Clin. exp. Immunol. (1989) 77, 211–214

Charge distribution of plasma IgA in IgA nephropathy

T. HARADA, P. HOBBY, M. COURTEAU, J. F. KNIGHT, D. GWYN WILLIAMS *Renal Unit, United Medical and Dental Schools of Guy's and St. Thomas's Hospitals, Guy's Hospital, London, England*

(Accepted for publication 4 May 1989)

SUMMARY

The spectrotype of plasma IgA in patients with IgA nephropathy was studied by isoelectric focussing. Densitometry of the gels showed a significant increase in the anionic region at isoelectric points (pI) 4·7–5·1 ($P=0·02$) and a reduction in the cationic region pI 5·8–6·0 ($P=0·03$) in patients ($n=15$) compared with controls ($n=8$). Measurement of the IgA concentration in eluates of sequential slices of the gels showed that the ratio of anionic-to-cationic IgA, using pI 5·6 as the dividing point, was significantly greater in patients ($n=10$) than in controls ($n=10$) ($P=0·03$). Two different methods of analysis have therefore demonstrated an increased proportion of anionic and decreased proportion of cationic IgA in the plasma of patients with IgA nephropathy.

Keywords IgA IgA nephropathy charge

INTRODUCTION

IgA nephrophathy is characterized by a predominant deposition of IgA in the glomerular mesangium. Although several abnormalities affecting IgA are recognized, such as an increase in IgA-bearing cells (Nomoto, 1979), a decrease of IgA-specific suppressor T cell activity (Sakai, Nomoto, & Arimori, 1979), an increase of IgA-specific T and B cell activity (Egido *et al.*, 1983; Hale *et al.*, 1986), an increase in the serum concentration of IgA and of its polymeric:monomeric ratio (Trascara *et al.*, 1980), and an excess in glomerular IgA of the dimeric form and of negative charge (Timino *et al.*, 1982; Monteiro *et al.*, 1985), the origin of the glomerular IgA and the mechanism of its deposition are unknown.

In order to examine further the role of the electrical charge of IgA in the pathogenesis of IgA nephropathy, we studied the charge distribution of plasma IgA in patients with this disease by isoelectric focusing (IEF) followed by densitometry or elution of the gels.

MATERIALS AND METHODS

Patients

Heparinized blood was collected from 15 patients with IgA nephropathy (age 13–60, mean 36·5 years) proven in each case by immunohistology of a renal biopsy and by the absence of clinical and serological evidence of systemic disease. At the time when the blood samples were taken, 13 of the 15 patients had microscopic haematuria, all had proteinuria of non-nephrotic range, a normal serum creatinine concentration and no macroscopic haematuria.

Correspondence: Dr D. G. Williams, Clinical Science Laboratories, 17th Floor, Guy's Tower, Guy's Hospital, London SE1 9RT, England.

Control subjects

Heparinized blood was taken from 12 healthy adults (age 29–43, mean 35·4 years) as controls. Separated plasma specimens were stored at −70°C.

Preparation of agarose gel plates

Agarose (0·8% isogel agarose-EF, LKB) and D-sorbitol (10%, BDH) were dissolved in 100 ml double-distilled water with constant stirring at 100°C. The solution was degassed under a slightly negative air pressure, and then the temperature was allowed to fall to 75°C. Ampholines (Pharmalyte 3–9, Pharmacia) were added to a final concentration of 2·5% at 75°C; 50 ml of the dissolved gel were poured onto a gelbond film on a horizontal table at 45°C and kept overnight at 4°C.

IEF

IEF was performed on a Multiphor II flatbed electrofocusing unit apparatus (LKB) cooled by a Multitemp II cooling circulator (LKB) at 10°C. All samples were focused with a constant power of 10 W with maximum current of 250 mA and a maximum voltage of 1600 V for 90 min. The anolyte and catholyte were 0·5 M ethanoic acid and 0·5 M sodium hydroxide, respectively. All samples were diluted to a concentration of 1 mg/ml IgA with double-distilled water, and 10 μl of each diluted sample were applied to a piece of filter paper (5 mm × 10 mm) which was placed on the surface of the gel. Marker proteins (isoelectric point (pI) range 4·7–10·6, BDH) were applied to the first and last tracks of each gel to define the gradient of pH across the gel and to demonstrate its uniformity across the width of the gel.

