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Immunoelectron microscopic study of childhood IgA nephropathy and Henoch-Schönlein nephritis

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Summary. Renal biopsy specimens from 11 children with Henoch-Schönlein nephritis and 14 with IgA nephropathy were examined by immunoelectron microscopy. The distribution of IgA reaction product (RP) was found to be similar to that of the electron-dense deposits seen with conventional electron microscopy. Deposits of IgA-RP were present in the mesangium and in the subendothelial region of the peripheral glomerular capillary wall in all patients. Subepithelial deposits of IgA-RP were seen in 12 patients. Deposits of IgG-RP were rare and no deposits of IgM-RP were observed. Deposits of C3-RP were found frequently, although they were smaller and less extensive than deposits of IgA-RP. There was no significant difference between Henoch-Schönlein nephritis and IgA nephropathy with regard to immunoelectron microscopy findings. These observations suggest that Henoch-Schönlein nephritis and IgA nephropathy are both forms of mesangiopathic glomerulonephritis caused by immune complexes, mainly composed of IgA.

Key words: Immunoelectron microscopy – Henoch-Schönlein nephritis – IgA nephropathy – Children

Introduction

Henoch-Schönlein (HS) nephritis occurs predominantly during childhood and is characterized by a purpuric rash, arthralgia, abdominal pain and gastro-intestinal bleeding (Yoshikawa et al. 1981). IgA nephropathy occurs in both children and adults, and affected patients usually have episodes of macroscopic haematuria with normal renal function and mild proteinuria (Yoshikawa et al. 1987a). Both conditions show the same type of glomerular changes characterized by various degrees of focal or diffuse mesangial proliferation on light microscopy (Yoshikawa et al. 1987b). Immunofluorescence microscopy shows diffuse deposition of IgA, and less frequently, less intense deposition of IgG and IgM in the glomerular mesangium. IgG, IgA and IgM have also been observed to show a segmental capillary distribution. Electron microscopy reveals numerous characteristic electron-dense deposits in the mesangium, and subendothelial and subepithelial deposits on the glomerular basement membrane are frequently found. Our previous studies have shown that subepithelial deposits are significantly associated with more severe clinical presentations, a worse outcome and more severe light microscopic glomerular changes (Yoshikawa et al. 1981, 1985) suggesting that subepithelial deposition might play a role in the formation of changes in the glomerular basement membrane which exert an influence on the progression of HS nephritis and IgA nephropathy (Yoshikawa et al. 1986).

The present study was conducted in order to determine whether the subepithelial deposits seen by electron microscopy contain IgA, and if so, to elucidate the mechanism of subepithelial deposition of IgA.

Materials and methods

At Kobe University Hospital 11 patients with HS nephritis and 14 with IgA nephropathy from whom renal biopsies had been obtained were studied. HS nephritis was diagnosed when haematuria and proteinuria were associated with a characteris-

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tic purpuric rash together with either abdominal pain or joint pain, or both (Yoshikawa et al. 1981). The diagnosis of IgA nephropathy was based on the presence of IgA as the sole or predominant immunoglobulin in the glomerular mesangium with no evidence of systemic disease such as HS syndrome and systemic lupus erythematosus (Yoshikawa et al. 1987a).

The biopsies were performed by the percutaneous technique using a Tru-Cut needle under X-ray control, as previously described (Yoshikawa et al. 1980). Biopsy specimens for light microscopy were fixed in phosphate-buffered 10% formalin, embedded in paraffin, sectioned at a thickness of 4 μm and stained with haematoxylin-eosin, periodic acid-Schiff and silver methenamine. Tissue for immunofluorescence was snap-frozen in acetone dry ice, cut at a thickness of 4 μm and stained with fluorescein-tagged commercial antisera to human IgG, IgA, IgM, C4, C3 and fibrinogen (Hoechst, Darmstadt, FRG). Sections were viewed with a Nikon Optiphot EF reflected fluorescence microscope. The portion of tissue used for electron microscopy was fixed in phosphate-buffered 5% glutaraldehyde for 2 h at 4° C and post-fixed for 1 h in 2% osmium tetroxide. It was then dehydrated through a graded ethanol series and embedded in Epon 812 resin. For orientation, 1-μm sections were cut and stained with 1% toluidine blue. Ultra-thin sections were cut on an LKB ultra-microtome, stained with uranyl acetate and lead citrate, and examined with a JEM-100S electron microscope.

