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Immunohistochemical demonstration of parietal epithelial cells and macrophages in human proliferative extra-capillary lesions

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Summary. The cellular composition of crescents in diffuse crescentic glomerulonephritis is still controversial. Ten renal biopsies were studied on serial sections by using antikeratin antibodies as specific markers of epithelial cells of Bowman's capsule and both anti-macrophage and anti-lymphocyte antibodies. Semiautomatic morphometry showed that cellular crescents consisted of epithelial cells of Bowman's capsule (24–61%), of macrophages (19–34%) and of unlabelled cells (12–53%). In each biopsy, parietal epithelial cells outnumbered macrophages within crescents.

Key words: Crescentic glomerulonephritis – Antikeratin antibody – Anti-macrophage antibody – Immunoperoxydase

The cellular composition of glomerular crescents in human crescentic glomerulopathies remains a matter of debate. On histologic and ultrastructural aspects, the crescentic lesions have been traditionally thought to arise from the proliferation of either parietal (Heptinstall 1966; Bacani et al. 1968) or visceral and parietal epithelial cells (Churg et al. 1973; Morita et al. 1973). More recently, the prominent participitation of monocytes/macrophages in crescentic proliferation as suggested by glomerular culture (Atkins et al. 1976), was demonstrated by histochemical and immunohistochemical studies (Atkins et al. 1979, 1981, 1982; Magil and Wadsworth 1982; Hancock and Atkins 1984). In Hancock's study, the 10% crescentic cells identified as epithelial cells were in fact visceral epithelial cells recognized by PHM 5 (Hancock and Atkins 1983) and anti-C3 b-receptor monoclonal antibodies; no markers were used for parietal epithelial cells. In the present study, biopsy specimens obtained from patients with diffuse crescentic glomerulopathies were investigated using two anti-keratin monoclonal antibodies as markers for parietal epithelial cells, as well as both anti-macrophage and anti-lymphocyte antibodies.

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Material and methods

Renal biopsies. Ten renal biopsies showing diffuse crescentic glomerulonephritis (GN) were obtained from eight patients. The biopsies were classified according to following pathological criteria: diffuse crescentic GN without endocapillary proliferation and without immunoglobulin deposits (four biopsies from three patients); diffuse crescentic GN with anti-GBM antibodies (two biopsies from two patients); diffuse crescentic GN with Wegener's granulomatosis (four biopsies from three patients). The time interval between the presumed clinical onset of renal disease and the biopsy ranged from about 20 to 40 days. In two patients, a second renal biopsy was performed respectively two and three months later (patients $n=4$ and $n=6$). Normal renal transplant biopsies obtained from four patients were used as controls. Each sample was processed by standard methods for light microscopy and routine immunofluorescence. Eight serial cryostat sections from stored frozen material were cut at 6 μm, allowed to dry over night at −4° C, fixed ten minutes in acetone at room temperature and then air-dried before immunostaining with monoclonal antibodies.

Monoclonal antibodies. Four mouse monoclonal antibodies were used. Their characteristics are indicated in Table 1. EBM 11 was shown by us to react with blood monocytes and histiocytes from lymph nodes, interstitial mononuclear infiltrates in transplant kidney, Kupffer cells and cells from histiocytic granulomas in sarcoidosis and cutaneous leprosy. KL1 and KL4 were both negative on tissular macrophages.

Immunostaining of tissue sections. Immunostaining was performed with a three-stage immunoperoxidase procedure (Gatter et al. 1983). Dry, acetone-fixed sections were sequentially reacted with primary monoclonal antibody, peroxidase conjugated rabbit anti-mouse Ig Dakopatts (Glostrup, DENMARK) peroxidase conjugated goat anti-rabbit Ig Nordic (Tilburg, THE NETHERLANDS) and diaminobenzidine/H202 substrate. Slides were counterstained with haematoxylin. The eight serial sections from each case were stained in the following order: 1. control by omission of monoclonal antibody, 2. methenamine-silver staining, 3. KL1 antibody, 4. EBM 11 antibody, 5. OKT 3 antibody, 6. methenamine-silver staining, 7. KL4 antibody, 8. EBM 11 antibody. Crescents were localized morphologically on immunostained sections by comparison with serial sections stained with methenamine-silver and with haematoxylin.