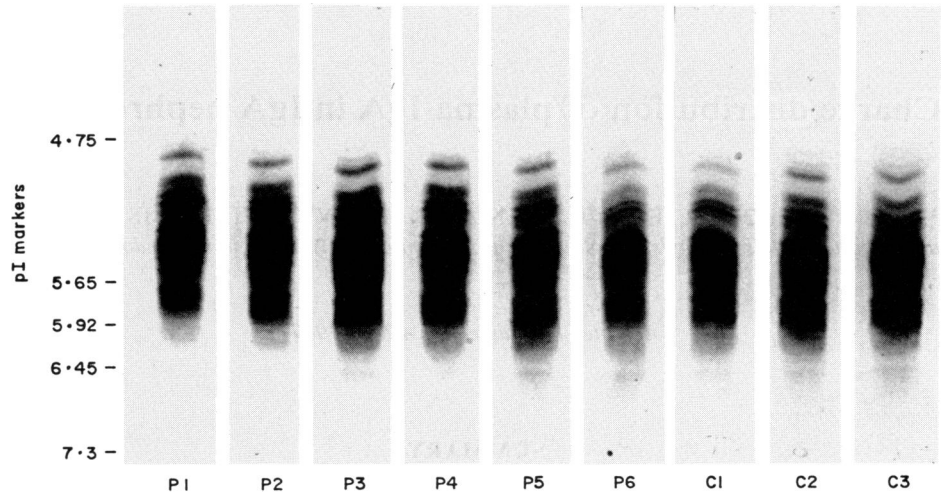

Fig. 1. Example of IEF gel showing spectrotypes of patients with IgA nephropathy (P1–6) and healthy control subjects (C1–3).

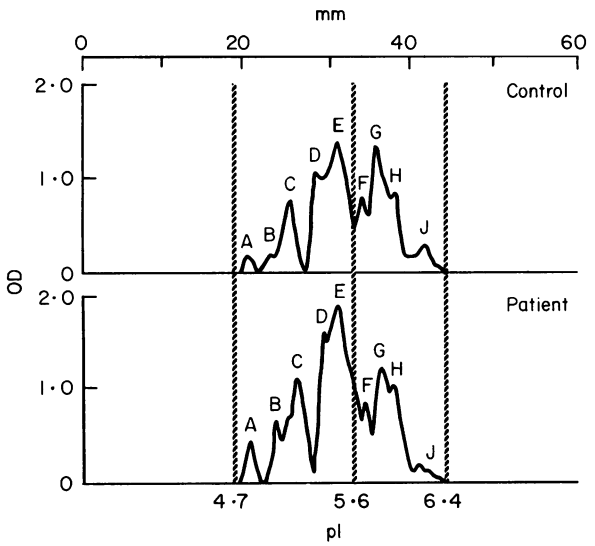

Fig. 2. Examples of densitometry read-out of IEF gel of plasma from a patient with IgA nephropathy and from a healthy control subject

Quantification of IgG spectrotypes

Densitometry. The focused gel was incubated with polyclonal goat anti-human IgA (Sigma), diluted 1:3 with phosphate-buffered saline (PBS) in a humidity chamber for 25 min at 25°C. The gel was washed in several changes of saline for 48 h with continuous gentle agitation. Immunofixed material remaining in the gel after washing was fixed with trichloroacetic acid and sulphosalicylic acid and, stained with Coomassie brilliant blue R250.

Analysis of immunofixed gel. The stained gels were analysed by densitometry (video-densitometer, Bio-Rad) and the relative areas of the individual peaks and their proportional contribution to the total IgA spectrotype were determined by computer.

Elution study

IEF was performed as above, using whole plasma from 10 randomly selected patients with IgA nephropathy, and 10 controls. The gels were then cut transversely into 2-mm slices, and each slice was agitated vigorously in 1 ml PBS-Tween (0·5%) for 24 h for elution. (In a separate series of experiments this method of elution was validated for completeness by showing that strips of gel did not stain for IgA after IEF of plasma). IgA concentrations in the eluates were measured by ELISA.

ELISA for IgA concentration

Multiwell plates (Dynatech) were coated with goat anti-human IgA antibody 1:1000 (Tago) diluted in carbonate-bicarbonate buffer, pH 9·8, and then left overnight at 4°C in a humidity chamber. The plates were washed three times with PBS-Tween and samples were added (75 μl/well) and incubated at room temperature for 90 min. The plates were washed three times with PBS-Tween and incubated with alkaline phosphatase-conjugated goat anti-human IgA antibody (Tago) at 1:4000, 75 μl/well at room temperature for 90 min. After washing with PBS-Tween, the plates were developed by adding phosphate substrate (Sigma, 104) diluted with diethanolamine buffer. The intensity of colour generated was read as absorbance by an automatic plate reader with a 420 mm filter after 30 min (maximum OD 1·2–1·5).