The portion of tissue for immunoelectron microscopy was fixed in periodate-lysine-paraformaldehyde at 4° C for 4–6 h (McLean and Nakane 1974), washed in increasing concentrations of sucrose in phosphate-buffered saline (PBS), and embedded in Tissue-Tek compound (Lab-Tek Products). Sections were cut on a cryostat microtome, placed on albumin-coated slides, and dried in room air. They were then reacted with periodic acid to inactivate endogenous peroxidase prior to reaction with the HRP-labeled antibodies in a moist chamber for 16 h at 4° C. The sections were subsequently washed in 10% sucrose in PBS, incubated in 2% glutaraldehyde-PBS for 10 min and washed again in PBS, followed by immersion in 3, 3′-diaminobenzidine (DAB) solution containing 1% dimethylsulfoxide for 30 min and then in DAB-H_2O_2 solution for 2–3 min (Davies et al. 1977). Finally, they were washed in PBS, post-fixed in 2% osmium tetroxide in PBS for 1 h, dehydrated in a graded ethanol series, and embedded in Epon 812 resin. Ultra-thin sections were cut on a Porter-Blum MT-2B ultra-microtome, either unstained or stained with lead citrate, and examined with a JEM-100S or Hitachi HS-9 electron microscope.

Goat anti-human IgG, IgA, IgM and C3 were purchased from TAGO, Inc. Antibody specificity was assessed by immunodiffusion in agarose gel and by immunoelectrophoresis. $F(ab')_2$ fragments of γ-globulin from each antiserum were labeled with horseradish peroxidase (HRP) (Wilson and Nakane 1978).

Renal biopsy specimens from patients with the minimal change nephrotic syndrome and those with benign haematuria were negative by immunoelectron microscopy for IgA-, IgM-, IgG- and C3-RP.

The glomerular reaction product was completely abolished by absorption with the corresponding antigen. The reaction product staining was blocked with unlabeled antiserum.

Results

The clinical findings are shown in Tables 1 and 2.

There were 7 boys and 4 girls with HS nephritis, and 10 boys and 4 girls with IgA nephropathy. The age at onset or discovery of nephropathy ranged from 4 to 9 years for HS nephritis and from 5 to 13 years for IgA nephropathy. In HS nephritis, one patient presented with nephrotic syndrome and hypertension, whereas the other 10 had proteinuria and haematuria. In IgA nephropathy, 2 patients presented with nephrotic syndrome and macroscopic haematuria, 8 with macroscopic haematuria and four with asymptomatic proteinuria and microscopic haematuria.

Renal biopsy was performed at 0.5–6 months after clinical presentation for HS nephritis and at 1–21 months for IgA nephropathy. The results of light microscopy are shown in Tables 1 and 2.

The glomerular changes in HS nephritis were graded according to the classification evolved by the pathologists of the International Study of Kidney Disease in Children as follows: (I) minor glomerular abnormalities; (II) pure mesangial proliferation; (III) minor glomerular abnormalities or mesangial proliferation, with crescents/segmental lesions (sclerosis, adhesion, thrombosis, necrosis) in $<50\%$ of glomeruli; (IV) as III, but with crescents/segmental lesions in 50–75% of glomeruli; (V) as III, but with crescents/segmental lesions in $>75\%$ of glomeruli; (VI) membranoproliferative-like lesions. Three patients showed grade II, 6 grade III and 2 grade IV.

