	Anti-rat ICAM-1 + anti-rat LFA-1	◄——►

* 0.5 mg i.v. and 0.5 mg i.p. three times a week.

Table 2. *Morphological, Immunohistochemical, and Serological Findings*

	Group					
	I	II	III	IV	V	VI
Glomerular cellularity at 2 wk	49 ± 1	116 ± 8	113 ± 9	49 ± 1*	49 ± 1*	116 ± 8
Crescent formation at 5 wk						
Circumferential (%)	0 ± 0	68 ± 11	64 ± 18	1 ± 2*	0 ± 0*	0 ± 0*
1/2 glomerulus (%)	0 ± 0	24 ± 5	24 ± 10	2 ± 4*	0 ± 0*	3 ± 3*
1/4 glomerulus (%)	0 ± 0	8 ± 7	8 ± 8	9 ± 7	7 ± 11	28 ± 15
Interstitial cellularity at 5 wk						
Monocyte/macrophages	2 ± 0	170 ± 31	160 ± 18	15 ± 3*	ND	ND
T lymphocytes	2 ± 1	61 ± 31	57 ± 12	35 ± 25*	ND	ND
Rat IgG in GBM at 2 wk	0	+ + +	+ + +	+ + +	+ + +	+ + +
Creatinine at 5 wk (mg/dl)	0.60 ± 0.05	1.60 ± 0.48	1.61 ± 0.59	0.87 ± 0.12*	0.75 ± 0.19*	0.89 ± 0.17*

* Significant difference when compared with groups II or III ($p < 0.01$).

Group I. Normal Rats and Rats Injected with CFA Only. Kidney tissue obtained at 10–14 d and 5 wk showed no histologic or immunohistochemical abnormalities. ICAM-1 was weakly expressed by the endothelial cells of large vessels, peritubular capillaries, and glomeruli. Tubule cells did not stain for ICAM-1. Rare LFA-1$^+$ cells were present in some large blood vessels. The urinary protein excretion was normal (20–40 mg/d). The PBL count was 5,190 ± 1,114/mm^3. Serum creatinine levels were normal and no circulating antibodies to the α3 chain of type IV collagen were found.

Group II. Rats Immunized with Bovine GBM in CFA. 7 d after immunization, renal specimens revealed no abnormal histologic or immunohistochemical findings. There was no abnormal proteinuria.

10–14 d after immunization, all rats had increased urinary protein excretion (Fig. 1), linear deposition of rat IgG in the GBM (+++) and in tubular basement membranes, and mild glomerular abnormalities characterized by irregular hypercellularity of tufts, adhesions, and early crescents (Fig. 2 *a*). Minimal deposition of C3 in the GBM was also observed. Mononuclear cells expressing markers of T lymphocytes and monocyte/macrophages (Fig. 2 *b*) and LFA-1 (Fig. 2 *c*) were found in glomerular capillaries, in Bowman's space, in areas of glomerulo-capsular adhesions, and in nascent crescents. Neutrophils were not present. The expression of ICAM-1 in the glomerular endothelial cells was greater than in normal rats or in immunized rats at day 7 (Fig. 2, *d* and *e*). Weak staining for ICAM-1 was also found in the brush border of proximal tubules. The sera of 16 rats in group II were tested for the antibodies to the α3 chain of type IV collagen, and all the sera contained antibodies (Fig. 3). In 15 of the rats the titers were relatively low (36.3 ± 21.8 U); however, one rat had a very high titer (350 U). The renal lesions of this rat did not differ from those of the others. The average PBL count was 7,513 ± 835/mm^3, and the differential count revealed 27 ± 5% polymorphonuclear cells and 73 ± 5% mononuclear cells.

5 wk after immunization, circumferential fibrocellular or fibrotic crescents with partial or complete sclerosis of tufts were observed in most glomeruli (Fig. 4). Linear GBM staining for IgG (+++) was seen in nonsclerotic portions of glomeruli. The crescents were stained intensely by antifibrinogen antibodies (Fig. 4, *inset*). There was irregular tubular atrophy and focal interstitial fibrosis. Markedly increased expression of ICAM-1 was detected in the endothelium and in the mesangial matrix of glomeruli (Fig. 5 *a*), and in the brush border of the proximal tubule cells. LFA-1-positive T lymphocytes and monocyte/macrophages were present in the tufts, crescents, in Bowman's capsule, and in the interstitium, especially around glomeruli (Fig. 5 *c–f*). All rats tested had circulating antibodies to the α3 chain of type IV collagen (Fig. 3), increased urinary protein and serum creatinine, and decreased urinary output (Fig. 1). The rats appeared sick, developed ascites, and died in uremia 5–6 wk after immunization.

