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Modified immunogold labelling applied to the study of protein droplets in glomerular disease

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Summary. Glomerular protein droplets have been observed in human glomerular epithelial cells, peripolar cells and mesangial cells, particularly in the preeclamptic lesion. Although morphologically similar, their functional role is uncertain. Whilst previous electron microscopic studies have shown these droplets to be generally uniform in electron density, our immunoelectron microscopy study of 10 biopsies, one showing lesions of preeclampsia, and the remaining 9 showing different forms of glomerular disease, generally displayed two distinct patterns of electron density and immunolabelling, when using a less severe fixation regime. An electron dense inner core was strongly positive to anti-fibrinogen, whilst a less dense peripheral zone was positive to anti-IgM. All other immunoglobulins, C_3c, and albumin showed a less specific immunolabelling pattern. Protein droplets were also observed in proximal tubules in 7 out of 10 biopsies. In droplets found in most tubular cells immunolabelling was not partitioned, the most prominent findings being diffuse labelling for fibrinogen and albumin.

In most instances where glomerular droplets were found adjacent to intraglomerular deposits, the marker identified within the deposit could also be seen in either the core area or the peripheral zone of the droplets. These findings raise the possibility that these droplets may be involved in protein absorption from the deposits through a damaged glomerular basement membrane.

Key words: Immunolabelling – Protein A-gold – Protein droplets – Glomerulonephritis

Introduction

Although protein droplets appear in glomerular epithelial cells and mesangial cells in a variety of glomerular lesions (Zollinger and Mihatsch 1978) major interest has focused on their presence in preeclampsia where they have been described as a characteristic feature (Faith and Trump 1966; Fisher et al. 1981; Hill et al. 1988; Hopper et al. 1961; Mautner et al. 1962; Pirani et al. 1963; Sheehan and Lynch 1973; Sheehan 1980). Droplets in epithelial podocytes have been assumed to be protein reabsorption droplets and Zollinger and Mihatsch (1978) found a correlation with the degree of proteinuria in various forms of glomerular disease and we have confirmed this in preeclampsia (Hill et al. 1988). Some of these droplets, in parietal epithelial cells are in a peripolar position and indistinguishable from the droplets in peripolar cells described by Ryan who proposed a secretory role for these cells (Gall et al. 1986; Hill et al. 1983; Ryan et al. 1979; Ryan et al. 1982).

In order to visualize the activity of these droplets more closely, serial sections from 10 biopsies, one showing lesions of preeclampsia, and the remaining 9 showing different forms of glomerular disease, were immunolabelled using protein A-gold. Bendayan et al. (1987) demonstrated that tissue processing conditions for optimal immunocytochemical labelling must be worked out for each class of binding site. In our preliminary immunocytochemistry experiments an evaluation of different tissue processing techniques and immunolabelling approaches were investigated. In our experience tissue routinely fixed in 1% OsO_4 for 2 h following initial PLP fixation showed good preservation of ultrastructural detail but a non specific immunolabelling pattern, therefore some modifications were necessary. Optimal immunoelectronmicroscopy conditions for renal antigens were determined after using a variety of tissue processing regimes. These conditions were then utilised in the investigation of glomerular protein droplets and adjacent intraglomerular deposits in an attempt to elucidate their role in glomerular disease.

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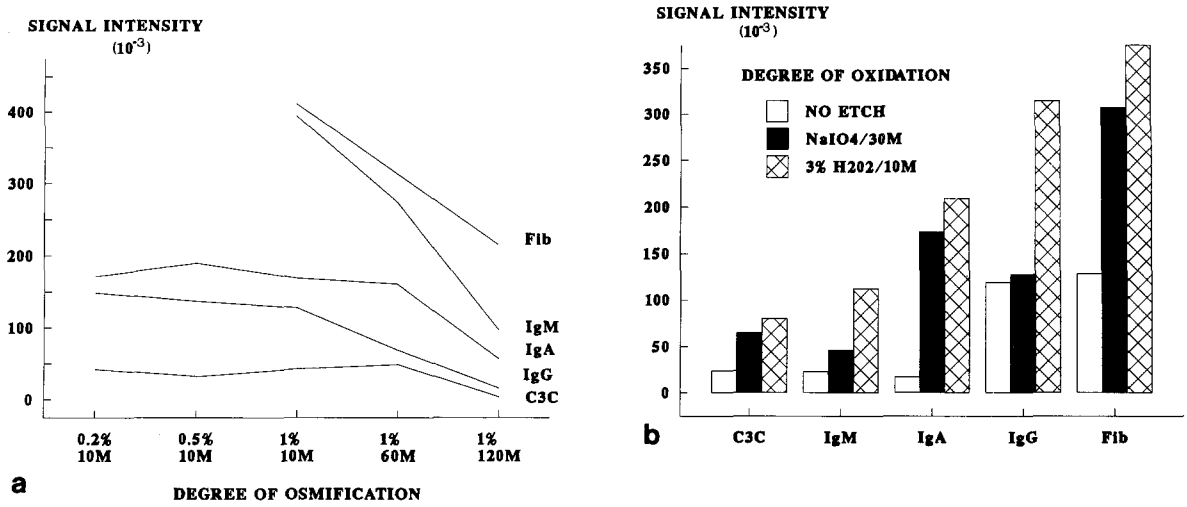

Fig. 1. Signal intensity (number of binding sites labelled with gold particles) is conditional upon both concentration and length of fixation time with OsO_4 (**a**); and the type of oxidant (etching agent) used (**b**)

Materials and methods

In order to evaluate optimum tissue processing conditions, renal tissue obtained by percutaneous needle biopsy, was fixed in periodate-lysine-paraformaldehyde (PLP) fixative (McLean and Nakane 1974) for 2 h at 4° C, washed for between 1 day and 1 week in sucrose buffer, and subjected to variable OsO_4 post-fixation, ranging from 0.2% aqueous solution for 10 min to 1% for 120 min. After fixation, the tissue was dehydrated in graded ethanol to 75%, embedded in L.R. White medium grade resin (London Resin Co., London, UK) and polymerized at 60° C for 2 h in sealed gelatin capsules. Ultrathin sections (gold in colour) were cut with a diamond knife and mounted on uncoated 300 mesh nickel grids prior to immunolabelling and heavy metal staining. Colloidal gold (15 nm) was prepared according to the method of Frens (1973) using 1% sodium citrate. The protein-A-gold complex (PAG) was prepared according to the method of Tanaka et al. (1984).