Table 1. Clinical and histological findings in children with Henoch-Schönlein nephritis

Patient No.	Sex	Age at onset (years)	Initial renal presentation	Duration from onset to biopsy (months)	Histological grade
1	M	6	P +H	2	III
2	F	7	P +h	6	IV
3	M	5	P +H	1	II
4	M	6	Ns+h+Hypertension	0.5	III
5	M	7	P +H	2	II
6	M	7	P +H	1	III
7	M	9	P +H+Edema	1	III
8	F	6	P +h	2	II
9	F	7	P +H	1	IV
10	F	9	P +H	1	III
11	M	4	P +h	1	III

P, proteinuria $\geqq 1$ g/day; Ns, nephrotic syndrome; H, macroscopic haematuria; h, microscopic haematuria

Table 2. Clinical and histological findings in children with IgA nephropathy

Patient No.	Sex	Age at onset or discovery (years)	Initial presentation	Duration from onset to biopsy (months)	Mesangial proliferation
1	M	7	Ns+H	2	Focal
2	M	10	p +H	20	Diffuse
3	F	11	Ns+H	1	Diffuse
4	M	5	P +H	4	Diffuse
5	M	8	p +h	2	Focal
6	M	7	P +H	2	Diffuse
7	F	8	p +H	3	Focal
8	M	9	p +H	2	Focal
9	M	9	P +h	21	Focal
10	M	6	p +H	1	Focal
11	M	6	p +H	5	Focal
12	F	12	p +h	17	Minimal
13	M	13	p +h	2	Focal
14	F	6	p +H	4	Focal

Ns, nephrotic syndrome; P, proteinuria $\geqq 1$ g/day; p, proteinuria < 1 g/day; H, macroscopic haematuria; h, microscopic haematuria

The biopsies from IgA nephropathy cases were graded according to the extent of mesangial cell proliferation as follows: (1) Minimal glomerular changes. The majority of glomeruli appeared normal, although a few showed slight enlargement of the mesangial matrix with or without mesangial hypercellularity. The number of mesangial cells per peripheral mesangial area did not exceed three. (2) Focal mesangial proliferation. Up to 80% of glomeruli showed moderate to severe mesangial cell proliferation, i.e. more than three mesangial cells per peripheral mesangial area. (3) Diffuse mesangial proliferation. More than 80% of glomeruli showed moderate to severe mesangial cell proliferation. One patient showed minimal glomerular change, 9 focal mesangial proliferation and 4 diffuse mesangial proliferation.

The results of electron microscopy are seen in Tables 3 and 4, which also show the immunoelectronmicroscopic findings.

Electron-dense deposits in the mesangium were the most prominent feature and were seen in all but one of the 25 patients. They appeared as granular masses situated immediately beneath the lamina densa in the perimesangial region and the size and extent of mesangial deposits varied from patient to patient. Peripheral capillary wall deposits were also frequently found. Subendothelial deposits were observed in 7 patients with HS nephritis and 6 with IgA nephropathy and subepithelial deposits were seen in 3 with HS nephritis and 8 with IgA nephropathy. These deposits were usually small and scanty. The subendothelial deposits occurred most frequently in the capillary wall adjacent to

Table 3. Electron-dense deposits and immunoelectron microscopic findings in children with Henoch-Schönlein nephritis

Patient No.	Electron-dense deposits			IgA-RP			IgG-RP			C3-RP		
	Mesan.	Subendo.	Subepi.	Mesan.	Subendo.	Subepi.	Mesan.	Subendo.	Subepi.	Mesan.	Subendo.	Subepi.
1	2+	1+	2+	3+	2+	2+	0	0	0	0	0	0
2	2+	1+	1+	3+	1+	1+	0	0	0	1+	1+	0
3	2+	1+	1+	2+	1+	1+	0	0	0	0	0	0
4	3+	1+	0	3+	2+	1+	0	0	0	0	0	0
5	3+	1+	0	3+	1+	0	1+	0	0	1+	0	0
6	3+	1+	0	3+	1+	0	0	0	0	0	0	0
7	2+	1+	0	3+	2+	0	0	0	0	0	0	0
8	3+	0	0	3+	1+	0	1+	1+	0	2+	1+	0
9	3+	0	0	2+	1+	0	0	0	0	0	0	0
10	2+	0	0	3+	2+	0	0	0	0	0	0	0
11	1+	0	0	3+	2+	0	0	0	0	2+	1+	0

