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Glomerular electron-dense deposits in childhood IgA nephropathy

N. Yoshikawa[1], H. Ito[2], C. Nakahara[2], S. Yoshiara[1], K. Yoshiya[1], T. Matsuo[1], O. Hasegawa[2], H. Hazikano[2], and S. Okada[1]

Departments of Pediatrics and Pathology[1], Kobe University School of Medicine, 7 Kusunoki-cho, Chuo-ku, Kobe, Japan and Tokyo Metropolitan Children's Hospital[2]

Summary. An electron-microscopic study of the glomeruli was made on 154 children with IgA nephropathy and no evidence of systemic disease, in whom immunofluorescence microscopy had shown diffuse mesangial deposition of IgA. Mesangial deposits were observed in all but eight children. Subepithelial deposits were observed in 40 children and were almost always accompanied by both mesangial and subendothelial deposits. Subepithelial deposits were significantly associated with more severe clinical presentations, a worse outcome and more severe light microscopic glomerular changes. These observations support the concept that IgA nephropathy is an immune complex disease.

Key words: IgA nephropathy-Dense deposits-Childhood

Introduction

IgA nephropathy is characterized by the diffuse deposition of IgA, and less frequently and less intensely of IgG and C3 in the glomerular mesangium. This is accompanied by various degrees of focal or diffuse mesangial proliferation. IgA deposits are seen by immunofluorescence microscopy in the mesangial regions of all the glomeruli even when the mesangial proliferation is focal by light microscopy. Patients with IgA nephropathy usually have macroscopic or microscopic hematuria with normal renal function and mild proteinuria which is frequently exacerbated by upper respiratory infection. Since its original description by Berger (1969), IgA nephropathy has increasingly been recognized as a distinct clinicopathological entity. It is the most common primary glomerulonephritis in children attending our renal units (Yoshikawa and Matsuo 1984).

There are many reports of the electron-microscopic findings in IgA nephropathy. Numerous electron-dense deposits in the mesangium are charac-

Offprint requests to: N. Yoshikawa at the above address

teristic (Davies et al. 1973; Levy et al. 1973; Lowance et al. 1973; McCoy et al. 1974; Van de Putte et al. 1974; Finlayson et al. 1975; Zimmerman and Burkholder 1975; Clarkson et al. 1977; Joshua et al. 1977; Hara et al. 1980; Michalk et al. 1980). However subepithelial deposits on the glomerular basement membrane are reported to be unusual. We have noted that subepithelial deposits are frequently found in children with severe IgA nephropathy. In this paper we describe the frequency, position and significance of electron-dense deposits in biopsies from 154 children with IgA nephropathy.

Materials and methods

All patients with IgA nephropathy who had had a renal biopsy examined by electron-microscopy at the Kobe University Hospital or Tokyo Metropolitan Children's Hospital during the years 1975–1983 inclusive were reviewed. The diagnosis 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 Henoch-Schoenlein syndrome. 154 patients were available for analysis.

The clinical and laboratory findings were obtained from the medical records after examination of the biopsy specimen was completed. Hematuria was defined as an erythrocyte excretion $\geqq 10/mm^3$ of uncentrifuged urine or 5/high-power field of centrifuged urine. The acute nephritic syndrome was defined as hematuria associated with glomerular filtration rate (GFR) <60 ml/min/1.73 m^2 and/or hypertension. The nephrotic syndrome was defined by heavy proteinuria and hypoalbuminemia $\leqq 25$ g/l. Heavy proteinuria was defined as a urinary protein excretion $\geqq 40$ mg/h/m^2 body surface area or 1.0 g/day. Hypertension was diagnosed if the diastolic blood pressure was persistently $\geqq 90$ mm Hg. The GFR was determined by endogenous creatinine clearance, the lower limit of normal for our laboratories being 80 ml/min/1.73 m^2.

Percutaneous renal biopsy was performed as previously described (Yoshikawa et al. 1980 and 1984). Biopsy specimens for light microscopy were fixed in phosphate-buffered 10% formalin, enbedded in paraffin, sectioned at 4 μm thickness and stained with hematoxylin-eosin, periodic acid-Schiff and silver methenamine. Tissue for immunofluorescence was snap-frozen in acetone dry ice, cut at 4 μm and stained with fluorescein-tagged commercial antisera to human IgG, IgA, IgM and C3 (Hoechst, Darmstadt, FRG). Sections were viewed with a Nikon Optiphot EF reflected fluorescence microscope. The immunofluorescence findings were characterized with regard to location and pattern, and intensity was graded as 0 to 3+. Specimens with 0 or 0/1 fluorescence were considered negative. Tissue for electron-microscopy was fixed in phosphate-buffered 2% glutaraldehyde for 2 h at 4° C and post-fixed for 1 h in 2% osmium tetroxide. It was subsequently processed through graded alcohol 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 a LKB ultra-microtome, stained with uranyl acetate and lead citrate, and examined with a JEM-T7S7 electron-microscope. One or two glomeruli from each specimen were examined.

