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Detection of monomeric and polymeric IgA containing immune complexes in serum and kidney from patients with alcoholic liver disease

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SUMMARY

The purpose of this study was to characterize circulating IgA and the IgA deposited in the glomeruli of patients with alcoholic liver disease. In the 6 patients studied there was an increased proportion in monomeric IgA (3·5 fold) and IgA between 9–13S (8·94 fold), 13–17S (4·49 fold) and 17–21S (1·63 fold) fractions on 5–40% sucrose density gradient ultracentrifugation at physiological pH. All fractions between 9–21S decreased at acid pH, however a 3·28 fold increase in fractions where polymeric IgA is expected to appear. IgA eluted at acid pH from autopsy kidney was studied by the same procedures. At pH 7·4 about 55% of that IgA have a molecular weight comprised between 9–21S, decreasing to around 25% at acid pH. The existence of true polymeric IgA in serum and kidney was based on the capacity of high molecular weight IgA to bind human secretory component. The amount of immune complexes with monomeric IgA were higher than those with polymeric IgA in serum as well as in kidney. However, the percentage of heavy IgA (probably polymeric IgA) in kidney were, in each patient, higher than those observed in serum. Our results show the presence of high amounts of monomeric and polymeric IgA, both partially as immune complexes, in serum and kidneys of patients with alcoholic liver disease and IgA glomerulonephritis. Furthermore, our data suggest a role for human liver in the clearance of serum IgA such as has been demonstrated in some animal species, especially in rats.

INTRODUCTION

High serum IgA levels have been frequently found in patients with alcoholic liver disease (Husby *et al.*, 1977; Iturriaga *et al.*, 1977). Deposits of IgA in a granular pattern have been observed in the glomerular mesangium in a large number of cases (Berger, Yaneva & Nabarra, 1978) and more rarely in the hepatic sinusoids and the capillaries of the skin and jejunum (Kater *et al.*, 1979). These facts suggested an abnormality in the IgA itself, even though the nature and biochemical characteristics of the serum IgA and the organ deposited IgA remained unknown.

Previously some authors have observed that sera of patients with alcoholic liver disease contain IgG and IgA immune complexes (IC) detectable by a variety of methods such as Raji cell immunofluorescence test, C1q binding test and anti-antibody neutralization test (Thomas, Potter & Sherlock, 1977; Penner, Albini & Milgrom, 1978; Andre, Druguet & Andre, 1978; Kaufman *et al.*, 1979). However, they could not completely rule out the possibility that aggregated IgA (or

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polymeric IgA) were detected with these assays (Penner *et al.*, 1978). Andre & Andre (1976) have also suggested that an increase of monomeric and dimeric IgA could exist in these patients.

Recently in two IgA-related diseases, IgA mesangial glomerulonephritis (Berger's disease) and Schönlein-Henoch syndrome, we have demonstrated the existence of a large amount of true IgA polymers, partially as immune complexes, in the serum of these patients (López–Trascasa *et al.*, 1979, 1980; Egido *et al.*, 1980a). Furthermore, the presence of polymeric IgA at the mesangial level was demonstrated by immunofluorescence in kidney biopsies based upon the affinity of IgA for secretory component (Egido *et al.*, 1980a).

The aim of this paper was to study the biochemical characteristic of the serum IgA and the IgA eluted by acid pH of autopsy kidneys from patients which presented simultaneously alcoholic liver disease and IgA mesangial glomerulonephritis.

MATERIALS AND METHODS

Patients were studied who had alcoholic liver disease and IgA glomerulonephritis both diagnosed by histological methods (Scheuer, 1973; Berger *et al.*, 1978). All patients had a long history of alcoholic ingestion. None had evidence of any systemic disease. Patients with HBsAg in the serum were discarded.

2 patients had evidence of an active nephropathy (proteinuria, hematuria and moderate renal insufficiency). The rest were asymptomatic. Sera studied were obtained within 3 months of death. Venous blood samples were allowed to clot at 37°C for 1 hr and centrifuged at 2000 ***g*** at room temperature for 15 min. The sera obtained plus 0·05% sodium azide were stored in small aliquots at −20°C and thawed only once. Sera from 10 medical students and staff members were used as controls. Kidneys taken at the moment of autopsy were handled as described below.

