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CLINICAL INVESTIGATION

The detection of monocytes in human glomerulonephritis

FRANCO FERRARIO, ALDO CASTIGLIONE, GUILIANO COLASANTI, GIOVANNI BARBIANO DI BELGIOIOSO, SILVIO BERTOLI, and GIUSEPPE D'AMICO, with technical assistance from STEFANIA NAVA

Divisions of Nephrology, San Carlo and Ca' Granda Hospitals, Milan, Italy

The detection of monocytes in human glomerulonephritis. Renal biosy specimens from 343 patients with primary or secondary glomerulonephritis (GN) were examined for monocytes by the non-specific esterase reaction. Large numbers of monocytes per glomerulus (M/G) were found in essential cryoglobulinemia GN (29 pts, M/G 30.6 ± 22.4), in acute post-infectious GN (27 pts, M/G 9.1 ± 8.3), in rapidly progressive crescentic GN (20 pts, M/G 5.6 ± 2.7), in systemic lupus GN (61 pts, M/G 5.0 ± 5.6), and in IgA-GN associated with chronic liver disease (5 pts, M/G 6.4 ± 5.9) or Schönlein-Henoch purpura (15 pts, M/G 3.3 ± 6.4). Clinico-histological correlation showed that monocyte infiltration was correlated with the extent of proteinuria (all groups), with the presence of endoluminal "thrombi" (cryoglobulinemia GN), of polymorphonuclear leukocyte infiltration (post-infectious GN), of cellular crescents (crescentic GN), of "active" lesions (lupus GN), and with the extension of lesions to the peripheral capillary walls (IgA-associated GN). The M/G index was negligible in renal amyloidosis (21 pts), in idiopathic membranoproliferative GN (10 pts), in idiopathic IgA mesangial GN (63 pts), in membranous GN (40 pts), in focal glomerulosclerosis (29 pts), in minimal change nephropathy (18 pts), and in diabetic glomerulosclerosis (5 pts). The results confirm the participation of cells of the monocyte-macrophage series in the genesis of proliferative lesions, both intracapillary and extracapillary, in immune-mediated human GN and suggest their direct involvement in glomerular injury.

Détection des monocytes lors de glomérulonéphrites humaines. Des échantillons de biopsie rénale provenant de 343 malades atteints de glomérulonéphrites (GN) primitives ou secondaries ont été examinés pour les monocytes par la réaction non spécifique à l'estérase. De grands nombres de monocytes par glomérule (M/G) ont été trouvés au cours de la GN de la cryoglobulinémie essentielle (29 malades, M/G 30,6 ± 22,4), de la GN aiguë post-infectueuse (27 mals., M/G 9,1 ± 8,3) dans la GN à croissants rapidement progressive (20 mals., M/G 5,6 ± 2,7), dans la GN du lupus systémique (61 mals., 5,0 ± 5,6) et dans la GN à IgA associée aux hépatopathies chroniques (5 mal., M/G 6,4 ± 5,9) ou au purpura de Schönlein-Henoch (15 mals., M/G 3,3 ± 6,4). Les corrélations histocliniques ont montré que l'infiltration monocytaire était corrélée à l'importance de la protéinurie (tous les groupes), à la présence de "thrombus" endoluminaux (GN avec cryoglobulinémie), à l'infiltration leucocytaire polynucléaire (GN post-infectieuse), aux croissants cellulaires (GN à croissants), aux lésions "actives" (GN du lupus), et à l'étendue des lésions des parois capillaires périphériques (GN associée à IgA). L'index M/G était négligeable lors de l'amylose rénale (21 mals.), de la GN membranoproliférative idiopathique (10 mals.), de la GN à IgA mésandiales idiopathique (63 mals.), de la GN extramembraneuse (40 mals.), de la glomérulosclérose focale (29 mals.), de la néphropathie à lésions glomérulaires minimes (18 mals.) et de la glomérulosclérose du diabète (5 mals.). Ces résultats confirment la participation des cellules de la série monocytaire-macrophagique dans la genèse de lésions prolifératives, intracapillaires et extracapillaires lors des GN humaines à médiation immune, et ils suggèrent leur implication directe dans les lésions glomérulaires.

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Several studies have documented the presence of monocytes/macrophages within the glomeruli in many types of immune-mediated experimental glomerulonephritis (GN) [1–12]. The cause of the accumulation of these cells in the glomerulus and their local role is still a matter of controversy. They might migrate as a non-specific inflammatory phagocytic response, acting as "scavengers" of immune complexes, cellular debris, or fibrin [12, 13], or play specific roles in humoral responses through their surface Fc and C3 receptors [14, 15], or in cell-mediated immune mechanisms [16]. Apart from their phagocytic function, monocytes participate directly in the glomerular injury through release of lytic enzymes that alter glomerular basement membranes [17] and of factors stimulating the proliferation of fibroblasts and other glomerular cells [18–21].

