Virchows Archiv A Pathol Anat (1988) 413:431–443

Virchows Archiv A
Pathological Anatomy and Histopathology

The juxtaglomerular apparatus in IgA nephropathy: an analysis of the transport and fate of IgA deposits at the glomerular hilus

Mitsuru Hara [1,4], Kazuo Honda [1], Shoji Matsuya [1], Yuzo Endo [2], Shigeko Hara [3], and Yoshio Suzuki [3]
Departments of Pathology [1], Immunology [2], and Nephrology [3], Toranomon Hospital, Toranomon 2-2-2, Minato-ku Tokyo 105, Japan
Okinaka Memorial Institute for Medical Research [4], Toranomon 2-2-2, Minato-ku, Tokyo 105, Japan

Summary. This study on 27 cases of IgA nephropathy has shown that IgA deposits are rare in the juxtaglomerular apparatus (JGA) despite large amounts of IgA deposits in the mesangium. Phagocytes are absent in JGA. A small number of ill-defined, IgA-positive substances are seen in the matrix of lacis cells, and their electron-density is decreased. These findings indicate dissolution of IgA deposits in the intercellular matrix. In addition, it is suggested that the transport of IgA deposits through the glomerular stalk toward JGA is prevented at the border area between the mesangium of glomerular hilus and the lacis cell region. The block is not complete, because small, IgA-positive substances are seen sparsely in the matrix of lacis cells. The structure of the lacis cell region is thought to restrict the passage of macromolecules such as IgA deposits. Frequently positive staining for C3 in the mesangium and lacis cell region and within the wall of afferent and efferent arterioles indicates that C3 is easily accessible to the arteriolar wall adjacent to JGA, by the route through the glomerular stalk and JGA. This may be concerned in the pathogenesis of arteriolar hyalinosis at the glomerular hilus.

Key words: Mesangium – Juxtaglomerular apparatus – Macromolecular transport – IgA nephropathy – Arteriolar hyalinosis

Introduction

Several studies carried out in mice and rats following administration of tracer substances such as aggregated proteins, colloidal carbon or iron-dextran complexes have shown that certain macromolecules which can not pass through the glomerular tuft capillary wall are taken up at the peripheral mesangium and thence are transported through the glomerular stalk to the region of the juxtaglomerular apparatus (JGA) (Michael et al. 1967, 1980; Mauer et al. 1972; Elema et al. 1976; Leiper et al. 1977; Lee and Vernier 1980). Assuming that this macromolecular transport mechanism is also operative in the human kidney, immune deposits in the mesangium could be thought to move to the JGA in human glomerulonephritides.

The JGA consists of the lacis cells (agranular cells or Goormaghtigh cells), the granular cells (epithelioid cells) of the afferent and efferent arterioles, and the macula densa of the distal convoluted tubule. The lacis cell region (extraglomerular mesangium), which forms a cell mass in direct continuity with the mesangium of glomerular hilus, is located between the afferent and efferent arterioles, and is in close and extensive contact with the granular cells and the macula densa (Latta and Maunsbach 1962; Barajas 1970; Bohle et al. 1982). The matrix material embedding lacis cells and granular cells is continuous with the mesangial matrix. Between the lacis cells and the macula densa, the tubular basement membrane loses its proper ultrastructure and fuses with the matrix material embedding lacis cells (Leiper et al. 1977). Thus, the lacis cell region is thought to be a main route for egress of mesangial macromolecules. In IgA nephropathy, glomerular IgA deposits are present predominantly in the mesangium (Berger 1969). Therefore, it seems reasonable to presume that mesangial IgA deposits are transported to the lacis cell region through the glomerular stalk. The present study on IgA nephropathy, however, shows that IgA deposits are rare in the lacis cell region, and describes the possible causes of this.

Offprint requests to: M. Hara

Materials and methods

This study is based on 27 renal biopsy cases selected from 203 biopsy cases of IgA nephropathy examined at the Department of Pathology, Toranomon Hospital between January 1980 and September 1987. The criteria of the selection were that a renal biopsy specimen for immunohistology contained more than 2 JGAs with glomeruli and that tissue for electron microscopy was sectioned through the glomerular stalk including the lacis cell region and through the macula densa.

In none of the cases studied was there any clinical or laboratory evidence to suggest an underlying disease, such as systemic lupus erythematosus, Henoch-Schönlein purpura, rheumatic fever, liver cirrhosis or chronic pulmonary inflammation.

For light microscopy renal tissue was fixed in 4% neutral buffered formaldehyde, embedded in paraffin, cut at 2 to 3 μ, and was stained with haematoxilin and eosin (H&E), periodic acid-Schiff (PAS), periodic acid-Schiff methenamine silver with H&E counterstain (PAM), and Weigert's elastica-van Gieson stain.

For immunofluorescence microscopy renal tissue was rapidly frozen in dry ice and acetone mixture and stored in a deep freezer at −70° C until use. Frozen sections were cut at 3 to 4 μ in a cryostat at −20° C. After fixation in acetone, the sections were incubated with FITC-labeled rabbit antiserum to human IgG, IgA, IgM, Clq, C3 or C4 (Medical Biological Lab., Japan), respectively, in a moist chamber for 30 min at 37° C. C3d was stained for indirectly, using rabbit antiserum to human C3d (Nordic, the Netherlands) followed by FITC-labeled goat antiserum to rabbit IgG (Medical Biological Lab., Japan) as the second antibody. The monospecificity of each antiserum was checked by Ouchterlony double immunodiffusion method. The sections were observed under a magnification X 200 with a fluorescence microscope. The intensity of fluorescence was arbitrarily graded as −(negative), ±(mild), 1+(moderate), 2+(marked).

To detect monocytes or macrophages, nonspecific esterase was examined on frozen sections cut in a cryostat at −20° C by the method of Yam et al. (1971) with a modification using alpha-naphthyl butyrate (Sigma, USA) as substrate. To detect monocytes, macrophages or leucocytes, further, lysozyme (muramidase) was examined by an indirect immunostaining (Klockars and Reitamo 1975; Spicer et al. 1977) of formalin-fixed and paraffin-embedded renal tissue, using rabbit antiserum to human lysozyme (DAKO, Denmark) and biotin-streptavidin-alkaline phosphatase complex kit (Bio Genex Lab., USA).

For electron microscopy small cubes of renal cortical tissue were fixed in 2.5% glutaraldehyde, buffered with 0.1 M phosphate (pH 7.2) and postfixed in 1.0% osmium tetroxide. After embedding in epoxy resin, sections were cut on a Porter-Blum ultramicrotome, stained with uranyl acetate and lead citrate. Observations were made in a Hitachi H-600A electron microscope. To distinguish between the afferent and the efferent arterioles in the findings by electron microscopy, the tissue embedded in epoxy resin was serially sectioned, stained with methylene blue and was observed with a light microscope.

Examination by immunoelectron microscopy was performed on frozen ultrathin sections by methods modified from the techniques of Tokuyasu (1980). Small cubes of renal cortical tissue were fixed in a mixture of 0.1% glutaraldehyde and 4.0% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3) for 1 h at 4° C. After thorough washing in 0.1 M phosphate buffer, the tissue was immersed in solution of 2.3 M sucrose in 0.1 M phosphate buffer for 10 h at 4° C. The tissue was then snap-frozen in liquid nitrogen and was stored in liquid nitrogen until use.