Before labelling, ultrathin sections from tissue optimally post-fixed with OsO_4, were oxidised (etched) with either hydrogen peroxide (H_2O_2) or sodium meta-periodate under variable time and concentration conditions. After washing thoroughly in distilled water, sections were incubated for 5 min on a drop of 0.01 M phosphate buffered saline, pH 7.4 containing 1% ovalbumin (ovalbumin/PBS). Sections were then transferred to a drop of rabbit antiserum specific to heavy chain human IgA, IgG, IgM, as well as C_3c and fibrinogen (Dakopatts, Denmark), diluted 1:200 with ovalbumin/PBS, prior to incubation for 16 h at 4° C. After 3 × 5 min washes with ovalbumin/PBS sections were reacted with protein A-gold complex for 30 min at room temperature. Following a further 3 × 5 min wash with ovalbumin/PBS sections were rinsed in distilled water and dried. Routine uranyl acetate/lead citrate staining was done prior to examination of sections under the electron microscope.

Control experiments were performed with the omission of the antiserum incubation step, but with the application of protein A-gold complex. In order to determine optimum conditions for tissue fixation and section etching, quantitative evaluation of the degree of immunolabelling was performed. Electron micrographs of all tissue were taken and magnified to 18000 times the original. At least six different areas of special interest (electron dense deposits), were set aside for quantitative evaluation using a MD-20 image analysis system (Logi-Tech Computers, Australia). The intensity of labelling obtained with anti-IgA, IgG, IgM, or C_3c antibody over the electron dense deposits and with anti-fibrinogen antibody over fibrillar fibrin in the glomeruli was evaluated and compared. The non specific labelling present over other areas (background) was also evaluated. Having calibrated the system to read a single 15 nm gold particle as 1 picture unit (pixel), our results were compiled according to the formula:

$$D = S_s/A_s - S_{ns}/A_{ns}$$

where,

D = density of labelling or signal intensity.
A_s = the surface area of the electron dense deposits or fibrillar fibrin labelled specifically with gold particles.
A_{ns} = the surface area without A_s, labelled non specifically with gold particles.
S_s = the number of gold particles present over A_s.
S_{ns} = the number of gold particles present over A_{ns}.

Assessment of variable OsO_4 post-fixation are summarised in Fig. 1a. An increase in concentration and/or length of immersion in OsO_4 decreased labelling intensity. One percent OsO_4 for 10 min offered adequate preservation of glomerular architecture without substantial loss of signal intensity. This optimal OsO_4 post-fixation was used for subsequent analysis of tissue oxidation (etching). Intensity of labelling was improved after etching with 3% H_2O_2 for 10 min (Fig. 1b). Etching with higher concentrations and/or longer times resulted in minor alterations to structural detail. The immunocytochemical techniques were applied to tissue samples from 10 renal biopsies used in this study; those showing protein droplets in epithelial cells in lesions of preeclamptic toxaemia, Goodpastures syndrome, mesangio-capillary glomerulonephritis (Type I), mesangial IgA glomerulonephritis, idiopathic crescentic glomerulonephritis, polyarteritis nodosa, focal and segmental hyalinosis and sclerosis, diabetic nephropathy, lupus nephritis, and membranous nephropathy. Forty-three biopsies from cases of minimal lesion nephrotic syndrome were examined for protein droplets in epithelial cells.

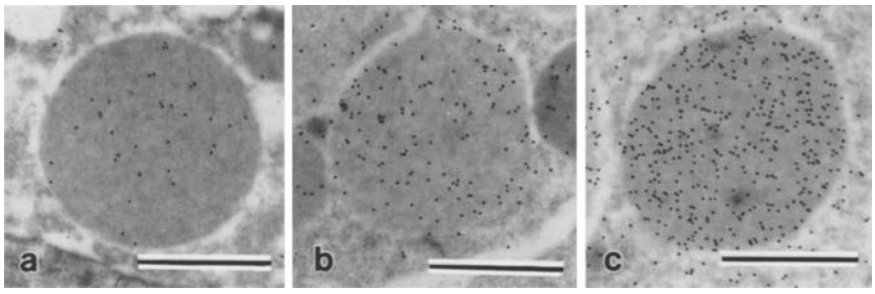

Fig. 2. Immunoelectron micrographs of visceral epithelial cell droplets demonstrating increasing degrees of positive labelling (**a**) +, (**b**) ++, (**c**) +++. ×18000. *Bar* = 1 μm

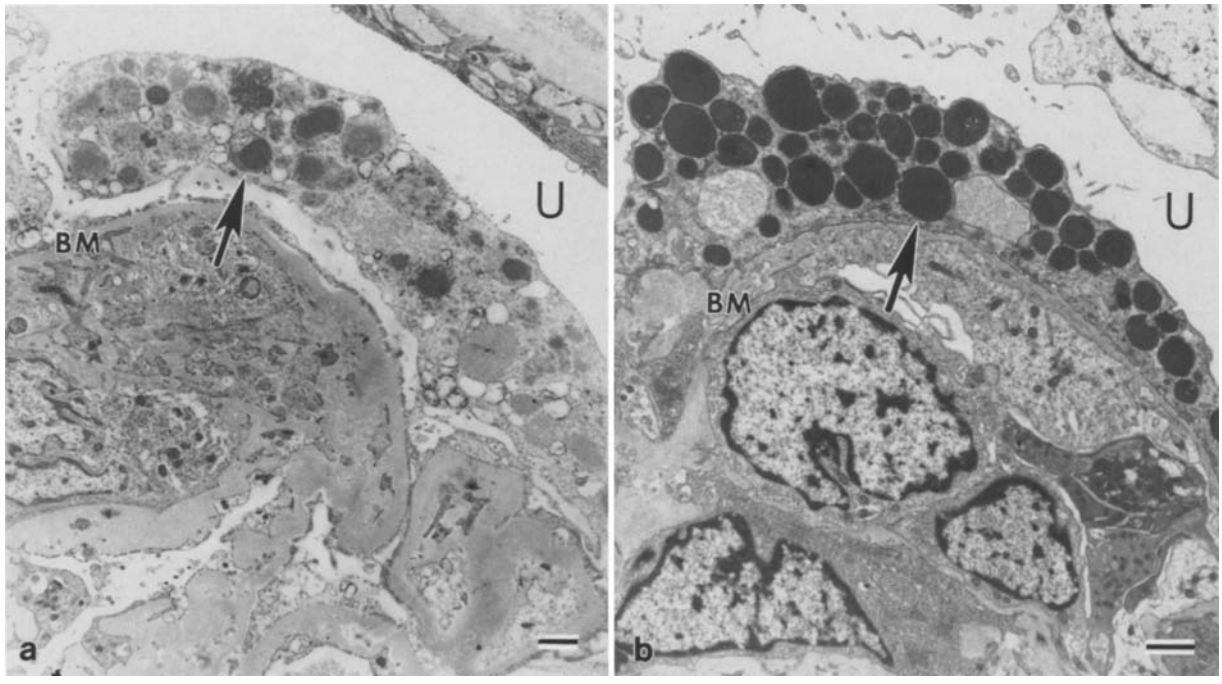

Fig. 3. Electron micrographs of droplets (*arrow*) in visceral epithelial cell in a case of lupus nephritis. Tissue processed for immunoelectron microscopy (**a**) shows a marked heterogeneity in electron density of droplets, whilst tissue processed for conventional microscopy (**b**) shows general uniformity in electron density of the droplets. BM = glomerular basement membrane, U = urinary space. (**a**) × 5000, (**b**) × 6000. *Bar* = 1 μm