Morphometric evaluation of immunostained sections. Sections were examined on magnification 250 using a NACHET microscope connected to a compact digitizer (HIPAD TM digitizer HOUSTON INSTRUMENTS), with a handy movable pointer. It sends coordinates of points into computer (microcomputer NACHET NS 1000). The NS 1000 software permits the display

Table 1. Monoclonal antibodies

Antibody	Subclass	Source	Specificity	Type of positivity	References
KL1	IgG1	IMMUNOTECH	Human cytokeratins PM 55.57000	Cytoplasm	Viac et al. 1983
KL4	IgG1	MAPI	Human cytokeratins PM 55.67000	Cytoplasm	not yet commercially available
OKT3	IgG2	ORTHO	Human peripheral T lymphocyte	Membrane	Kung et al. 1979
EBM 11	IgG1	from Pr McGEE Oxford	Monocytes Macrophages	Membrane	Theaker et al. 1985

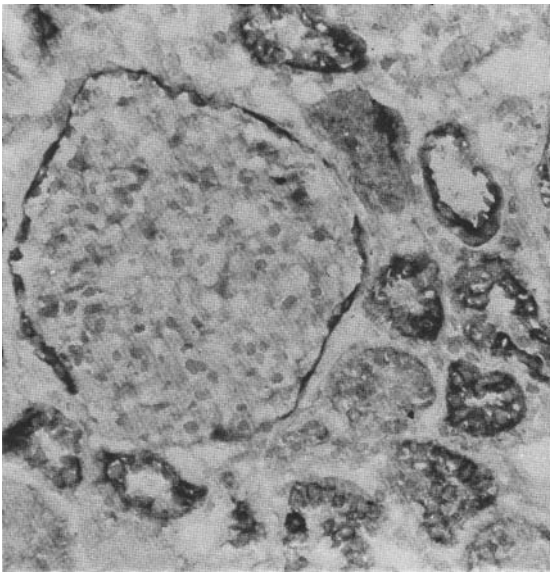

Fig. 1. Immunoperoxydase labelling of normal kidney with KL4 monoclonal antibody. ×250

on CRT of the pointed image on a real time basis. The constant visual control of the observer allows to cancell wrong measurements before putting in memory.

On stained sections, five parameters were evaluated: K1: number of crescentic cells positive with anti-keratin antibodies per glomerular section (KL1 and KL4 provided identical staining in serial sections of each case); K2: number of crescentic cells negative with anti-keratin antibodies per glomerular section; M1: number of crescentic cells positive with EBM 11 antibody per glomerular section; M2: number of crescentic cells negative with EBM 11 antibody per glomerular section; M3: number of cells positive with EBM 11 antibody infiltrating the glomerular tuft per glomerular section. Only cells with visible nuclei were included in the count of positive cells (K1–M1–M3). Crescentic negative cells (K2–M2) were identified with the haematoxylin stained nuclei. The weighted mean percentage of crescentic cells positive with a given antibody per glomerular section equals to sum of all crescentic cells reactive with this antibody divided by the sum of all positive and negative crescentic cells $\left(\frac{\varepsilon K1}{\varepsilon(K1+K2)} \text{ or } \frac{\varepsilon M1}{\varepsilon(M1+M2)}\right)$.

The mean number of macrophages reactive with EBM 11 antibody and of T-lymphocytes reactive with T3 antibody infiltrating glomerular tufts was expressed per glomerular section on each biopsy.

Insterstitial inflammatory cells reactive with EBM 11 and T3 antibodies were semi-quantitatively evaluated and graded 0 to 3+.

Results

Normal kidney

KL1 and KL4 antibodies were found to react with parietal epithelial cells of Bowman's capsule. In addition, KL1 reacted with epithelial cells of proximal and distal tubules while KL4 reacted only with distal tubular cells. In glomeruli, no other cellular positivity was observed (Fig. 1).