Mesan., mesangium; subendo., subendothelial region; subepi., subepithelial region; 0, 1+, 2+, 3+, quantity of electron-dense deposits and reaction product (RP)

Table 4. Electron-dense deposits and immunoelectron microscopic findings in children with IgA nephropathy

Patient No.	Electron-dense deposits			IgA-RP			IgG-RP			C3-RP		
	Mesan.	Subendo.	Subepi.	Mesan.	Subendo.	Subepi.	Mesan.	Subendo.	Subepi.	Mesan.	Subendo.	Subepi.
1	3+	1+	1+	3+	2+	1+	0	0	0	1+	1+	0
2	3+	1+	1+	3+	1+	1+	0	0	0	2+	1+	0
3	1+	1+	1+	2+	1+	2+	1+	0	0	0	0	0
4	1+	1+	1+	2+	1+	2+	0	0	0	1+	1+	1+
5	1+	1+	1+	2+	1+	1+	0	0	0	0	0	0
6	3+	0	1+	3+	1+	2+	1+	1+	1+	3+	2+	0
7	3+	0	1+	2+	2+	1+	2+	1+	1+	2+	1+	1+
8	1+	0	2+	3+	1+	1+	0	0	0	0	0	0
9	3+	1+	0	3+	2+	0	1+	2+	0	1+	1+	0
10	3+	0	0	3+	1+	0	1+	0	0	2+	1+	0
11	3+	0	0	3+	2+	0	0	0	0	0	0	0
12	2+	0	0	3+	1+	0	1+	0	0	1+	1+	0
13	1+	0	0	3+	1+	0	0	0	0	1+	0	0
14	0	0	0	2+	1+	0	0	0	0	1+	1+	0

Mesan., mesangium; subendo., subendothelial region; subepi., subepithelial region; 0, 1+, 2+, 3+, quantity of electron-dense deposits and reaction product (RP)

the mesangium, although they were also observed in the peripheral part of the loops. The subepithelial deposits were often surrounded by replicated lamina densa.

Changes in the glomerular basement membrane such as thinning and lamination of the lamina densa, and expansion of the lamina rara externa were noted frequently.

An immunoelectron microscopy IgA reaction product (IgA-RP) was found to have a similar distribution to the electron-dense deposits seen with conventional electron microscopy, although deposits of IgA-RP were generally more extensive. Deposits of IgA-RP in the mesangium (Fig. 1) and in the subendothelial region of the peripheral glomerular capillary wall (Fig. 2) were present in all patients with HS nephritis and IgA nephropathy. Deposits of IgA-RP in the subepithelial region of the glomerular basement membrane were seen in 4 patients with HS nephritis and 8 with IgA nephropathy (Figs. 3 and 4). Subepithelial deposits of IgA-RP were usually small and scanty. Deposition of IgA-RP was also frequently observed in the lamina densa of the glomerular basement membrane beneath the subepithelial deposits of IgA-RP, although it was usually less intense than subepithelial deposits. The lamina densa beneath, or close to, subepithelial deposits of IgA-RP was thin and irregular. The epithelial cells over deposits of IgA-RP were swollen and lacked foot processes,