Calculation of anionic:cationic ratio in eluates

The anionic:cationic ratio (A:C ratio) was calculated as follows:

$$\text{A:C ratio} = \frac{\text{Total IgA level (pI} < 5{\cdot}6\text{) in eluates}}{\text{Total IgA level (pI} > 5{\cdot}6\text{) in eluates}}$$

A dividing pI of 5·6 was chosen for its being in the middle of the range as well as being the upper limit of pI found in the glomerular eluates from patients with IgA nephropathy (Monteiro *et al.*, 1985).

Statistical analysis

Significant differences were analysed by the Mann-Whitney *U*-test and adjusted for multiple comparison when appropriate.

Table 1. Percentage contribution of peaks identified in spectrotype of patients with IgA nephropathy and controls.

		Patients ($n=15$)		Controls ($n=8$)		
Peaks (pI range)		Range	Mean (s.d.)	Range	Mean (s.d.)	*P*
A+B+C	(4·7–5·1)	14·0–23·6	18·8 (2·6)	9·7–22·8	16·0 (3·6)	0·02
D+E	(5·1–5·5)	40·3–46·9	43·1 (2·6)	42·0–49·6	44·0 (1·9)	NS
F	(5·5–5·8)	6·4–12·9	9·0 (1·7)	5·1–11·8	8·3 (4·1)	NS
G+H	(5·8–6·0)	15·7–26·4	21·3 (3·2)	18·6–30·3	24·1 (4·3)	0·03
J	(6·0–6·4)	2·4–11·6	7·6 (3·1)	6·4– 3·0	6·7 (3·7)	NS

NS = not significant.

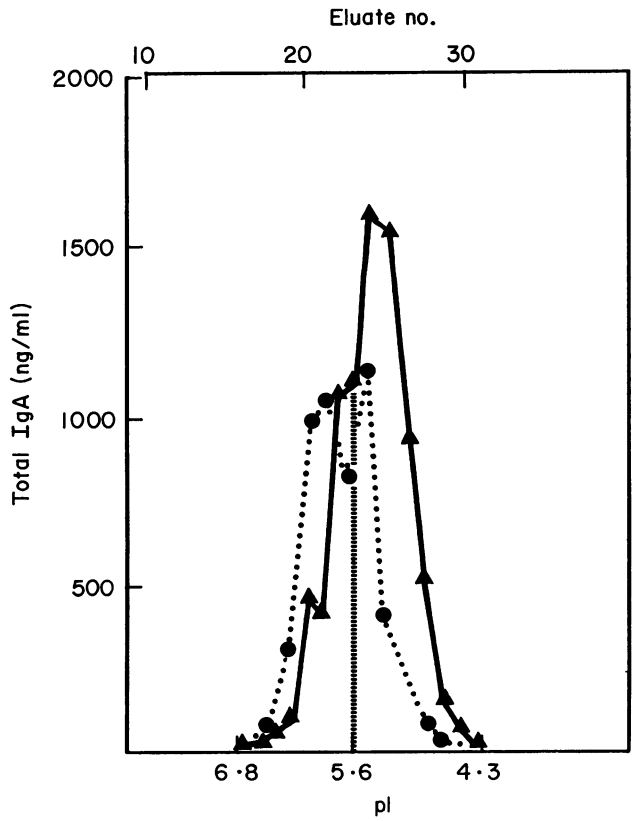

Fig. 3. Concentration and charge distribution of IgA in eluates from IEF gel in a patient with IgA nephropathy (▲) and in a healthy control subject (●).

