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Localisation of complement components in association with glomerular extracellular particles in various renal diseases

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Summary. Intraglomerular extracellular microparticles including so-called virus-like particles and striated membranous structures have been observed in various renal diseases. The presence and localisation of complement components in these extracellular bodies was studied using the protein A-gold electron microscopy method. Ultrastructurally these particles were differentiated into microspherical structures (MSS) and thread-like structures (TS). Both structures showed weak to moderate diffuse labelling with C1s, whilst the intense labelling found with C3d and C9 was confined to individual membrane-like structures of both MSS and TS. Labelling with IgA, IgG, fibrinogen and the complement components C1q, C1r, C3c C4 and C5 showed negative or trace results. There were no differences between the immunolabelling patterns of MSS and TS, nor among different renal diseases in which these structures were found. These findings raise the possibility that formation of so-called virus-like particles such as MSS and TS may be associated with complement activation.

Key words: Complement – Glomerular deposits – Immunohistochemistry

Introduction

Several previous investigators have reported the presence of small coarse, round/oval or thread-like extracellular glomerular particles in a variety of renal diseases (Bariety and Callard 1972, 1975; Burkholder et al. 1973; Carlson and Surerus 1986; Churg and Grishman 1972; Duncan et al. 1965; Mazzucco et al. 1990; Nagel et al. 1969; Olsen et al. 1974; Zollinger and Mihatsch 1978). Generally, classification of these electron-dense bodies has been based on ultrastructural appearance, with a number of descriptive terms being used, including virus-like particles (Bariety and Callard 1972; Churg and Grishman 1972), spherical microparticles (Burkholder et al. 1973) and striated membrane structures (Bariety and Callard 1975; Carlson and Surerus 1986; Nagel et al. 1969; Olsen et al. 1974). Previous investigations using conventional electron microscopy have concentrated on their shape, size (diameter), density and location. Although these structures often resemble viruses morphologically, there has only been an occasional positive identification of viruses by using cytochemical methods (Burkholder et al. 1973; Zollinger and Mihatsch 1978). The origin and nature of these particles in renal glomeruli is still the subject of considerable speculation.

We have been using an immunogold labelling method to investigate the subcellular localisation of immunoglobulins, fibrinogen and complement components in the glomeruli. In this study we demonstrate that complement components are uniformly associated with these glomerular extracellular particles in various types of renal disease.

Materials and methods

Renal biopsy tissue from 48 patients was examined ultrastructurally for the presence of round/oval or thread-like extracellular particles. The following primary diagnostic categories were considered: diabetic nephropathy (17 cases), IgA glomerulonephritis (8 cases), lupus nephritis (7 cases), idiopathic membranous nephropathy (5 cases), crescentic glomerulonephritis with or without Goodpasture's syndrome (4 cases), mesangiocapillary glomerulonephritis type I (1 case), thrombotic microangiopathy (2 cases), focal and segmental hyalinosis and sclerosis (2 cases), and 1 case each of mesangial proliferative glomerulonephritis (non-IgA) and pre-eclamptic toxaemia.

Renal biopsy tissue was initially fixed in periodate-lysine-paraformaldehyde (PLP) fixative (McLean and Nakane 1974) and post-fixed in 1% osmium tetroxide for 10 min. After dehydration in graded ethanol, the tissue was embedded in L.R. White resin (London Resin, London, UK).

Immunoelectron microscopy using the protein A-gold technique was performed on serial sections in 8 of these patients. In brief the method consisted of mounting sections consecutively on top of a drop of the following reagents: (a) 1% ovalbumin in 0.01 M phosphate-buffered saline pH 7.4 (ovalbumin/PBS) for 5 min; (b) rabbit anti-human IgA, IgG, IgM, C1q C3c, C5, fibrino-

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gen (Dakopatts, Copenhagen, Denmark) or rabbit anti-human C1r, C1s (Calbiochem, San Diego, CA, USA) or rabbit anti-human C4, C9 (Wako, Tokyo, Japan) for 16 h at 4° C; (c) ovalbumin/PBS (3 times for 5 min); (d) protein A-gold complex solution (diameter 15 nm) for 30 min min; (e) ovalbumin/PBS (3 times for 5 min). After washing with distilled water and drying, sections were stained with uranyl acetate and then examined under the electron microscope.

Colloidal gold was prepared according to the method of Frens (1973) and was conjugated with protein A (Pharmacia, Uppsala, Sweden) according to the method of Tanaka et al. (1984).

