Mesangial cells in membranous glomerulonephritis

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SYNOPSIS Renal biopsies of seven patients with membranous glomerulonephritis were examined by light, electron, and immunofluorescence microscopy. All had characteristic changes of glomerular basement membranes, typically with bright granular membranous fluorescence of IgG and variable deposits of complement. Cellularity was normal or slightly increased due largely to mesangial cell proliferation. These cells assumed a variety of appearances referred to as resting, hyperactive, and dark. Resting forms occurred in cases with normal cellularity and limited complement deposition. Hyperactive cells showed ultrastructural evidence of increased secretory and digestive activity and were associated with proliferation and more pronounced complement deposition. Dark cells, interpreted as undergoing shrinkage and degeneration, were found in small numbers in all biopsies.

The characteristic glomerular lesion in membranous glomerulonephritis is a diffuse and fairly uniform thickening of the capillary walls without significant cellular proliferation. This results from the deposition of complexes in which immunoglobulins and complement can be demonstrated by immunofluorescence (McCluskey, 1969). These immune complexes occur mainly in a subepithelial position, separated by outward projections of the basement membrane which give spiky profiles to the membranes in sections stained with methenamine silver (Brewer, 1964). These prominent membrane changes have overshadowed the less obvious cellular ones in the glomeruli in this nephropathy. In this study of seven cases of membranous glomerular disease, the morphology of the mesangial cells has been investigated.

Materials and Methods

Kidney tissue was obtained by percutaneous needle

biopsy from seven patients (table I) with membranous glomerulonephritis. In all cases, clinical indications of systemic lupus erythematosus were absent; in four, the onset was insidious with no preceding infections. One however, had a sore throat, and another a long history of tonsillitis before onset. In the other case intrarenal vein thrombosis was found at biopsy. Only one patient had a family history of renal disease.

Small pieces of tissue from each end of a biopsy core were processed for electron microscopy; the remainder was bisected longitudinally, one piece being used for light and the other for immunofluorescence microscopy.

LIGHT MICROSCOPY

Tissue was embedded in paraplast by normal histological methods, and 1-2 μ m-thick sections were cut after both block and knife had been cooled in a refrigerator. Sections were stained with haematoxylin and eosin (HE), periodic acid Schiff (PAS), Martius yellow, soluble blue, brilliant scarlet (MSB), and methenamine silver (MS).

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Patient	Sex	Age at Biopsy (yr)	Duration of Renal Symptoms	Onset of Nephrotic Syndrome
1	M	35	6 months	Recurrent tonsillitis for 18 years before onset; family history of renal disease
2	M	56	3 years	Insidious
3	F	34	10 years	Insidious
4	F	50	1½ years	Insidious
5	M	31	6 months	Six weeks' sore throat before onset
6	F	17	2 years	Insidious
7	M	64	4 months	Insidious; thrombosis of intrarenal veins in biopsy

Table I *Histories of seven cases of membranous glomerulonephritis*

Fig 1 Case 1: glomerulus showing diffuse capillary wall thickening without cellular proliferation (HE $\times$ 300).

IMMUNOFLUORESCENCE MICROSCOPY

Cryostat sections, 3–4 μ m thick, were prepared from tissue snap frozen in a mixture of isopentane and cardice, and were fixed for one minute in acetone. The sections were stained by the direct technique (Coons and Kaplan, 1950), with commercially prepared FITC-conjugated antisera to IgG, IgA, IgM, β 2C/IA, and fibrinogen. Unconjugated blocking control antisera were also used. Glomerular fluorescence was scored semiquantitatively on a six-point scale (0, +/–, +, ++, +++, +++++).