Quantitative measurement of IgA from serum and autopsy kidney eluates. Sucrose density gradients (5–40% w/v) were prepared in 5 ml polyallomer tubes. Sucrose was dissolved in 0·15 M Tris-HCl pH 7·4 or in 0·15 M glycine-HCl pH 2·8 buffer. 500 μl aliquots of a concentrated renal eluate (see below) or 50 μl serum samples diluted 1/10 were applied. The conditions of analytical ultracentrifugation and IgA radioimmunoassay were performed as previously described (López-Trascasa *et al.*, 1980).

Immunofluorescence studies. Kidney samples were mounted in Ames OCT compound snap-frozen in liquid nitrogen and stored at −70°C for further processing. Cryostat sections were cut at 3 microns and stained with commercially obtained antisera to human IgG, IgA, IgM, C3, C4. Fibrinogen and Albumin (Meloy Laboratories, Springfield, Virginia). The monospecificity of the antisera was confirmed by immunoelectrophoresis. Rabbit antihuman secretory component (SC) (FICT conjugated) was obtained from Dakopatts (Denmark). The purity and specificity of this reagent were proven as follows: immunoelectrophoresis and Ouchterlony double diffusion analysis showed no precipitation reaction against normal human serum, IgA, IgG and both light chains, but reacted with free human SC isolated as described by Underdown *et al.* (1977) and with human colostrum. This antiserum was further purified by passing it subsequently through a Sepharose 4B column (Pharmacia Fine Chemicals, Uppsala) conjugated to human IgA, IgG and both light chain types. The resulting antiserum gave a single line precipitation with SC.

To examine the presence of polymeric IgA in the mesangium, kidney sections as described above were incubated with purified SC in a moist chamber at room temperature and then washed repeatedly with PBS. The optimal conditions previously obtained were with 150 μg/ml of SC and 30 min incubation time (Egido *et al.*, 1980a). Slides were then incubated with the fluorescent anti–SC antiserum (at 1/15 dilution) for another 30 min. Patients with other nephropathies, with or without IgA deposits, and sections from normal human kidneys were used as controls. A group of patients with diffuse IgM mesangial deposits constituted a positive control group because only J chain containing 19S IgM and polymeric or dimeric IgA show specific non-covalent affinity for free SC *in vitro*.

Elution of immunoglobulins deposited in kidneys. Kidneys of autopsies done within 10 hr after death from patients with proven alcoholic liver disease were used for this study. In six cases with IgA

deposits in the mesangium demonstrated by immunofluorescence technique (some also with IgG and/or C3, but not IgM, deposits) the following elution technique was performed: renal cortex was separated from medulla, weighed, homogenized and washed in PBS, pH 7·4 until supernatant became clear. Afterwards sediment containing the cortical material was mixed with 0·1 M Glycine-HCl buffer, pH 2·8 (1:10, v/v) and incubated at 4°C with constant stirring for 1 hr. After centrifugation at 4°C and 2000 × *g* during 15 min the supernatant was neutralized with 0·5 M NaOH and then dialysed against several changes of PBS. Precipitates were removed and the supernatant concentrated to 1 or 2 volumes. The total protein was measured by the Lowry method (Lowry *et al.*, 1951). The presence of immunoglobulins in the concentrated eluate were assessed by radial immunodiffusion, immunoelectrophoresis, and double immunodiffusion. To eliminate the non-globulin proteins, the eluates were subsequently precipitated by the addition of an equal volume of saturated ammonium sulphate. Afterwards the samples were dialysed overnight against PBS and stored in aliquots at −20°C until their use. As a control the kidney from an autopsy of a patient with hepatic cirrhosis but without immunoglobulin deposits in the glomeruli was treated in the same manner.