Control experiments were performed with the omission of the primary antiserum incubation step, but with the application of the secondary antiserum and/or protein A-gold.

Intensity of immunolabelling was graded semi-quantitatively according to density of gold particles on photographs with equivalent final magnification (×12000). This was represented as: (−) not significantly different from background staining, (±) barely above background, (+), (++), (+++) increasing degrees of positive labelling with 10–20, 21–50 and greater than 50 gold particles/cm^2 respectively.

Table 1. Incidence of microspherical structures (MSS) and thread-like structures (TS) in various renal diseases

Diagnosis	Cases (*n*)	MSS	TS	MSS and TS
Diabetic nephropathy	17	9	11	6
IgA glomerulonephritis	8	2	1	1
Lupus nephritis	7	2	1	0
Idiopathic membranous	5	0	3	0
Crescentic GN	4	0	2	0
MCGN	1	0	1	0
FSHS	2	0	2	0
Mesangial proliferative GN	1	1	0	0
PET	1	0	0	0
Thrombotic microangiopathy	2	0	0	0
Total	48	14	21	7

MCGN, Mesangiocapillary glomerulonephritis; FSHS, focal and segmental hyalinosis and sclerosis; PET, pre-eclamptic toxaemia

Results

To preserve antigenicity for immunoelectron microscopy, tissue was fixed in PLP and prolonged post-fixation in osmium tetroxide was avoided (Nakajima et al. 1989). Preservation of ultrastructure was sufficient to identify these particles but the detailed membrane structures were blurred, so we differentiated generally between microspherical structures (MSS) (often referred to as virus-like particles) and thread-like structures (TS).

Either MSS or TS were observed extracellularly in 28 out of 48 renal biopsies. In 7 of 48 cases, both MSS and TS were seen. The incidence of these extracellular particles in various renal diseases is shown in Table 1. MSS were observed as spherical homogeneous electron-dense structures (Fig. 1a), whilst TS appeared to form circular or whorled patterns (Fig. 1b). Both types were either present in isolation or grouped in a single mass, often in association with a matrix material that resembled protein deposit. MSS, which usually occurred in clusters and sometimes in association with TS, were located in the subepithelial space of the basement membrane, often between two contiguous basement membranes lying over the mesangial area (Fig. 1a). This phenomenon occurred in 14 out of 48 patients. In 20 out of 48 patients, TS were observed in association with MSS in sclerotic tissue. In a patient with idiopathic membranous nephropathy, the basement membrane contained many areas of TS encapsulated within deposits as well as degradation granules (Fig. 2). Neither MSS nor TS were observed in subendothelial location. These data are summarized in Table 1.

The intensity of immunogold labelling of MSS and TS found in serial sections with 12 different antisera is detailed in Table 2. All MSS and TS showed intense labelling with antiserum to C3d and C9 and weak to moderate labelling with antiserum against C1s (Figs. 3,