Frozen sections were cut at a temperature between −110° C and −120° C with glass knives on a Reichert-Jung cryo-ultramicrotome, Ultracut E/FC4D. Ultrathin sections were collected by a platinum loop with a droplet of 2.3 M sucrose solution, and were transferred to polyvinyl formver- and carbon-coated copper grids of 150-mesh, which had been made hydrophilic before use by ionization in a vacuum evaporator. Sucrose was removed by inverting the grids onto a layer of 1.0% gelatin with 0.3% agarose for 15 min at room temperature. The grids were rinsed in phosphate-buffered saline containing 0.01 M glycine (PBS-glycine), four times for 1 min. The grids were then incubated in PBS-glycine with 0.5% bovine serum albumin. The grids were next incubated in goat antiserum to human IgA labeled with 20 nm colloidal gold particles (E-Y Laboratories, USA) for 30 min at room temperature, and then thorough washing was made by stream of PBS-glycine containing 2% trioxyethylene (20) sorbitan monolaurate (ICI, USA). The grids were rinsed again in 0.1 M phosphate buffer, six times for 1 min, and finally in distilled water, four times for 1 min. Sections on the grids were stained in 2.0% neutral uranyl acetate solution for 10 min. They were then washed in distilled water, four times for several sec, and were dried in air at room temperature. Observations were made in a Hitachi H-600A electron microscope.

Monospecificity of the antiserum was checked by immunodiffusion and immunoelectrophoresis. The optimal dilution of the antiserum was determined on known positive specimens.

Clinically blood pressure, urinalysis, urinary protein excretion per 24 h, serum creatinine, endogenous creatinine clearance, serum levels of immunoglobulins, and plasma renin activity of peripheral blood were examined. Hypertension was defined as systolic blood pressure above 150 mmHg or diastolic pressure above 90 mmHg.

Results

Histological changes in the JGA were usually minimal (Fig. 1), but the juxtaglomerular cell mass consisting of lacis cells and granular cells was enlarged in 3 cases with the nephrotic syndrome (Cases 1, 9, 20 in Table 1). Hyalinosis of the afferent or efferent arteriole was seen in 13 of 27 cases, 9 of these 13 cases were normotensive. In 6 of the 13 cases with arteriolohyalinosis, the hyalinosis was confined to the arteriolar wall adjacent to the JGA (Fig. 2).

Because there was a mild variation among the intensities of immunostaining of the JGAs in a biopsy specimen, the findings listed in Table 1 represent the most positive. Staining for IgA in the lacis cell region was negative in 15 of 27 cases, whereas staining for IgA in the mesangium was always prominent (Fig. 3A, Table 1). Any positive staining for IgA in the lacis cell region was fainter by far than that in the mesangium. The transition from prominent staining for IgA in the mesangium of glomerular hilus to the poor or negative staining for IgA in the lacis cell region was abrupt in most cases (Fig. 3A). However, staining for C3 was pos-

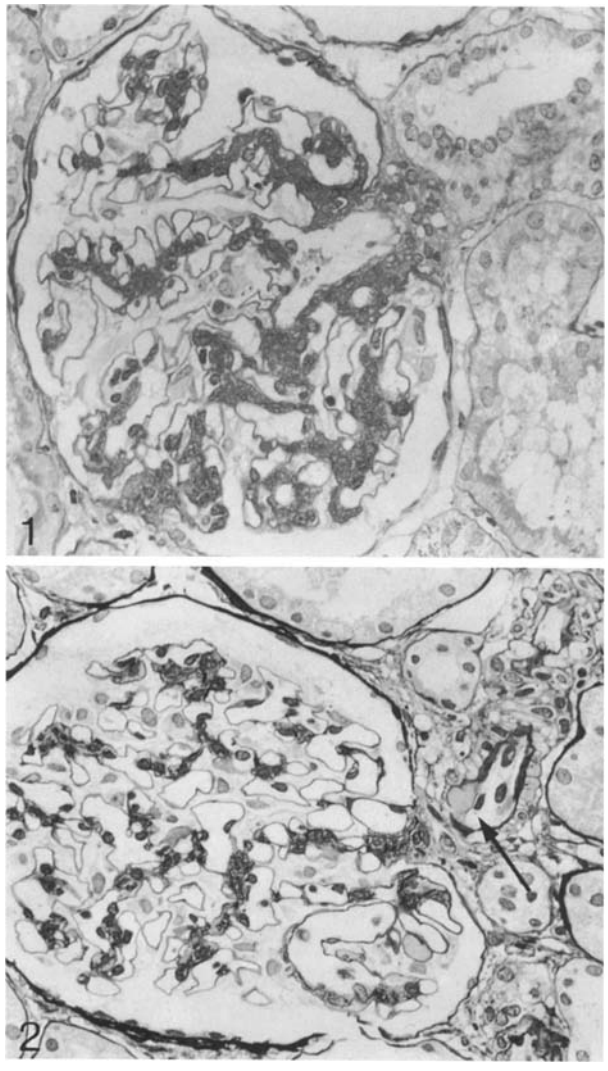


Fig. 1. Histological changes of the lacis cell region and the macula densa are minimal, whereas the mesangium is mildly enlarged (Case 22). PAS (×120)

Fig. 2. Hyalinosis of the afferent arteriolar wall adjacent to the lacis cell region (*arrow*) (Case 16). PAM (×120)

itive in both the mesangium and the lacis cell region in 23 of 27 cases (Fig. 3B), though the staining in the lacis cell region tended to be a little fainter than that in the mesangium (Tables 1, 2). In the lacis cell region, the incidence of positive staining for C3 was significantly higher than that for IgA ($P<0.01$, χ^2 test).

Staining for IgA within the wall of the afferent or efferent arteriole was positive in 3 of 21 cases, whereas staining for C3 within the arteriolar wall was positive in 13 of 21 cases (Fig. 3C, Table 1). The difference between the incidences was significant ($P<0.01$, χ^2 test). Both IgA and C3 were negative in the macula densa.

Positive staining for IgG in the mesangium was less frequent and fainter than that for IgA in the mesangium (Table 2), and IgG was slightly positive in the lacis cell region in only one of 27 cases. IgG was negative within the arteriolar wall. Staining for IgM in the lacis cell region was similar to that for IgA in the lacis cell region (Table 2). IgM was positive within the arteriolar wall in 2 of 21 cases (Cases 5, 27 in Table 1). Both IgG and IgM were negative in the macula densa.

Stainings for Clq and C4 were negative, respectively, both in the glomerulus and in the JGA. The pattern and intensity of staining for C3d were almost the same as those for C3, not only in the mesangium but also in the lacis cell region and arterioles. However, C3d was positive in the glomerular tuft capillary wall more often than C3.

Those portions of the arteriolar walls stained positive for IgA, IgM or C3 often corresponded to the areas of hyalinosis of the arteriolar wall in the findings by light microscopy.

Mononuclear cells positive for nonspecific esterase or for lysozyme were sometimes present in glomerular tuft capillaries, in the urinary space of Bowman's capsule and in the renal interstitium. However, they were rare in the mesangium and were not found in the lacis cell region in 27 cases studied. Phagocytes were thus absent in the lacis cell region.

Large amounts of electron-dense deposits were present in the mesangial matrix in 27 cases studied, and very large electron-dense deposits were often seen in the peripheral mesangium. Small electron-dense deposits were seen sparsely in the subendothelial space of the glomerular tuft capillaries in 12 of 27 cases. Subepithelial or intramembranous dense deposits were not seen in the tuft capillaries.

