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Detection of polymeric IgA in glomeruli from patients with IgA nephropathy

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SUMMARY

A study on the detection of polymeric IgA in glomeruli from renal biopsy specimens in patients with IgA nephropathy is described. Renal biopsy specimens were obtained from patients with IgA nephropathy. These specimens were stained with FITC-labelled anti-human J chain antisera and then examined with a fluorescent microscope. The J chain was observed in the glomerular mesangium by immunofluorescent staining. In parallel studies, renal biopsy specimens were treated with citrate buffer (pH 3·2) and the 'eluate' was neutralized by sodium hydroxide. The eluate was labelled with iodine-125, and the radiolabelled 'eluate' was fractionated by sucrose density-gradient ultracentrifugation. Polymerized IgA in the 'eluate' obtained from patients with IgA nephropathy was found to sediment predominantly as 9S to 11S using a sucrose density gradient analysis. Polymeric IgA in the fractions of the density gradient analysis was determined by anti-human IgA and anti-human J chain antisera. It was demonstrated that IgA and J chain were eluted from the glomeruli in some patients with IgA nephropathy. It is concluded that IgA deposited in the glomeruli is composed of dimers and/or larger polymers of circulating IgA in some patients with IgA nephropathy.

INTRODUCTION

IgA nephropathy is characterized by mesangial deposition of IgA with less intense deposition of IgG, IgM and C3 in patients without evidence of systemic diseases (Berger, 1969; McCoy, Abramowsky & Tisher 1974). Although the pathogenesis of IgA nephropathy is still obscure, immune complex mediated glomerulonephritis is presumed to be a cause of this disease (McCoy *et al.*, 1974; Berger *et al.*, 1975). It has been postulated that IgA may play a role in pathogenesis and development of this disorder (Lowance *et al.*, 1977). Lopez-Trascasa *et al.* (1980) reported the presence of a large amount of IgA polymers, partially as immune complexes, in the serum of patients with IgA nephropathy. Clarkson *et al.* (1980) reported that sucrose density analysis of serum samples identified monomeric (8S) IgA in patients with IgA nephropathy.

The purpose of this study was to determine the deposition of J ('joining') chain in renal biopsy specimens by immunofluorescence and the molecular size of the 'eluate' obtained from renal biopsy specimens by sucrose density analysis in patients with IgA nephropathy.

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MATERIALS AND METHODS

Patients. Renal biopsy specimens were obtained from 12 patients with IgA nephropathy. Routine microscopic, immunofluorescent and electron microscopic analysis were performed for the diagnosis of IgA nephropathy. Patients whose biopsy specimens stained predominantly for IgA in mesangial areas were included in this study after exclusion of patients with systemic lupus erythematosus and Henoch-Schoenlein purpura (HSP) nephritis.

Immunofluorescent study. Renal biopsy specimens were embedded in the Tissue-Tek® II (Lab-Tek Products, Division Miles Laboratories, Naperville, Illinois, USA, Lot No. 0125068) and rapidly frozen in acetone dry ice, sectioned to 3–4 μm with a rotary microtome in a cryostat at about −25°C, and air dried. Cryostat sections were fixed with cold acetone and cold ethanol (95%). Other sections were not fixed before staining. Heavy chain specific fluorescein-labelled anti-human IgG, IgM, IgA and C3 antisera were obtained from the Behringwerke AG (Marburg-Lahn, West Germany) (F/P molar ratios ranged from 1·8 to 2·9). Rabbit anti-human J chain antisera was kindly provided by Dr Kobayashi, Yamaguchi University, Yamaguchi-ken, Japan. Antisera against J chain were prepared by injecting rabbits with purified proteins suspended in Freund's complete adjuvant (FCA) as previously described (Kobayashi *et al.*, 1973a, 1973b). Anti-human J chain antisera were conjugated with FITC using the method of Kawamura (1977). The F/P molar ratio of this FITC-conjugated antisera was 2·0. These antisera were absorbed three times with mouse liver acetone powder. Specificities of these antisera were determined by immunodiffusion and immunoelectrophoresis. Positive staining of the J chain was confirmed in the tonsillar cells obtained from tonsillectomized tissues of a patient with acute glomerulonephritis. Dilution of antisera was 1:10 in phosphate-buffered isotonic saline (PBS, pH 7·2) unless indicated otherwise.