Group III. Rats Immunized with Bovine GBM in CFA and Injected with Irrelevant anti-DTPA and Human CD44 mAbs. The histological, immunohistochemical, functional, and serological findings were similar to those observed in rats of group II.

Group IV. Rats Immunized with Bovine GBM in CFA and Injected with Anti-ICAM-1 and LFA-1 mAbs from Day 2 before Immunization to Day 14. The renal abnormalities were strikingly less in this group than in GBM-immunized rats not given anti-ICAM-1 or anti-LFA-1 antibodies (groups II and III). At 14 d, mouse IgG (presumably anti-ICAM-1) was found on the glomerular endothelium (Fig. 5 *g*). Thus, it was not feasible to detect ICAM-1 in glomeruli by indirect immunofluorescence. The levels of antibodies to the α3 chain of type IV collagen (23.0 ± 6.2 U) were slightly lower than those in 15 of 16 rats in group II whose antibodies were measured (Fig. 3), but the intensity of staining for rat IgG (+++) and C3 in the GBM was similar to that of group II. Nevertheless, the glomeruli, tubules, and interstitium appeared histologically normal (Fig. 6). In particular, there was no mononuclear cell infiltration of glomerular tufts. Urinary protein excretion (Fig. 1) was also normal. The total PBL count was 7,570 ± 1,143/mm^3, with 27 ± 4% polymorphonuclear and 73 ± 4% mononuclear leukocytes.

5 wk after immunization, and 3 wk after cessation of injections of antibodies to ICAM-1 and LFA-1, there was still intense staining for rat IgG (+++) in the GBM. However, levels of circulating antibodies to the α3 chain of type IV collagen were lower than those of rats in group II (Fig. 3). Mouse IgG was no longer detectable in glomeruli. Antibodies to ICAM-1 reacted intensely with the glomerular endothelium and with the brush border of proximal tubules. LFA-1$^+$ cells were now present in glomeruli and in the interstitium. The glomerular and tubulointerstitial abnormalities were strikingly less than those seen at 5 wk in group II (Fig. 7 *a*). In particular, the formation of crescents was remarkably reduced. Small deposits of fibrinogen/fibrin were found immediately around some glomeruli, but not in Bowman's space (Fig. 7 *a*, inset), and sclerosis of glomerular tufts was not present. Proteinuria (Fig. 1) and serum creatinine levels were less than in groups II and III. The PBL counts were normal. 12 wk after immunization the rats were still alive.

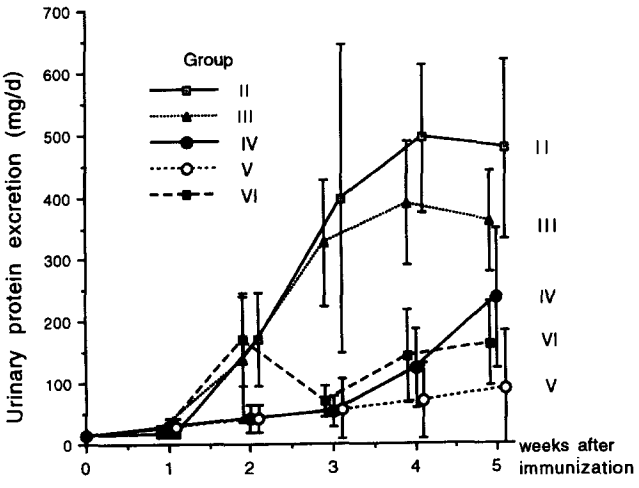

Figure 1. Urinary protein excretion in treated and untreated rats.