Large electron-dense deposits were absent in the lacis cell region except the area adjacent to the mesangium, where electron-dense deposits were seen in some cases (Figs. 4A, 8A). A small number of ill-defined, small, electron-dense substances similar in granularity to mesangial deposits were seen sparsely in the matrix of the lacis cell region in 15 of 27 cases (Fig. 5). The electron-density of these substances was decreased as compared with that of electron-dense deposits in the mesangium. These substances were also seen sparsely in the matrix between lacis cells and the macula densa (Fig. 4B). They were not found either in the cytoplasm of lacis cells, granular cells and macula densa cells, or in the intercellular spaces of the macula densa. Neither electron-dense deposit nor

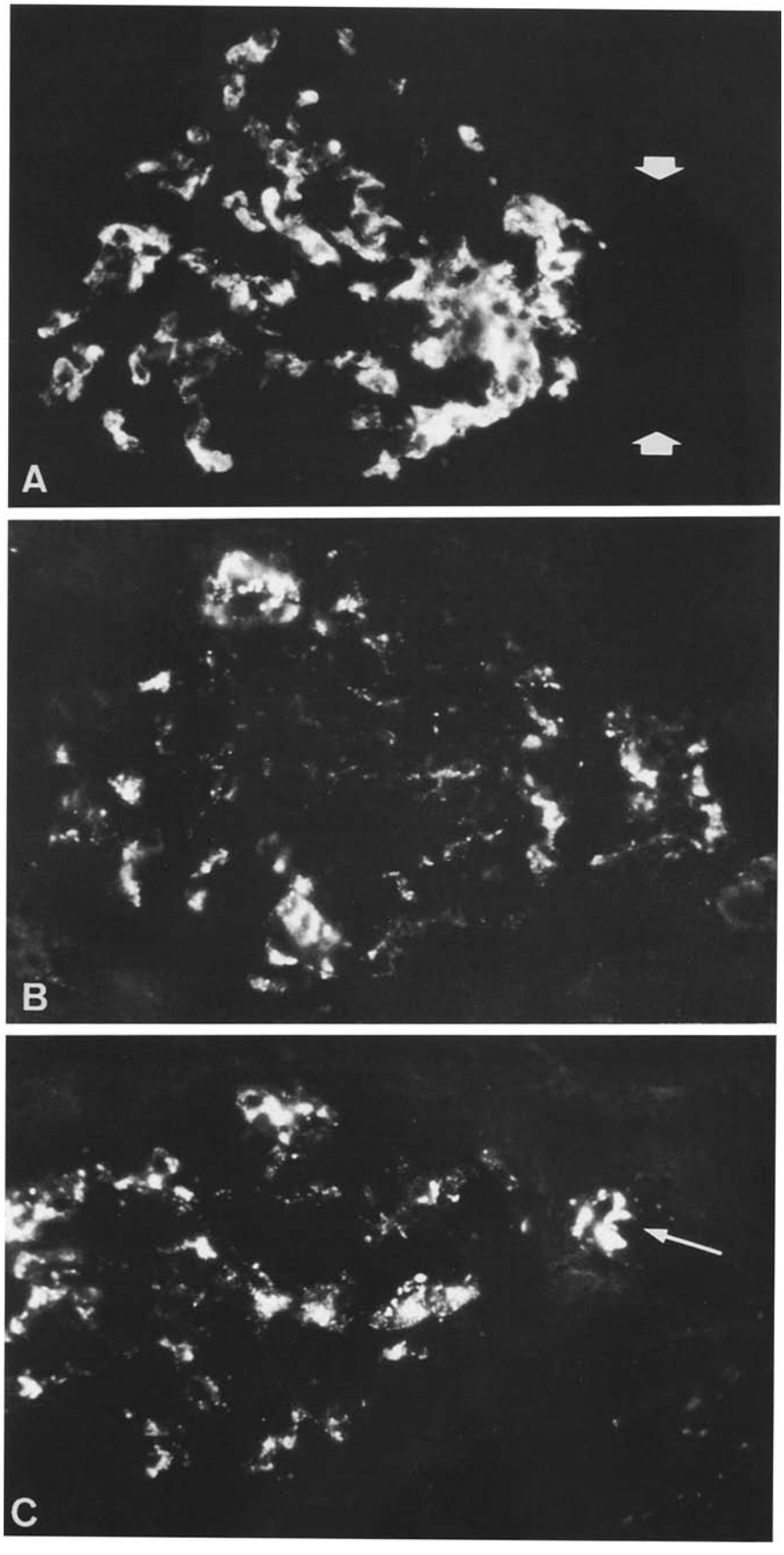


Fig. 3. Immunostainings of a glomerulus with JGA (Case 5). (**A**) Staining for IgA. IgA is prominent both in the peripheral mesangium and in the mesangium of glomerular hilus, but IgA is negative in the lacis cell region (*between arrows*). (×200). (**B**) Staining for C3. C3 is positive not only in the mesangium but in the lacis cell region. (×200). (**C**) Staining for C3. C3 is positive also within the arteriolar wall (*arrow*). (×200)

Table 1. Immunohistological and ultrastructural findings in the mesangium and the lacis cell region in study cases. Examination by immunoelectron microscopy was made in Case 22, 24, 25, 26 and 27. mes. = mesangium, lacis = lacis cell region, art. = afferent or efferent arteriole, ++ = marked, + = moderate, ± = mild, − = negative, D = presence of large electron-dense deposit, d = presence of small electron-dense deposit, *n* = absence of electron-dense deposit

Case	Age	Sex	Staining for IgA			Staining for C3			Electron Microscopy	
	(years)		mes.	lacis	art.	mes.	lacis	art.	mes.	lacis
1	36	M	++	−		+	±		D	d
2	25	F	++	±	+	+	±	+	D	d
3	49	F	+	−		+	+		D	d
4	34	F	++	−	−	+	−	−	D	n
5	39	M	++	−	−	+	+	+	D	n
6	48	F	++	+	−	++	+	±	D	d
7	16	F	++	±		+	+		D	n
8	22	M	++	−	−	+	−	±	D	d
9	30	M	++	−	−	++	+	−	D	n
10	45	M	+	±	−	+	+	+	D	n
11	16	F	++	±	−	+	±	−	D	n
12	16	M	+	−	+	±	−	±	D	n
13	22	F	++	±		+	±		D	d
14	16	M	++	−		++	±		D	n
15	30	M	++	−	−	+	±	±	D	n
16	41	M	++	+	−	+	±	±	D	d
17	41	M	++	−	−	++	±	±	D	d
18	35	M	++	±		+	+		D	d
19	54	M	+	±	−	+	±	−	D	d
20	34	M	++	−	−	+	±	−	D	n
21	25	F	++	+	−	++	+	±	D	d
22	24	F	++	+	−	+	+	−	D (IgA+)	d (IgA+)
23	32	F	++	−	−	+	±	±	D	d
24	21	F	++	−	±	+	+	±	D (IgA+)	d (IgA+)
25	36	M	++	±	−	+	±	−	D (IgA+)	d (IgA+)
26	23	M	++	−	−	++	±	+	D (IgA+)	n (IgA−)
27	45	F	+	−	−	+	−	−	D (IgA+)	n (IgA−)

Table 2. Summary of immunohistological stainings of the mesangium and the lacis cell region in study cases

	Staining	Mesangium	Lacis cell region
IgA	−	0	15
	±	0	8
	+	5	4
	++	22	0
IgG	−	18	26
	±	6	1
	+	3	0
	++	0	0
IgM	−	6	19
	±	13	5
	+	8	3
	++	0	0
C3	−	0	4
	±	1	13
	+	20	10
	++	6	0

ill-defined, small electron-dense substance was found in the lacis cell region in 12 of 27 cases (Fig. 6).

Electron-dense deposits were present in the smooth muscle layer or in the vascular basement membrane of the afferent or efferent arteriole in 8 of 12 cases (Figs. 6B, 7B).

The electron-density of the intercellular matrix was lower in the lacis cell region than in the mesangium of glomerular hilus, and the decreased electron-density was especially marked in the matrix near the macula densa (Figs. 4, 5, 6).