Affinity of the secretory component for the polymeric IgA in whole serum and kidney eluates. Human secretory component (SC) was isolated from human whey by affinity chromatography on IgM Sepharose adsorbent (Underdown *et al.*, 1977). The measurement of the affinity of the secretory component for polymeric IgA in whole sera and kidney eluates was based upon the method of Brandtzaeg (1974) partially modified. To eliminate IgM that could interfere with the assay by binding SC, the samples to be tested were previously incubated with an excess of insolubilized anti-IgM Sepharose 4B immunoadsorbent. Briefly, the assay was performed as follows: serum samples of 100 μl (diluted ½ with PBS) or eluted IgA from kidneys (both without containing IgM) were incubated for 2 hr at room temperature with ^{125}I-SC, labelled by the lactoperoxide (Thorell & Johansson, 1971) at an initial specific activity of 0·5–1 mCi/mg, in a final molar SC:IgA ratio higher than 1:75. After incubation the IgA ^{125}I-SC complexes formed were separated from free ^{125}I-SC by ultracentrifugation on a 5–40% sucrose density gradient as described above, measuring the c.p.m. in each fraction.

RESULTS

Serum studies

The mean absolute and percentual values of serum IgA from patients and controls according to the different molecular weight, established by sucrose density gradient ultracentrifugation at pH 7·4 are shown in Table 1. In patients with alcoholic liver disease there was a significant increase in serum concentrations of IgA in fractions with sedimentation constants between 9–13S (8·94 fold than controls), 13–17S (4.49 fold than controls), 17–21S (1·63 fold than controls), where the polymeric IgA is expected. There was also an increase in monomeric IgA (5–9S fractions) in patients (3·5 fold) in relation to a group of ten normal sera. To observe how much of that IgA with high molecular weight was forming part of immune complexes, the same samples were analyzed by sucrose density gradient ultracentrifugation at pH 2·8. As can be seen in Table 2 there was an important decrease in the levels of IgA comprised between 9–13S, 13–17S and 17–21S, and an increase of IgA in 5–9S fractions. These results are compatible with the presence of IgA immune complexes in the serum of these patients. In addition, the comparison of absolute IgA amounts found in 9–13S fractions after centrifugation at pH 2·8 in relation to normal controls (Table 2) suggests the presence of high levels of true polymeric IgA in these patients. To observe whether these high IgA levels were merely a reflect of the total increase of serum IgA, the results were also represented in percentual values (Table 1). As can be seen, there was a significant increase in 9–13S fractions and a decrease in 5–9S fractions confirming the presence of an absolute and percentual increase of high molecular weight IgA, partially as immune complexes, in the serum of the patients.

To further verify whether this IgA with high molecular weight correspond to true IgA polymers the affinity of whole serum IgA for free labelled secretory component was studied. As can be observed in Table 3 & Fig. 1, there was an increase in the binding affinity to secretory component in

Table 1. Distribution of absolute and percentual values of IgA in the sera after fractionation of sucrose gradient at pH 7·4

	Normal sera ($n=10$)	ALD[(a)] sera (n = 6)	P Value†
5–9S	(A) 147·03 ± 56·31*	515·6 ± 196·3	$P<0.0005$
	(P) 72·09 ± 6·5	65·07 ± 21·41	$P<0.0025$
9–13S	51·1 ± 30·88	457·0 ± 434·9	$P<0.01$
	23·11 ± 6·96	33·23 ± 21·23	$P<0.05$
13–17S	6·26 ± 2·72	28·1 ± 14·8	$P<0.0005$
	2·98 ± 0·83	1·47 ± 0·88	$P<0.0025$
17–21S	0·57 ± 0·47	0·9 ± 0·9	n.s.
	0·48 ± 0·48	0·12 ± 0·10	$P<0.05$

* Mean ± 1 standard deviation.
† Unpaired t-test.
(A) = Absolute values are expressed in mg% of IgA in each zone.
(P) = Percentual values respect to total IgA.
n.s. = Not significant.
ALD[(a)] = Patients with alcoholic liver disease.

comparison with that of a pool of 10 normal sera. These data suggest that patients with alcoholic liver disease present high serum levels of true polymeric IgA that could be in part as immune complexes.

Kidney studies

To characterize the IgA deposited in the glomerular mesangium of these patients we planned two types of approaches. The first one consisted in the examination of the IgA eluted from autopsy kidneys and the second one was based upon the affinity of polymeric IgA for the SC that could be studied either by immunofluorescence or ultracentrifugation after incubation with ^{125}I-SC.