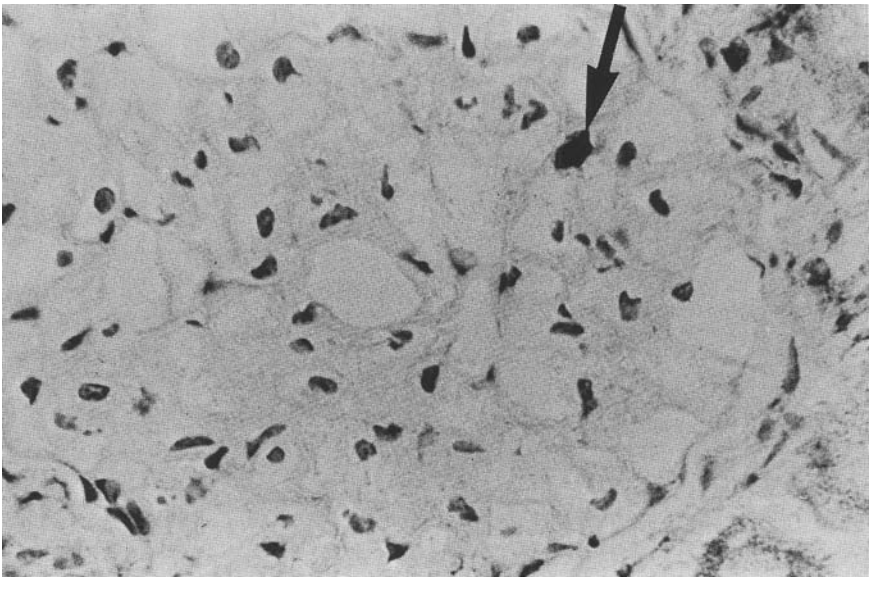

Fig. 2. Immunoperoxydase labelling of normal kidney with EBM 11 antibody showing only one circulating monocyte (*arrow*). × 600

EBM 11 antibody was negative for resident glomerular cells; few EBM 11 positive cells (0.8 cells/glomerular section) were found within glomerular capillaries. A weak staining of proximal tubular cells was also observed (Fig. 2).

Crescentic Glomerulopathies

Results are expressed in Table 2.

Light microscopy. In each specimen at least 10 glomeruli were available for examination. Five biopsies ($n=1$, 2, 3, 4a, 7) showed circumferential cellular crescents with necrotic fibrin-like material. Mitoses were seen occasionally in these crescents. Ruptures of Bowman's capsule with periglomerular granuloma were observed in the two cases of anti-GBM GN and in two of the three cases of Wegener's granulomatosis. The other biopsies ($n=5$, 6a, 8) showed segmental cellular crescents. The second biopsies ($n=$ 4b and 6b) contained only scarry fibrotic crescents. In all these biopsies, there was no endocapillary proliferation.

Immunostaining (Fig. 3): Identical results were obtained in the three groups of crescentic GN.

Immunostaining with anti-keratin antibodies. Proliferative cellular crescents contained 24%–69% cells labelled with the two anti-keratin antibodies.

Table 2. Relevant histologic and immunohistochemical findings in the eight cases of cellular crescentic glomerulonephritis

Case *n*	Patho-genesis	Interval (days)	Number of glomeruli	Glomeruli with cellular crescents	Glomeruli with fibrous crescents	Cellular crescents			Flocculus		Interstitium	
						Mean number of crescentic cells/glomerulus	% of anti-keratine +crescentic cells*	% of EBM 11 +crescentic cells*	EBM 11 **	T3 **	EBM 11	T3
1	anti-GBM	30	13	12	1	80	34% (2–69)	34% (15–47)	22	2	+++	++
2	anti-GBM	20	11	4	5	53	61% (49–69)	19% (2–35)	3.5	0	+	+
3	Wegener	25	20	18	0	95	40% (12–74)	30% (18–51)	7.9	0	++	0
4a	Wegener	30	12	10	2	58	43% (33–72)	29% (16–49)	3.5	0	+	0
4b	Wegener	90	15	0	11				2.4	0	±	0
5	Wegener	20	16	3	3	27	44% (36–50)		not available			
6a	IF (−)	30	12	5	5	67	69% (43–79)	19% (2–48)	3.2	0	+	+
6b	IF (−)	120	7	0	2				0.3	0	0	0
7	IF (−)	25	16	6	0	75	24% (11–46)	23% (16–28)	2.7	ND	+	ND
8	IF (−)	45	18	6	5	63	43% (26–48)		not available			

ND: not done

Interval refers to the estimated time between clinical onset of renal disease and the biopsy.

* Weighted mean percentage of positive crescentic cells with each antibody per glomerular section. Range is the variation in the percentage of positive cells in crescents.