A small number of studies has documented the presence of intracapillary and extracapillary monocytes in some types of human GN also [22–28]. However, these studies have usually been performed with small and/or unselected populations of patients. The aim of our study was to quantitate the presence and distribution of monocytes in a large number of primary and secondary GN, classified histologically, and to evaluate the relationships between the degree of monocytic infiltration and the main clinico-histological features of these diseases.

Methods

Patients

Renal biopsy specimens from 343 patients with primary or secondary GN were selected for this study. (Table 1). All cases studied by us after June, 1979, were included, except when there was not enough material for either light or immunofluorescence microscopy. The study was prospective from 1982 and retrospective for patients biopsied before that time.

The patients were classified according to generally accepted clinico-histological criteria as follows: IgA-associated GN: 83 cases, of which 63 were idiopathic (Berger's disease), 15 were

Table 1. Analysis of intraglomerular monocytes in human glomerulonephritis

Disease	Number of cases	M/G (Mean ± SD)
Essential cryoglobulinemia-associated GN	29	30.6 ± 22.4
Acute post-infectious GN	27	9.1 ± 8.3
Rapidly progressive crescentic GN	20	5.6 ± 2.7
Systemic lupus-associated GN	61	5.0 ± 5.6
IgA-associated GN		
Chronic liver disease	5	6.4 ± 5.9
Henoch-Schönlein purpura	15	3.3 ± 6.4
Berger's disease	63	0.3 ± 0.8
Renal amyloidosis	21	2.2 ± 2.3
Idiopathic membranoproliferative GN	10	0.2 ± 0.5
Membranous GN	40	0
Focal glomerulosclerosis	29	0
Minimal change nephropathy	18	0
Diabetic glomerulosclerosis	5	0
	Total 343	

associated with Henoch-Schönlein purpura and five were associated with liver diseases; Systemic Lupus (SLE) GN: 61 cases; Membranous GN: 40 cases; Focal glomerulosclerosis: 29 cases; Essential cryoglobulinemia-associated GN: 29 cases; Acute post-infectious GN: 27 cases; Renal amyloidosis: 21 cases; Rapidly Progressive Crescentic GN: 20 cases, of which 12 were idiopathic, four were associated with SLE and four with Systemic Vasculitis; Minimal change GN: 18 cases; Idiopathic Membranoproliferative GN (MPGN): 10 cases; Diabetic glomerulosclerosis: five cases.

All patients with circumferential crescents in more than 80% of glomeruli (with or without associated lesions of the glomerular tuft and a downhill clinical course were arbitrarily classified as a distinct subgroup, as proposed recently [29], because of their peculiar histological and clinical features. Six other patients, three with Berger's disease and three with diffuse proliferative SLE-GN showed non-circumferential crescents in 30 to 50% of glomeruli and a less rapid decline of renal function: they were not classified as rapidly progressive crescentic GN.

Biopsies

The specimens were routinely processed for light and immunofluorescence microscopy as described previously [30].

Methods

The tissue was snap-frozen in liquid nitrogen-cooled isopentane at the time of biopsy and stored at −80°C until processed. The duration of storage varied from 1 week to 5 years. In the processing, 4 μ cryostatic sections were stained immediately by immunohistochemical means for non-specific esterase (NSE), according to the method of Nachlas and Seligman [31], modified by Pearse [32], using α-naphthyl acetate. With this method, monocytes, renal tubular epithelial cells, and megakaryocytes stain diffusely, whereas the normal cells present in the glomeruli are negative. To check whether the duration of storage could in some way affect the intensity and distinctness of the reaction, in the last months we stained unstained frozen sections from 10 patients already studied at the time of biopsy in 1982 with NSE and found no substantial differences in the number of monocytes per glomerulus. For each biopsy the total numbers of intraglomerular intensely red-brown NSE-positive cells were counted in two sections, at least, by two independent observers (FF and AC), and then divided by the number of glomeruli (all the biopsy samples contained more than five glomeruli), obtaining an index (M/G) (Table 2), which is the mean number of intra-capillary monocytes per glomerulus. When two investigators found different counts, a joint examination of the specimens was made, until agreement was reached. Small fragments of NSE-positive material, possibly representing portions of cells, were not counted. Clinico-histological correlations between each disease and the different values of M/G were determined.

Results

The M/G in the different types of GN are shown in Table 1. NSE-positive cells were found mainly in proliferative GN. Patients with cryoglobulinemia-associated GN, acute post-infectious GN, crescentic GN, SLE-GN, liver disease-associated IgA GN and Henoch-Schönlein purpura GN had the largest numbers of monocytes per glomerulus, while patients with idiopathic (pure mesangial) IgA-GN (Berger's disease) and idiopathic MPGN had lesser monocytic infiltration.