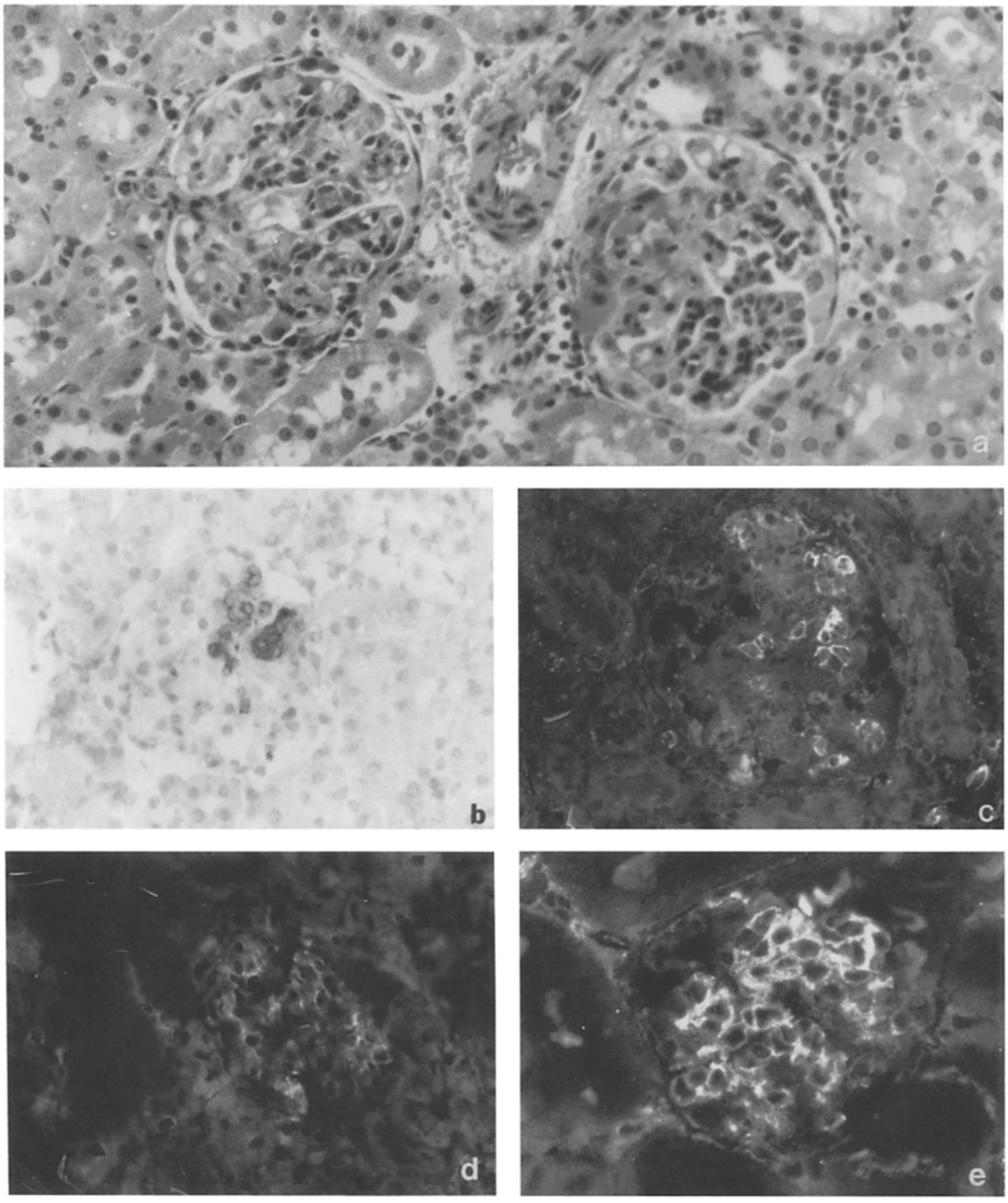

Figure 2. Glomerular lesions 14 d after immunization of rats with GBM in CFA (group II). (*a*) Irregular hypercellularity of glomerular tufts is seen (H&E; ×800); (*b*) cells reactive with ED1 (monocyte/macrophages) are seen in the glomerulus; (*c*) infiltration of LFA-1$^+$ cells in the glomerulus and focally in the interstitium. There is only faint staining for ICAM-1 in the glomerular capillary walls on day 7 (*d*), and markedly increased expression on day 14 (*e*); the section in *e* is contiguous to that in *c*. (*b–e*) ×400.