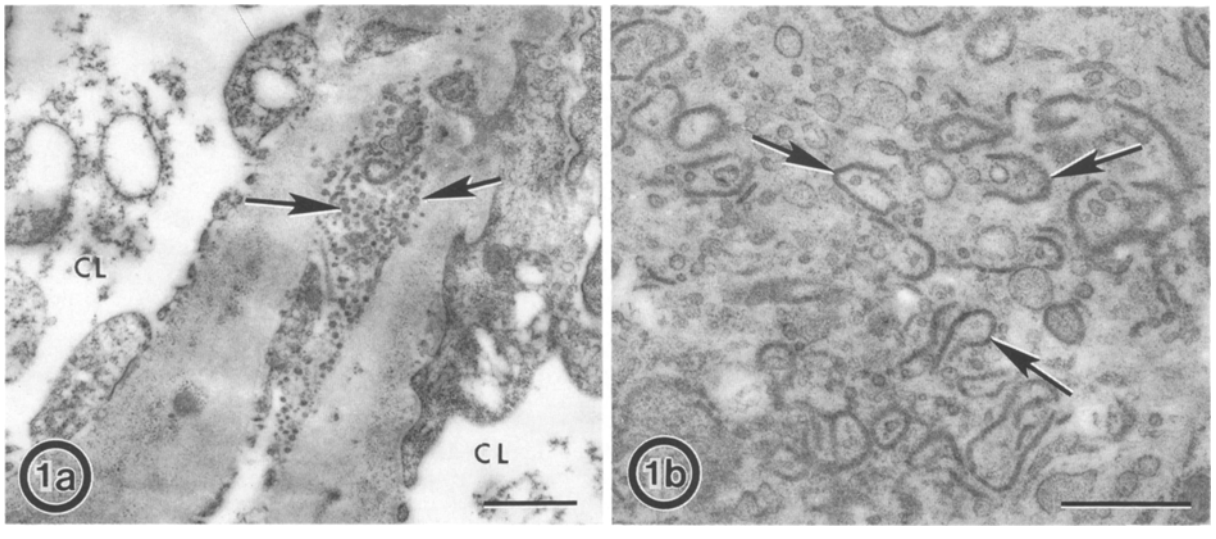

Fig. 1. Electron micrographs of sub-epithelial microspherical structures (*arrows*) surrounded by folds of glomerular basement membrane in a case of diabetic nephropathy (**a**). Thread-like structures (*arrows*) are seen in the mesangial matrix in a case of diabetic nephropathy (**b**). *CL*, Capillary lumen. *Bar* = 1 μm

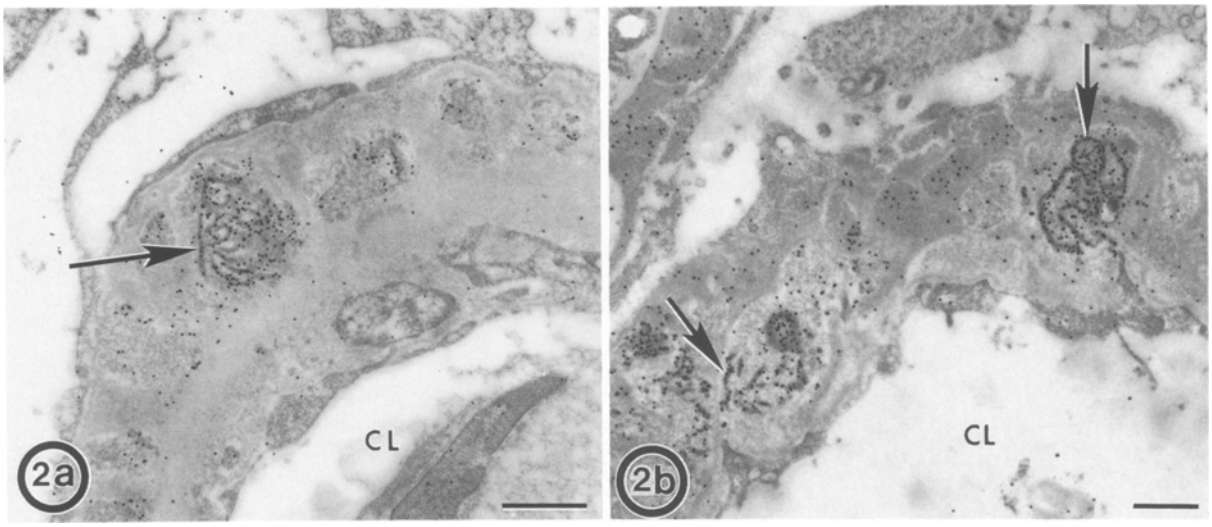

Fig. 2. a, b Immunoelectron micrographs of ultra-thin sections from a patient with membranous nephropathy labelled for C9. Some electron-dense deposits are replaced by thread-like structures (*arrows*), which show intense labelling with gold particles. *CL*, Capillary lumen. *Bar* = 1 μm

Table 2. Immunolabelling of microspherical and thread-like structures in various renal diseases

Case no.	Primary diagnosis	C1q	C1r	C1s	C4	C3c	C3d	C5	C9	IgA	IgG	IgM	Fg
1	Diabetic nephropathy	–	–	++	–	–	+++	–	+++	–	–	–	–
2	Diabetic nephropathy	–	–	+	–	–	+++	–	+++	NF	NF	NF	–
3	Diabetic nephropathy	–	–	++	–	–	+++	–	+++	NF	NF	NF	NF
4	Diabetic nephropathy	–	–	+	–	–	+++	–	+++	–	–	–	–
5	Diabetic nephropathy	–	–	+	–	NF	+++	–	+++	NF	NF	NF	NF
6	Diabetic nephropathy	–	–	++	–	–	+++	–	+++	–	–	–	–
7	Diabetic nephropathy	–	–	+	–	–	+++	–	+++	–	–	–	–
8	Diabetic nephropathy	–	–	+	–	–	+++	–	+++	ND	ND	ND	ND
9	Idiopathic MN	–	–	+	+	–	+++	–	+++	–	–	–	–
10[a]	Idiopathic MN	–	–	++	–	–	+++	–	+++	–	–	–	–
11	Idiopathic MN	–	–	+	–	ND	+++	ND	+++	–	–	–	–
12	FSHS	–	–	+	–	–	+++	–	+++	–	–	–	–
13	FSHS	–	–	+	–	–	+++	–	+++	–	–	–	–