In non-proliferative GN, NSE-positive cells were found only in patients with amyloidosis. Membranous GN, focal glomerulosclerosis, and minimal change GN were completely negative.

Essential cryoglobulinemia-associated GN (29 cases); M/G 30.6 ± 22.4

The largest number of monocytes (as many as 80 to 100 per glomerulus in some cases) was found in this GN, mostly in intracapillary locations (Fig. 1) Their cytoplasm sometimes insinuated into the double-contoured peripheral capillary wall. The cells were often in close contact with the endoluminal thrombi, when present, and sometimes seemed to phagocytose them (Fig. 2). In fact, the largest number of monocytes per glomerulus (M/G = 54.2 ± 12.5) was found in cases with diffuse proliferative glomerular lesions and massive deposition of thrombi (Table 2), the average M/G declining progressively in cases with diffuse proliferative GN without thrombi (mean M/G 32.1 ± 14.2), in those with lobular GN (mean M/G 8.4 ± 5.8), and in those with focal GN (mean M/G 6.6 ± 6.5). In cases with massive deposition of endoluminal thrombi, there was great variability in the numbers of NSE-positive cells in the different glomeruli, which seemed to correlate with the large variability in the number and dimensions of such thrombi in the different glomeruli. Two patients with massive thrombi and high M/G index in the first biopsy specimen showed their total disappearance, together with great histological improvement, in the second biopsy specimen taken after 4 or 5 months. These findings confirm the strict relationship between monocytes and endoluminal thrombi.

The M/G index was not correlated with the degree of renal functional impairment, with the amount of circulating cryoglobulins, with the level of serum complement, nor with the positivity of the different antisera at immunofluorescence. Proteinuria was significantly higher (4.9 ± 5 g/24 hr) in the 12 patients with M/G > 40 than in the eight patients with M/G < 10 (1.2 ± 1.5 g/24 hr; $P < 0.05$), and to a lesser degree, in the nine patients with intermediate M/G (3.4 ± 2.3; $P < 0.02$).

Table 2. Analysis of intraglomerular monocytes in NSE-positive glomerulonephritides according to different histological subgroups

	Number of cases	M/G (Mean ± SD)	
Essential cryoglobulinemia-associated GN	29		
A) Diffuse proliferative with thrombi	9	54.2 ± 12.5	A – B, B – C $P < 0.01$
B) Diffuse proliferative without thrombi	10	32.1 ± 14.2	A – C, A – D, B – D $P < 0.001$
C) Lobular	4	8.4 ± 5.8	
D) Focal proliferative	6	6.6 ± 6.5	C – D NS
Acute post-infectious GN	27		
Proliferative exudative	19	12.6 ± 7.4	$P < 0.001$
Proliferative non-exudative	8	1.0 ± 2.4	
Crescentic GN (> 80% of circumferential crescents)	20		
A) Cellular crescents	11	7.2 ± 2.3	A – B $P < 0.05$
B) Fibrocellular crescents	5	4.7 ± 1.0	A – C, B – C $P < 0.01$
C) Fibrotic crescents	4	2.2 ± 0.9	
Systemic lupus-associated GN	61		
A) Diffuse active proliferative	23	10.6 ± 5.2	A – B $P < 0.005$
B) Diffuse sclerosing proliferative	5	2.5 ± 2.0	
C) Focal proliferative	17	1.9 ± 2.3	A – C, A – D $P < 0.0005$
D) Mesangial	9	1.1 ± 1.9	A – E
E) Membranous	7	1.0 ± 1.0	
Membranoproliferative GN	17		
With prevalent IgA deposits (five with liver diseases and two with Henoch-Schönlein purpura)	7	10.0 ± 7.8	$P < 0.01$
Idiopathic	10	0.2 ± 0.5	

Acute post-infectious GN (27 cases); M/G 9.1 ± 8.3

As Table 2 shows, the M/G ratio was significantly higher ($P < 0.005$) in the 19 cases with intracapillary hypercellularity and granulocyte exudation (M/G = 12.6 ± 7.4) than in the eight cases with prominent mesangial hypercellularity (M/G = 1.0 ± 2.4). This difference could not be accounted for by the duration of the disease, since the interval from clinical onset to biopsy was similar in the two groups (22 vs. 21 days as a mean).

Patients with M/G > 5 presented more severe proteinuria (2.4 ± 2.0 vs 0.8 ± 0.5 g/24 hr) and more frequent hypocomplementemia than patients with M/G < 5. (Table 3).

Rapidly progressive crescentic GN (20 cases); M/G 5.6 ± 2.7

By definition, these are cases with > 80% of glomeruli with circumferential crescents. Twelve cases were primary idiopathic forms and eight were associated with systemic diseases (four SLE and four vasculitis).