(a) *Study of the IgA eluted from autopsy kidneys.* 6 autopsy kidneys, which showed IgA deposits by immunofluorescence at the mesangial level, were available for study. The IgA was eluted at acid pH relatively easily as stated in Methods. Between 6 and 68 μg of IgA/g of renal cortex could be recovered (mean 24·56). To rule out that the IgA eluted from the autopsy kidneys were a serum IgA

Table 2. Distribution of absolute values of serum IgA after ultracentrifugation in sucrose density gradients in patients with alcoholic hepatic disease and controls

	5–9S	9–13S	13–17S	17–21S
pH 7·4	558 ± 229(a)*	620·7 ± 326·5	18·8 ± 13·8	0·8 ± 0·9
	$P<0.05$†	$P<0.05$	$P<0.05$	n.s.
pH 2·8	1045·7 ± 374·9	172·4 ± 221·3	3 ± 1·7	0·09 ± 0·1
	(7·24)‡	(3·28)	(0·67)	(0·12)
NHS (pH 2·8)§	144·4 ± 16·9	52·5 ± 15·5	4·4 ± 1·7	0·7 ± 0·2

(a) Results are expressed in mg% of IgA in each zone.
* = Mean ± standard deviation of three patients. + = Paired t-test; n.s. = not significant (b) ‡ Fold increases of mean values in patients in relation with the mean values in controls; § = No significant changes were seen between normal sera fractionated at 7·4 and 2·8 pHs.

Table 3. Affinity of the secretory component for the serum polymeric IgA*

	% binding of ^{125}I SC
Patient 1	54·4
Patient 2	47·1
Patient 3	56·7
Patient 4	47·5
Control pool sera	23·0

* Serum samples of 100 μl (diluted $\frac{1}{2}$ with PBS) from patients and pool control sera were incubated with ^{125}I SC for 2 hr at room temperature, centrifuged on 5–40% sucrose density gradients and the binding to ^{125}I SC counted. The values express the % of total ^{125}I SC added and found in the fractions when the polymeric IgA is expected to appear (see Materials and Methods).

contaminant, a cirrhotic kidney without deposits of IgA by immunofluorescence was submitted to the same elution conditions as the other ones. Only trace amounts of IgA could be detected. For this reason the IgA eluted in the other patients was considered true mesangial IgA. This eluted IgA enabled us to fraction the IgA deposited in the kidney and to study the different molecular weights. As shown in Fig. 2 at physiological pH more than 50% of eluted IgA has a molecular weight higher than 9S. After treatment with acid there was a significant decrease in 9–13S and a significant

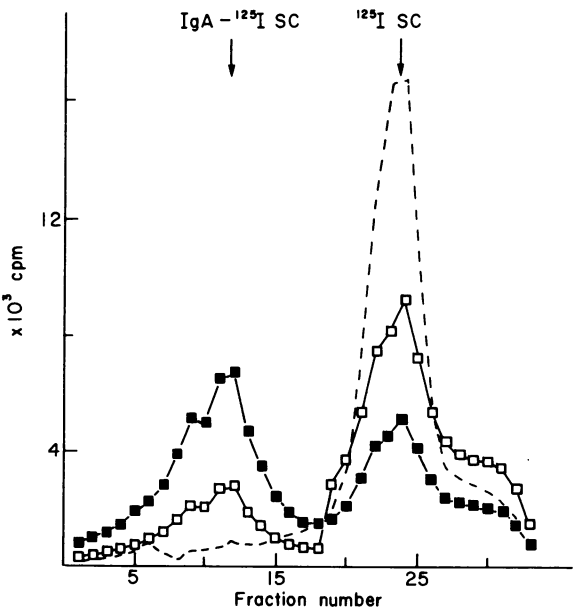

Fig. 1. Representative distribution of radioactivity after sucrose-density gradient ultracentrifugation at pH 7·4 of control and patient serum incubated with ^{125}I-SC (secretory component). See legend Table 3. Patient 3 (■———■); pool control sera (□———□); free ^{125}I-SC (- - - -). Bottom of the gradient to the left. The position of markers in the fraction number was as follows: IgM (No. 3); IgG (No. 18); BSA (No. 21).