** Mean number of positive cells infiltrating the tuft per glomerular section.

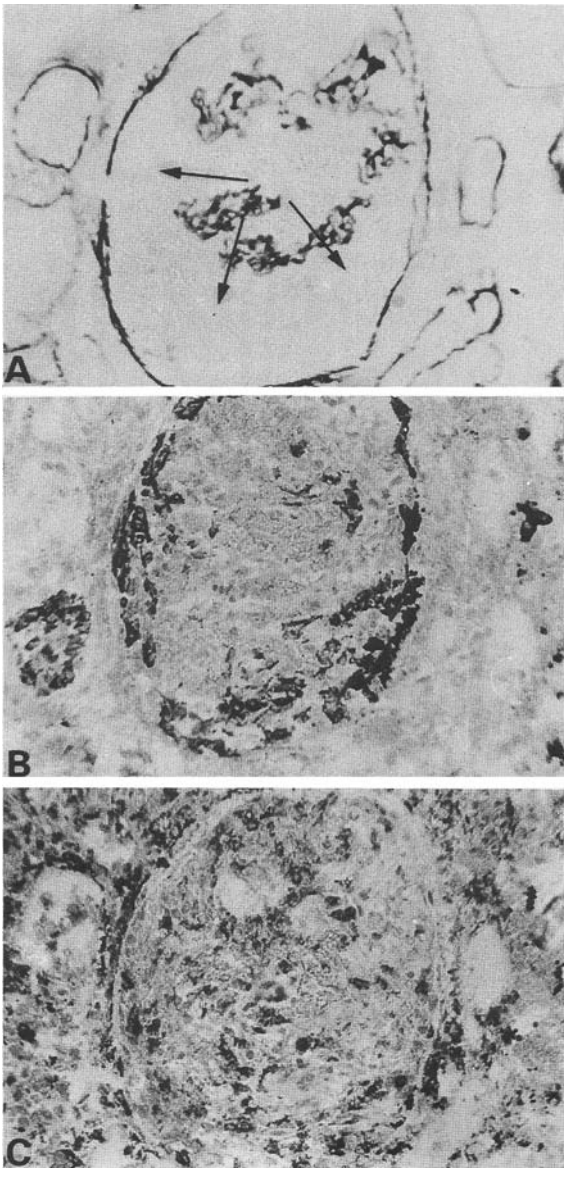

Fig. 3. Diffuse crescentic glomerulonephritis: serial sections A, B, C and D, E, F of the same glomerulus from two renal biopsies, successively stained with methenamine silver, anti-keratin and anti-macrophage monoclonal antibodies. In crescentic proliferation (*arrows*), both keratin and EBM 11 positive cells are seen. ×350

These positive crescentic cells were therefore identified as Bowman's capsule parietal cells. These florid positive lesions were observed in the eight biopsies performed within a month after the apparent clinical onset of the renal disease. Fibrotic crescents observed in the two late biopsies ($n=4$b and 6b) were totally areactive with anti-keratin antibodies.

Immunostaining with anti-macrophage and anti-lymphocyte antibodies. All proliferative cellular crescents contained cells positive with EBM 11 antibody identified as macrophages. The mean percentage of EBM 11 positive