Neither monocytes nor leucocytes were present in the JGA. Breakdown products of cells and striated membranous structures (Bariety and Callard 1975) were often seen in the matrix of the lacis cell region, irrespective of presence or absence of electron-dense substances in the lacis cell region.

Structures suggestive of lymphatic capillaries were not found in the lacis cell region.

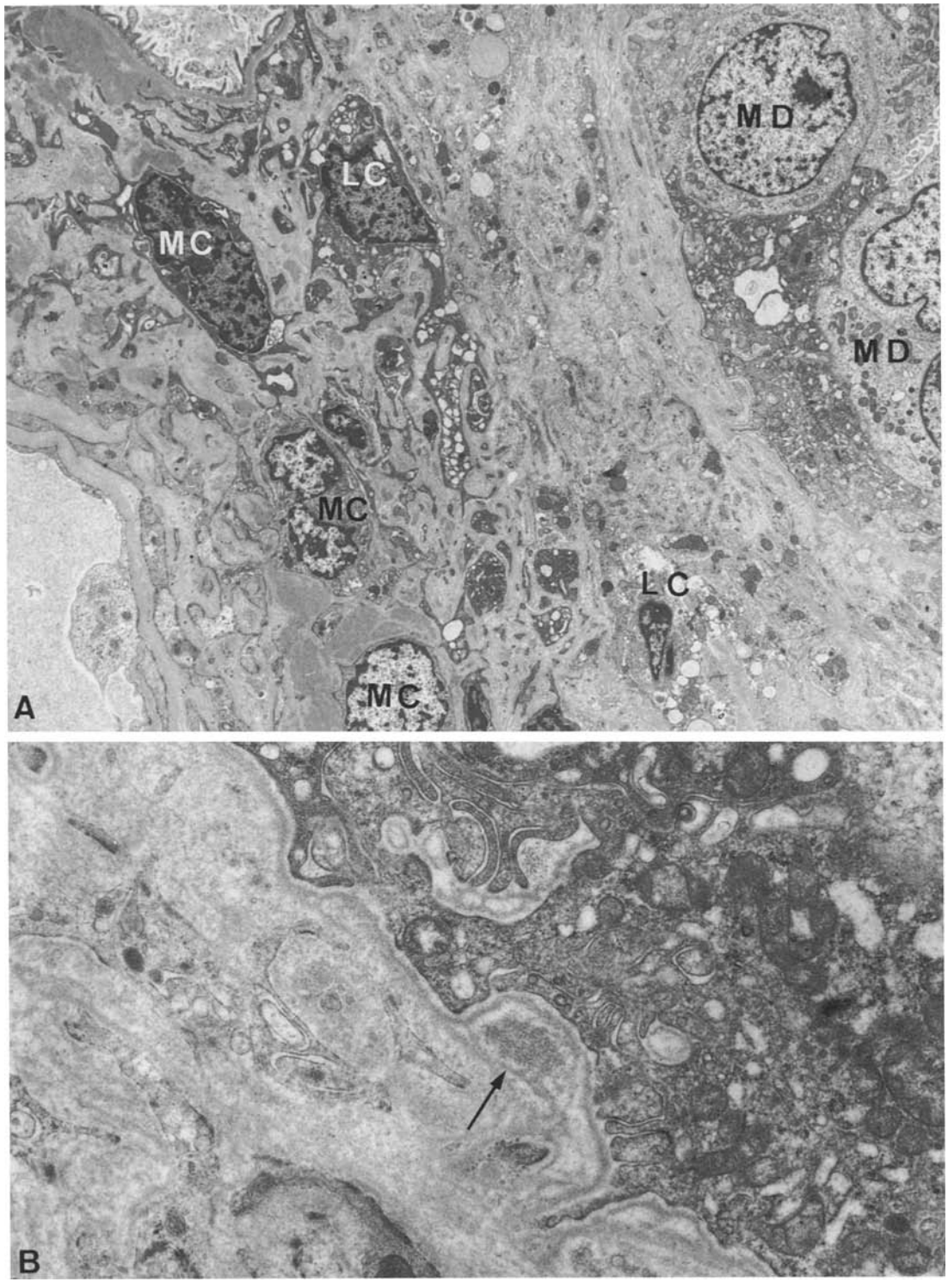


Fig. 4. Electron micrograph of the mesangium of glomerular hilus and the lacis cell region (Case 13). **(A)** Large electron-dense deposits are seen in the mesangial matrix, but they are not seen in the matrix of lacis cells. Small dense deposits are seen in the matrix of lacis cells adjacent to the mesangium. Note that the electron-density of the intercellular matrix is lower in the lacis cell region than in the mesangium. *MC* = mesangial cell, *LC* = lacis cell, *MD* = macula densa. (× 4000). **(B)** Higher magnification of Fig. 4A, showing decreased electron-density of the matrix of the lacis cell region. A small, ill-defined electron-dense substance is seen in the matrix near the macula densa. (*arrow*) (× 18000)

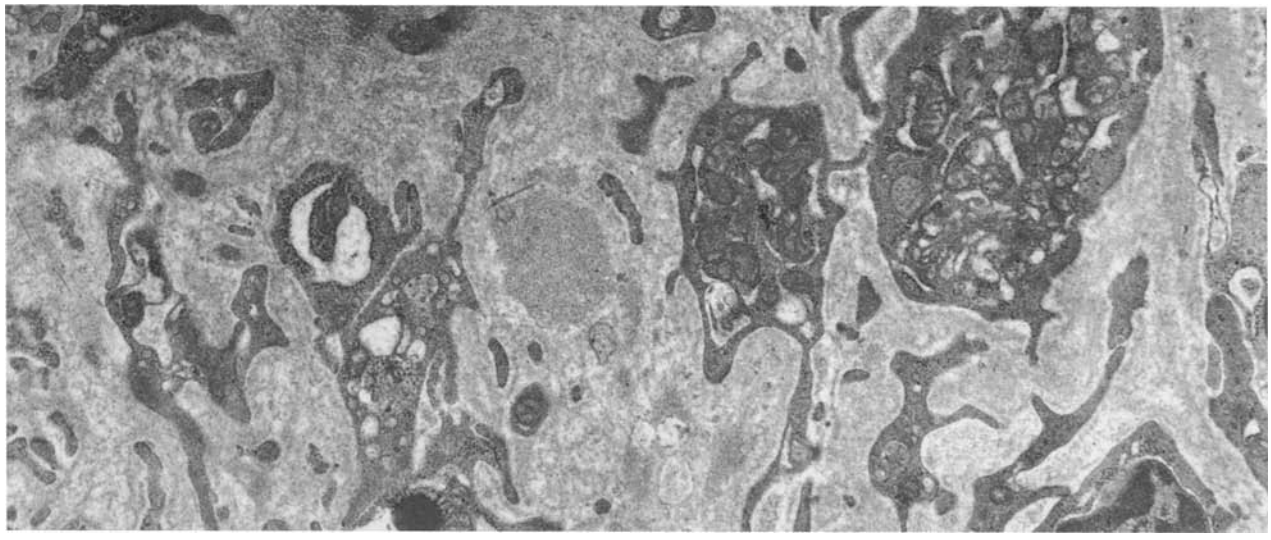


Fig. 5. Ill-defined, electron-dense substances in the matrix of the lacis cell region (Case 1). The electron density of the substances is a little decreased as compared with that of mesangial dense deposits (×15000)

Examination by immunoelectron microscopy was performed in 5 cases (Cases 22, 24, 25, 26, 27). Most of electron-dense deposits in the mesangium were positive for IgA (Fig. 7A). IgA-positive substance was apparently absent in the cytoplasm of mesangial cells, endothelial cells or epithelial cells of the glomeruli. In hyalinizing glomeruli, electron-dense deposits negative for IgA were often seen in the mesangial matrix.