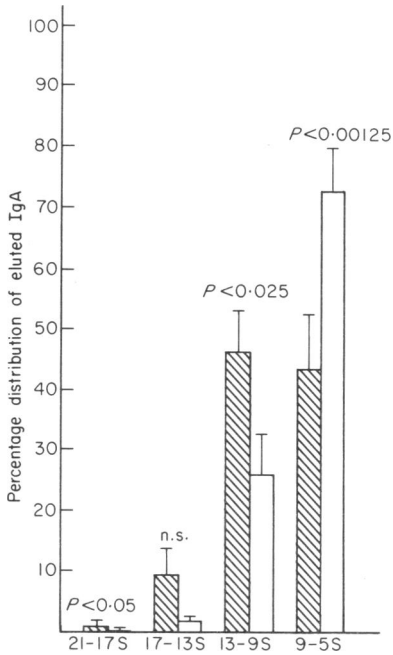

Fig. 2. Percentage distribution of kidney eluted IgA after ultracentrifugation in sucrose density gradients at pH 7·4 (▨) and 2·8 (□) Mean ± standard deviation.

increase in 5–9S fractions. These results are compatible with the presence of polymeric and monomeric IgA containing immune complexes. To further verify if the eluted IgA with high molecular weight possessed some other characteristic of polymeric IgA the binding to SC was studied. In the 6 eluates the ranges of SC binding percentages were between 17–32% (mean 22·84 ± 7·06) suggesting that a part of deposited mesangial IgA was true polymeric IgA.

(b) *Immunofluorescence study of kidney IgA.* In 3 autopsy kidneys the secretory component binding test could be studied by immunofluorescence. There was a positive fixation of SC with a granular pattern similar to that of IgA. 3 patients with lupus nephropathy and 5 mesangiocapillary glomerulonephritis with IgA glomerular deposits and 4 normal human kidneys showed no fixation to SC. 4 patients with IgM mesangial glomerulonephritis were used as positive controls because J chain from polymeric IgM show specific non-covalent affinity for free SC *in vitro* (Brandtzaeg, 1974). These experiments further confirm that apart from other situations in which polymeric IgA has been demonstrated in the kidney (Egido *et al.*, 1980a) only glomerular IgA from patients with alcoholic liver disease shows the characteristics of polymeric or dimeric IgA.

Comparison between molecular sizes of eluted and serum IgA

As we have seen the most dramatic changes in serum IgA in these patients were observed in the absolute values either of monomeric or of IgA in the 9–12S fractions. However, in an attempt to know whether the IgA deposited in the mesangium was merely a reflection of the serum IgA, or whether the heavy IgA was predominant at that site, a comparison between the amounts of IgA of different molecular weights was made. The IgA percentages between 9–21S fractions in the serum of the 6 patients studied at physiological pH were less than 40% of total IgA, versus around 55% in the same fractions of the kidney eluates (Table 1, Fig. 2). These data suggest that heavy IgA was predominantly deposited in the mesangium. The results in the 3 patients in which the serum and eluates could be studied simultaneously at 7·4 and 2·8 pHs are shown in Fig. 3. As can be observed, at pH 2·8 there was an increase in monomeric IgA and a decrease in 9–21S fractions compared with

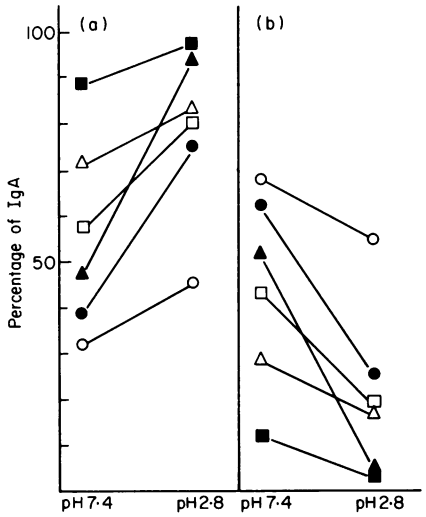

Fig. 3. Comparison of IgA percentages established by sucrose density gradients ultracentrifugation of serum and eluates from 3 patients in whom it was possible to study it simultaneously (■ ▲ ● = serum; □ △ ○ = eluates; each symbol represents one patient). As can be observed at pH 2·8 there was an increase in the 5–9S (a) fractions and a decrease in 9–21S (b) fractions in relation to the studies performed at pH 7·4. This is compatible with the presence of IgA containing immune complexes in both serum and eluates. Note that in each patient the IgA in fractions 9–21S (where polymeric IgA is expected) is higher in eluates than in sera.

the studies performed at pH 7·4. This is compatible with the presence of IgA containing immune complexes in both serum and eluates. However, in each patient the IgA in the 9–21S fractions (where polymeric IgA is expected) was higher in the eluate than in serum.