The 11 cases with cellular crescents, whether primary or associated with systemic disease and regardless of the immunohistological pattern (negative, granular, or linear), showed many NSE-positive cells within the extracapillary proliferation, confirming the participation of monocytes in the formation of the crescent (Fig. 3). These cases also showed intracapillary NSE-positive cells, with an M/G significantly higher than that found in the five cases with fibrocellular crescents or in the four cases with already fibrotic crescents (7.2 ± 2.3 vs 4.7 ± 1.0 vs 2.2 ± 0.9; Table 2).

The amount of proteinuria was the only clinical parameter significantly correlated with the degree of monocyte infiltration (Table 3).

SLE-GN (61 cases); M/G 5.0 ± 5.6

The M/G index in this secondary GN also correlated with the extent of active proliferative lesions (Table 2). The number of NSE-positive cells per glomerulus was greater (M/G 10.6 ± 5.2) in patients with diffuse "active" proliferative GN and decreased progressively in those with diffuse "sclerosing" proliferative GN (M/G 2.5 ± 2.0), with focal proliferative GN (M/G 1.9 ± 2.3; Fig. 4), with mesangial GN (M/G 1.1 ± 1.9) and with pure membranous GN (1.0 ± 1.0).

Three patients of the active group had crescents in 20, 30, and 50% of their glomeruli: their average M/G was 8.1 ± 4.2, slightly smaller than that of the whole group. Correlations with some laboratory findings are shown in Table 3. As in all other forms of NSE-positive GN, patients with M/G > 5 tended to have more marked proteinuria ($P < 0.01$) than patients with lower M/G.

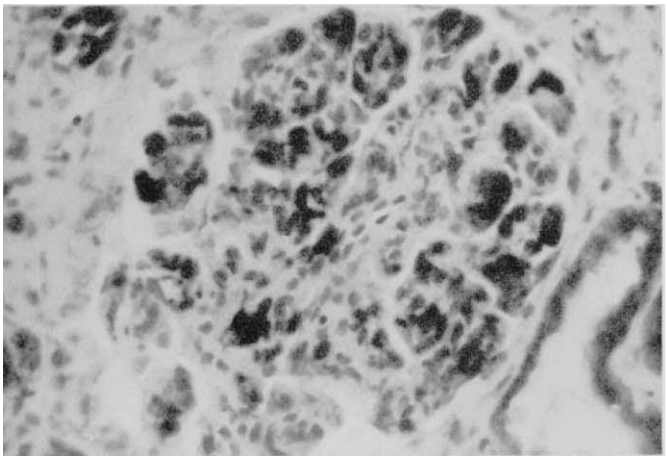

Fig. 1. *Cryoglobulinemia-associated GN: a high number of cells in the glomerulus show an intense NSE reaction* (dark cells). (NSE, ×245)

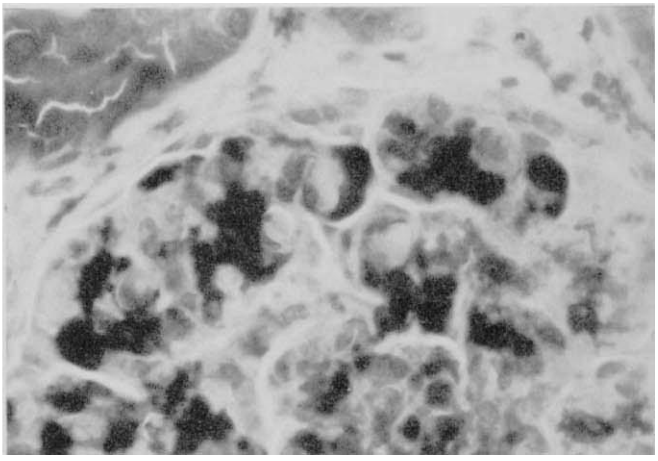

Fig. 2. *Cryoglobulinemia-associated GN: intraglomerular monocytes show a close contact with endoluminal thrombi.* (NSE, ×390)

IgA-associated GN (83 cases); M/G 1.2 ± 3.5

The M/G index was markedly different (Table 1) in the three subgroups represented by *a*) membranoproliferative GN associated with chronic liver diseases (five cases, M/G 6.4 ± 5.9); *b*) GN associated with Henoch-Schönlein purpura (15 cases, M/G 3.3 ± 6.4); and *c*) idiopathic IgA-GN (63 cases, M/G 0.3 ± 0.8). The very large standard deviation from the mean in the Henoch-Schönlein purpura-GN subgroup is due to the fact that two patients with histological patterns of MPGN had an M/G index of 20 and 18, while in the remaining 13 cases, with a prevalent mesangial involvement, the average M/G index was 0.9 ± 1.0.