In some experiments, cryostat sections were treated with 6M urea in glycine-HCL buffer (pH 3·2) for 30 min, 60 min, 3 hr and 20 hr at 4°C as described previously (Nagura *et al.*, 1979; Korsrud & Brandtzaeg, 1981). The treated sections were washed in PBS as described above. Other sections were not treated with acid-urea before staining. Immediately before staining, sections were washed three times in PBS for 15 min. Crysostat sections of the renal biopsy specimens were stained with these FITC-labelled antisera in a moist chamber at 4°C overnight. The optimum conditions of immunofluorescence was determined by our previous studies (Tomino *et al.*, 1981). The sections were washed with PBS and then covered with buffered glycerol and a cover slip, and examined with a Zeiss Orthoflux microscope (Model 9902; Carl Zeiss Inc., New York). The intensity of the fluorescence was graded as none (−), trace (±), 1(+), 2(+) and 3(+).

Density gradient ultracentrifugation. Elution procedures were performed as follows. Renal specimens obtained from open renal biopsies were treated with citrate buffer (pH 3·2) and the 'eluate' was neutralized by sodium hydroxide as previously described (Tomino *et al.*, 1982). Approximately 80 glomeruli were observed in the 10 serial sections placed on a single glass slide. Mean concentration of total proteins in the 'eluate' from five patients with IgA nephropathy was 5·6 μg/ml as measured by Lowry's method (Lowry *et al.*, 1951), and that of IgA in the 'eluate' was 1·7 μg/ml as measured using a laser nephelometer. The 'eluate' was labelled with ^{125}I by the Chloramine T method (McConahey & Dixon, 1960). Specific activity of this radiolabelled protein was $1{\cdot}41 \times 10^5$ c.p.m./μg protein. Density gradient ultracentrifugation was performed by layering 50 μl of ^{125}I-labelled 'eluate' over 5 ml of 5–40% continuous sucrose density gradient in Beckman cellulose nitrate tubes (Beckman Instruments, Mountainside, New Jersey, USA). Each sample was analysed by quadruplicate tubes.

Sucrose was dissolved in 0·15 M Tris-HCl, pH 7·4. The gradients were then centrifuged at 170,000 *g* for 16 hr at 4°C in a Spinco L-2 ultracentrifuge with a SW 50 RI rotor. IgM(19S), IgG(7S) and bovine serum albumin (BSA; 4·5S) were used as markers. The centrifuged tubes were punctured and 40 fractions were collected from the bottom using an Absorbance Monitor (ISCO, Model UA, USA). Each fraction was measured by γ counter (Aloka JDC-755, Tokyo, Japan). Each fraction was absorbed with aliquots of anti-human IgA, IgA1, IgA2 or J chain antisera, as well as anti-human IgG or IgM, before measuring the radioactivity. Anti-human IgA, IgG and IgM antisera were obtained from the Behringwerke AG (Marburg-Lahn, West Germany). Anti-human IgA1 and IgA2 antisera were obtained from the Nordic Laboratories (Tilburg, The Netherlands).