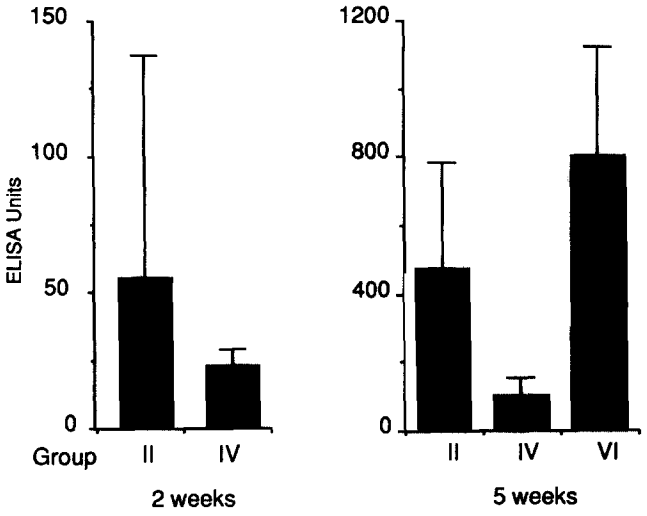

Figure 3. Circulating antibodies to the α3 chain of type IV collagen detected by ELISA. The columns represent the average of the results of 16 randomly selected rats at 2 wk, and seven at 5 wk in group II, all rats at 2 wk, and seven at 5 wk in group IV, and all rats at 5 wk in group VI. At 2 wk the difference in titers between groups II and IV was not statistically significant. The average titer in rats of group IV was 23.0 ± 6.2 U. One rat in group II had an unusually high titer of antibodies (350 U), and all the other 15 rats had relatively low titers (36.3 ± 21.8 U). At 5 wk, however, the titer of antibodies in group IV was significantly lower than in group II ($p < 0.05$). At 5 wk, the titers of antibodies in group VI were comparable to those in group II.

Group V. Rats Immunized and Injected with Anti-ICAM-1 and LFA-1 mAbs from Day 2 before Immunization to Day 35. On day 14, the kidneys appeared normal in histologic sections despite accumulation of rat IgG in the GBM (+++). At 5 wk, the histologic and functional changes were strikingly less severe than those seen in groups II and III, and were even less severe than in group IV; in particular there was almost complete absence of crescent formation. By way of contrast, the intensity of staining for IgG in the GBM (+++) was comparable to that seen in groups II and III. All the rats in group V were alive 20 wk after immunization.

Group VI. Rats Immunized with Bovine GBM in CFA and Injected with Anti-ICAM-1 and LFA-1 mAbs from Days 14–35. On day 14, at the start of injections of anti-ICAM-1 and LFA-1 antibodies, renal tissue showed histologic and immunofluorescence findings similar to those in immunized rats in groups II and III. At 5 wk, the intensity of staining for rat IgG (+++) in the GBM and the titers of the antibodies to the α3 chain of type IV collagen (Fig. 3) were comparable to those rats in group II. Nevertheless, the histologic abnormalities in group VI were considerably less severe; they were, however, more severe than in group V. The rats of group VI had developed mild acute, focal, glomerulonephritis (Fig. 7 *b*), but had only a few small crescents, minimal periglomerular deposits of fibrinogen/fibrin (Fig. 7 *c*), only mild proteinuria, and mild increase of serum creatinine levels.

Discussion

In the present study, we showed that injections of mAbs against ICAM-1 and LFA-1 largely suppressed the development of autoimmune anti-GBM glomerulonephritis in rats, when treatment was started shortly before immunization with the GBM preparation and continued for 2 wk or more. Of particular importance, crescent formation was largely prevented. In addition, even when treatment was started shortly