Applying the technique previously outlined biopsy tissue was fixed initially in PLP, post fixed in 1% OsO_4 for 10 min and embedded in LR White resin.

Survey sections were cut and stained to locate the granulated cells prior to ultrathin sectioning. After etching with 3% H_2O_2 for 10 min grids were immunolabelled with anti-human IgA, IgG, IgM, C_3c, fibrinogen or albumin (Dakopatts, Denmark). The intensity of labelling was graded (−ve to +++ve) as shown in Fig. 2.

For double immunolabelling with IgM and fibrin/fibrinogen, one side of the tissue section was immunoreacted with anti-IgM serum/15 nm-PAG as described above, whilst the other side was immunoreacted with anti-fibrinogen serum/5 nm-PAG.

Results

In the sections scanned in this study, glomerular protein droplets were found in the visceral epithelial cell in 9 cases of glomerular disease, in the parietal epithelial cell in 3 cases, in a peripolar cell in 1 case and in a mesangial cell in 1 case. These protein droplets showed a remarkable morphological difference after processing with this modified technique (Fig. 3a) compared to the droplets examined after conventional electron microscopy techniques (Fig. 3b). Closer examination of droplets showed that, regardless of their cell type, the degree of electron density could vary from droplet to droplet, and that in some droplets a distinctive inner electron dense core was partitioned from a peripheral zone of less electron density. In a survey of 43 cases of minimal lesion nephrotic syndrome, no glomerular epithelial cell droplets were seen. Protein droplets were found in proximal tubules in 7 out of 10 cases showing glomerular epithelial

Table 1. Relationship between immunolabelling pattern of glomerular droplets in visceral epithelial cells and adjacent intraglomerular deposits

Case examined	Area examined	IgA	IgG	IgM	C_3c	Fib	Alb
Crescentic GN	Visceral C(P)	− (++)	− (++)	− (+)	+ (+)	+++ (−)	+++ (+++)
	deposit	−	−	+++	+	++	+
Goodpastures syndrome	Visceral C(P)	− (−)	− (−)	− (−)	+ (+)	+++ (−)	+++ (+++)
	deposit	−	−	++	+	+	−
Systemic lupus erythematosus	Visceral C(P)	+++ (+++)	− (+++)	− (+++)	+ (+)	+++ (−)	+++ (+++)
	deposit	−	+	++	+	+	−
Mesangial IgA disease	Visceral C(P)	++ (++)	+ (+)	− (+++)	− (−)	+++ (−)	+++ (+++)
	deposit	+	−	++	−	+	+
Membranous nephropathy	Visceral C(P)	+ (+)	− (−)	− (+)	+ (+)	+++ (−)	+++ (+++)
	deposit	−	+	+	−	−	+
Polyarteritis nodosa	Visceral C(P)	+ (+)	− (−)	− (−)	− (−)	++ (−)	+++ (+++)
	deposit	n/a	n/a	n/a	n/a	n/a	n/a
Diabetic nephropathy	Visceral C(P)	− (++)	− (+)	− (+++)	− (−)	+++ (−)	+++ (+++)
	deposit	−	−	++	−	++	+
Focal & seg. hyal./scler.	Visceral C(P)	+ (+)	− (−)	− (−)	+ (+)	+++ (−)	+++ (+++)
	deposit	−	−	+++	+	+	−

C(P), glomerular cell droplet; core area (peripheral zone); Deposit, adjacent intraglomerular deposit; Fib, fibrinogen; Alb, albumin; n/a, not applicable

Table 2. Immunolabelling pattern of glomerular droplets in parietal epithelial cells

Case examined	Area examined	IgA	IgG	IgM	C_3c	Fib	Alb
Mesangio-capillary, Type 1	Parietal C(P)	− (−)	+ (+)	− (++)	+ (+)	++ (−)	+++ (+++)
Goodpastures syndrome	Parietal C(P)	− (−)	− (−)	− (++)	− (−)	+++ (−)	+++ (+++)
Membranous nephropathy	Parietal C(P)	+ (+)	− (−)	− (+)	+ (+)	+++ (−)	+++ (+++)

C(P), glomerular cell droplet; core area (peripheral zone); Fib, fibrinogen; Alb, albumin

droplets. Morphologically they were different from the glomerular droplets, being smaller in size, fragmented on occasions, and displaying a less consistent zonal partition.

Immunocytochemistry using the protein A-gold technique with antiserum specific to human IgA, IgG, IgM, C_3c, fibrinogen and albumin, demonstrated specific labelling patterns within the droplets. In the glomerulus the distribution pattern and labelling intensity of the visceral epithelial cell droplets and parietal cell droplets is shown in Table 1 and Table 2 respectively.

In all 9 cases of glomerular disease, binding sites for fibrin/fibrinogen were located in and restricted to the electron dense inner core area of the droplet (Fig. 4a, 4b) found in visceral and parietal epithelial cells, and in the one mesangial cell examined. In 5 cases, binding sites for IgM were located in and restricted to the peripheral zone of droplets found in visceral cells (Fig. 4c) whilst in 3 cases the same pattern for IgM was observed in parietal cell droplets (Fig. 4d). The other immunoglobulins, albumin and C_3c showed a different distribution pattern within droplets of both visceral and parietal cells with gold particles randomly binding the sites of the peripheral zone and the inner core; labelling for albumin was intense (Fig. 4e), IgA and IgG was variable (Fig. 4f) and C_3c was sparse.