A small number of IgA-positive substances were seen in the matrix of the lacis cell region in 3 of 5 cases (Cases 22, 24, 25). Most of them were ill-defined and small, and their electron-density was decreased when compared with IgA deposits in the mesangial matrix (Fig. 8A, B). Some of the electron-dense substances in the lacis cell region were negative for IgA (Fig. 8B). IgA-negative, small electron-dense deposits were also seen sparsely in the matrix of mesangium. IgA-positive substance was not found either in the cytoplasm of lacis cells, granular cells and macula densa cells, or in the intercellular spaces of the macula densa.

Electron-dense deposits in the basement membrane of the terminal portion of the afferent arteriole were negative for IgA (Fig. 7B). IgA-positive, small electron-dense substances were seen sparsely in the matrix of granular cells around the wall of afferent arteriole adjacent to the JGA in 1 of 5 cases (Case 22).

The patients of the 27 cases consisted of 15 males and 12 females from the age of 16 to 54 years. Hypertension was present in 5 of 27 cases. Elevated plasma renin activity was observed in 4 of 27 cases, and 2 of the 4 cases were hypertensive. Seven cases revealed an impairment of renal function, and 3 of the 7 cases were hypertensive. Heavy proteinuria above 3.0 g of protein per 24 h was found in 5 of the 27 cases, and 2 of the 5 cases were in a state of elevated plasma renin activity.

Discussion

It has been shown that IgA deposits are rare in the lacis cell region, despite the presence of a large amount of IgA deposits in the mesangium. This result is similar to the observation by Latta and Maunsbach (1962) in the work carried out in rats following administration of thorotrast, in which thorotrast particles were rarely found in the lacis cell region, whereas clumps of the particles were present in the mesangium. Silva et al. (1986) have reported the paucity of demonstrable electron-dense deposits in the JGA, and the abrupt ending of electron-dense deposits in the border area between the mesangium of glomerular hilus and the lacis cell region in the kidney of systemic lupus erythematosus. They noted a number of inflammatory cells including polymorphonuclear leucocytes in the lacis cell region. In the present study, however, neither leucocytes nor monocytes are present in the lacis cell region. In addition, IgA-positive substances are not found in the cytoplasm of lacis cells or granular cells. It is therefore unlikely that IgA deposits are removed by phagocytosis in the lacis cell region. As structures suggestive of lym-

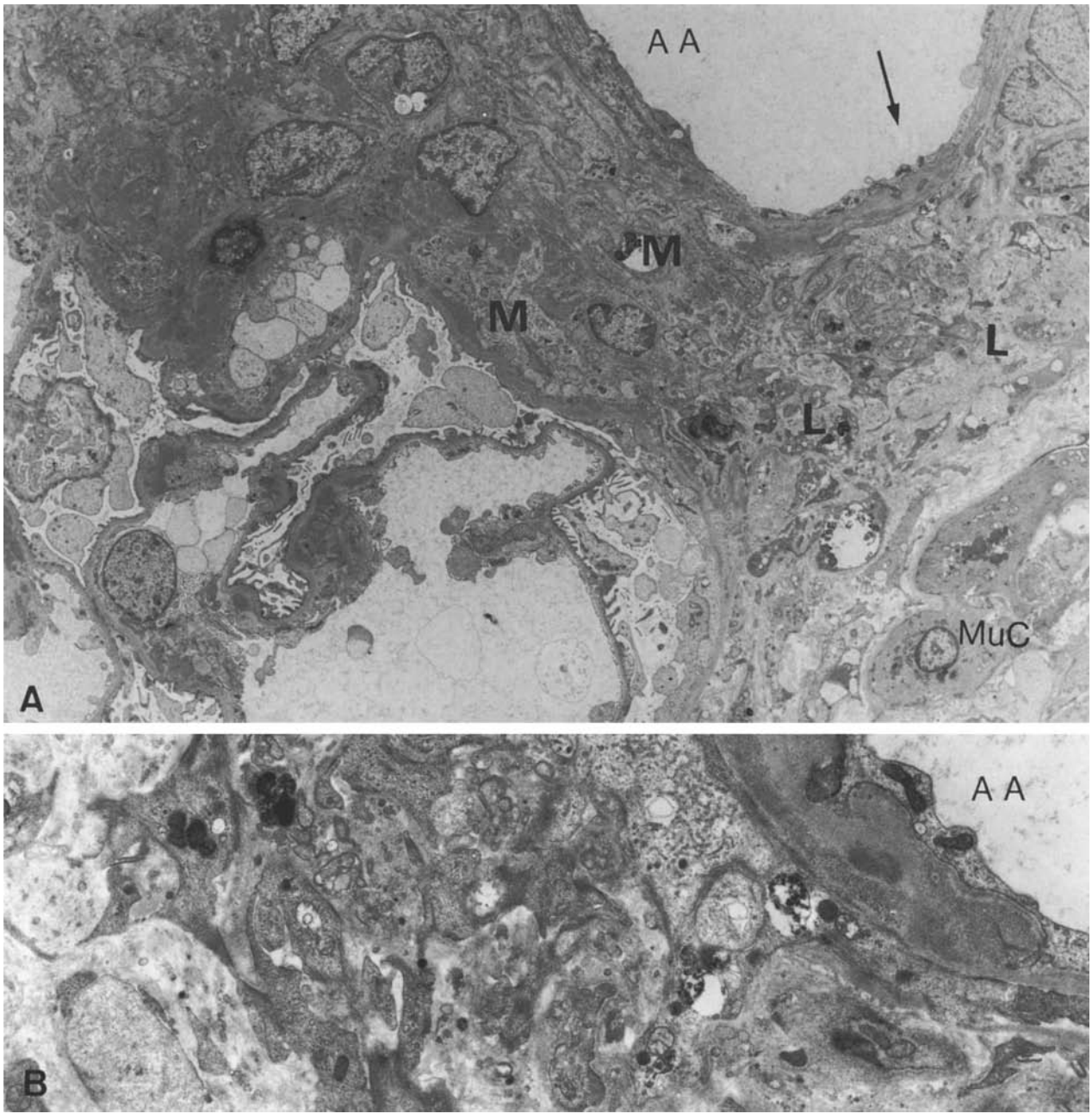


Fig. 6. Electron micrograph of the glomerular hilus (Case 4). **(A)** Electron-dense deposits are present in the matrix of the mesangium and in the basement membrane of the terminal portion of the afferent arteriole (*arrow*), but they are not seen in the matrix of lacis cells. Note that the basement membrane of the afferent arteriole is continuous with the mesangial matrix of glomerular hilus. *AA*=terminal portion of afferent arteriole. *L*=lacis cell region, *M*=mesangium of glomerular hilus, *MuC*=muscle cell of afferent arteriole. (×3000). **(B)** Higher magnification of the lacis cell region in Fig. 6A. (×9000)

phatic capillaries are not found in the lacis cell region, it is also unlikely that IgA deposits are drained away from the lacis cell region through lymphatics.