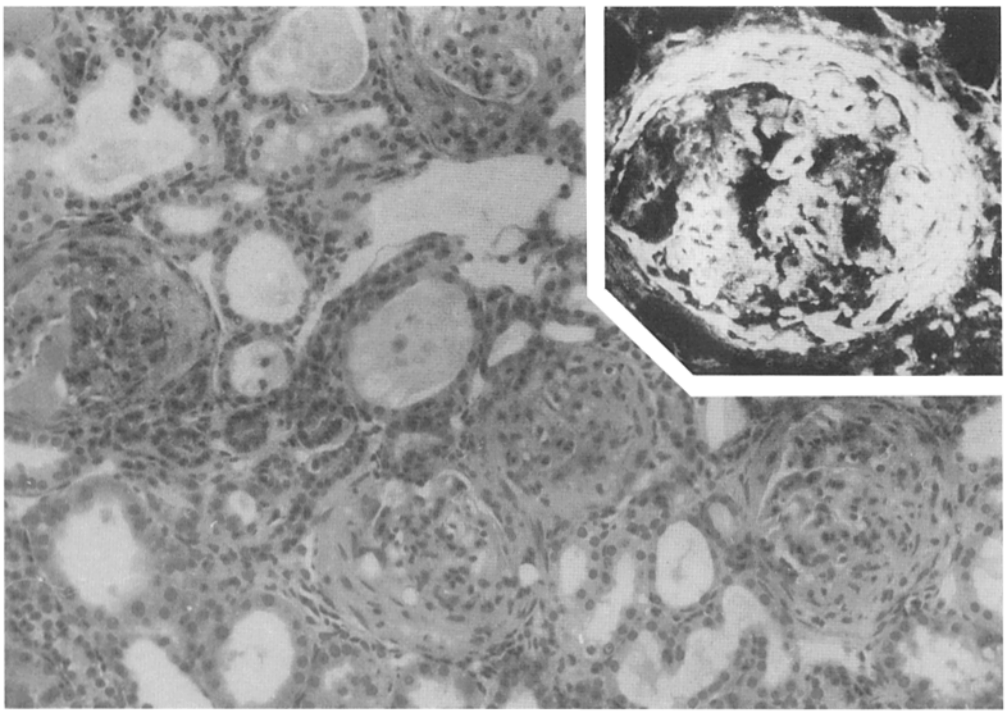

Figure 4. Rat of group II 5 wk after immunization with GBM. Crescentic, sclerosing, glomerulonephritis, and tubular atrophy is shown (H&E, ×350). The inset (×500) shows deposition of fibrinogen/fibrin in glomerular tufts and in a crescent.