Statistical analyses were performed using chi-square test.

Results

Electron microscopy

Electron-dense deposits in the mesangium were the most constant and prominent feature and were seen in all but 8 of the 154 children examined (Figs. 1 and 2). They were granular masses situated immediately beneath the lamina densa in the perimesangial region and expanded the mesangium. The size

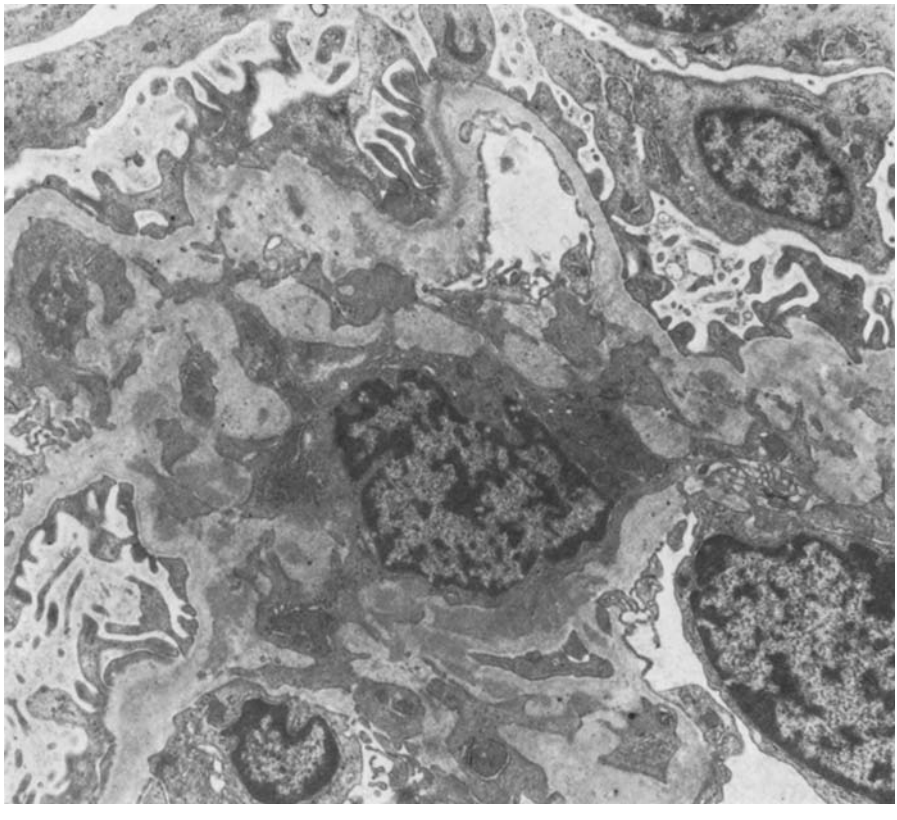

Fig. 1. Mesangial dense deposits. × 7,500

and extent of mesangial deposits varied from patient to patient and in several they were so large as to produce localized protrusions (Fig. 2).

Subendothelial deposits were also found in 53 children (Fig. 3). They occurred most frequently in the capillary wall adjacent to the mesangium, although they were also observed in the peripheral part of the loops. The subendothelial deposits were usually small and few.

Subepithelial dense deposits were observed in 40 patients and were always associated with mesangial deposits. They were also associated with subendothelial deposits in 34. They were scanty and usually localized to a few capillary loops (Fig. 4). They were generally small and flat and the humps typical of acute poststreptococcal glomerulonephritis were not observed. The subepithelial deposits were often surrounded by replicated lamina densa (Fig. 5).