Protein identity and labelling intensity of the droplets in visceral epithelial cells was compared with that of adjacent intraglomerular deposits (Fig. 5) in the subendothelial and subepithelial situation in 7 cases. Table 1 shows that the protein marker identified within the deposit seen in cases of crescentic glomerulonephritis and diabetic nephropathy could also be seen in either the core area or the peripheral zone of the droplets. In the remaining 5 cases of glomerular disease, the immunolabelling pattern was not so clear cut, but it was apparent that the same protein, albeit in different concentration, could be detected in both deposits and droplets in most instances.

Lesions of preeclampsia were studied and drop-

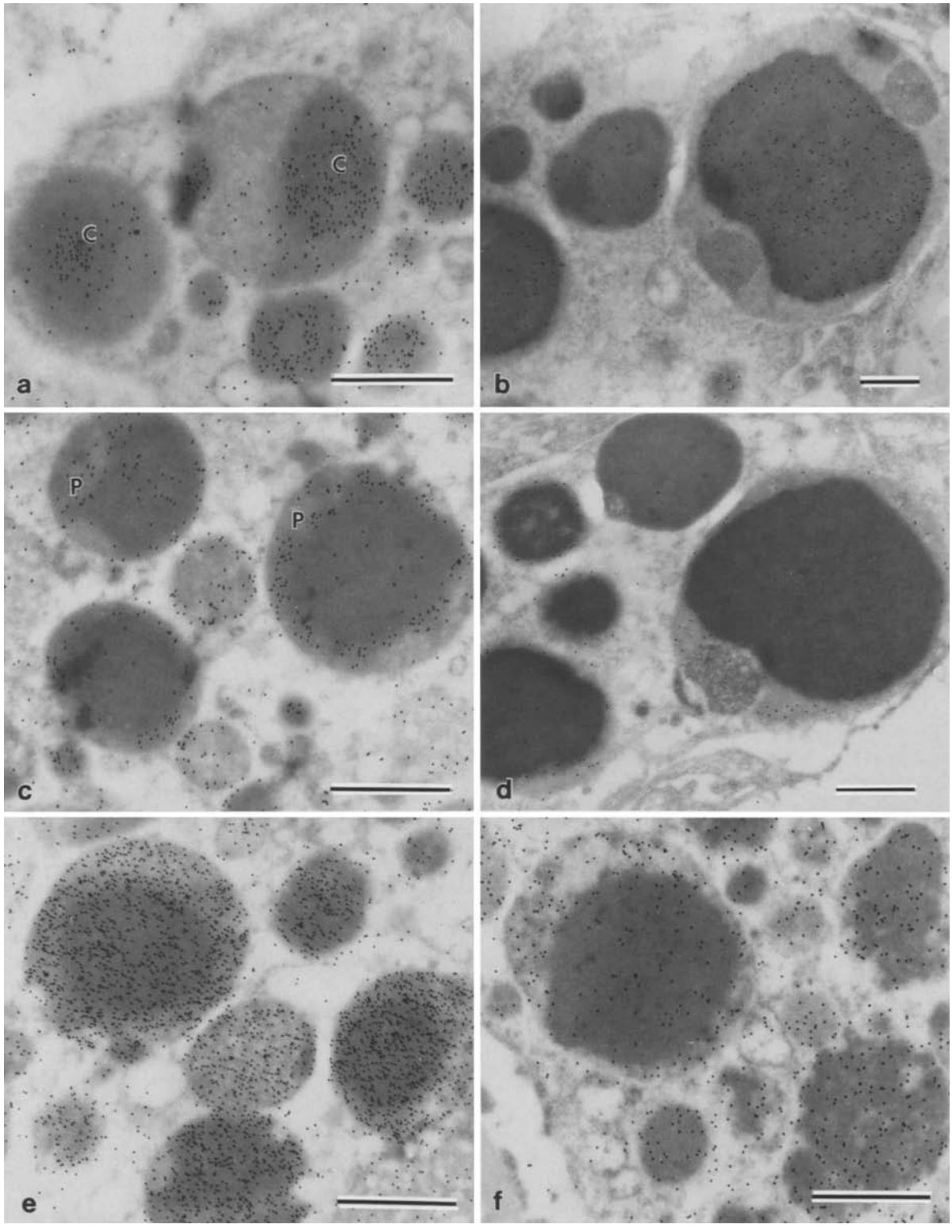

Fig. 4. Immunoelectron micrographs of droplets in visceral or parietal epithelial cells in various types of glomerular disease. (**a**) Intense labelling for fibrin/fibrinogen is seen only in the central electron dense core area of droplets in a visceral epithelial cell in the case of diabetic nephropathy. C = core area. × 20000. *Bar* = 1 μm (**b**) Intense labelling for fibrin/fibrinogen is confined to the central electron dense core area of droplets in a parietal epithelial cell in the case of Goodpastures syndrome. × 10000. *Bar* = 1 μm. (**c**) Intense labelling for IgM is confined to the peripheral semi-translucent zone of droplets in a visceral epithelial cell from the same patient as in Fig. 4a. P = peripheral zone. × 20000. *Bar* = 1 μm. (**d**) Mild-moderate labelling for IgM is confined to the semi-translucent zone of droplets in a parietal epithelial cell from the same patient as in Fig. 4b. × 13000. *Bar* = 1 μm. (**e**) Intense labelling for albumin is randomly distributed throughout both the central electron dense core area and the semi-translucent peripheral zone of droplets in a visceral epithelial cell from the same case as in Fig. 4a. × 20000. *Bar* = 1 μm. (**f**) Moderate labelling for IgA is randomly distributed in the same pattern as Fig. 4e within droplets in a visceral epithelial cell in a case of lupus nephritis. × 20000. *Bar* = 1 μm