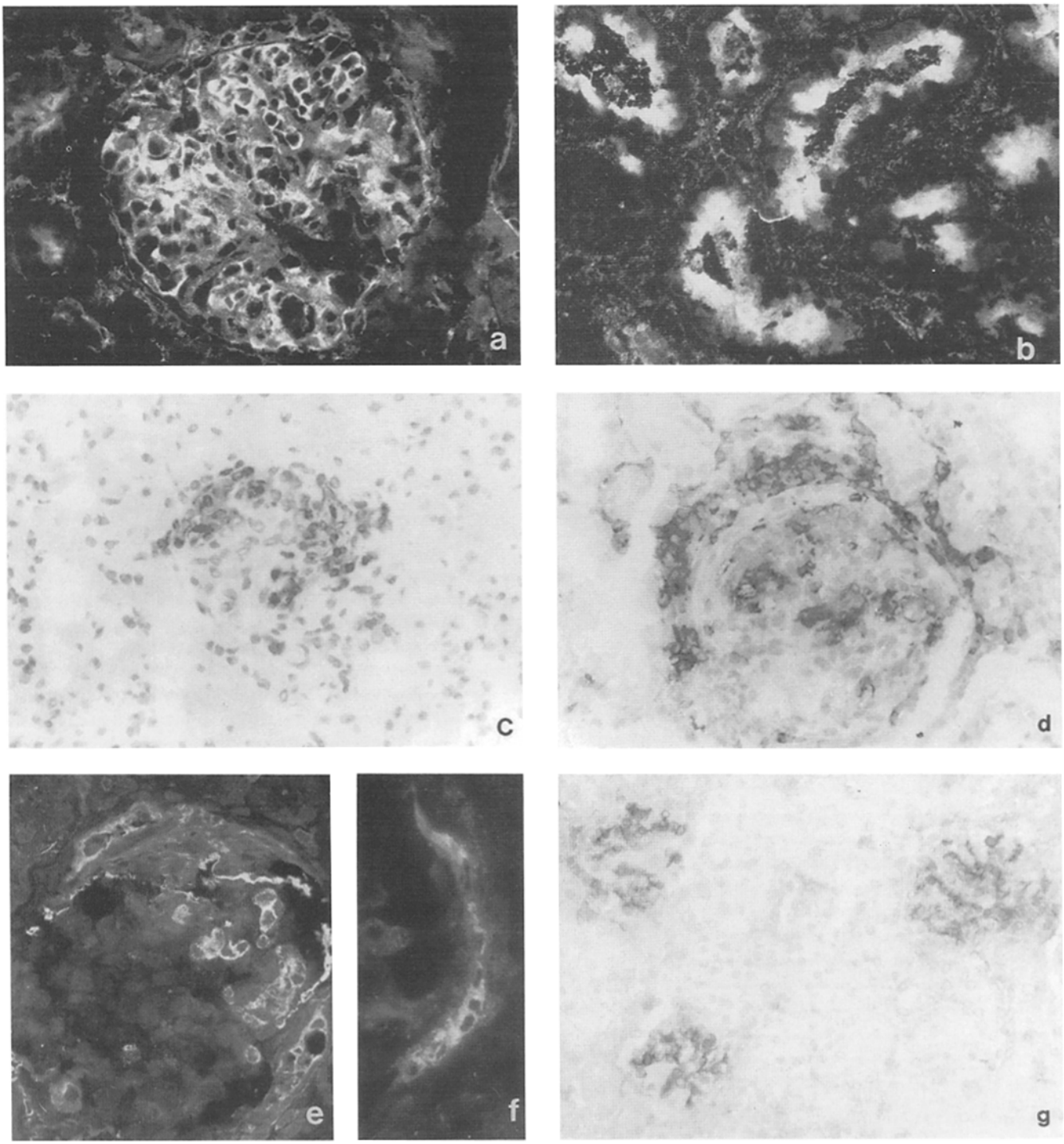

Figure 5. (*a–f*) Findings 5 wk after immunization with GBM (group II). (*a*) ICAM-1 in the capillary walls and in the mesangial matrix of a glomerulus, and (*b*) in the brush border of the proximal tubules; (*c*) infiltration of W3/13$^+$ cells (T lymphocytes) in a glomerulus and in the interstitium; (*d*) infiltration of ED1$^+$ cells (monocyte/macrophages) in and around a glomerulus, and in the interstitium; (*e*) LFA-1$^+$ cells in a crescent, and (*f*) infiltrating Bowman's capsule. (*g*) Deposition of mouse IgG in the glomerular capillaries of a rat of group IV after 16 d of injections with anti-ICAM-1 and LFA-1 antibodies. (*a–f*) ×500; (*g*) ×150.

after the onset of disease, at 14 d, progression of glomerular lesions was markedly reduced.

Two general types of mechanisms may explain the results. The mAbs may interfere with the autoimmune anti-GBM response; or, treatment may block effector mechanisms, either by causing lysis of effector leukocytes or by preventing the intraglomerular attachment and emigration of leukocytes. Experiments with other models have provided evidence that

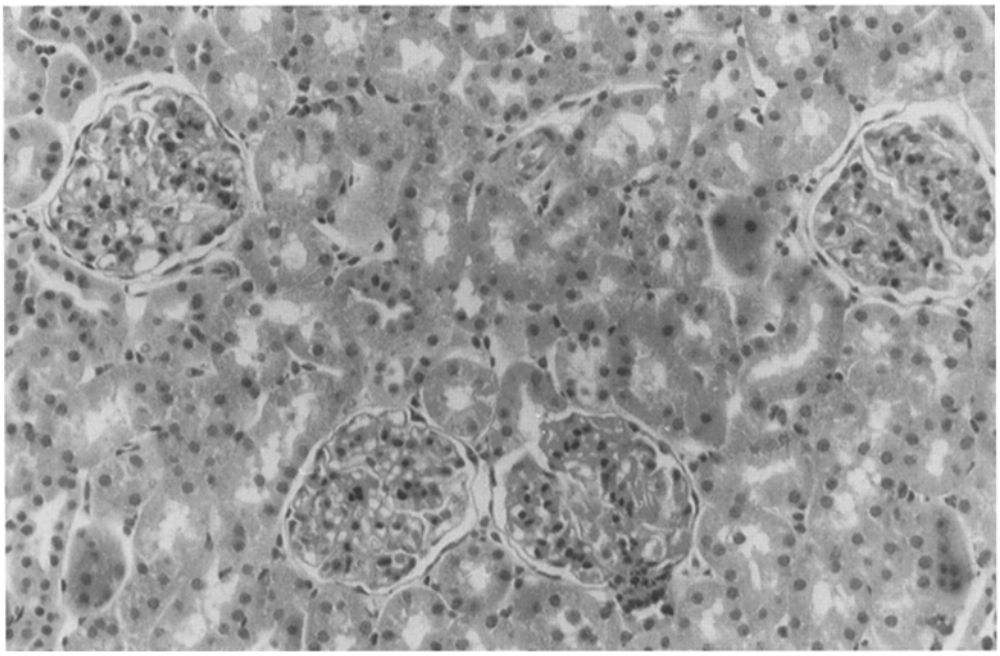

Figure 6. Photomicrograph showing a representative section of renal tissue obtained 14 d after immunization from a rat injected with anti-ICAM-1 and LFA-1 antibodies (group IV). Glomeruli, tubules, and interstitium appear normal. H&E; ×300.

these two types of mechanisms may account for protective effects of anti-ICAM-1 and/or anti-LFA-1 antibodies, which have been observed in mice with collagen-induced arthritis (23), in mice with cardiac allografts (24), in rats with adjuvant arthritis (25), in rabbits with Shwartzman-like reactions (26) or with lung inflammation induced by phorbol ester (27), in nonhuman primates with renal allografts (28), and in humans with bone marrow allografts (29). A synergistic effect was obtained in mice with cardiac allografts by simultaneous administration of anti-ICAM-1 and LFA-1 antibodies. Thus, although the injection of either antibody slightly prolonged the survival of cardiac allografts, the injection of both antibodies induced permanent and specific tolerance of the graft (24).