A variety of other glomerular alterations were noted, such as proliferation of mesangial cells and increase of matrix, foot process fusion, degeneration and ulceration of foot process, replication and crenation of the lamina densa, granular and lucent expansion of the lamina rara interna, irregularity

Fig. 2. Mesangial dense deposits producing localized protrusion. × 12,000

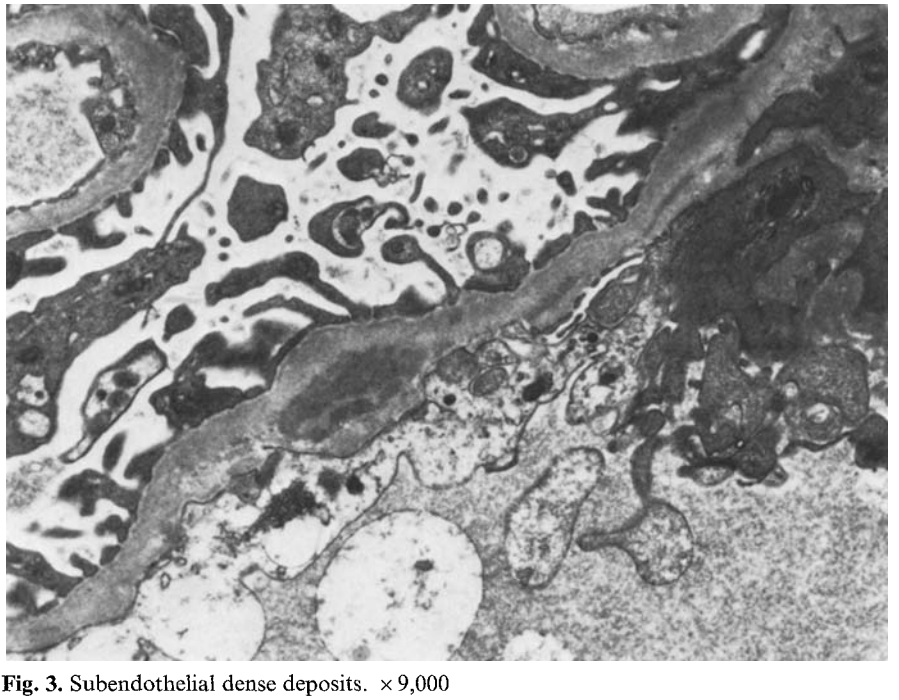

Fig. 3. Subendothelial dense deposits. × 9,000

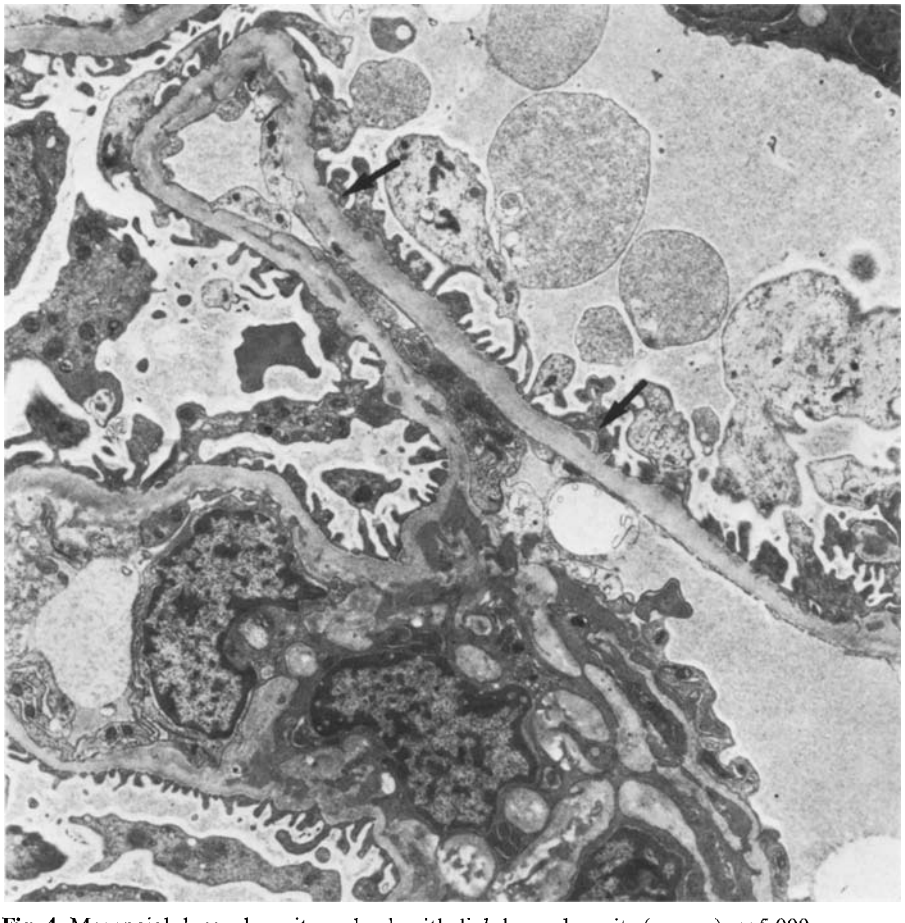

Fig. 4. Mesangial dense deposits and subepithelial dense deposits (*arrows*). × 5,000

in outline of the external aspect of the basement membrane, and circumferential mesangial interposition.