[a] Were seen in basement membrane
NF, not found; ND, not done; Fg, fibrinogen; MN, membranous nephropathy; FSHS, focal and segmental hyalinosis and sclerosis

4). The labelling patterns for C1s were diffuse and random (Fig. 3a, 4a), whilst immunolabelling for C9 was confined to individual membrane-like structures within the mass of MSS and TS (Figs. 3c, 4c). C3d labelling of MSS and TS showed a distribution pattern intermediate to that of C1s and C9 (Figs. 3b, 4b). Immunogold labelling with other antisera resulted in negative labelling of both MSS and TS, except for weak positive labelling of C4 in one case of idiopathic membranous nephropathy.

None of the control experiments showed any specific labelling pattern.

Discussion

Since these extracellular bodies were first reported in Goodpasture's syndrome by Duncan et al. (1965) they have been observed in a number of renal diseases (Bariety and Callard 1972, 1975; Burkholder et al. 1973; Carlson and Surerus 1986; Churg and Grishman 1972; Mazzucco et al. 1990; Nagel et al. 1969; Olsen et al. 1974; Zollinger and Mihatsch 1978). These extracellular particles include so-called round/oval virus-like particles, spherical microparticles and striated membranous structures.

In our preliminary investigation we frequently found MSS within the subepithelial region of glomerular capillary walls. These structures correspond to the round/oval virus-like particles seen by other investigators. Although these structures have been traditionally referred to as virus-like particles, in the majority of renal diseases there is no direct evidence that they are in fact viruses (Burkholder et al. 1973; Zollinger and Mihatsch 1978). On the contrary, they are thought to be of a degenerative cytoplasmic, rather than viral origin on the basis of their appearance, location and association with membrane profiles (Bariety and Callard 1972). Striated membrane