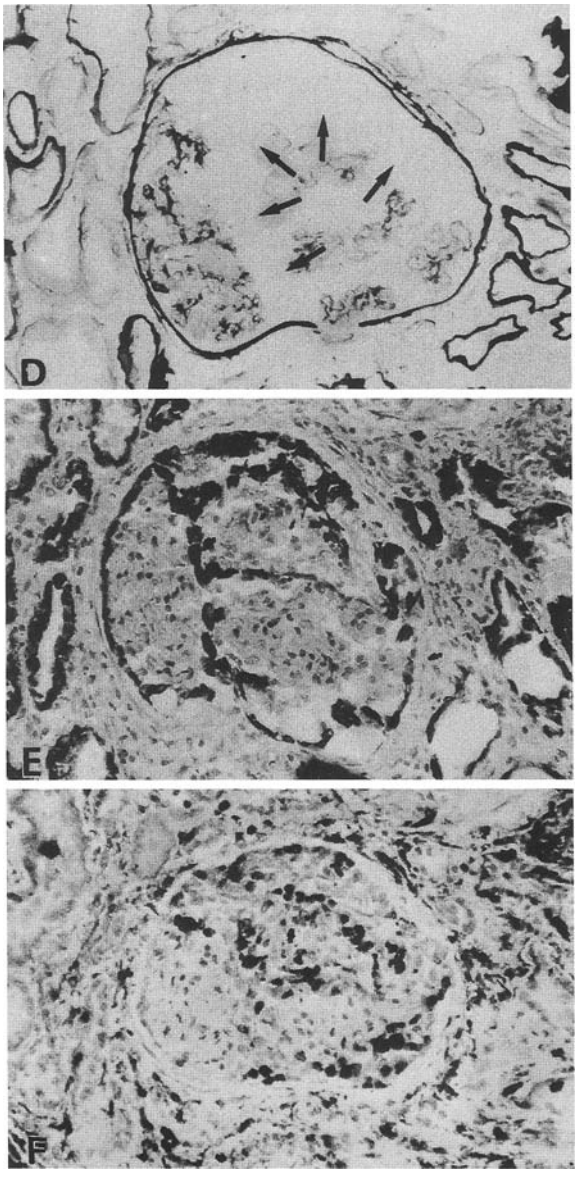

Fig. 3

crescentic cells per glomerular section ranged from 19%–34%. In each specimen, the mean percentage of crescentic proliferated epithelial cells exceeded the mean percentage of crescentic macrophages. No T-lymphocyte was found in cellular crescents. Fibrotic lesions were unlabelled. The number of EBM 11 positive cells infiltrating glomerular tufts ranged from 0.3 to 22 per glomerular section. The highest numbers of infiltrating macrophages were associated with visible necrosis. T-lymphocytes were found within glomerular tufts only in biopsy $n=1$.

Interstitial foci of macrophages and, to a less extent, of T-lymphocytes, were found mostly around glomeruli containing necrotic lesions. They were absent in fibrotic lesions.

Discussion

The present study shows the participation of both parietal epithelial cells and monocytes/macrophages in crescent formation. Cellular crescents contained between 24 and 61% cells positive with the two antikeratin monoclonal antibodies (KL1, KL4) and between 19 and 34% monocytes/macrophages. A mean percentage of 29% crescentic cells remained unreactive with the different antibodies. KL1 and KL4 recognize epitopes present on human cytokeratins of respectively 55, 57 and 55, 67 kilodaltons. A strong reproducible staining of glomerular parietal epithelial cells was found on our normal controls excluding the other glomerular cell types. Holthofer et al. (Holthofer et al. 1983, 1984), using another monoclonal antibody (PKK1) directed against epitopes from human cytokeratins of 41, 45, 48 and 56 kilodaltons, found discontinuous positivity of parietal glomerular epithelium and strong staining of collecting, proximal and distal tubular epithelium in normal kidney. With the same antibody, PKK1, Magil (Magil 1985) showed a significant proportion of positive cells within crescentic proliferation. These results are quite in agreement with ours: epithelial cells of parietal origin contribute significantly to crescent formation. The presence of cytokeratins, a characteristic intermediate filament of epithelial cells, does not support the hypothesis that crescentic cells could be epithelioid cells derived from monocyte transformation (Atkins et al. 1979). The large participation of parietal epithelial cells within crescents can account for the production of connective material in these lesions.

The presence of monocytes/macrophages in crescent formation was claimed by Atkins et al. (Atkins et al. 1979, 1981) and Hancock et al. (Hancock and Atkins 1984) from histochemical and immunohistochemical studies. In the present study, monocytes/macrophages were identified within crescents by EBM 11 monoclonal antibody. In previous studies on similar crescentic glomerulopathies, we showed that extracapillary proliferation did not express Ia-antigen and C3b-receptor antigen (Hinglais et al. 1984; Kazatchkine et al. 1982). These two surface membrane antigens are known to be present on the large majority of human peripheral blood monocytes (Winchester and Kunkel 1979; Charron 1981; Cook and Kazatchkine 1983). Lack of detectable Ia and C3b-receptor antigens on crescentic monocytes/macrophages could reflect defective synthesis or expression. No difference in the respective percentages of monocytes/macrophages and epithelial cells appears in the three groups of crescentic GN. So the predominance of monocytes/macrophages in anti-GBM GN versus other crescentic GN showed by Magil (Churg et al. 1973) in histochemical study, is not confirmed here.

Monocytes/macrophages could accumulate in urinary space either by egress from glomerular capillaries through gaps in glomerular basement

membrane or through breaks in Bowman's capsule. Experimental studies have showed two basic mechanism of macrophage accumulation within glomeruli: passive accumulation by chemotaxis or immune adherence (Atkins et al. 1982; Holdsworth 1983; Schreiner et al. 1982) and T cell directed accumulation (Bhan et al. 1978, 1979). Tipping et al. (Tipping et al. 1985) demonstrated the early presence of T cells in murine anti-GBM proliferative GN. In case $n=1$, a mean number of 2 T cells in tuft per glomerular section was found; in the other cases, glomeruli were negative with T3 antibody. But in human pathology, kidney biopsies are always performed long after the immunologically mediated onset of the lesion. Whatever way for their glomerular accumulation, macrophages could play a role in epithelial cell proliferation: macrophages can stimulate endothelial and mesangial cell proliferation in glomerular culture (Dubois et al. 1981). Whether they have a similar effect on parietal epithelial cells, remains to be established.

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