ELECTRON MICROSCOPY

Tissue was fixed in 2.5% buffered glutaraldehyde for four hours, postfixed in cold 1% osmium tetroxide for one hour, dehydrated in graded alcohols, and embedded in araldite according to the method of Luft (1961). Sections 0.5 μ m thick were stained with toluidine blue for tissue recognition and ultrathin (silver/gold) sections were cut from suitable blocks. These were mounted on uncoated copper grids and double stained with uranyl acetate (Watson, 1958) and lead citrate (Reynolds, 1963). Alternatively, some sections were stained with alcoholic 0.5% phosphotungstic acid (PTA) for collagen (Pease,

Fig 2 Case 5: glomerulus with diffuse capillary wall thickening and hypercellularity particularly involving the mesangial cells. The mesangial matrix is also widened (arrows) (HE $\times$ 300).

1964). The grids were examined with an AEI EM6B electron microscope. When possible at least two glomeruli from each case were studied.

Results

LIGHT MICROSCOPY

Characteristic thickening of the glomerular capillary wall (Heptinstall, 1966; Rosen, 1971) was seen in all the biopsies (figs 1 and 2), with numerous sub-epithelial humps and small intramembranous vesicles visible after MS staining. Toluidine blue showed dark spots in the glomerular basement membrane corresponding to the vesicles and the areas between the humps. Some splitting of the glomerular basement membrane by material giving staining reactions suggestive of cytoplasm was seen, especially in cases 5 and 6.

The glomeruli in two cases (1 and 2) had normal, or possibly slightly reduced cellularity (fig 1), and a slight increase of mesangial matrix. Cell counts gave values within the normal limits calculated by

Fig 3 Case 4: glomerulus treated with FITC conjugated anti IgG serum showing characteristic diffuse granular, membranous fluorescence. There is also some mesangial deposition (arrow) ($\times 350$).

Fig 4 Glomerulus from the same case as fig 3 treated with FITC conjugated anticomplement serum. The pattern of deposition corresponds closely with that of IgG ($\times 350$).

Kawano, Arakawa, McCoy, Porch, and Kimmelstiel (1969). In one case, however (3), there was a trend towards sclerosis of the tuft and reduced cellularity.

Mild cellular proliferation was detectable in the glomeruli of two cases (4 and 7) while the remaining cases showed a moderate proliferation (fig 2) affecting the mesangial cells in particular. There was also thickening of the matrix which was rather focal in case 6 and diffuse in case 5. Lobulation was accentuated in some of the glomeruli in the latter biopsy but the changes were incompatible with those of membrano-proliferative glomerulonephritis (Mandalenakis, Mendoza, Pirani, and Pollak, 1971).

IMMUNOFLOUORESCENCE MICROSCOPY

The most striking feature was the bright immunofluorescence of IgG seen in all except one biopsy (table II). The deposits were diffuse and granular (fig 3) with a similar distribution to those seen in the histological preparations. There was often some mesangial fluorescence.

Fluorescence with complement was variable, being brightest in the biopsies with cellular proliferation (fig 4). The type of deposit corresponded closely with that of IgG. IgM occurred in most cases, especially in the mesangia, and occasional granular deposits of IgA were detected in the membranes and mesangia. Fibrinogen, however, was identified in one biopsy only, where it was distributed in the mesangia and capillary lumens.

ELECTRON MICROSCOPY

Mesangial cells

These were characterized by their centrolobular position (fig 5), and by their cytoplasm which contained characteristic bundles of fine filaments (Trump and Bulger, 1968; Simon and Chatelanat, 1969), 4-7 nm in diameter, with dense attachment areas at cell surfaces (fig 6) similar to those of smooth muscle cells (Rhodin, 1962).