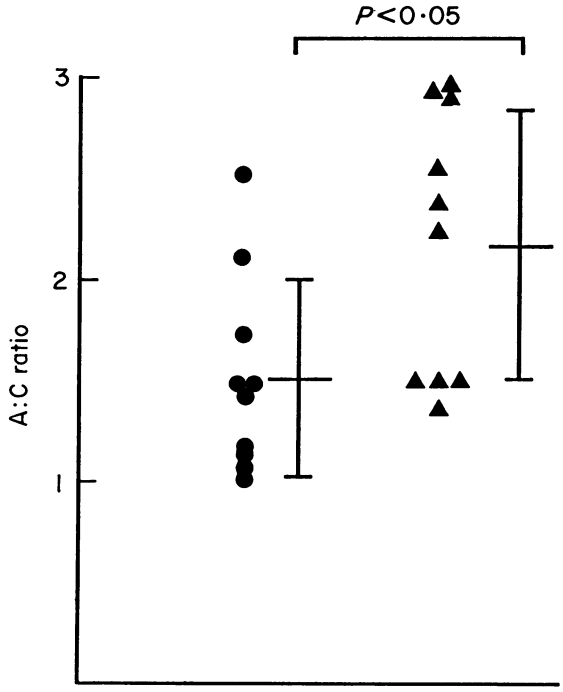

Fig. 4. Anionic:cationic (A:C) ratio of IgA concentrations less than pI 5·6 (anionic) and greater than pI 5·6 (cationic) in eluates from IEF gel of patients with IgA nephropathy (▲) and from healthy control subjects (●).

RESULTS

IEF with immunofixation

Fifteen patients and eight controls were studied. An example of an immunofixed agarose gel is shown in Fig. 1. The bulk of IgA focused in the pI range 4·7–6·4 in patients and controls, and a slight excess anionic distribution of IgA was recognized in several patients with IgA nephropathy (e.g. patients 1, 2 and 3) compared with controls.

Examples of the distribution of IgA in a patient and in a control subject, analysed by microdensitometry, are shown in Fig. 2. Well-defined peaks were common to all the spectrotypes and for the purpose of analysis were used as A+B+C; D+E; F; G+H; and J, reading from the anionic end of the pI range.

The percentage contribution of each of these peaks to the total spectrotype is shown in Table 1. The areas of peaks A+B+C in IgA nephropathy were higher in patients than in controls ($P<0·05$) and those of G+H were lower than controls ($P<0·05$).

Elution

Ten patients and ten controls were studied. The IgA eluted from the gels prepared by IEF was distributed from pI 4·3 to 6·8 in patients, and from pI 4·6 to 6·5 in controls. Figure 3 shows the distribution and concentration of IgA in the eluates in examples from a patient and a control. The A:C ratios of the eluted fractions are shown in Fig. 4; the A:C ratio in IgA nephropathy was significantly higher than in controls ($P<0·05$). The four

patients with an A:C ratio <2·0 formed an apparent cluster, but they were not distinguishable from the remaining patients on either clinical, histological or serological (concentration and charge distribution of plama IgA) grounds (data not shown).

DISCUSSION

Our studies have shown by two different methods that there is an increased proportion of anionic IgA, coupled with a reduced cationic proportion, in the plasma of patients with IgA nephropathy. This shift in charge occurred mainly within the normal range of pI of IgA, and was not related to the age of the patients nor their age at presentation (data not shown).

The charge of IgA eluted from renal biopsies from patients with IgA nephropathy has been found to be in the pI range 4·5–5·6 (Monteiro *et al.*, 1985). Although this finding was not related to the pI of plasma IgA, which was not measured, it is reasonable to assume that the mesangial deposition of IgA was selective for the more negatively charged molecules. Our data, together with a preliminary report of an increased amount of binding of anionic IgA of undefined pI range in sera of patients with IgA nephropathy (Monteiro *et al.*, 1988), suggest that a factor predisposing to mesangial deposition of anionic IgA is an increased availability of negatively charged IgA in the plasma. It must be emphasized, however, that the bulk of plasma IgA in the patients we studied was in the normal range of pI.

The reason for the increased anionic and reduced cationic production of IgA in IgA nephropathy is not known. There are several possible explanations: firstly, it may represent an antibody response to an antigen(s) of predominantly positive charge, in accordance with the observation of reciprocal charges in an antigen–antibody response (Sela & Mozes, 1965). Although IgA nephropathy has been considered an immune-complex disease, for which there has been some evidence, no antigens have been identified. However, recent reports of mesangial cytomegalovirus antigens (Gregory, Hammond & Brewer, 1988) and of circulating antibodies to collagen epitopes (Cederholm *et al.*, 1986) in IgA nephropathy may allow this hypothesis to be tested. Secondly, IgA nephropathy is characterized by an increase in polymeric/dimeric forms of IgA in the plasma and in the glomeruli, and whether the predominance of these IgA molecules would result in a shift of charge from cationic to anionic is not yet known. Thirdly, the production of an increased proportion of anionic IgA, which is probably a reflection of either its glycosylation or amino acid content, or both, may be a marker of predisposition to the disease, either because the anionic IgA itself is involved in the pathogenesis of the nephritis, as discussed below, or because it is linked genetically to some other predisposing factor.