Staining for C3 is positive not only in the mesangium but in the lacis cell region in most cases, though the staining in the lacis cell region tends to be a little fainter than that in the mesangium. The presence of C3d as well as C3 in the mesangium and in the lacis cell region indicates the presence of several fragments of C3 in these regions (Müller-Eberhard and Schreiber 1980). As the antiserum to human C3 used in the present study is polyclonal, not only C3 but several fragments

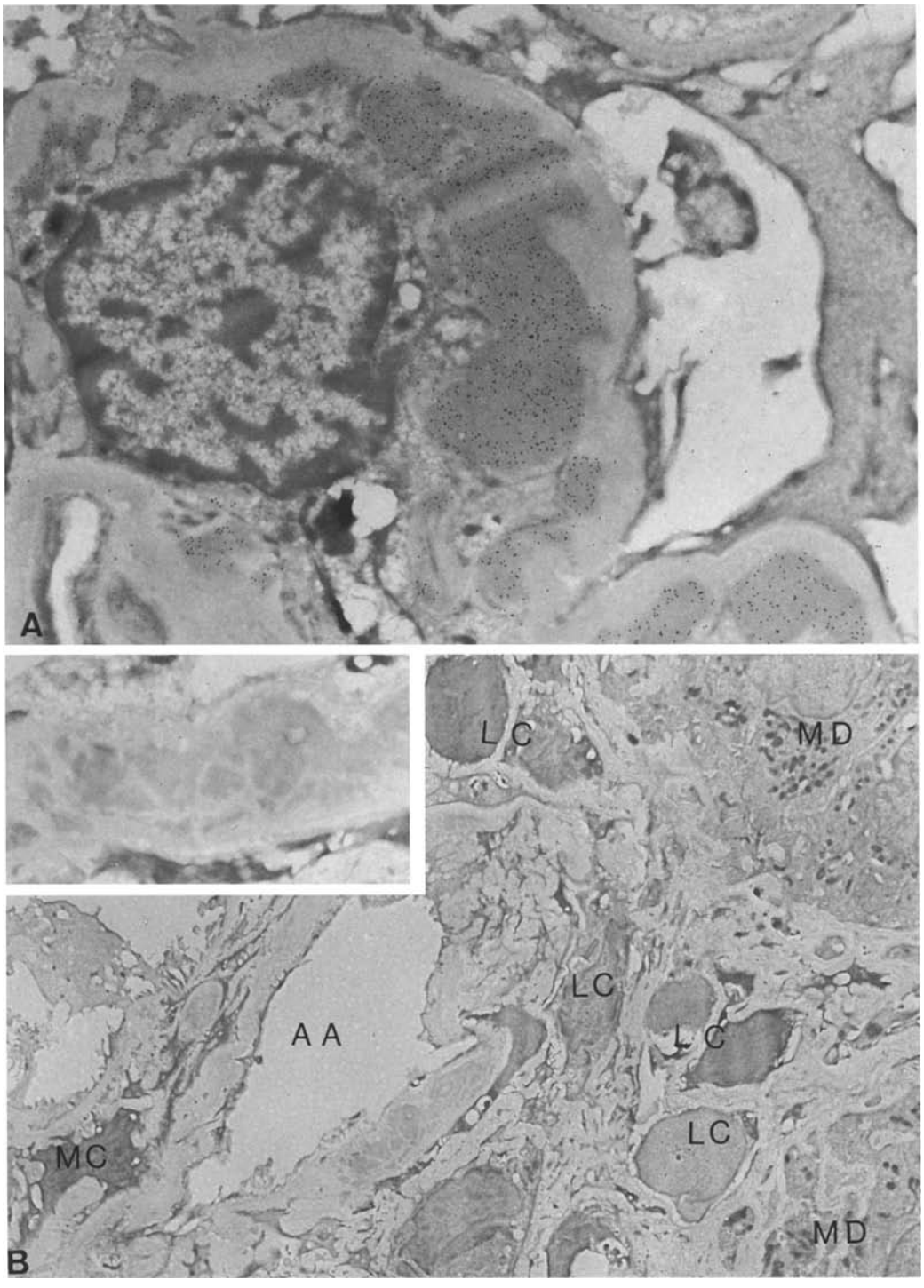
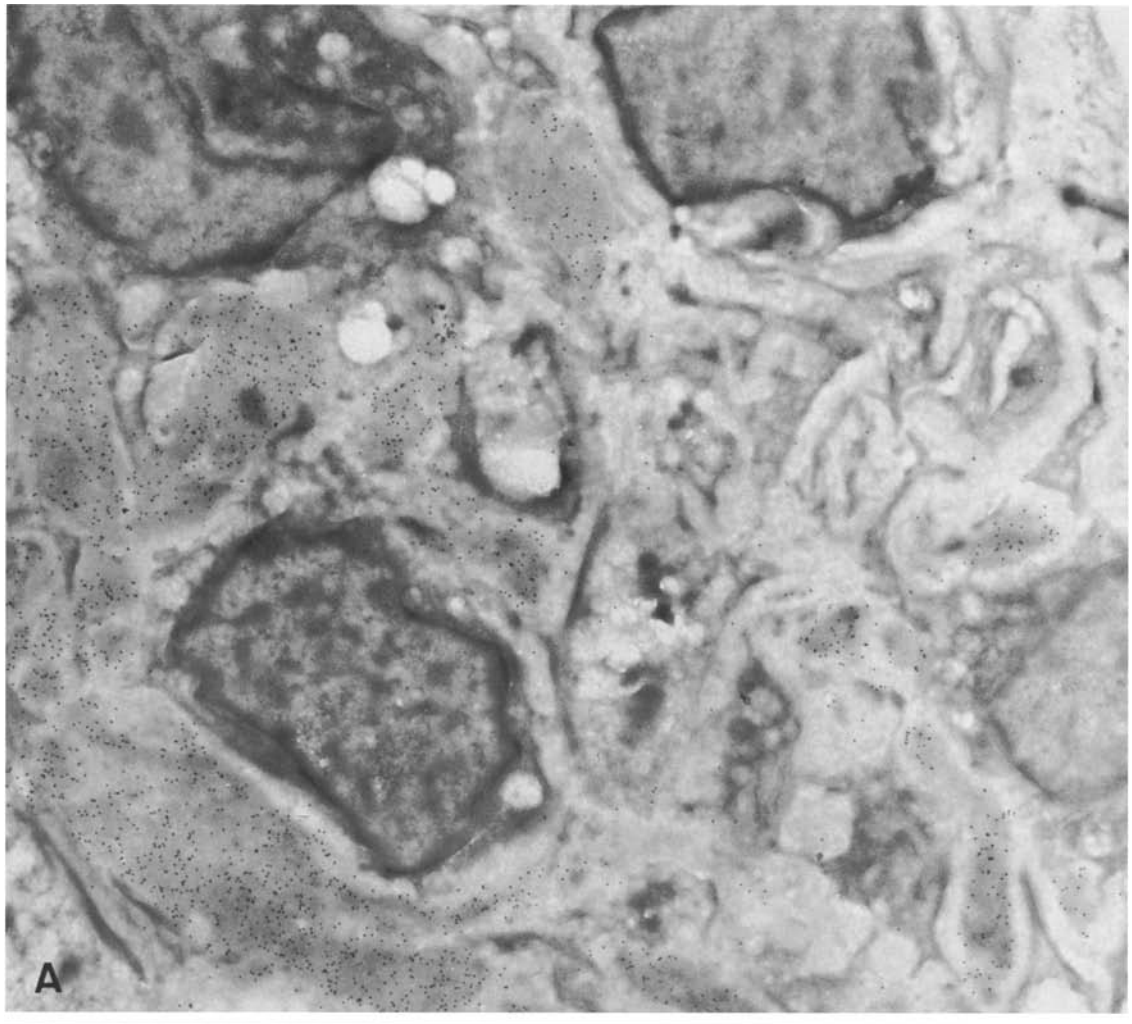
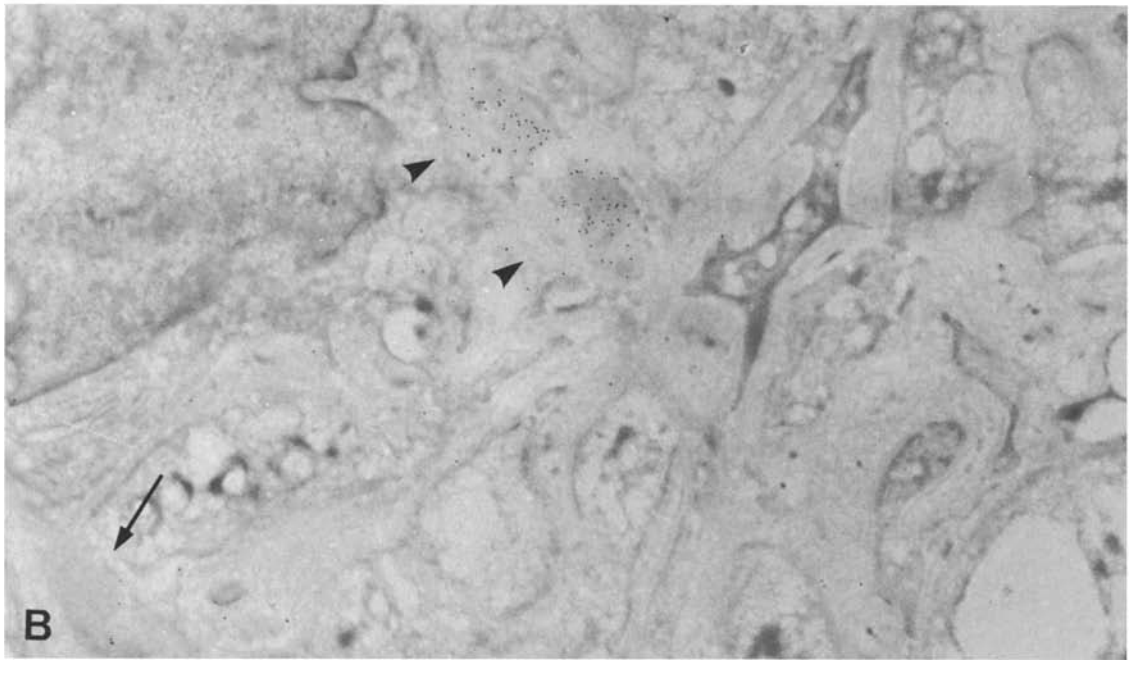