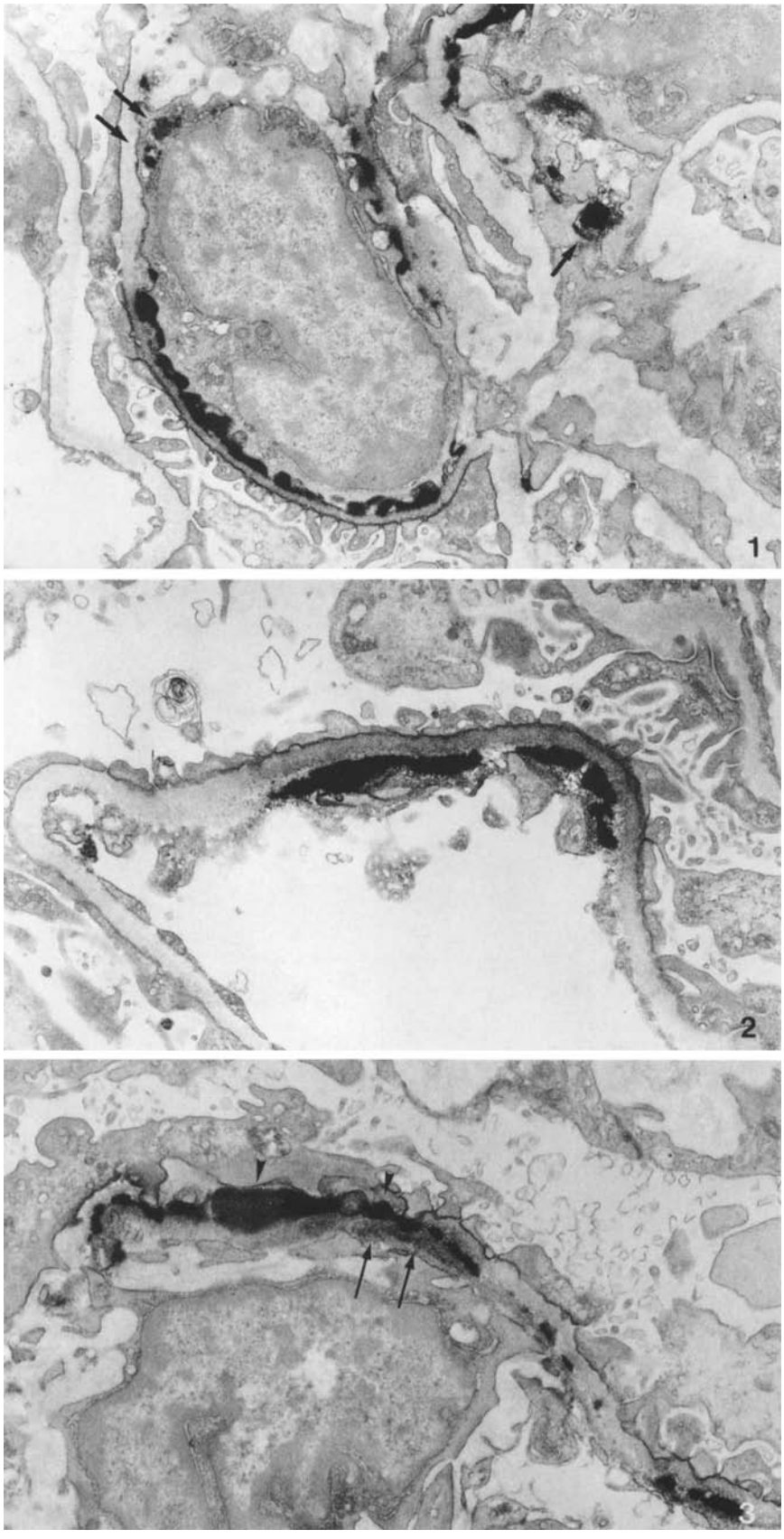

Fig. 1. Deposits of IgA-RP in the mesangium. Some phagosomes in the mesangial cell contain IgA-RP (*arrows*). HS nephritis. ×11000

Fig. 2. Deposits of IgA-RP in the subendothelial region of the glomerular basement membrane. HS nephritis. ×13000

Fig. 3. Deposits of IgA-RP in the subepithelial region (*arrow heads*) and lamina densa (*arrows*) of the glomerular basement membrane. HS nephritis. ×14000