Evidence obtained in the present study favors the interpretation that the principal mechanism by which the mouse anti-ICAM-1 and LFA-1 antibodies protected against anti-GBM glomerulonephritis was by interference with attachment and emigration of leukocytes in glomeruli. First, in untreated rats there was markedly increased expression of ICAM-1 in glomerular endothelium at days 10–14, associated with infiltration of LFA-1$^+$ T lymphocytes and monocyte/macrophages. In treated rats, mouse IgG (presumably anti-ICAM-1) was detected transiently on glomerular endothelium, and glomerular infiltration of T lymphocytes and monocyte/macrophages was reduced or eradicated. Furthermore, 3 wk after discontinuing injection of anti-ICAM-1 and LFA-1 antibodies (group IV), when mouse IgG was no longer detected in glomeruli, T lymphocytes and monocyte/macrophages were seen in glomeruli. In addition, the finding that anti-ICAM-1 and LFA-1 antibodies given after onset of disease, on day 14 (group VI), reduced the progression of glomerular lesions supports the role of interference with effector mechanisms.

In other models, anti-ICAM-1 and/or LFA-1 antibodies have been shown to suppress immune responses, as measured either in terms of specific T cell responses (23, 25) or antibody production (30, 31). It is possible that in our experiments reduction in the anti-GBM response contributed to the beneficial effects of the anti-ICAM-1 and LFA-1 antibodies. Thus, at 5 wk there was clear-cut reduction in group IV in the levels of circulating antibodies to the $\alpha 3$ chain of type IV collagen. Nevertheless, other findings suggest that reduction in anti-GBM antibody production played at most a minor role. The levels of anti-GBM antibodies in treated rats (group IV) were about the same as those found in 15 of 16 untreated rats (group II) at 2 wk. Moreover, in rats treated shortly after onset of the renal disease (group VI), anti-GBM antibodies were not decreased at 5 wk, although the severity of the renal disease at that time was considerably less than in group II. In addition, at both 2 and 5 wk, there was intense linear staining for rat IgG in the GBM in treated rats. We have not investigated the possibility that rats treated with the anti-ICAM-1 and LFA-1 antibodies have fewer T lymphocytes specifically reactive with the GBM antigen than in untreated immunized rats.

We failed to find evidence that the injections of the mAbs resulted in elimination of circulating LFA-1$^+$ cells, since total counts and percentages of polymorphonuclear and mononuclear cells were unchanged.

Our results are clearly different from those of experiments in which the administration of anti-ICAM-1 and LFA-1 antibodies induced a state of specific tolerance (24, 30, 31). It may be that in our model tolerance is difficult or impossible to achieve because of the intense stimulation provided by a persistent depot of GBM antigen in CFA. It is of interest that in human anti-GBM nephritis, the autoimmune response is generally self-limited (32), which suggests that the antigenic stimulus ceases to operate.