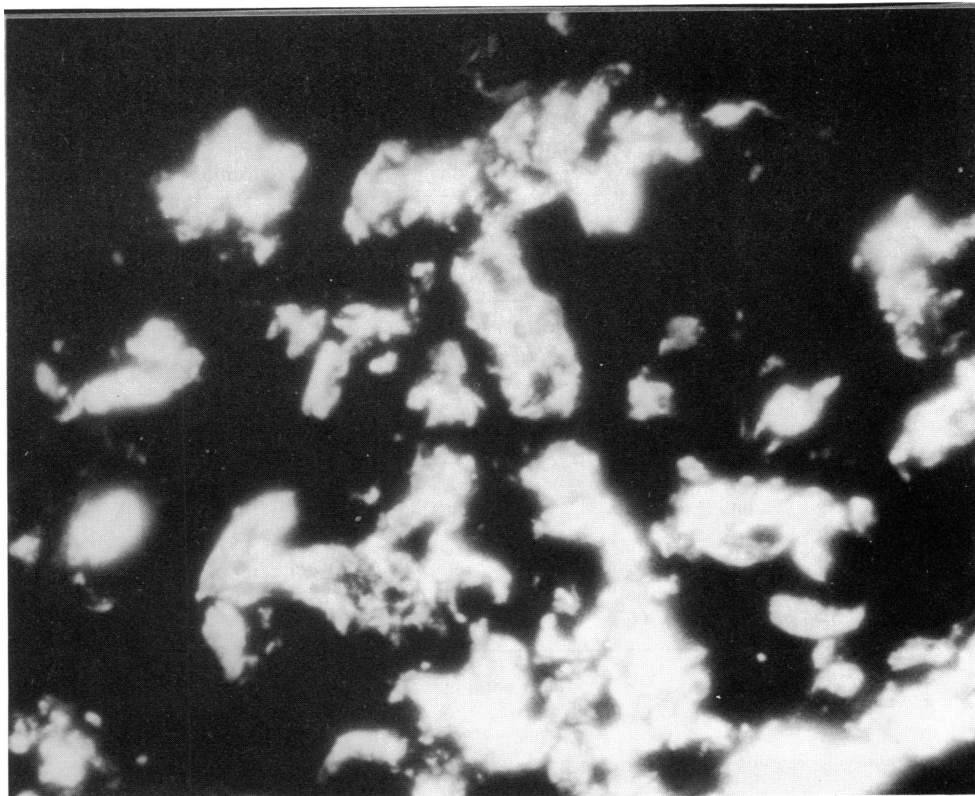

Fig. 1. Immunofluorescent location of IgA in glomeruli from a patient with IgA nephropathy.

RESULTS

Immunofluorescent study

Immunoglobulin A was the prominent class of immunoglobulin deposited in the glomeruli of all patients with IgA nephropathy (Fig. 1). The deposition of IgA was mainly observed in the glomerular mesangium. The intensity of IgG, IgM and C3 deposition was always less than that of IgA deposition. The distribution of IgG, IgM and C3 was similar to that of IgA although that of IgM was focal and/or segmental in some cases. The results from the immunofluorescent studies on patients with IgA nephropathy are summarized in Tables 1 and 2. After fixation with cold acetone followed by treatment with PBS before staining, the J chain was observed in two out of 10 patients examined. A trace amount of J chain deposition was also observed in five out of 10 patients examined. After fixation with cold acetone followed by treatment with urea before staining, a trace amount of J chain deposition was observed in two out of 10 patients. After fixation with cold ethanol followed by treatment with PBS before staining, the J chain was observed in one out of five patients. A trace amount of J chain deposition was observed in three out of five patients. After fixation with cold ethanol followed by treatment with urea before staining, a trace amount of J chain deposition was observed in one out of five patients. After treatment with PBS and urea without previous fixation, J chain was not observed in any patients examined. Results from preliminary studies on the effect of incubation time with treatment of urea, the degree of intensity of J chain deposition showed that treatment for 30 min yielded more intense staining than that for 60 min, 3 hr and 20 hr (Fig. 2).

Table 1. Immunofluorescent staining in kidneys of patients with IgA nephropathy. Demonstration of presence of J chain under various conditions

	Fixation					
	Cold acetone		Cold ethanol		No fixation	
	Treatment					
	With PBS	With urea	With PBS	With urea	With PBS	With urea
1	(+)	(±)	(±)	(−)	(−)	(−)
2	(+)	(±)	(+)	(−)	(−)	(−)
3	(±)	(−)	(−)	(−)	(−)	(−)
4	(±)	(−)	(±)	(±)	(−)	(−)
5	(±)	(−)	(±)	(−)	(−)	(−)
6	nd	nd	nd	nd	(−)	(−)
7	(±)	(−)	nd	nd	(−)	(−)
8	nd	nd	nd	nd	(−)	(−)
9	(±)	(−)	nd	nd	(−)	(−)
10	(−)	(−)	nd	nd	nd	nd
11	(−)	(−)	nd	nd	nd	nd
12	(−)	(−)	nd	nd	nd	nd

nd = not done.