Three types of mesangial cell (resting, hyperactive, and dark) were distinguished. Resting cells (fig 6)

Patient	Degree of Fluorescence ¹				
	IgG	IgA	IgM	Beta 1C/1A	Fibrinogen
1	++++	-	-	+/-	-
2	++++	++	+	+/-	nt
3	++	-	+	+/-	nt
4	++++	+/-	+	+++	-
5	++++	+/-	+	++	+/-
6	+++	+	+++	+	+/-
7	+/-	+/-	+/-	+	+

Table II Summary of immunofluorescence results for seven patients with membranous glomerulonephritis

¹nt = not tested, +, ++, +++, +++++ = positive result, +/- = equivocal positivity, - = negative result

had irregularly shaped, sometimes deeply notched nuclei and cytoplasm of medium density with thin cell processes confined to the axial region. Golgi apparatus and rough endoplasmic reticulum were

poorly developed. Other cytoplasmic organelles, including a few dense bodies resembling lysosomes (Miller and Palade, 1964), were confined mainly to the juxtanuclear cytoplasm and larger cell processes.

Fig 5 Case 1: low-power electron micrograph showing the deposits (arrows) in the capillary walls and the resulting lumpy thickening of the glomerular basement membrane. Mesangial cells (M) occupy a centrolobular position, and those depicted here are resting forms ($\times 5000$).

Cells of this type were found predominantly in cases with normal cell numbers.

Hyperactive mesangial cells had abundant ribosomes (both free and attached), and well developed Golgi apparatus in cytoplasm which ranged in appearance from very pale to rather dense. Pale cells had rounded nuclei and plump cell processes which sometimes extended towards the periphery of capillary loops (fig 7), while images suggestive of phagocytosis were common and numbers of dense bodies were often increased. These cells were found in the cases with the highest cellularity and complement deposition.

With increase in cytoplasmic density (fig 8), cell processes became thinner and more tortuous and

were largely confined to the axial regions, while nuclear density increased and nuclear outlines became more irregular. Dense bodies were fewer than in the pale cells although profiles suggestive of phagocytosis were identifiable. Vesicles similar to those surrounding the Golgi apparatus very occasionally appeared to fuse with cell membranes and faint vesicular profiles (50-70 nm in diameter) lay in the mesangial matrix against the plasmalemma (fig 8). Ribosomes were abundant and there were clusters of larger (20-30 nm), probably glycogen, granules. Dense hyperactive cells were prominent in biopsies showing mild mesangial cell proliferation, although they also occurred in cases with the highest cellularity.

Fig 6 Resting mesangial cell showing irregularly shaped nucleus and poor development of the Golgi apparatus (G) and rough endoplasmic reticulum (r). Occasional dense bodies (D) may be detected. The prominent bundles of fine filaments with dense attachment areas (arrows), and the lack of fenestrated cytoplasm (f) distinguish mesangial cells from endothelial cells parts of which line capillary loops in the upper right and lower left of this micrograph ($\times 18\,750$).

Dark cells (figs 7 and 9) were found in small numbers in most biopsies but were commonest in cases with the largest deposits of complement. They had dense crenellated nuclei and condensed cytoplasm and their organelles included swollen mitochondria and numerous 15-30 nm particles. In none of the biopsies were cells of haematogenous origin identified either within the mesangium or in abnormal numbers within the capillary lumens.

Mesangial matrix and glomerular basement membrane

Mesangial matrices comprising irregularly packed filaments (2-4 nm diameter) were thickened according to the duration of the disease and the patient's age. Deposits, although often occurring in the lamina densa where it crossed the axial region, could not be identified in the intercellular channels.

The glomerular basement membrane was irregularly thickened in all cases with the lamina densa split and frayed around numerous deposits. The latter could be roughly divided into dense, intermediate, and lucent. Dense deposits occurred immediately below the podocytes (fig 10), sometimes as humps or separated from the epithelium by a strand of lamina densa. Intermediate deposits comprised more loosely packed granular or flocculent material often surrounded by an electron-lucent halo and separated from the podocytes by strands of lamina densa (fig 11). Lucent deposits occurred subendothelially or intramembranously; they were often large, irregularly shaped (figs 10 and 11) and sometimes contained scattered flocculent material and small granules with dense rims and lucent cores containing dense particles 60-70 nm in diameter.