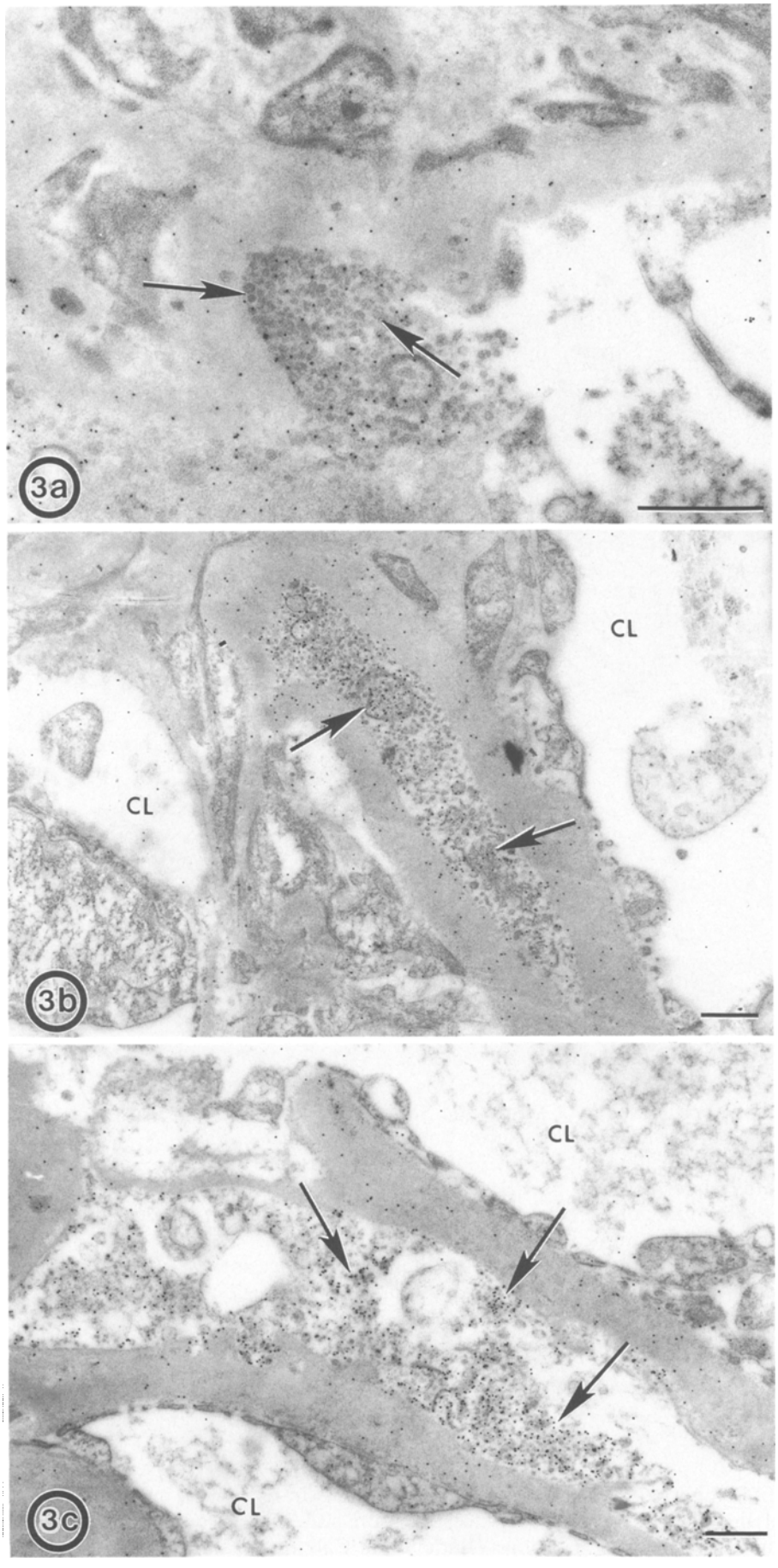

Fig. 3a–c. Immunoelectron micrographs of ultra-thin sections from patients with diabetic nephropathy. Microspherical structures show weak labelling for C1s (**a**) and intense labelling for C3d (**b**) and C9 (**c**). *Bar* = 1 μm