DISCUSSION

The relation between IgA and liver diseases, more precisely alcoholic liver disease, has been discussed during the last few years (Andre & Andre, 1976; Iturriaga *et al.*, 1977; Berger *et al.*, 1978; Kater *et al.*, 1979). A polyclonal increase in serum immunoglobulin levels, predominantly IgG and IgA are frequently found in these patients, probably reflecting the occurrence of antibodies to substances absorbed from the gut (Stobo, 1979). Levels of serum IgA correlated with the severity of liver damage (Iturriaga *et al.*, 1977). The occurrence of glomerulonephritis in patients with liver diseases has been known for a long time (Callard *et al.*, 1975; Nochy *et al.*, 1976). The predominance of glomerular IgA deposits in patients with alcoholic liver disease is relevant; Berger *et al.* (1978) found that 61 out of 100 patients with cirrhosis of the liver (90 of whom had alcoholic cirrhosis) presented, at the time of autopsy, granular deposits of IgA in the glomeruli.

IgA in the serum could theoretically circulate in the form of immune complexes (IC) or aggregates. The presence of IgG-IC in sera of patients with alcoholic liver disease has been demonstrated by several authors (Thomas *et al.*, 1977; Andre *et al.*, 1978; Penner *et al.*, 1978; Kaufman *et al.*, 1979). However, most current assays for detection of circulating IC are not suitable for the detection of IgA-IC, because they do not distinguish between non-specifically aggregated IgA, polymeric IgA and complexed IgA (Penner *et al.*, 1978). The fractionation of sera by sucrose density ultracentrifugation at physiological and acid pHs, though it is not a technique to employ routinely, allows the differentiation between IgA-IC and aggregated IgA and to determine the amount of IgA of different molecular sizes (Benveniste, J. & Bruneau, C., 1979; López-Trascasa *et al.*, 1980).

In this paper we show that patients with alcoholic liver disease and IgA nephropathy have an

increase in monomeric IgA and IgA with a molecular weight between 9–21S in relation to controls. Of this heavy IgA approximately two thirds were in form of IC. The ability for binding the secretory component permitted us to confirm the existence of polymeric IgA, whose serum levels are about three times higher than those found in normal control sera (Table 2).

The origin of these high levels of monomeric and polymeric IgA are not completely elucidated. A large amount of evidence has been published in the last years concerning the pivotal role of the liver, at least in some animal species (Hall, Gyure & Payne, 1980), in the blood clearance of IgA probably through the secretory component (SC) as a receptor (Lemaitre-Coelho, I., Jackson, G.D.F. & Vaerman, J.P., 1977; Hopf *et al.*, 1978; Orlans *et al.*, 1979; Socken *et al.*, 1979; Fisher *et al.*, 1979). In fact blood retention of IgA has been observed in rats with ligated bile ducts (Orlans *et al.*, 1978) and with liver damage (Kaartinen, 1978). Although a similar mechanism has not been demonstrated in man the existence in serum of large amounts of monomeric and polymeric IgA found in the patients with important liver disfunction suggests a role for the human liver in IgA clearance.

The biochemical characteristics, size and class of IgA deposited in the glomeruli remained to be established. The granular aspect of the deposits suggested immune complex deposition. That we were able to elute the IgA from kidney at acid pH, which is known to elute the antibody part of IC, suggest that we are dealing with true IC. Although the data obtained on eluted proteins should always be considered with caution, the diminution of heavy IgA in the eluate samples after submitting them again to acid pH (Table 2) suggests that part of the IgA might be eluted from glomeruli in an IC with the corresponding antigens. The ability of kidney IgA to bind SC by the two methods employed confirms that a part of the IgA deposited in the mesangium is true polymeric IgA. The SC binding assays used in this paper are based upon the specific fixation of SC to J chain containing immunoglobulins (polymeric IgA and IgM) and has been successfully applied to study immune cells in the intestinal epithelium, circulating B cells and polymeric IgA in fluid phase (Brandtzaeg, 1974, 1976, 1978).