Fig. 7. Immunoelectron micrograph of the mesangium (**A**) and the lacis cell region (**B**) (Case 26). (**A**) Mesangial electron-dense deposits are labelled for IgA with gold particles. (×9000). (**B**) Substances labelled with gold particles are not seen in the lacis cell region. Electron dense deposits in the basement membrane of the terminal portion of the afferent arteriole are not labelled with gold particles (*inset*). *AA*=terminal portion of afferent arteriole, *LC*=lacis cell, *MC*=mesangial cell, *MD*=macula densa. (×3000, inset: ×9000)


A


B

of C3 may be stained positive for C3 here. Thus, the dissociation between the staining for IgA and C3 in the lacis cell region does not necessarily mean that C3 or antigenic determinants of C3 are retained after degradation of IgA in the lacis cell region. The positive staining for C3 not only in the mesangium but also in the lacis cell region accords with the observations made in mice and rats that certain macromolecules are transported from the mesangium to the JGA region (Michael et al. 1967, 1980; Mauer et al. 1972; Elema et al. 1976; Leiper et al. 1977; Lee and Vernier 1980). However, the problem arises of why IgA deposits are rare in the lacis cell region in IgA nephropathy.

The presence of C3 and the absence of Clq and C4 in the lacis cell region as well as in the mesangium suggest the participation of the alternative complement pathway in these regions (Müller-Eberhard and Schreiber 1980). Immune deposits are dissolved by mediation of complement system, and this phenomenon depends essentially on the alternative complement pathway (Miller and Nussenzweig 1975; Miller 1976). Accordingly, IgA deposits are thought to be dissolved in the mesangium and in the lacis cell region bathed in the flow of blood plasma through mesangial channels toward the JGA. However, this should be underemphasized because of the report by Tomino et al. (1983) that the solubilization of glomerular immune deposits is impaired in sera from patients with IgA nephropathy. On the other hand, degradation of immune deposits may occur extracellularly in the mesangium and in the lacis cell region because IgA-negative, electron-dense substances are seen sparsely in the matrix of the JGA and in the matrix of the mesangium in the present study.

The electron-density of the intercellular matrix is lower in the lacis cell region than in the mesangium of the glomerular hilus. Leiper et al. (1977) proposed that the matrix of mesangium and JGA and the basement membrane of the macula densa act as a continuous gel column running from the high pressure in the glomerulus to the low pressure at the macula densa. We agree with them on this point. In this gel column, however, the concentration of the gelling substances must be lower in the lacis cell region than in the mesangium of glomerular hilus, judging from the decreased electron-density of the matrix of lacis cells. Therefore, the diffusion of dissolved substances in the matrix is thought to be speedier in the lacis cell region than in the mesangium of glomerular hilus according to the theory of diffusion in gelified media (Ouchterlony and Nilsson 1973). This could facilitate the dissolution of immune deposits in the matrix of the lacis cell region than in the matrix of the mesangium. The presence of ill-defined, IgA-positive substances of decreased electron-density in the matrix of lacis cells supports this inference. The ultimate fate of dissolved IgA deposits in the lacis cell region remains to be clarified.

Mesangial changes in IgA nephropathy, such as large amounts of IgA deposits, increased matrix and hypercellularity, may impair the transport of macromolecules through mesangial channels. Therefore, the paucity of IgA deposits in the lacis cell region may be a result of impaired transport of IgA deposits through mesangial channels in cases of severe mesangial changes, which are rare in the present study.

We have shown that the transition from the prominent staining for IgA in the mesangium of glomerular hilus to the poor staining in the lacis cell region is abrupt in most cases. This result cannot be explained either by dissolution and diffusion of IgA deposits in the matrix of lacis cells or by impaired transport of macromolecules through mesangial channels. Thus, it is suggested that the transport of IgA deposits through the glomerular stalk toward the JGA is prevented at the border area between the mesangium of glomerular hilus and the lacis cell region, where cells are packed close together with narrow matrix between them, as Latta and Maunsbach (1962) pointed out. The block of the passage is not complete, because small number of IgA-positive substances are present sparsely in the matrix of the lacis cell region. It seems that the structure of the lacis cell region acts like a narrow corridor and restricts the passage of macromolecules such as IgA deposits. The juxtaglomerular cell mass consisting of lacis cells and granular cells enlarges in the nephrotic syndrome (Hara and Meyer 1975). Therefore, egress of mesangial macromolecules may be more severely restricted at the glomerular hilus in situations of heavy proteinuria.