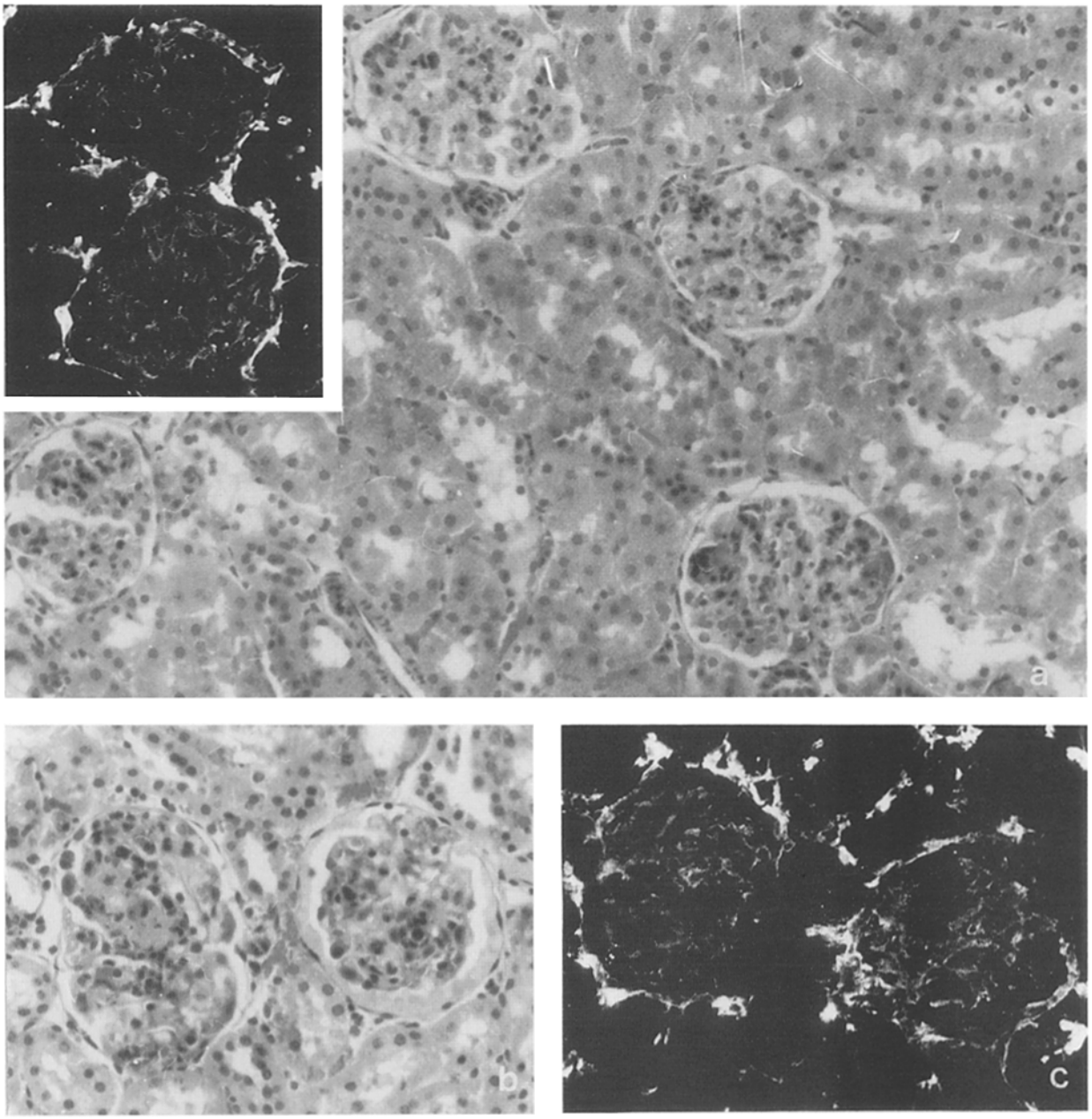

Figure 7. (*a*) Section of kidney from a rat in group IV at 5 wk, 3 wk after discontinuation of the injections of anti-ICAM-1 and LFA-1 antibodies. Slight, irregular hypercellularity of glomerular tufts is seen, but no crescents. The inset shows moderate deposits of fibrinogen/fibrin in the periglomerular capillaries of the same rat. (*b* and *c*) Sections from a rat in group VI at 5 wk, that was treated with anti-ICAM-1 and LFA-1 antibodies from days 14 to 35. Glomerular hypercellularity is seen but not crescents (*b*). Periglomerular deposits of fibrinogen/fibrin are seen (*c*). ×400.

Although a major beneficial effect of anti-ICAM-1 and LFA-1 treatment was to reduce glomerular damage, especially crescent formation, there was also lessening of tubulointerstitial damage, which is a feature of experimental and human anti-GBM nephritis. In control immunized rats (groups II and III) early infiltration of T lymphocytes and monocyte/macrophages in glomeruli was associated with or followed by interstitial infiltration of T lymphocytes and monocyte/macrophages, first around glomeruli and then more extensively in the cortical interstitium. Concomitantly, a marked expression of ICAM-1 became evident in the apical part of the epithelial cells of the proximal tubules. The abnormal tubular expression of ICAM-1, possibly induced by cytokines produced within inflamed glomeruli and reabsorbed by the tubules (33), may render this segment of the nephron vulnerable to LFA-1$^+$ effector cells. Leukocytes that cross the tubular basement membrane and then migrate between tubular cells or leukocytes that enter Bowman's space in the course of cres-

cent formation may gain access to the apical part of the tubules (34). Thus, interference with the ICAM-1/LFA-1 interaction may help prevent the development of tubulointerstitial changes, which contribute appreciably to the severity of anti-GBM nephritis.

In patients with anti-GBM nephritis the prognosis is poor unless aggressive immunosuppressive treatment is initiated before development of diffuse circumferential crescents and oliguria (1). The results of our study suggest that early administration of mAbs to ICAM-1 and LFA-1 (possibly in association with "pulses" of intravenous methylprednisolone [35] and plasmapheresis [36]) may constitute a new mode of therapy. In humans, blocking of the ICAM-1/LFA-1 interaction might be even more effective than in the rat model because the anti-GBM response is generally transient and the recurrence of the disease is rare (32).

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