Fig 7 Pale hyperactive cell with cytoplasmic processes which have spread into the glomerular basement membrane (arrows). Note also part of a dark mesangial cell (Md) ($\times 5000$).

Fig 8 Dense hyperactive mesangial cell. Pseudopods are reduced, and the nuclear outline is irregular, but rough endoplasmic reticulum and Golgi apparatus are still well developed. Faint vesicular profiles (V) lie in the mesangial matrix against the plasmalemma, and the cytoplasm contains dense particles which are probably glycogen granules (arrow) ($\times 18\,750$).

Discussion

All the patients in this study were judged to have membranous glomerulonephritis by histological criteria. None had evidence of systemic lupus erythematosus.

The irregular, lumpy thickening of the glomerular basement membrane was visible with the light microscope, and ultrastructurally dense, intermediate, and lucent deposits could be detected. It was thought that initial dense, subepithelial, often hump-like deposits became covered by a layer of lamina densa and evolved into intermediate deposits and finally formed lucent areas. Similar findings have been reported by Churg, Grishman, Goldstein, Yunis, and Porush (1965) and Ehrenreich and Churg (1968)

although the deposits did not appear to be replaced by basement membrane as these workers suggested.

There was good correlation between the light immunofluorescence and electron microscopic results: bright granular membranous fluorescence of IgG was seen in all but one of the biopsies, and corresponded particularly with the intermediate and dense deposits. This is in accord with the findings in experimental models (reviewed by McCluskey and Vassalli, 1969), and in human membranous glomerulonephritis (McCluskey, Vassalli, Gallo and Baldwin, 1966; Rosen, 1971), and is compatible with the theory of immune complex deposition as the basis of the disease (Dixon, Feldman, and Vazquez 1961; McCluskey, 1970; Combes, Stastny, Shorey Eigenbrodt, Barrera, Hull, and Carter, 1971; Lewis

Fig 9 Dark mesangial cell (Md) with dense crenellated nucleus and dark cytoplasm. A hyperactive mesangial cell (M) is also present ($\times 12\,500$).

Loughridge, and Phillips, 1971; Nowoslawski, Krawczynski, Brzosko, and Madalinski, 1972). In one case, however, in which IgG deposition was equivocal but some fibrinogen was detected, thrombosis of intrarenal venous radicles was seen at biopsy. The association between renal vein thrombosis and membranous glomerular disease is well known (Pollak, Kark, Pirani, Schafer, and Muehrcke, 1956; Tublin, 1968; Giard, Paget, Saout, Crocchel, Leroy, and Cousin, 1969), and the present finding suggests a pathogenic mechanism based on the glomerular localization of fibrinogen complexes or aggregates. The coagulation abnormality responsible for the formation of such macromolecules might then be responsible for the thrombosis. Experimental work on this syndrome has, however, concentrated on the production of models with thrombosis as the prime event and membranous

glomerulonephritis has not been initiated (Omae, Masson, and Corcoran 1958; Fisher, Sharkey, Pardo, and Vuzevski, 1968; Harris, Ehrenfeld, and Wylie, 1968; Sturgill and Munsie, 1969).

In several cases, immunofluorescence gave positive results for IgG while complement was only equivocally positive, a phenomenon occasionally observed elsewhere (McIntosh, Tinglof, Kaufman, Dornfeld, Gonick, Smith, and Vernier, 1971).

Most workers report minimal mesangial changes in membranous glomerulonephritis (Churg *et al*, 1965; Rosen, 1971) although Combes *et al* (1971) noted focal mesangial hypercellularity following membranous deposition of Australia antigen-antibody complexes. In the present study, slight or moderate mesangial cell proliferation was often found, and the cellular ultrastructure varied considerably—resting, hyperactive, and dark forms

Fig 10 Capillary loop showing subepithelial dense deposits (d) and subendothelial and intramembranous lucent areas (l). Dense granules are scattered within the latter ($\times 12\,500$).

being recognized. It could be argued that the shape and density differences were fixation artefacts, for perfusion *in situ* which gives the best results is not applicable to human biopsy tissues. However, while there was probably some distortion, the variations of cell shape and constituent organelles in different biopsies subject to identical fixation methods supports the contention that mesangial cells exist in several forms.