The mechanism of IgA deposition and its long persistence in the glomeruli are unknown. The impairment of hepatic clearance of polymeric IgA, such as observed in rats with liver damage (Kaartinen, 1978) or ligated bile ducts (Orlans *et al.*, 1979), could also be true for patients with alcoholic liver disease allowing the persistence and subsequent deposition in tissues of IgA-IC.

The amounts of IC with monomeric IgA were higher than those with polymeric IgA in serum, as well as in kidney. However, the fraction of heavy IgA (probably polymeric IgA) in the kidney were, in each patient, higher than those observed in serum. The ultracentrifugation studies on both serum and eluted IgA at acid pH does not exclude completely that a proportion of polymeric IgA could circulate uncomplexed to any antigen and be deposited in the kidney in such a form.

The immunopathogenetic role of polymeric IgA in the induction of IgA nephropathy is unclear. In this regard in two human nephritides such as Berger's disease and Schönlein–Henoch syndrome, characterized by the predominance of IgA deposits in glomeruli, high levels of polymeric IgA have been found in the serum and kidneys of these patients suggesting a common pathogenesis for the two entities (Egido *et al.*, 1980a, b). Furthermore, in a recent animal model of IgA nephropathy, polymeric IgA was observed to be critical for renal deposition of complexes and induction of nephritic histological changes. Immune complexes formed either *in vivo* or *in vitro* with monomeric IgA failed to induce glomerulonephritis (Rifai *et al.*, 1979).

Although many aspects remain to be elucidated, the presence of polymeric IgA, partially as immune complexes, in the serum and kidneys from patients with alcoholic liver disease. Berger's disease and Schönlein–Henoch syndrome suggests a common pathogenesis for kidney involvement. In that sense the term polymeric IgA disease is suggested.

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REFERENCES

ANDRE, F. & ANDRE, C. (1976) Participation de l'immunoglobuline A monomerique et dimerique à l'hypergammaglobulinemie de la cirrhose ethylique. *Biol. Gastroenterol.* **9,** 147.

ANDRE, F., DRUGUET, M. & ANDRE, C. (1978) Effect of food in circulating antigen–antibody complexes in patients with alcoholic liver cirrhosis. *Digestion*, **17,** 554.

BENVENISTE, J. & BRUNEAU, C. (1979) Detection and characterization of circulating immune complexes by ultracentrifugation. I. Technical aspect. *J. Immunol. Methods*, **26,** 99.

BERGER, J., YANEVA, H. & NABARRA, B. (1978) Glomerular changes in patients with cirrhosis of the liver. *Adv. Nephrol.* **7,** 3.

BRANDTZAEG, P. (1974) Characteristics of SC–IgA complexes formed *in vitro*. *Adv. exp. Med. Biol.* **45,** 87.

BRANDTZAEG, P. (1976) Studies on J chain and binding site for secretory component in circulating human B cells. II. The cytoplasm. *Clin. exp. Immunol.* **25,** 59.

BRANDTZAEG, P. (1978) Polymeric IgA is complexed with secretory component (SC) on the surface of human intestinal epithelial cells. *Scand. J. Immunol.* **8,** 39.

CALLARD, P., FELDMAN, G., PRANDI, D., BELAIR, M.F., MANDET, C., WEISS, Y., DRUET, P., BENHAMON, J.P. & BARIETY, J. (1975) Immune complex type glomerulonephritis in cirrhosis of the liver. *Am. J. Pathol.* **80,** 329.

EGIDO, J., SANCHO, J., MAMPASO, F., LÓPEZ-TRASCASA, M., SÁNCHEZ-CRESPO, M., BLASCO, R. & HERNANDO, L. (1980a) A possible common pathogenesis of the mesangial IgA glomerulonephritis in patients with Berger's disease and Schönlein–Henoch syndrome. *Proc. Eur. Dial. Transplant Ass.* **17,** 660.

EGIDO, J., LÓPEZ-TRASCASA, M., MAMPASO, F., SANCHO, J. & HERNANDO, L. (1980b) Polymeric IgA glomerulonephritis. *4th International Congress of Immunology, Paris. Abs. 18.5.09.*

FISHER, M.M., NAGY, B., BAZIN, H. & UNDERDOWN, B.J. (1979) Biliary transport of IgA: role of secretory component. *Proc. Natl. Acad. Sci. USA*, **76,** 2008.