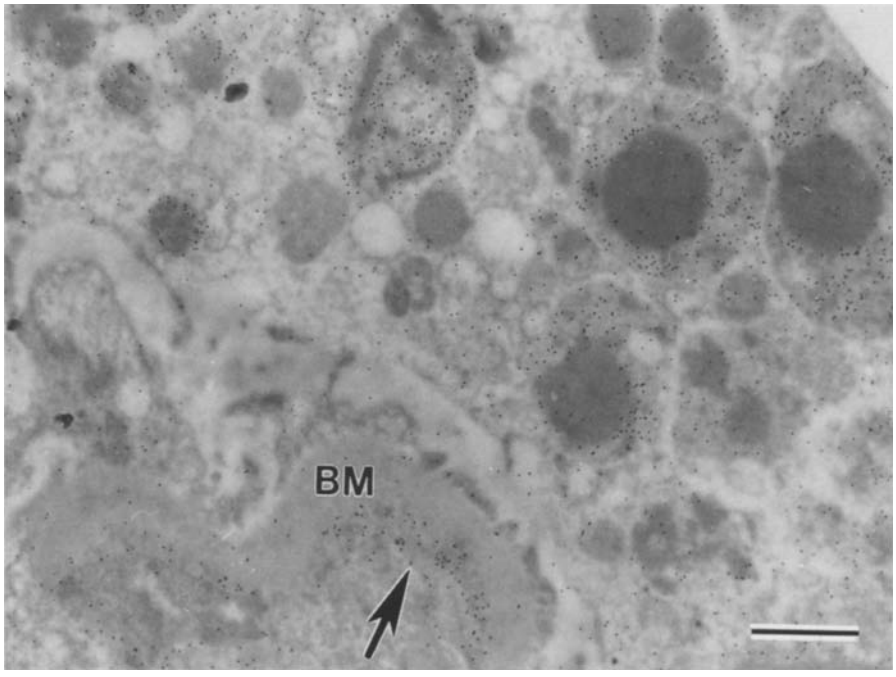

Fig. 5. Immunoelectron micrograph of visceral epithelial cell droplets labelled for IgM in a case of lupus nephritis. Intense labelling can be seen in the peripheral zone of the droplets whilst moderate labelling is visualized in the binding sites of the subendothelial deposits (*arrows*) of the glomerular capillary. BM = basement membrane; ×14000. *Bar* = 1 μm

Table 3. Relationship between immunolabelling pattern of glomerular droplets in visceral epithelial cells, mesangial cells and adjacent intraglomerular deposits

Case examined	Area examined	IgA	IgG	IgM	C_3c	Fib	Alb
Preeclamptic toxaemia	Visceral C(P)	+ (+)	+ (+)	+ (+)	+ (+)	+ + (−)	+ + + (+ + +)
	deposit	+	+	+ + +	+	+ + +	+
Preeclamptic toxaemia	Mesangial C(P)	− (+ + +)	− (+ + +)	− (+ + +)	+ (+)	+ + + (−)	+ + (+ + +)
	deposit	+	+	+ + +	+	+ + +	+

C(P), glomerular cell droplet; core area (peripheral zone); Deposit, adjacent intraglomerular deposit; Fib, fibrinogen; Alb, albumin

lets were found in both mesangial cells and visceral epithelial cells. The distribution pattern and labelling intensity are shown in Table 3. Closer examination of the immunolabelling pattern of the mesangial cell droplets revealed that for all immunoglobulins (IgA, IgG, IgM), labelling was confined to the peripheral zone (Fig. 6a, 6b) whilst fibrin/fibrinogen labelling was confined to the electron dense inner core (Fig. 6c). C_3c and albumin labelled particles were distributed throughout both the electron dense inner core and the peripheral semi-translucent zone. Double labelling for IgM and fibrin/fibrinogen confirmed the separate distribution of these two proteins within the droplets (Fig. 6d). In droplets found in the visceral epithelial cell, albumin, immunoglobulins (IgA, IgG, IgM) and C_3c were all evenly distributed throughout both the peripheral zone and the electron dense core. Fibrin/fibrinogen showed moderate labelling confined to central electron dense core only. Labelling for albumin was intense whilst labelling for immunoglobulins and C_3c was sparse. Adjacent mesangial deposits and subendothelial deposits were also examined, and protein identification within the deposit in preeclampsia was identical, albeit in different intensity, to that seen in either the core area or peripheral zone of the droplets regardless of cell type.

In the case of membranous nephropathy, protein droplets were identified in a parietal cell which was clearly in a peripolar position (Fig. 7a). They were also identified in other parietal cells along Bowman's capsule, and in visceral epithelial cells. The same immunolabelling pattern was identified in all cell types (Fig. 7b, 7c), and this data is summarized in Table 4. Whilst subepithelial deposits were immunolabelled with both IgG and IgM, the droplets of adjacent visceral cells contained IgM only in the peripheral zone.