It is interesting to note that the three patients with Berger's disease who had noncircumferential crescents in > 30% of glomeruli had an M/G index of 2.3 ± 1.8, which is definitely larger than the average index for the total group of 63 patients with this type of primary GN. If all seven patients with IgA-related GN showing the histologic features typical of MPGN (five cirrhotic and two Henoch-Schönlein patients) are combined and compared with the group of ten patients with idiopathic MPGN, there is a significant difference in the amount of monocyte infiltration (Table 2). For the whole group of 17 patients with MPGN, no correlations were found between the main clinical parameters and the M/G values, with the exception of proteinuria (Table 3). We have not looked for correlations in Berger's disease and in the other types of GN listed in Table 1 because of the negligible numbers of NSE-positive cells.

Discussion

A number of monoclonal antibody markers are available for precise identification of marrow-derived cells in tissue; nevertheless, still unresolved problems of cross reactivity and of correct interpretation make them unsuitable for clinico-pathological correlation. On the other hand, the α-naphthyl acetate reaction for NSE seems to be a reliable method for identifying and counting monocytes [5, 6, 27, 33] within glomeruli in human biopsy specimens. No NSE-positive cells are found in normal subjects or in patients with non-proliferative types of GN, which means that normal resident glomerular cells do not give false positive reactions and that the resident macrophage-like cells with Ia-antigens recently described in rats [34], if present in normal humans, also do not stain with NSE. We found glomerular infiltration of mononuclear phagocytes in human renal biopsy specimens from a large number of patients with some (but not all) types of proliferative GN, in overall agreement with other studies in which the same [23, 27, 33, 35] or other methods [25, 26] were used to identify these cells.

Monocytes were found in increasing numbers in biopsy samples with increasing hypercellularity so that, in general, mild mesangial hypercellularity was associated with few monocytes and severe diffuse hypercellularity was associated with larger numbers of monocytes. However, we found marked differences, in spite of comparable degrees and locations of hypercellularity, among the various types of primary and secondary GN. In detail, the average number of intracapillary monocytes was much larger in diffuse proliferative GN associated with SLE, cryoglobulinemia, liver diseases, and Henoch-Schönlein purpura than in idiopathic MPGN. These findings are at variance with those of Magil, Wadsworth, and Loewen [27] and of Laohapand, Cattell, and Gabriel [26], who demonstrated a strong association of monocyte infiltration with the presence of subendothelial deposits, a very characteristic lesion in MPGN. We found such an association only in SLE, in cryoglobulinemia, and, rather surprisingly, in IgA-containing MPGN of cirrhotics and of Henoch-Schönlein patients, but not in the idiopathic form of MPGN, suggesting that the type of immune deposits and their possible inflammatory nature, more than their location, are the stimuli for glomerular accumulation of monocytes.

The extraordinarily large number of intraglomerular monocytes found in diffuse proliferative GN associated with essential cryoglobulinemia seems to confirm this assumption. The correlation of the number of NSE-positive cells with the presence of endoluminal thrombi, and the location of the cells in close contact with these massive immune deposits, suggest that the cryoprecipitable immune complexes typical of this disease are particularly capable of recruiting monocytes to the glomerulus, where they can eventually phagocytose cryoprecipitates [36]. Experimental observations showing that monocytes can fix to

Table 3. Correlations between M/G and the main laboratory findings in some types of human glomerulonephritis

	Acute post-infectious GN		Crescentic GN		SLE–GN		Membranoproliferative GN	
M/G (number of cases)	> 5 (18)	< 5 (9)	> 5 (13)	< 5 (7)	> 5 (27)	< 5 (34)	> 5 (5)	< 5 (12)
Mean serum creatinine, *mg %*	2.0 ± 1.7	1.2 ± 0.3	7.2 ± 5.4	8.7 ± 4.2	1.9 ± 2.2	1.2 ± 1.6	3.1 ± 1.0	2.5 ± 3.5
	NS		NS		NS		NS	
Serum hypo-C_3	15/8	3/9	5/11	1/5	18/26	16/30	1/4	5/12
	$P < 0.01$		NS		NS		NS	
Serum hypo-C_4	2/18	3/9	1/11	1/5	23/26	22/30	0/4	4/12
	NS		NS		NS		NS	
Mean proteinuria, *g/24 hr*	2.4 ± 2.0	0.8 ± 0.5	5.3 ± 2.0	1.5 ± 1.0	2.9 ± 2.5	1.4 ± 1.3	7.3 ± 5.9	3.1 ± 1.8
	$P < 0.005$		$P < 0.001$		$P < 0.01$		$P < 0.025$	