Light microscopy

Of the 154 patients reviewed, biopsy specimens from 3 contained less than 5 glomeruli and were excluded from analysis. The biopsies were graded according to extent of mesangial cell proliferation as follows:
(1) Minimal glomerular changes (24 patients). The majority of glomeruli were optically normal, although a few showed slight enlargement of the mesangial matrix with or without slight mesangial hypercellularity. The number of mesangial cells per peripheral mesangial area did not exceed three. There were small foci of tubular atrophy and interstitial lymphocyte infiltration in two patients.

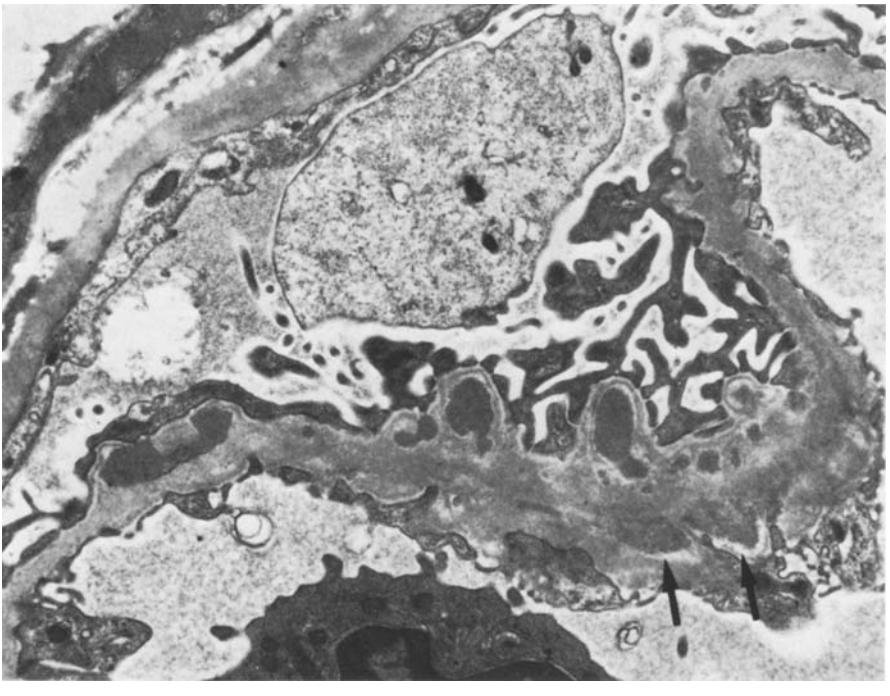

Fig. 5. Subepithelial dense deposits surrounded by the replicated lamina densa and subendothelial dense deposits (*arrows*). ×9,000

(2) Focal mesangial proliferation (89 patients). Up to 80% of glomeruli showed moderate or severe mesangial cell proliferation, i.e., more than three mesangial cells per peripheral mesangial area. The degree of mesangial cell proliferation varied considerably among glomeruli as well as among different mesangial regions of a single glomerulus. Mesangial cell proliferation was usually associated with an increase of matrix. Cellular or fibrocellular crescents were found in 43 patients. Crescents were usually present in less than 20% of the glomeruli, although in five patients, they were encountered in 20–60% of the glomeruli. Capsular adhesions were frequently seen, and in these areas there was usually mesangial proliferation. Segmental areas of capillary collapse, usually associated with crescents, were frequently found. A small number of glomeruli showing global sclerosis were often present. Tubular atrophy and interstitial fibrosis were present in 54 patients but were not extensive. Small foci of interstitial lymphocyte infiltration were observed in 18 patients. There was no vascular lesion.