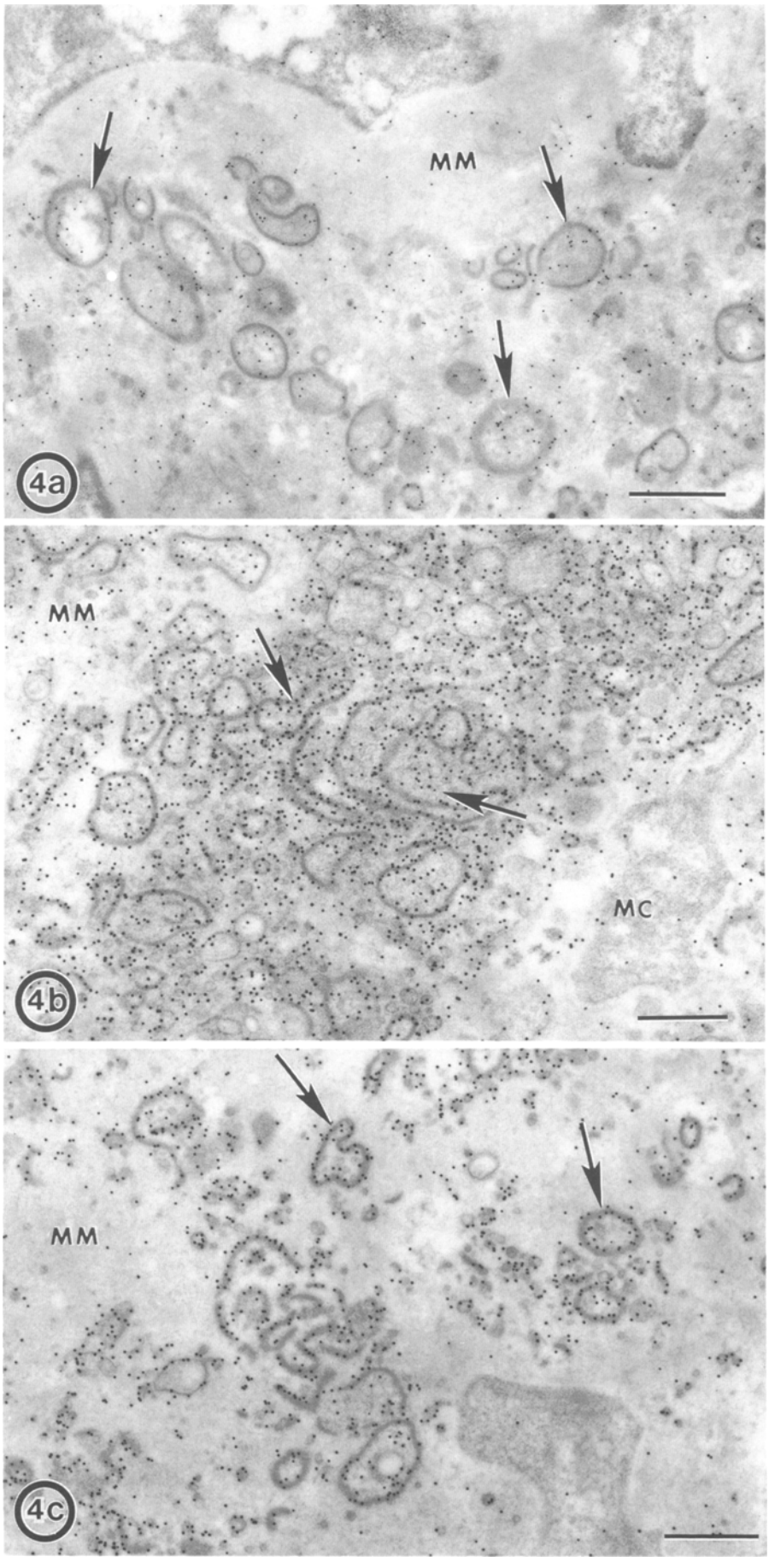

Fig. 4a–c. Immunoelectron micrographs of ultra-thin sections in a case of diabetic nephropathy. Thread-like structures show weak labelling for C1s (**a**) and intense labelling for C3d (**b**) and C9 (**c**). C1s and C3d labelling demonstrate a random distribution pattern whilst gold particles for C9 are confined to individual threads. *MM*, Mesangial matrix; *MC*, mesangial cell. *Bar* = 1 μm

structures, which are the same as our TS, are also thought to represent fragmented cellular processes, from their ultrastructural appearance (Ferrans et al. 1976; Nagel et al. 1969). The occurrence of these inclusions in aetiologically unrelated renal conditions suggests the view that they occur as a cellular response, rather than of viral nature.

In the present study, MSS and TS found in various renal diseases were consistently labelled for the complement components C1s, C3d and C9, suggesting that formation of these structures may be non-specific to each type of renal disorder. Furthermore, universal positive labelling of C1s, C3d and C9 was observed in association with these structures, regardless of variations in their structure and intraglomerular location. These findings imply that these structures may be derived from the same origin and that complement components may be associated with the occurrence of so-called virus-like particles in glomeruli.

Gold particles labelling against C1s within a mass of MSS/TS were not found isolated to these structures, but rather showed a scattered distribution pattern in the overall mass of particles. On the other hand, gold particles against C9 were always found confined to individual membrane-like structures. The distribution of gold particles against C3d was in between that of C1s and C9. Although speculative, these findings raise the possibility that in situ activation of the complement pathway may result in the formation of these structures, and that membrane-like structures within a mass of MSS/TS could be poly C9 complex or membrane attack complex (MAC; C5b-9 complex). In fact, the ultrastructure of poly C9 or MAC, as demonstrated by Podak (1986), in many respects resembles that of our MSS/TS. Furthermore, MAC was demonstrated recently using a pre-embedding immunoperoxidase electron microscopy method to label structures similar to our MSS and TS (Hinglais et al. 1986; Miyamoto et al. 1988). At the same time the observation of complement in cell remnants in normal human kidneys has led to the suggestion that complement plays a role in the shedding process of plasma membranes from aging cells (Hinglais et al. 1986).

In the present study, other complement components (C1q, C1r, C3c, C4, and C5) gave either negative or almost negative labelling of MSS/TS. This may relate to the relative stability of the various antigens under the present processing conditions. On the other hand, we have previously demonstrated the preservation of IgA, IgG, IgM and fibrinogen antigenicity when using this fixation procedure (Nakajima et al. 1989). The negative labelling for immunoglobulins and fibrinogen suggests that the association of complement with MSS and TS may not be due to immune complex or coagulation processes.

In conclusion, so-called virus-like particles found in glomeruli are possibly associated with complement activation rather than of a viral nature or due to fragmented cellular processes. However, the aetiology of these structures remains unclear.

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