NS = not significant

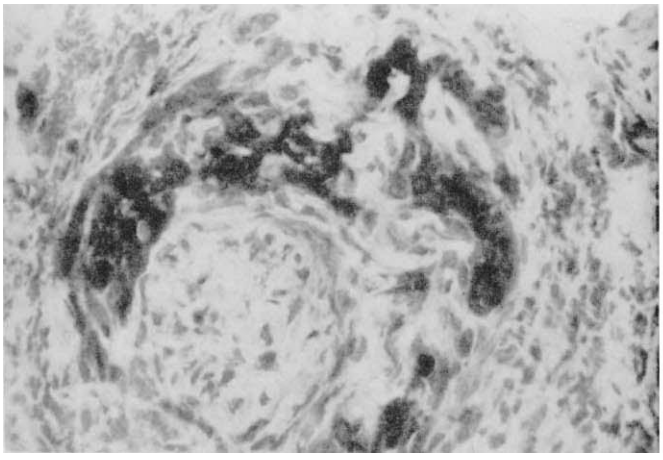

Fig. 3. *Crescentic GN: NSE-positive cells are present in the site of extracapillary proliferation.* (NSE, ×245)

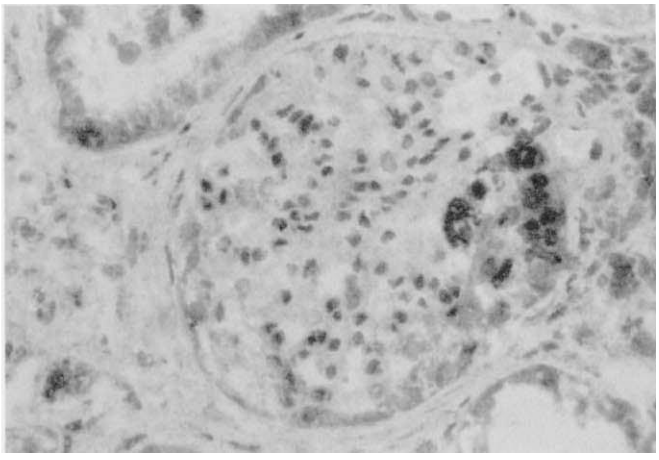

Fig. 4. *Focal proliferative SLE-GN: intraglomerular monocytes are present only in a segmental position.* (NSE, ×245)

circulating or deposited immune complexes via their surface Fc receptors [14, 15] confirm the possibility that such mechanism is active in human GN.

Animal experiments suggest that complement activation and fibrin deposition at the site of antibody deposition within the glomerulus [12] can also mediate the participation of monocyte in the pathogenesis of glomerular injury. In acute post-infectious GN, we found a good correlation between the decrease in serum C3 and the amount of intraglomerular monocytes. Furthermore, there were significant correlations in SLE-GN and in crescentic GN between the extent of fibrin deposition (at immunofluorescence) and the M/G index. However, it is difficult to say whether these correlations between hypocomplementemia and fibrin deposition on one hand, and extent of glomerular monocyte accumulation on the other, are due to a direct stimulating effect of these two factors or possibly an indirect consequence of a more severe injurious mechanism, which in some patients independently produces more marked complement activation, fibrin deposition, and intraglomerular monocyte accumulation. The marked difference in the M/G index that we found in two diseases with similar activation of the complement system (SLE-GN and idiopathic MPGN) and the finding that depletion of complement does not lessen macrophage accumulation in experimental GN [15] are in agreement with this latter assumption.

We found a strongly positive correlation between number of monocytes and amount of proteinuria in all types of NSE-positive GN; this may also be a non-specific consequence of the more severe renal damage, although data indicate that peak proteinuria coincides with the time of intraglomerular monocyte accumulation in different experimental models of GN [4, 6, 10] and that prevention of this accumulation with an anti-macrophage serum reduces the accompanying proteinuria [5]. The large number of NSE-positive cells found in acute post-infectious GN confirms previous data [24, 27, 28, 37, 38]. The strict correlation between the degree of monocyte accumulation and the extent of the exudative involvement of the glomeruli suggests that the recruitment of these cells is related to the intensity of the local inflammatory response rather than to the injury's immunological mechanism and, together with polymorphonuclear leukocytes, contributes to its induction. Proof for such a direct causal role of macrophages in inducing renal injury comes from studies in experimental GN, in which these cells have been specifically suppressed [15]. Since in non-exudative post-infectious GN, in idiopathic IgA mesangial GN, and in idiopathic MPGN there is a significant, often marked, proliferation of intrinsic glomerular cells even in the absence of intraluminal monocytes, macrophages seem not to be responsible in these diseases for this proliferation, as they are in certain models of experimental immune-mediated GN in which administration of anti-macrophage antiserum is able to abolish proliferation [15].

Our findings in crescentic GN confirm previous reports for experimental GN [12] that monocytes are present in intracapillary positions as well as in crescents, supporting the idea [7, 12] that these cells are first attracted intraluminally into the glomerular loops and subsequently migrate from there into Bowman's space through breaks in the glomerular basement membrane. In cases of crescentic GN with linear, granular, or negative patterns by immunofluorescence, we found similar numbers of NSE-positive cells, in accordance with the findings of Atkins et al [37], whereas Magil and Wadsworth [33] found them to be especially high in the anti-GBM type of crescentic GN. The M/G tended to decrease in fibrosed crescentic GN, as described by Magil and Wadsworth [33], suggesting that the presence of monocytes is an early event. The correlation between the NSE-index and the extent of fibrin deposition in the crescents confirms in humans the findings of Holdsworth et al [39] that in experimental crescentic GN fibrin acts as the stimulus to the migration of monocytes towards Bowman's space.