(3) Diffuse mesangial proliferation (38 patients). More than 80% of glomeruli showed moderate or severe mesangial cell proliferation. Intensity of the proliferation varied in different regions of the mesangium in a given glomerulus, as well as from one glomerulus to another. Mesangial cell proliferation was always accompanied by an increase of mesangial matrix. Cellular and fibrocellular crescents were present in less than 20% of the glomeruli in

Table 1. Relationship between histological grade and site of electron-dense deposits

Mesangial proliferation	No. of patients	Site of electron-dense deposits				
		None	Mesangial alone	Mesangial and subendothelial	Mesangial and subepithelial	All three sites
Minimal	24	2	18	1	2	1
Focal	89	5	57	13	3	11
Diffuse	38		12	4	1	21
Total	151	7	87	18	6	33

10 patients, 20% to 40% in eight, and 40–80% in five. Capsular adhesions were frequently seen. A small number of glomeruli showing global sclerosis were often present. Tubular atrophy and interstitial fibrosis were present in 27 patients and were extensive in four. Interstitial lymphocyte infiltration was observed in 19 patients and was extensive in one. Interstitial foam cells were seen in two patients who had persistent heavy proteinuria. There was no vascular lesion.

Table 1 shows the relationship between the extent of mesangial cell proliferation and site of electron-dense deposits. Of the 38 patients with diffuse mesangial proliferation, 22 (58%) showed subepithelial dense deposits. In contrast, only 3 of the 24 children (13%) with minimal change and 14 of the 89 (16%) with focal mesangial proliferation showed subepithelial deposits ($p < 0.01$).

Immunofluorescence microscopy

The diagnostic immunopathological pattern of IgA nephropathy was diffuse deposits of IgA in the mesangial areas, often extending into adjacent capillary walls (Fig. 6). IgA deposits were associated with IgG in 49 patients, IgM in 12, and both IgG and IgM in 17, although these were usually less intense. In 76 patients IgA was the only immunoglobulin detected. C3 in a similar distribution pattern was observed in 99 patients, though it was usually less intense than IgA. There was no relationship between the composition of the immune deposits and the location of electron-dense deposits.

Clinical and laboratory findings

There were 95 boys and 59 girls. The age at onset or discovery of urinary abnormalities ranged from 3 to 14 years, the mean being 9.9 years. Ninety-seven patients were found to have microscopic hematuria and/or proteinuria by a school screening program and 13 were referred for investigation of microscopic hematuria after a routine medical examination. Thirty-one children had macroscopic hematuria and 13 had edema at onset. One hundred

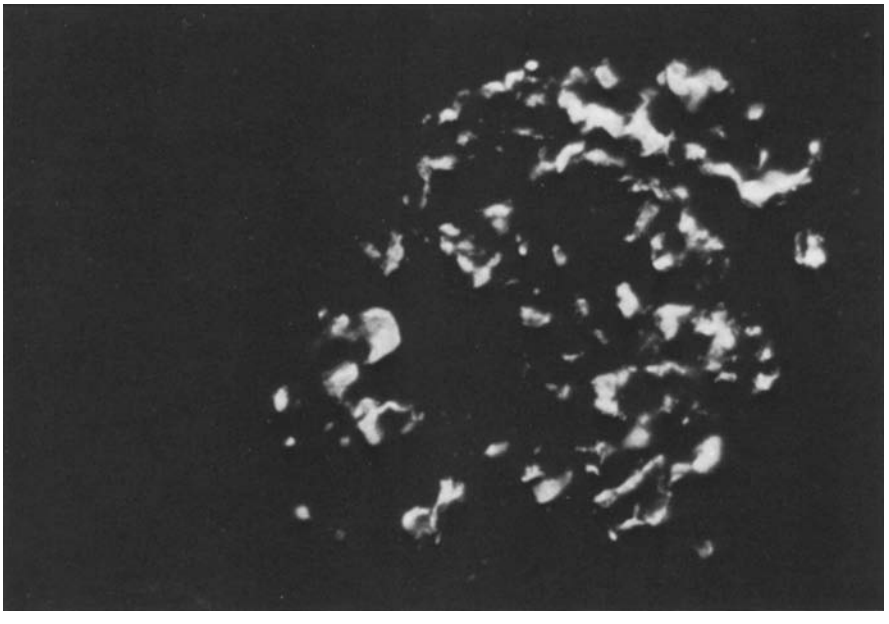

Fig. 6. IgA deposits in the mesangium. ×400

forty-six patients had hematuria and 8 had proteinuria without hematuria initially. The interval between the onset or discovery of nephropathy and first biopsy ranged from 1 month to 7.9 years, the mean being 18 months. There was no relationship between the interval and the site of electron-dense deposits.