Examination of glomerular droplet formation

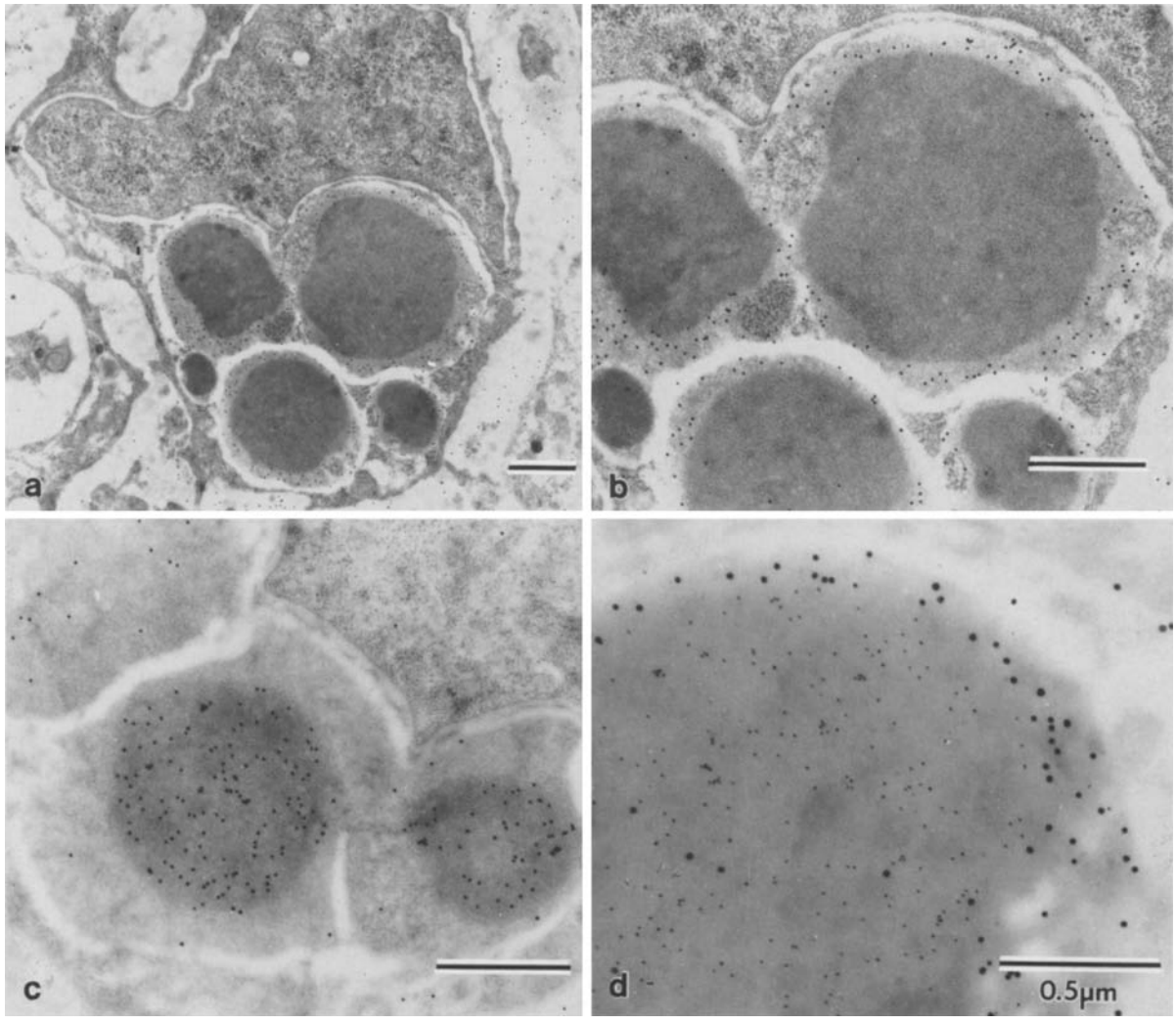

Fig. 6. Immunoelectron micrographs of droplets in a mesangial cell in a case of preeclampsia. (a & b) Intense labelling for IgM is confined to the peripheral semi-translucent zone of the droplets. (**a**) ×9000; (**b**) ×16000. *Bar* = 1 μm. (**c**) Intense labelling for fibrin/fibrinogen is confined to the central electron dense core of the droplets. ×18000. *Bar* = 1 μm. (**d**) Double labelling for IgM (*large gold particles*) and fibrin/fibrinogen (*small gold particles*) shows the different distribution pattern of both factors within the droplet, IgM in the peripheral zone and fibrin/fibrinogen in the central electron dense core. ×43000. *Bar* = 0.5 μm

in all cell types from all 10 biopsies showed no morphological evidence of extrusion of protein from droplets into the urinary space although some varying degree of degranulation was seen.

Protein droplets were also observed in proximal tubules in 7 out of 10 biopsies. Immunolabelling of most tubular droplets was not partitioned. The immunolabelling pattern for all immunoglobulins was both less distinct and intense in tubular droplets, whilst C_3c displayed a similar pattern. The most prominent finding was a diffuse labelling pattern for both fibrin/fibrinogen and albumin (Table 5).

In all biopsies, and for all cell types, control tests were carried out using the method described with the omission of the antiserum incubation step, but with the application of protein A-gold complex, and no specific immunolabelling was seen.

Discussion

Although protein droplets are observed in glomerular podocytes in different glomerular diseases they are predominantly associated with the deposition of intracapillary fibrin particularly in malignant hypertension and preeclampsia (Kincaid-Smith et al. 1984, 1985). In our study of 46 renal biopsies carried out during pregnancy, all 22 in which patients showed clinical features of preeclampsia, showed protein droplet formation in epi-

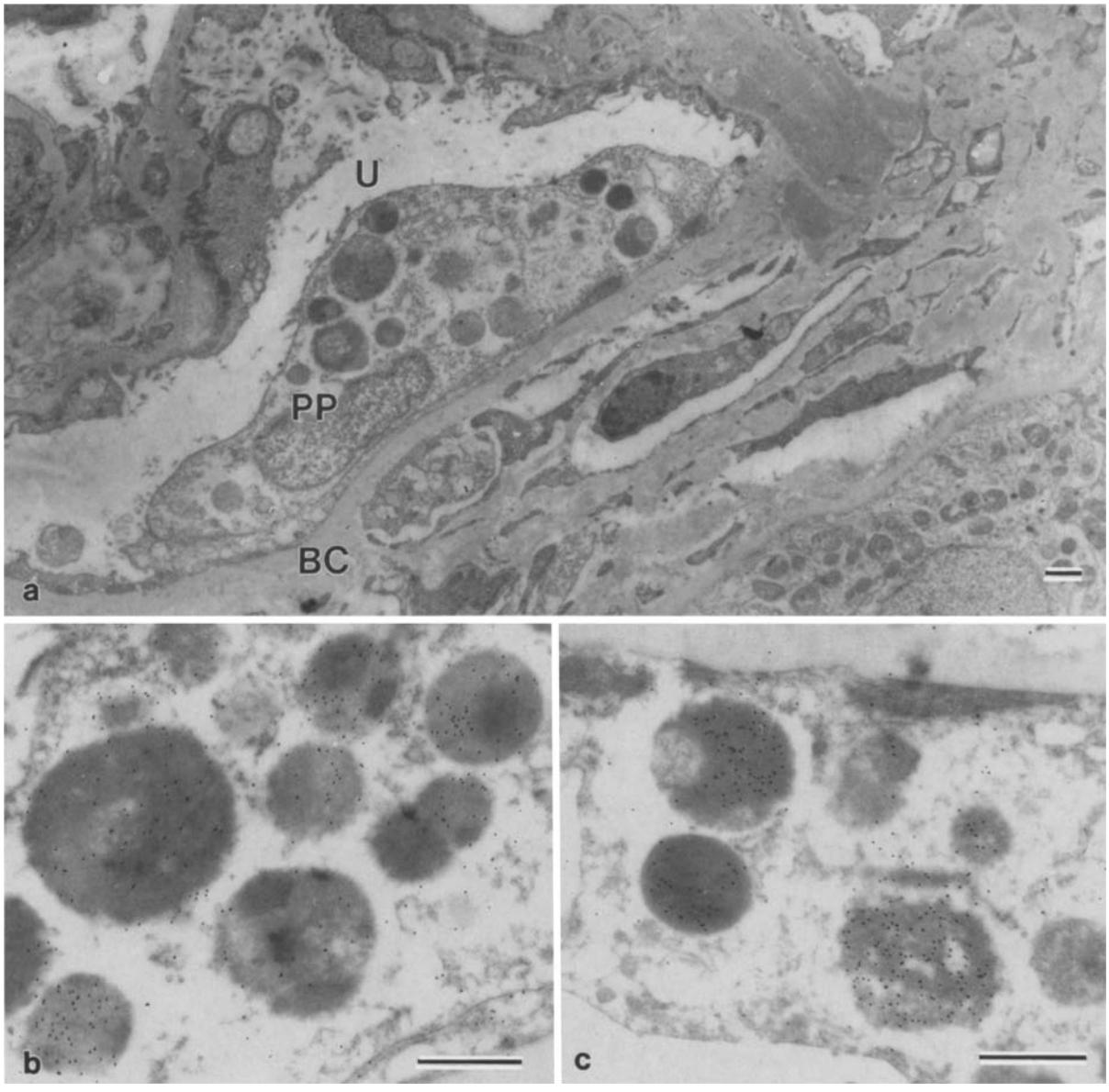

Fig. 7. Immunoelectron micrograph of droplets in a peripolar cell in the case of membranous nephropathy. (**a**) A parietal cell with numerous droplets is seen in the peripolar position. PP = peripolar cell; BC = bowmans capsule; U = urinary space. × 5000, *Bar* = 1 μm (**b**) Moderate labelling for IgM is seen in the electron translucent peripheral zone of the droplets. × 15000, *Bar* = 1 μm. (**c**) Moderate to intense labelling for fibrin/fibrinogen is seen in the electron dense inner core area of the droplets. × 15000, *Bar* = 1 μm