The recently demonstrated pro-coagulant activity inducible on monocyte membranes could contribute in generating fibrin deposits, and thus the association between the presence of monocytes and fibrin deposition could be more than a chemotactic one [40].

In conclusion, our data confirm that there may be an active role of cells of the monocyte-macrophage series in glomerular damage. Consistent numbers of these cells can be identified within the glomeruli only in immunologically-mediated GN characterized by a significant proliferation of resident glomerular cells. This accumulation shows some correlation with the prevalent site of proliferation, being more marked when peripheral capillary walls are involved, and also with the degree of activation of complement and with the amount of intraglomerular fibrin deposition. However, none of these factors can explain by itself the different degrees of monocyte accumulation found in various types of GN even when histological features are similar, suggesting that there are several mechanisms, many of them still unknown, responsible for the macrophage accumulation in these diseases.

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Reprint requests to Prof. G. D'Amico, Division of Nephrology, Ospedale San Carlo Borromeo, Via Pio II n° 3, Milano, Italy

References

1. Bradfield JWB, Cattell V, Smith J: The mesangial cell in glomerulonephritis. II. Mesangial proliferation caused by habu snake venom in the rat. *Lab Invest* 36:487–492, 1977
2. Cattell V, Jamieson SW: The origin of glomerular crescents in experimental nephrotoxic serum nephritis in the rabbit. *Lab Invest* 39:584–590, 1978
3. Holdsworth SR, Thompson NM, Glasgow EF, Dowling JP, Atkins RC: Tissue culture of isolated glomeruli in experimental crescentic glomerulonephritis. *J Exp Med* 147:98–109, 1978
4. Holdsworth SR, Neale TJ, Wilson CB: The participation of macrophages and monocytes in experimental immune complexes glomerulonephritis. *Clin Immunol Immunopathol* 15:510–524, 1980
5. Holdsworth SR, Allen DE, Thompson NM, Glasgow EF, Atkins RC: Histochemistry of glomerular cells in animal models of crescentic glomerulonephritis. *Pathology* 12:339–346, 1980
6. Hunsicker LG, Shearer TP, Plattern SB, Weisenburger D: The role of monocytes in serum sickness nephritis. *J Exp Med* 150:413–425, 1979
7. Kondo Y, Shigematsu H, Kobayashi Y: Cellular aspects of rabbit Masugi nephritis. II. Progressive glomerular injuries with crescent formation. *Lab Invest* 27:620–631, 1972
8. Morita T, Kihara I, Oite T, Yamamoto T: Participation of blood born cells in rat Masugi nephritis. *Acta Pathol Jpn* 26:409–422, 1976
9. Sano M: Participation of monocytes in glomerulonephritis in acute serum sickness of rabbit. *Acta Pathol Jpn* 26:423–433, 1976
10. Schreiner GF, Cotran RS, Pardo V, Unanue ER: A mononuclear cell component in experimental immunological glomerulonephritis. *J Exp Med* 147:369–384, 1978
11. Shigematsu H, Kobayashi Y: Accelerated serum sickness in the rabbit. II. Glomerular ultrastructural lesions in transient proliferative and progressive disorganizing glomerulonephritis. *Virchows Arch [Pathol Anat]* 369:269–278, 1976
12. Thompson NM, Holdsworth SR, Glasgow EF, Atkins RC: The macrophage in the development of experimental crescentic glomerulonephritis. *Am J Pathol* 94:223–240, 1979
13. Striker GE, Mannik M, Tung MY: Role of marrow-derived monocytes and mesangial cells in removal of immune complexes from renal glomeruli. *J Exp Med* 149:127–136, 1979
14. Haakenstad AO, Mannik M: The disappearance kinetics of soluble immunocomplexes prepared with reduced and alkylated antibodies in mice. *Lab Invest* 35:283–292, 1976
15. Holdsworth SR: Fc dependence of macrophage accumulation and subsequent injury in experimental glomerulonephritis. *J Immunol* 130:735–739, 1983
16. Bhan AK, Collins AB, Schneeberger EE, Mc Cluskey RT: A cell-mediated reaction against glomerular-bound immune complexes. *J Exp Med* 150:1410–1420, 1979
17. Kreisberg JI, Wayne DB, Karnowsky MJ: Rapid and focal loss of negative charge associated with mononuclear cell infiltration early in nephrotoxic serum nephritis. *Kidney Int* 16:290–300, 1979
18. Dubois CH, Foidart JB, Hautier MB, Dechenne CA, Lemaire MJ, Mahieu PR: Proliferative glomerulonephritis in rats: evidence that mononuclear phagocytes infiltrating the glomeruli stimulate the proliferation of endothelial and mesangial cells. *Eur J Clin Invest* 11:91–104, 1981
19. Leibovich SJ, Ross R: A macrophage-dependent factor that stimulates the proliferation of fibroblasts in vitro. *Am J Pathol* 84:501–513, 1976
20. Nathan CF, Murray HW, Cohen ZA: The macrophage as an effector cell. *N Engl J Med* 303:622–626, 1980
21. Unanue ER: Secretory function of mononuclear phagocytes. *Am J Pathol* 83:396–416, 1976
22. Atkins RC, Glasgow EF, Holdsworth SR, Matthews FE: The macrophage in human rapidly progressive glomerulonephritis. *Lancet* 1:830–832, 1976
23. Harry T, Bryant D, Coles GA, Davies M, Fortt W: The detection of monocytes in human renal biopsies: a prospective study. *Clin Nephrol* 18:29–33, 1982
24. Jones DB: Inflammation and repair of the glomerulus. *Am J Pathol* 27:991–999, 1951
25. Jothy S, Sawka RJ: Presence of monocytes in systemic lupus erythematosus-associated glomerulonephritis. *Arch Pathol Lab Med* 105:590–593, 1981
26. Laohapand T, Cattell V, Gabriel JRT: Monocyte infiltration in human glomerulonephritis: alpha-1-antitrypsin as a marker for mononuclear phagocytes in renal biopsies. *Clin Nephrol* 19:309–316, 1983
27. Magil AB, Wadsworth LD, Loewen M: Monocytes and human renal glomerular disease. A quantitative evaluation. *Lab Invest* 44:27–31, 1981
28. Monga G, Mazzucco G, Barbiano di Belgioioso G, Busnach G: Monocyte infiltration and glomerular hypercellularity in human acute and persistent glomerulonephritis. Light and electron microscopic, immunofluorescence and histochemical investigation on twenty-eight cases. *Lab Invest* 44:381–387, 1981