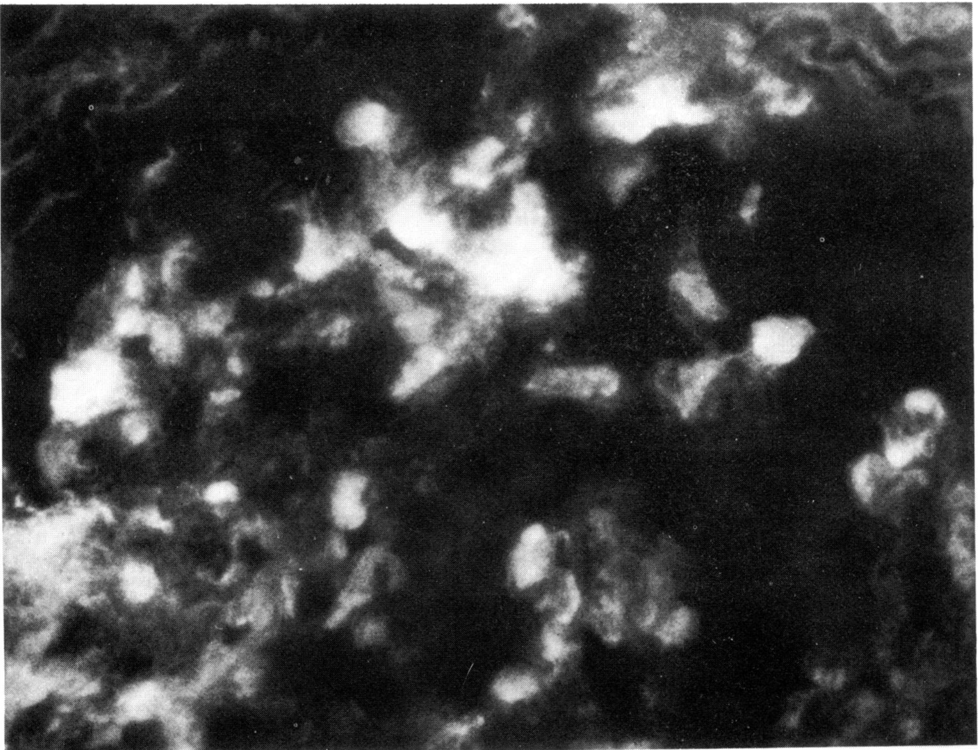

Fig. 2. Immunofluorescent location of J chain in glomeruli from a patient with IgA nephropathy.

Table 2. Immunofluorescent staining in kidneys of patients with IgA nephropathy. Presence of immunoglobulin classes, complement and J chain

	Intensity of immunofluorescent staining				
Case No.	IgA	IgG	IgM	C3	J chain*
1	+++	+	++	++	+
2	+++	+	++	+++	+
3	+++	+	++	+++	±
4	++	−	+	++	±
5	+++	−	+	+	±
6	+++	−	+	++	nd
7	+++	−	+	++	±
8	+++	±	+	+	nd
9	+++	−	++	++	±
10	+++	+	+	+	−
11	+++	−	±	++	−
12	+++	+	++	+++	−

* The conditions were fixation with cold acetone for 30 min and treatment with PBS before staining.

nd = not done.

The intensity of the deposition of the J chain was always less than that of IgA, IgG and IgM deposition (Table 2). The distribution of J chain was similar to that of IgA although some patients showed co-existence of the J chain with both IgA and IgM.

Density gradient ultracentrifugation

The 'eluates' obtained from five patients with IgA nephropathy were studied using sucrose density gradient ultracentrifugation. Fig. 3 shows the pattern of ^{125}I-labelled 'eluate' fractionated by

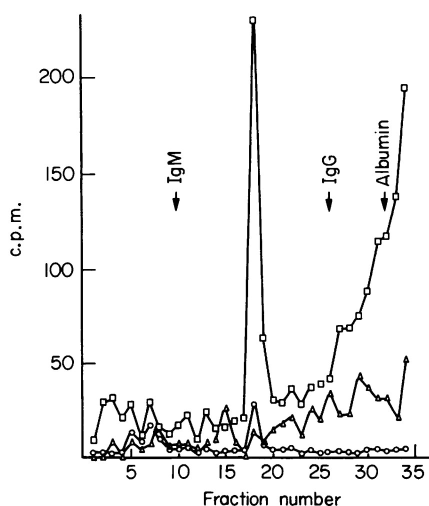

Fig. 3. The pattern of ^{125}I-labelled 'eluate' obtained from a patient with IgA nephropathy fractionated by sucrose density gradient ultracentrifugation. (□—□ = eluate; △—△ = eluate absorbed with anti-IgA antisera; ○—○ = eluate absorbed with anti-J chain antisera).

sucrose density gradient ultracentrifugation. A protein peak was observed at fraction numbers 18 and 19 in three out of five patients examined. This peak was located between 9S and 11S as determined by the location of marker proteins. The peak was absorbed by either heavy chain specific anti-human IgA or anti-human J chain antisera, but was not absorbed by either heavy chain specific anti-human IgG or IgM antisera. The peak was prominently absorbed by anti-human IgA1 antisera when compared with the use of anti-human IgA2 antisera in patients with IgA nephropathy.