Fig. 8. Immunoelectron micrograph of the lacis cell region (Case 25). (**A**) The lacis cell region adjacent to the mesangium of glomerular hilus (Glomerulus is leftward in the photograph). Electron-dense deposits in the matrix are labelled for IgA with gold particles. In the right half of the photograph, substances labelled with gold particles are small and ill-defined, and their electron-density is decreased (×9000). (**B**) The lacis cell region. Substances labelled for IgA with gold particles are seen in the matrix (*arrowhead*). They are ill-defined and the electron-density is decreased. An electron-dense substance negative for IgA is also seen (*arrow*). (×9000)

Portions of the arteriolar wall stained positive for IgA, IgM or C3 often correspond to hyalinosis of the arteriolar wall in the findings by light microscopy. The staining for IgA or for IgM within the arteriolar wall is positive in only small number of cases, whereas the staining for C3 within the arteriolar wall is often positive in the present study. This result of the stainings within the arteriolar wall is similar to that of the stainings for IgA, IgM and C3 in the lacis cell region. Dysart et al. (1983) have reported the presence of substances positive for IgA or C3 in the mesangial matrix, in the matrix of lacis cells and within the arteriolar wall near the glomerular hilus in immunoelectron microscopy of IgA nephropathy. Experimental studies have revealed that tracer substances such as aggregated proteins or colloidal carbon are transported from the peripheral mesangium through the glomerular stalk to the JGA and into the wall of arterioles adjacent to the JGA (Mauer et al. 1972; Elema et al. 1976; Lee and Vernier 1980). These findings accord well with the fact that the basement membrane and intercellular matrix of the arteriolar wall adjacent to the JGA are continuous with the mesangial matrix of glomerular hilus directly or through the matrix embedding lacis cells and granular cells. Arteriolar hyalinosis has been the subject of many studies and brought several controversies especially on the source and composition of "hyaline" (McGee and Ashworth 1963; Biava et al. 1964). Positive staining for C3 in arteriolar hyalinosis has been reported (Valenzuela et al. 1980; Gamble 1986). In addition, Gamble (1986) has demonstrated that iC3b, an inactivated form of C3b, is a major component of arteriolar hyaline. The endothelium of arterioles may be permeable to plasma proteins of the size of C3, as Gamble described. However, C3 and fragments of C3 can be more easily accessible to the arteriolar wall adjacent to the JGA by the route through the glomerular stalk and the lacis cell region, where these substances have been shown to be present in IgA nephropathy. Thus, it is conceivable that IgA nephropathy tends to induce hyalinosis of the afferent and efferent arteriolar wall adjacent to the JGA even in the absence of hypertension. Actually in the present study, the majority of cases with arteriolar hyalinosis are normotensive, and the hyalinosis is often confined to the arteriolar wall adjacent to the JGA. The frequent occurrence of hyalinosis of the afferent and efferent arterioles in normotensive patients with IgA nephropathy has been pointed out by several investigators (Kincaid-Smith 1975; Clarkson et al. 1977; Nakamoto et al. 1986). Arteriolar hyalinosis at the glomerular hilus could lead to the development of renal failure by affecting the haemodynamics of the glomeruli.

References

Barajas L (1970) The ultrastructure of the juxtaglomerular apparatus as disclosed by three-dimensional reconstructions from serial sections. The anatomical relationship between the tubular and vascular components. J Ultrastruct Res 33:116–147

Bariety J, Callard P (1975) Striated membranous structures in renal glomerular tufts. An electron microscopy study of 340 human renal biopsies. Lab Invest 32:636–641

Berger J (1969) IgA glomerular deposits in renal disease. Transplant Proc 1:939–944

Biava CG, Dyrda I, Genest J, Bencosme SA (1964) Renal hyaline arteriolosclerosis. An electron microscope study. Am J Pathol 44:349–363

Bohle A, Christensen J, Meyer DS, Laberke HG, Strauch M (1982) Juxtaglomerular apparatus of the human kidney: Correlation between structure and function. Kidney Int 22 (Suppl. 12):S18–S23

Clarkson AR, Seymour AE, Thompson AJ, Haynes WDG, Chan Y-L, Jackson B (1977) IgA nephropathy: a syndrome of uniform morphology, diverse clinical features and uncertain prognosis. Clin Nephrol 8:459–471

Dysart NK Jr., Sisson S, Vernier RL (1983) Immunoelectron microscopy of IgA nephropathy. Clin Immunol Immunopathol 29:254–270

Elema JD, Hoyer JR, Vernier RL (1976) The glomerular mesangium: Uptake and transport of intravenously injected colloidal carbon in rats. Kidney Int 9:395–406

Gamble CN (1986) The pathogenesis of hyaline arteriolosclerosis. Am J Pathol 122:410–420

Hara M, Meyer D (1975) The size of the juxtaglomerular apparatus in glomerulonephritis with the nephrotic syndrome. Virchows Arch A 367:1–14

Kincaid-Smith P (1975) The kidney. A clinico-pathological study. Blackwell, Oxford London Edinburgh Melbourne, pp 193–202

Klockars M, Reitamo S (1975) Tissue distribution of lysozyme in man. J Histochem Cytochem 23:932–940

Latta H, Maunsbach AB (1962) Relations of the centrolobular region of the glomerulus to the juxtaglomerular apparatus. J Ultrastruct Res 6:562–578

Lee S, Vernier RL (1980) Immunoelectron microscopy of the glomerular mesangial uptake and transport of aggregated human albumin in the mouse. Lab Invest 42:44–58

Leiper JM, Thomson D, MacDonald MK (1977) Uptake and transport of imposil by the glomerular mesangium in the mouse. Lab Invest 37:526–533

Mauer SM, Fish AJ, Blau EB, Michael AF (1972) The glomerular mesangium. I. Kinetic studies of macromolecular uptake in normal and nephrotic rats. J Clin Invest 51:1092–1101

McGee WG, Ashworth CT (1963) Fine structure of chronic hypertensive arteriopathy in the human kidney. Am J Pathol 43:273–299

Michael AF, Fish AJ, Good RA (1967) Glomerular localization and transport of aggregated proteins in mice. Lab Invest 17:14–29

Michael AF, Keane WF, Raij L, Vernier RL, Mauer SM (1980) The glomerular mesangium. Kidney Int 17:141–154

Miller GW, Nussenzweig V (1975) A new complement function: Solubilization of antigen-antibody aggregates. Proc Natl Acad Sci USA 72:418–422

Miller GW (1976) Solubilization of IgA immune precipitates by complement. J Immunol 117:1374–1377

Müller-Eberhard HJ, Schreiber RD (1980) Molecular biology and chemistry of the alternative pathway of complement. In: Kunkel HG, Dixon FJ (eds) Advances in Immunology. Vol 29. Academic Press, New York London Toronto Sydney San Francisco, pp 1–53

Nakamoto Y, Miki K, Miura AB, Dohi K, Nomura G (1986) Hypertension and intrarenal small vessel lesions in patients with IgA nephritis. A subset of cases mimicking essential hypertension. Jpn J Nephrol 28:1211–1220

Ouchterlony Ö, Nilsson LÅ (1973) Immunodiffusion and Immunoelectrophoresis. In: Weir DM (ed) Handbook of experimental immunology. Vol 1. Immunochemistry. Blackwell, Oxford London Edinburgh Melbourne, pp 19.1–19.39

Silva FG, Eigenbrodt EH, Glass M, Taft E (1986) An ultrastructural study of the renal juxtaglomerular apparatus and extraglomerular mesangium in patients with systemic lupus erythematosus. Am J Kidney Dis 7:47–57

Spicer SS, Frayser R, Virella G, Hall BJ (1977) Immunocytochemical localization of lysozymes in respiratory and other tissues. Lab Invest 36:282–295

Tokuyasu KT (1980) Immunochemistry on ultrathin frozen sections. Histochem J 12:381–403

Tomino Y, Sakai H, Suga T, Miura M, Kaneshige H, Endoh M, Nomoto Y (1983) Impaired solubilization of glomerular immune deposits by sera from patients with IgA nephropathy. Am J Kidney Dis 3:48–53

Valenzuela R, Gogate PA, Deodhar SD, Gifford RW (1980) Hyaline arteriolar nephrosclerosis. Immunofluorescence findings in the vascular lesions. Lab Invest 43:530–534

Yam LT, Li CY, Crosby WH (1971) Cytochemical identification of monocytes and granulocytes. Am J Clin Pathol 55:283–290

Accepted June 23, 1988