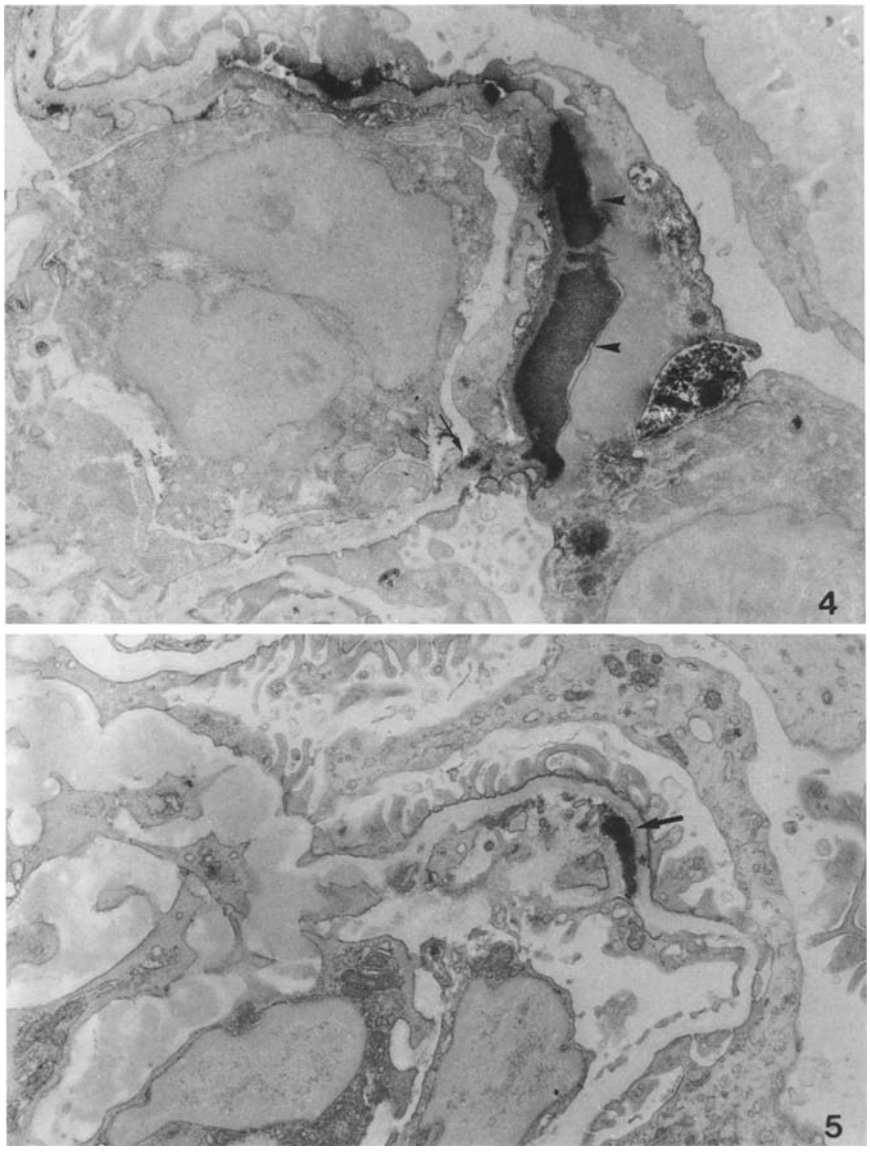

Fig. 4. Deposits of IgA-RP in the subepithelial (*arrow head*) and subendothelial (*arrow*) regions of the glomerular basement membrane. Deposits of IgA-RP also can be seen in the epithelium (*asterisk*). IgA nephropathy. ×10000

Fig. 5. Small deposits of C3-RP in the mesangium (*arrow*). Numerous electron-dense deposits can be seen in the mesangium. IgA nephropathy. ×12000

while the cytoplasm bordering deposits of IgA-RP usually contained a zone of condensed microfibrils. The epithelial, mesangial and endothelial cells often had IgA-containing phagosomes in the cytoplasm (Figs. 1 and 4).