DISCUSSION

The results obtained from this study showed that the J chain was detected in the glomeruli in the glomerular mesangium by immunofluorescence. The localization of J chain in the glomeruli was almost identical to that of IgA, although a few patients showed co-existence of the J chain with both IgA and IgM. The J chain has been described as a polypeptide linked by disulphide bridges to polymeric IgA and IgM since it is probably involved in the process of intracellular polymerization of IgA and IgM (Koshland, 1975). Because the secretory component was not detected in glomeruli from patients with IgA nephropathy by immunofluorescence as described previously (McCoy *et al.*, 1974; Shirai *et al.*, 1977), IgA deposited in glomeruli might be dimers and/or larger polymers of circulating IgA. However, some J chain detected in glomeruli might be linked to pentameric IgM. However, some J chain detected in glomeruli might be linked to pentameric IgM since the distribution of J chain deposition was similar to that of IgM in some patients with IgA nephropathy as indicated by the present study.

The results from the present study indicated that the J chain was more frequently stained after fixation with cold acetone without subsequent treatment with urea before staining. Under the condition of pre-treatment with urea before staining, the polymerized IgA might be easily dissolved from renal tissues in patients with IgA nephropathy. Moreover, IgA might be easily released from tissues by washings with PBS using non-fixed renal biopsy specimens in this study. Brandtzaeg (1976) has reported that acid-urea treatment of tissue sections could detect J chains masked by various classes of immunoglobulins. Previous method for detection of J chain always included fixation before staining with immunofluorescence and immunohistochemistry in various types of diseases (Nagura *et al.*, 1979; Laurent *et al.*, 1981; Korsrud & Brandtzaeg, 1981). It is indicated that selection of fixation procedure before immunofluorescent staining is critical for detection of J chain in renal tissues from patients with IgA nephropathy. Recently the deposition of J chain in glomeruli from some patients with IgA nephropathy has been observed using immunoelectron microscopy (Nagura *et al.*, unpublished observations).

Polymerized IgA in the 'eluate' obtained from renal biopsy specimens in some patients with IgA nephropathy was found to sediment predominantly as 9S–11S using sucrose density gradient analysis. The peak of protein in the 'eluate' obtained from renal biopsy specimens was completely absorbed with anti-human IgA or anti-human J chain antisera in some patients with IgA nephropathy. Recently, Lopez-Trascasa *et al.* (1980) reported the presence of a large amount of IgA polymers in sera from patients with IgA nephropathy. On the other hand, Clarkson *et al.* (1980) reported that monomeric (8S) IgA in sera was identified using a sucrose density analysis in patients with IgA nephropathy. However, it is not known whether IgA deposited in the glomeruli of patients with IgA nephropathy is a monomer or dimers. The results from the present study indicate that the majority of IgA eluted from such kidneys is polymers. Recently, Conley, Cooper & Michael (1980) and the authors have reported that IgA1 deposition was mainly observed in glomeruli from patients with IgA nephropathy (Tomino *et al.*, 1982b). Conley *et al.* (1980) noted that IgA deposited in glomeruli was mainly composed of IgA1 monomers since the intensity of J chain deposition was not correlated with IgA by immunofluorescent staining. The difference between their results and ours might be due to the different procedure used for immunofluorescence. Furthermore, the complete absorption of eluted IgA with both anti-human IgA1 antisera and anti-human J chain antisera supports the assumption that such eluted IgA consists of polymers of IgA1.

It is suggested that IgA deposited in glomeruli is composed of dimers and/or larger polymers of

circulating IgA (mainly, IgA1) in some patients with IgA nephropathy. Further study is required to determine whether these observations are ubiquitous findings in all patients with IgA nephropathy.

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