thelial podocytes whereas only one of 24 biopsies from patients with no clinical features of preeclampsia, showed droplet formation (Kincaid-Smith et al. 1985). We also found a correlation between the number of cells with protein droplet formation and the degree of preeclampsia (Kincaid-Smith et al. 1985). In a small number of biopsies from patients with malignant hypertension, we have also noted protein droplet formation. In experimental animal models of hypertension, protein droplet distribution was assessed, and we found that the number of cells with protein droplets correlates with the severity of hypertension and the degree of vascular damage (Hewitson et al. 1988). However, in a survey of 43 patients with the nephrotic syndrome due to minimal change disease no glomerular droplet formation was observed.

Conventional electronmicroscopy examination of these droplets have in the past been mainly focused on cellular origin with little regard for their

Table 4. Immunolabelling pattern of glomerular droplets in visceral and parietal epithelial cells, peripolar cells and their relationship to adjacent intraglomerular deposits

Case examined	Area examined	IgA	IgG	IgM	C_3c	Fib	Alb
Membranous nephropathy	Visceral C(P)	+ (+)	− (−)	− (+)	+ (+)	+++ (−)	+++ (+++)
	deposit	−	+	+	−	−	+
Membranous nephropathy	Parietal C(P)	+ (+)	− (−)	− (+)	+ (+)	+++ (−)	+++ (+++)
	deposit	n/a	n/a	n/a	n/a	n/a	n/a
Membranous nephropathy	Peripolar C(P)	+ (+)	− (−)	− (++)	+ (+)	++ (−)	+++ (+++)
	deposit	n/a	n/a	n/a	n/a	n/a	n/a

C(P), glomerular cell droplet; core area (peripheral zone); Deposit, adjacent intraglomerular deposit; Fib, fibrinogen; Alb, albumin; n/a, not applicable

Table 5. Immunolabelling pattern of glomerular and tubular droplets

Case examined	Droplet type	IgA	IgG	IgM	C_3c	Fib	Alb
Crescentic GN	Glom. cell C(P)	− (++)	− (++)	− (+)	+ (+)	+++ (−)	+++ (+++)
	PT cell C(P)	+ (+)	++ (++)	+ (+)	+ (+)	++ (++)	+++ (+++)
Goodpastures syndrome	Glom. cell C(P)	− (−)	− (−)	− (−)	+ (+)	+++ (−)	+++ (+++)
	PT cell C(P)	− (−)	− (−)	nf	− (−)	+++ (+++)	+++ (+++)
Systemic lupus erythematosus	Glom. cell C(P)	+++ (+++)	− (+++)	− (+++)	+ (+)	+++ (−)	+++ (+++)
	PT cell C(P)	− (−)	+ (+)	+ (+)	+ (+)	+++ (+++)	nf
Mesangial IgA disease	Glom. cell C(P)	++ (++)	+ (+)	− (+++)	− (−)	+++ (−)	+++ (+++)
	PT cell C(P)	+ (+)	− (−)	− (−)	− (−)	++ (++)	++ (++)
Membranous nephropathy	Glom. cell C(P)	+ (+)	− (−)	− (+)	+ (+)	+++ (−)	+++ (+++)
	PT cell C(P)	+ (+)	+ (+)	− (−)	+ (+)	++ (++)	+++ (+++)
Polyarteritis nodosa	Glom. cell C(P)	+ (+)	− (−)	− (−)	− (−)	++ (−)	+++ (+++)
	PT cell C(P)	− (−)	− (−)	− (−)	− (−)	− (−)	+++ (+++)
Preeclamptic toxaemia	Glom. cell C(P)	+ (+)	+ (+)	+ (+)	+ (+)	++ (−)	+++ (+++)
	PT cell C(P)	− (−)	− (−)	+ (+)	− (−)	+ (+)	+++ (+++)

Glom. cell C(P), glomerular cell droplet; core area (peripheral zone); PT cell, proximal tubule cell droplet; core area (peripheral zone); Fib, fibrinogen; alb, albumin; nf, not found

heterogenecity of electron density. Gardiner et al. (1986) in their study on the granules of peripolar cells from human nephrectomized kidneys, stated that "the majority were homogeneous in their electron density but a few appeared pale with some collapse of the investing membrane". Morild et al. (1988) however recently reported that the granules from peripolar cells in the avian kidney contained material with variable electron density, often with a more electron dense area in the centre of the granules.

In preparation for an ultrastructural assessment of droplet formation using immunolabelling techniques, we discovered that tissue fixation with PLP and 1% OsO_4 for 10 min, followed by section etching with 3% H_2O_2, resulted in a different morphological appearance of the droplets, and emphasized the heterogenecity of the droplet electron density. Whereas using conventional fixation procedures (i.e. 2.5% glutaraldehyde for 2 h, buffer wash and post fixation with 1% OsO_4 for 2 h) most droplets had uniform electron density, tissue processed as described for immunolabelling showed varying degrees of electron density within droplets, particularly in glomerular cells. The most common appearance was that of a peripheral semi-translucent zone surrounding an electron dense core. This morphological appearance is very similar to that reported by Morild et al. (1988). This partition was also noted immunologically by the visualization of gold particles labelled for fibrin/fibrinogen adhering to the binding sites of the inner core, whilst gold particles labelled for IgM adhered to the binding sites of the peripheral zone. Gold particles specific to other immunoglobulins, C_3c, and albumin could be seen adhering to binding

sites of the droplets in a more random pattern. The recognition of this distribution pattern within protein droplets has not been reported previously.