Deposits of IgG-RP and C3-RP were also found, although they were smaller and less extensive than deposits of IgA-RP (Fig. 5). IgM-RP was not observed.

There was thus no significant difference between HS nephritis and IgA nephropathy with regard to immunoelectron microscopy findings.

Discussion

Electron microscopy of renal biopsy specimens from patients with HS nephritis and IgA nephropathy shows electron-dense deposits in the mesangium and in the subendothelial and subepithelial region of the glomerular capillary wall. It has been widely assumed that the electron-dense deposits revealed by electron microscopy correspond to the immunoglobulin and complement seen by immunofluorescence. Immunofluorescence microscopy in HS nephritis and IgA nephropathy shows IgG, IgA, IgM and C3 deposits, and subendothelial and subepithelial electron-dense deposits are small and scanty. Immunoelectron microscopy is therefore necessary in order to determine whether IgG, IgA, IgM or C3 is deposited in the glomerular capillary wall. The present immunoelectron microscopic study showed that the electron-dense deposits observed in HS nephritis and IgA nephropathy always contain IgA, and IgG and C3 deposits were

also observed, although subepithelial deposition of IgG and C3 was rare.

Deposits of IgA-RP in the mesangium and subendothelial region were found in all patients with HS nephritis and IgA nephropathy. The frequent presence of IgA-RP in the lamina densa of the glomerular basement membrane beneath subepithelial deposits of IgA-RP strongly suggests that IgA is transported into the subepithelial region of the glomerular basement membrane across the lamina densa. No evidence for another route was found. These observations support the concept of Germuth and Rodriguez (Germuth and Rodriguez 1975) that HS nephritis and IgA nephropathy are both forms of mesangiopathic glomerulonephritis caused by immune complexes of intermediate size. Their location presumably depends on the balance between the amount of the immune complexes and mesangial capacity. If the complex deposition does not exceed the mesangial capacity, the deposits remain confined to the mesangium. If, on the other hand, the complex deposition is excessive and is thus greater than the capacity of the mesangium, complexes may also be deposited in the subendothelial space of the glomerular capillary wall, subsequently crossing into the subepithelial region. Immune complexes may be composed mainly of IgA and, to a lesser degree, IgG.

Subepithelial deposits of IgA-RP were frequently associated with a type of lesion which we consider to be caused by lysis of the glomerular basement membrane in both HS nephritis and IgA nephropathy. Our previous electron microscopic study (Yoshikawa et al. 1986) showed that lysis of the glomerular basement membrane was frequently associated with subepithelial electron-dense deposits and polymorphonuclear leukocytes, the latter possibly being attracted via Fc receptor interaction with deposited IgA (Naish et al. 1975; Sindrey and Naish 1979). These cells invade the subendothelial space and come into direct contact with the glomerular basement membrane, which they damage by releasing proteolytic enzymes. Leukocyte-derived proteases are known to digest the glomerular basement membrane (Davies et al. 1978). It is thus possible that damaged portions of the glomerular basement membrane provide a pathway by which protein molecules can enter the urinary space and create a point of weakness which is liable to perforate. Increased permeability of the glomerular capillary wall has been shown to enhance mesangial uptake of biologically active substances (Couser and Salant 1980; Michael et al. 1980) and crescent formation is thought to correlate with fractures of the glomerular basement membrane through which intravascular contents escape, causing precipitation of fibrin in Bowman's space (Kondo et al. 1972; Morita et al. 1973). Thus, changes in the glomerular basement membrane may lead to increasing degrees of mesangial change and crescent formation.

This study suggests two mechanisms whereby subepithelial deposits are resolved in HS nephritis and IgA nephropathy. First, the presence of IgA in epithelial phagosomes suggests that deposits are removed by epithelial phagosomes, perhaps in the course of the endocytotic activity of the epithelial cells. Second, deposits are surrounded by a new thin lamina densa, become incorporated within the basement membrane and seem to undergo dissolution, appearing more lucent or granular.

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