Table 2 shows the relationship between the main presentation recorded within 6 months of onset and the site of deposits. Nine of the 15 patients (60%) with acute nephritic and/or nephrotic syndrome and 13 of the 30 (43%) with heavy proteinuria showed subepithelial deposits. In contrast, of the 109 patients with slight proteinuria and/or hematuria only 18 (17%) showed subepithelial deposits ($p<0.01$). Ninety-one children had one or more episodes of macroscopic hematuria. There is no relationship between the severity of hematuria and the site of deposits.

The mean duration of follow-up was 3.8 years (range 0.6 to 11.3 years). At the latest observation the clinical status of the 151 patients followed more than one year was classified as follows:

State A-normal. physical examination (including blood pressure), urine and renal function were all normal;

State B-minor urinary abnormalities. normal physical examination and renal function, with hematuria or proteinuria <40 mg/h/m^2 (<1.0 g/24 h) or both;

Table 2. Relationship between initial presentation and site of electron-dense deposits

Initial presentation	No. of patients	Site of electron-dense deposits				
		None	Mesangial alone	Mesangial and subendothelial	Mesangial and subepithelial	All three sites
Acute nephritic syndrome and nephrotic syndrome	7		1	1		5
Acute nephritic syndrome and proteinuria	1		1			
Nephrotic syndrome and hematuria	7	1		2		4
Heavy proteinuria without nephrotic syndrome	30	2	12	3	3	10
Slight proteinura and/or hematuria	109	5	73	13	3	15
Total	154	8	87	19	6	34

Table 3. Relationship between outcome and site of electron-dense deposits

Clinical state	No. of patients	Site of electron-dense deposits				
		None	Mesangial alone	Mesangial and subendothelial	Mesangial and subepithelial	All three sites
A	44	1	27	6	1	9
B	85	5	54	10	4	12
C	16	2	3	2	1	8
D	6		2	1		3
Total	151	8	86	19	6	32

State C-active renal disease. heavy proteinuria or hypertension, or both, with GFR $\geqq 60$ ml/min/1.73 m^2.

State D-renal insufficiency. active renal disease but with GFR < 60 ml/min/1.73 m^2.

At the end of the observation period 44 patients were in state A and 85 in state B. Of the 16 in state C, 4 had both heavy proteinuria and hypertension. Six were in state D. Table 3 shows the relation between the outcome

and the site of electron-dense deposits. Of the 22 patients in state C or D, 12 (55%) showed subepithelial deposits and of the 129 patients in state A or B, 26 (20%) showed subepithelial deposits ($p < 0.001$).

Discussion

This study confirms our impression that subepithelial deposits are frequently observed in IgA nephropathy. As in other reports the most characteristic ultrastructural finding was mesangial deposits and they were observed in all but 8 of the 154 patients. Subepithelial deposits were almost always accompanied by both mesangial and subendothelial deposits and subendothelial deposits were always associated with mesangial deposits. These findings support the concept of Germuth and Rodriguez (1975) that IgA nephropathy is a mesangiopathic glomerulonephritis caused by immune complexes of intermediate size. Their location presumably depends on the balance between the amount of immune complexes and the 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 exceeds the capacity of the mesangium, complexes may also be deposited in the subendothelial space of the glomerular capillary wall and then cross into the subepithelial region.

Subepithelial deposits were more frequently observed in patients with heavy proteinuria than in those with slight or no proteinuria (Table 2). It is therefore possible that immune complexes may easily cross the glomerular basement membrane from the subendothelial to subepithelial site in a situation of increased permeability.

The significance of electron-dense deposits in IgA nephropathy has not been well appreciated. In our study subepithelial deposits were significantly associated with more severe glomerular changes on light microscopy, more severe clinical presentation and a worse outcome. These findings suggest that the immune complex deposition exerts an important influence on the severity and prognosis in IgA nephropathy.

Our previous report of children with Henoch-Schoenlein nephritis showed many similarities to the children in this series (Yoshikawa et al. 1981). Mesangial deposits are again characteristic and are always seen in glomeruli. Subendothelial deposits are always associated with mesangial deposits and subepithelial deposits are always accompanied by both mesangial and subendothelial deposits. As in IgA nephropathy, subepithelial deposits are correlated with more severe light microscopic glomerular changes, more severe clinical findings and a worse outcome. Although the relationship between IgA nephropathy and Henoch-Schoenlein nephritis is complex, this investigation leads us to conclude that common pathogenetic mechanisms may operate and that both conditions may be immune complex diseases.

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