Our findings confirm previous light microscopy observations that the droplets contain albumin, immunoglobulins and complement (Hill et al. 1988; Zollinger and Mihatsch 1978). The distribution of particles labelled with immunogold suggests that mesangial cells and podocytes actively take up immunoglobulins, fibrin/fibrinogen, C_3c and albumin which are present only within the droplets and not in the adjacent cytoplasm of the epithelial or mesangial cells. The reason for the segregation of immunoglobulins and fibrin/fibrinogen within the droplets is unknown, but it suggests that the cells process these different proteins in a different manner. In mesangial cells labelling for immunoglobulins was concentrated in a peripheral semi-translucent zone whilst fibrin/fibrinogen was concentrated in a dense central core area. In all cells, intense immunolabelling, may reflect an active process of protein uptake within the cell. Intense labelling of glomerular droplets was most common in the case of fibrin/fibrinogen, albumin and IgM, and in each biopsy studied, IgM labelling was confined to the semi-translucent peripheral area whilst fibrin/fibrinogen labelling was confined to the more central electron dense areas.

In one parietal cell in a peripolar position described by Ryan (Ryan et al. 1979), not only was the morphological appearance similar, but the distribution patterns of fibrin/fibrinogen and IgM were identical to those in other parietal cells. Ultrastructural studies of peripolar cells by previous investigators (Gall et al. 1986; Hill et al. 1988; Ryan et al. 1979; Ryan et al. 1982) have suggested a possible secretory role for this cell on the basis of an exocytotic release phenomena. Morild et al. (1988), however, in their recent study of peripolar cells in the avian kidney questioned whether the peripolar cell was indeed a specific cell type, because the droplets in the peripolar cell did not differ morphologically from those of the visceral or parietal epithelial cell. Furthermore, their preliminary immunological study did not show any positive labelling of droplets in peripolar cells against cathepsins B, D, L and H and renin. In our study, immunogold labelling revealed that the droplets in the peripolar cell contain immunoglobulins, C_3c, fibrin/fibrinogen and albumin in the same distribution pattern as those in visceral or parietal epithelial cells, suggesting that this so called peripolar cell, probably has the same characteristics as mesangial cells and epithelial podocytes.

The relationship between the nature of the proteins in mesangial, subendothelial and subepithelial deposits and those in the adjacent droplet formation, together with their segregation into separate compartments within the droplets, supports the view that these droplets have a resorption role for immunoglobulins, fibrin/fibrinogen and complement which may pass across the damaged basement membrane into podocytes or into the mesangium during resolution of intra glomerular deposits. Perhaps the strongest argument favouring this view is the absence of any protein droplets in the minimal lesion nephrotic syndrome in which there is consistently very heavy proteinuria.

References

Bendayan M, Nanci A, Kan F (1987) Effect of tissue processing on colloidal gold cytochemistry. J Histochem Cytochem 35:983–996

Faith GG, Trump BF (1966) The glomerular capillary wall in human kidney disease, acute glomerulonephritis, systemic lupus erythematosus and preeclampsia-eclampsia. Lab Invest 15:1682–1719

Fisher KA, Luger A, Spargo BH, Lindheimer MD (1981) Hypertension in pregnancy: clinicopathological correlations and remote prognosis. Medicine (Baltimore) 60:267–276

Frens G (1973) Controlled nucleation for the regulation of the particle size in monodisperse gold solutions. Nature Phys Sci 20:241–242

Gall JAM, Alcorn D, Butkus A, Coghlan JP, Ryan GB (1986) Distribution of glomerular peripolar cells in different mammalian species. Cell Tissue Res 244:203–208

Gardiner DS, More IAR, Lindop GBM (1986) The granular peripolar cell of the human glomerulus: an ultrastructural study. J Anat 146:31–43

Hewitson T, Kamitsuji H, Whitworth JA, Kincaid-Smith P (1988) Glomerular and vascular lesions in DOCA-salt hypertension. The role of anticoagulation. Clin Exper Hyper – Theory and Practice A10(4):649–666

Hill PA, Coghlan JP, Scoggins BA, Ryan GB (1983) Ultrastructural changes in the sheep renal juxtaglomerular apparatus in response to sodium depletion or loading. Pathology 15:463–473

Hill PA, Fairley KF, Kincaid-Smith P, Zimmerman M, Ryan GB (1988) Morphologic changes in the renal glomerulus and the juxtaglomerular apparatus in human preeclampsia. J Pathol 1956:291–303

Hopper J, Farquahar MG, Yamauchi H, Moon HD, Page EW (1961) Renal lesions in pregnancy. Obstet Gynecol 17:271–293

Kincaid-Smith P, North RA, Becker GJ, Fairley KF (1985) Proteinuria during pregnancy. In: Andreucci VE (eds) The Kidney in Pregnancy. Martinus Nijhoff Publishing USA, pp 133–164

Kincaid-Smith P, Owen J, Whitworth JA, Walter MA, Doyle A, Woods RA (1984) Glomerular & vascular changes in malignant hypertension. Clin Exper Hyper – Theory and Practice A6(1 and 2):471–491

Mautner W, Churg J, Grishman E, Dachs S (1962) Preeclamptic nephropathy. Lab Invest 11:518–530

MacLean I, Nakane PK (1974) Periodate-lysine-paraformaldehyde fixative. J Histochem Cytochem 22:1077–1083

Morild I, Christensen JA, Mikeler E, Bohle A (1988) Peripolar cells in the avian kidney. Virchows Arch [A] 412:471–477

Pirani CL, Pollak VE, Lannigan R, Folli G (1963) The renal glomerular lesions of preeclampsia: electron microscopic studies. Am J Obstet Gynecol 87:1047–1070

Ryan GB, Coghlan JP, Scoggins BA (1979) The granulated peripolar epithelial cell: a potential secretory component of the renal juxtaglomerular apparatus. Nature 277:655–656

Ryan GB, Alcorn D, Coghlan JP, Hill PA, Jacobs R (1982) Ultrastructural morphology of granule release from juxtaglomerular myoepithelioid and peripolar cells. Kidney Int 22(Suppl 12):S3–S8

Sheehan HL, Lynch JB (1973) Pathology of toxaemia in pregnancy. Churchill Livingstone, Edinburgh London

Sheehan HL (1980) Renal morphology in preeclampsia. Kidney Int 18:241–252

Tanaka H, Haga S, Takatsuki K, Yamaguchi K (1984) Localization of adult T-cell leukemia-associated antigen by the immuno-colloidal gold method. Cancer Res 44:3493–3504

Zollinger HU, Mihatsch MJ (1978) Renal pathology in biopsy. Light, electron and immunofluorescent microscopy and clinical aspects. Springer, Berlin Heidelberg New York, pp 164

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