The explanation for the alteration in charge of plasma IgA in IgA nephropathy is obviously related to the question whether it is involved in the pathogenesis of the disease. Experimental work has shown that anionic antigens, antibodies, and immune complexes are more likely to be deposited in the glomerular mesangium (Gallo *et al.*, 1983; Gauthier, Striker & Mannik, 1984; Isaacs & Miller, 1987). The excess amount of anionic IgA may therefore contribute to the pathogenesis of IgA nephropathy by its predeposition to mesangial deposition as a part of circulating immune complexes, or by binding with antigens *in situ*.

ACKNOWLEDGMENTS

We are grateful to the National Kidney Research Fund (P.H.), the Canadian Kidney Foundation (M.C.), the Canteen Committee of the Children's Hospital, Camperdown, Australia (J.F.K.), and the Special Trustees of Guy's Hospital for financial support. We are indebted to Miss J. M. Simpson for her skill and patience in preparing the manuscript.

REFERENCES

Cederholm, B., Wieslander, J., Bygren, P. & Heinegaard, D. (1986) Patients with IgA nephropathy have circulating antibasement membrane antibodies reacting with structures common to collagen I, II, and IV. *Proc. natl. Acad. Sci. USA*, **83**, 6151.

Egido, J., Blasco, R., Sancho, J. & Lozano, L. (1983) T-cell dysfunctions in IgA nephropathy: specific abnormalities in the regulation of IgA synthesis. *Clin. Immunol. Immunopathol.* **26**, 201.

Gallo, G.R., Caulin-Glaser, T., Emancipator, S.N. & Lamm, M.E. (1983) Nephritogenicity and differential distribution of glomerular immune complexes related to immunogen change. *Lab. Invest.* **48**, 353.

Gauthier, V.J., Striker, G.E. & Mannik, M. (1984) Glomerular localization of preformed immune complexes prepared with anionic antibodies or with cationic antigens. *Lab. Invest.* **50**, 636.

Gregory, M.C., Hammond, M.E. & Brewer, E.D. (1988) Renal deposition of cytomegalovirus antigen in immunoglobulin A nephropathy. *Lancet*, **i**, 11.

Hale, G.M., McIntosh, S.K., Hiki, Y., Clarkson, A.R. & Woodroffe, A.J. (1986) Evidence for IgA-specific B-cell hyperactivity in patients with IgA nephropathy. *Kidney Int.* **29**, 718.

Isaacs, K.L. & Miller, F. (1982) Role of antigen size and charge in immune complex glomerulonephritis. *Lab. Invest.* **47**, 198.

Monteiro, R.C., Chevailler, A., Noel, L.H. & Lesavre, P. (1988) Serum IgA preferentially binds to cationic polypeptides in IgA nephropathy. *Clin. exp. Immunol.* **73**, 300.

Monteiro, R.C., Halbwachs-Mecarelli, L., Roque-Barreira, M.C., Noel, L.H., Berger, J. & Lesavre, P. (1985) Charge and size of mesangial IgA in IgA nephropathy. *Kidney Int.* **28**, 666.

Nomoto, Y. (1979) Increase of IgA-bearing lymphocytes in peripheral blood from patients with IgA nephropathy. *Am. J. clin. Pathol.* **71**, 158.

Sakai, H., Nomoto, Y. & Arimori, S. (1979) Decrease of IgA-specific suppressor T-cell activity in patients with IgA nephropathy. *Clin. exp. Immunol.* **38**, 243.

Sela, M. & Mozes, E. (1966) Dependence of the chemical nature of antibodies on the net electrical charge of antigens. *Proc. natl. Acad. Sci. USA*, **55**, 445.

Tomino, Y., Sakai, H., Miura, M., Endoh, M. & Nomoto, Y. (1982) Detection of polymeric IgA in glomeruli from patient with IgA nephropathy. *Clin. exp. Immunol.* **49**, 419.

Trascasa, M.L., Egido, J., Sancho, J. & Hernando, L. (1980) IgA glomerulonephritis: evidence of high serum levels of polymeric IgA. *Clin. exp. Immunol.* **42**, 247.