29. GLASSOCK RJ: A clinical and immunopathologic dissection of rapidly progressive glomerulonephritis. *Nephron* 22:253–264, 1978
30. D'AMICO G, FERRARIO F, COLASANTI G, RAGNI A, BESTETTI BOSISIO M: IgA mesangial nephropathy (Berger's disease) with rapid decline in renal function. *Clin Nephrol* 16:251–257, 1981
31. NACLAS MM, SELIGMAN AM: Histochemical demonstration of esterase. *J Natl Cancer Inst* 9:415–419, 1949
32. PEARSE AGE: *Histochemistry: theoretical and applied.* Edinburgh & London, Churchill Livingstone, 1972, vol 2, p 773
33. MAGIL AB, WADSWORTH LD: Monocyte involvement in glomerular crescents. A histochemical and ultrastructural study. *Lab Invest* 47:160–166, 1982
34. SCHREINER GF, KIELY JM, COTRAN RS, UNANUE ER: Characterization of resident glomerular cells in the rat expressing Ia determinants and manifesting genetically restricted interactions with lymphocytes. *J Clin Invest* 68:920–931, 1981
35. MONGA G, MAZZUCCO G, BARBIANO DI BELGIOIOSO G, BUSNACH G: The presence and possible role of monocyte infiltration in human chronic proliferative glomerulonephritides. *Am J Pathol* 94:271–284, 1979
36. MONGA G, MAZZUCCO G, COPPO R, PICCOLI G, CODA R: Glomerular findings in mixed IgG-IgM cryoglobulinemia: light, electron microscopic, immunofluorescence and histochemical correlations. *Virchows Arch [Cell Pathol]* 20:185–191, 1976
37. ATKINS RC, HANCOCK WW, STOW J, BECKER GJ, THOMPSON NM, GLASGOW EF: Macrophage identification in human and experimental glomerulonephritis, in *Proc 8th Int Congr of Nephrol,* edited by ZURUKZOGLU W, PAPADIMITRIOV M, PYRPASOPOULOS M, SION M, ZAMBOULIS T, Basel, Karger, 1981, pp 865–871
38. SHIGEMATSU H, SHISHIDO H, KUMARA K, TSUCHIDA H, SUZUKI H, HIROSE K, TOJO S: Participation of monocytes in transient glomerular hypercellularity in poststreptococcal glomerulonephritis. *Virchows Arch [Cell Pathol]* 12:367–370, 1973
39. HOLDSWORTH SR, THOMPSON NM, GLASGOW EF, ATKINS RC: The effect of defibrination on macrophage participation in rabbit nephrotoxic nephritis: studies using glomerular culture and electronmicroscopy. *Clin Exp Immunol* 37:38–43, 1979
40. EDGINGTON TS: Recognition coupled response of the monocyte: activation of coagulation pathways. *Nouv Rev Fr Hematol* 25:1–6, 1983