Knowledge of the ultrastructure of 'normal' mesangial cells has been derived mainly from study of laboratory animals (Latta, Maunsbach, and Madden, 1960; Simon and Chatelanat, 1969). The state of the organelles in normal human mesangial cells (Portch, 1972) indicates low metabolic activity, and in the present investigation comparable cells were found, particularly in biopsies with normal cell numbers and minimal complement deposition. The

disease in these cases tended to be of long duration.

Cells regarded as hyperactive had cytoplasm of various densities. The main function of the pale forms was thought to be removal of material from the glomerular basement membrane and mesangial channels and they had extensive processes, some of which spread peripherally into the glomerular basement membrane. The fine structure of the denser cells suggested a secretory role, although evidence of phagocytosis was also seen. Their processes were confined largely to the axial region. Hyperactive cells were found in cases involving proliferation and complement deposition.

Mesangial cells can remove material, such as ferritin and myeloperoxidase, from the glomerular basement membrane and mesangial matrix (Miller and Palade, 1964; Graham and Karnovsky, 1966), and Farquhar and Palade (1962) thought that the

Fig 11 Part of the thickened glomerular basement membrane showing extensive lucent areas, which in this case (no. 2) contain small clumps of fibrin (f). Fibrin can also be detected in the capillary lumen (L). i, intermediate deposit ($\times 30\,000$).

chief function was to unclog the glomerular basement membrane, during which activity pseudopodial extensions and cytoplasmic dense body content are increased. It is also believed that mesangial cells secrete the surrounding matrix (Jones, 1963; Simon and Chatelanat, 1969) and that secretion is stimulated by the deposition of materials such as heavy metals or fibrinoid within the mesangial channels (reviewed by Simon and Chatelanat, 1969). The matrix is apparently composed of loosely packed basement membrane-like material, and Foster and Riad (1963) suggested that both structures were composed of collagen surrounded by mucoid material. The former, according to Misra and Berman (1966), is probably in the form of tropocollagen. Profiles of vesicles fusing with the mesangial cell surface as has been described in fibroblasts during collagen synthesis (Sheldon and Kimball, 1962; Goldberg and Green, 1964; Ross and Benditt, 1965; Bloom and Fawcett, 1968) were not widespread in the present study and were seen only in dense hyperactive cells.

The degree of mesangial thickening was related to the duration of the disease and the patient's age, a phenomenon noted in rats by Latta (1961). Endo-

thelial cells may also contribute to the matrix as they are reputed to secrete basement membrane elsewhere in the body (Ham, 1969).

Dark mesangial cells, which were found in all biopsies, especially in those with large complement deposits, were interpreted as undergoing shrinkage and degeneration. The role of complement in the production of glomerular damage is poorly understood, although complement fixation is known to promote the release of lysosomal enzymes (Henson, 1972). Free activated components and fragments of complement may also be released during or following complement fixation (Ingram, 1972), and substances inducing contraction of smooth muscle may be produced (Nelson, 1965). Furthermore, cells of the reticuloendothelial system, which probably includes mesangial cells, are stimulated to phagocytose complexes partly by complement-dependent adherence (Henson, 1972).

In the present study, mesangial cell proliferation was generally associated with complement deposition but it is not clear why this should be so. It might represent a response to an increased phagocytic workload, or to mild damage stimulating the cells to

divide, with more severe damage leading to shrinkage and degeneration.

Membranous glomerulonephritis is a chronic disorder of insidious onset. By the time it has produced overt clinical symptoms, there is usually significant glomerular damage mainly in the form of thickened capillary walls. Our findings suggest, however, that the mesangial cells, hitherto largely overlooked in this nephropathy, have a contribution to make to its evolution.

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