HALL, J.G., GYURE, L.A. & PAYNE, W.R. (1980) Comparative aspects of the transport of immunoglobulin A from blood to bile. *Immunology*, **41,** 899.

HOPF, V., BRANDTZAEG, P., HUTTEROTH, T.H. & MEYER ZUM BUSCHENFELDS, K.H. (1978) *In vivo* and *in vitro* binding of IgA to the plasma membranes of hepatocytes. *Scand. J. Immunol.* **8,** 543.

HUSBY, G., SKREDE, S., BLOMHOFF, J.P., JACOBSON, C.T., BERG, K. & GJONE, E. (1977) Serum immunoglobulins and organ nonspecific antibodies in diseases of the liver. *Scand. J. Gastroent.* **12,** 297.

ITURRIAGA, H., PEREDA, T., ESTÉVEZ, A. & UGARTE, G. (1977) Serum immunoglobulin A changes in alcoholic patients. *Ann. Clin. Res.* **9,** 39.

KAARTINEN, M. (1978) Liver damage in mice and rats causes tenfold increase of blood immunoglobulin A. *Scand. J. Immunol.* **7,** 519.

KATER, L., JOBSIS, A.C., BAART DE LA FAILLE KUYPER, E.H., VOGTEN, A.J.M. & GRIJM, R. (1979) Alcoholic hepatic disease. Specificity of IgA deposits in liver. *Am. J. clin. Pathol.* **71,** 51.

KAUFMAN, R.L., HOEFS, J.C., QUISMORIO, F.P. & TONG, M. (1979) Circulating immune complexes in patients with alcoholic liver disease. *Clin. Res.* **27,** 38A.

LEMAITRE-COELHO, I., JACKSON, G.D.F. & VAERMAN, J.P. (1977) Rat bile as a convenient source of secretory component. *Europ. J. Immunol.* **8,** 588.

LÓPEZ-TRASCASA, M., EGIDO, J., SANCHO, J. & HERNANDO, L. (1979) Evidence of high polymeric IgA levels in serum of patients with Berger's disease and its modification with phenytoin treatment. *Proc. eur. dial. Transplant Assoc.* **16,** 513.

LÓPEZ-TRASCASA, M., EGIDO, J., SANCHO, J. & HERNANDO, L. (1980) IgA glomerulonephritis (Berger's disease): evidence of high serum levels of polymeric IgA. *Clin. exp. Immunol.* **42,** 247.

LOWRY, O.H., ROSENBROUGH, N.S., FARR, A.L. & RANDALL, R.J. (1951) Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **142,** 265.

NOCHY, D., CALLARD, P., BELLON, B., BARIETY, J. & DRUET, P. (1976) Association of overt glomerulonephritis and liver disease: a study of 34 patients. *Clin. Nephrol.* **6,** 422.

ORLANS, E., PEPPARD, J., REYNOLDS, J. & HALL, J.G. (1978) Rapid active transport of IgA from blood to bile in rats. *J. exp. Med.* **147,** 588.

ORLANS, E., PEPPARD, J., FRY, J.F., HINTON, R.H. & MULLOCK, B.M. (1979) Secretory component as the receptor for IgA on rat hepatocytes. *J. exp. Med.* **150,** 1577.

PENNER, E., ALBINI, B. & MILGROM, F. (1978) Detection of circulating immune complexes in alcoholic liver disease. *Clin. exp. Immunol.* **34,** 28.

RIFAI, A., SMALL, P.A., PERRY, J., TEAGUE, O. & AYOUB, E.M. (1979) Experimental IgA nephropathy. *J. exp. Med.* **150,** 1161.

SCHEUER, P.J. (1973) Liver biopsy interpretation. 2nd ed. Baillière and Tindall, London.

SOCKEN, D.J., JEFFERSON, K.N., BAZIN, H. & UNDERDOWN, B.J. (1979) Identification of secretory component as an IgA receptor on rat hepatocytes. *J. exp. Med.* **150,** 1538.

STOBO, J.D. (1979) Cirrhosis and hypergammaglobulinemia. *Digestive Diseases and Sciences*, **24,** 737.

THOMAS, H.C., POTTER, B.J. & SHERLOCK, S. (1977) Is primary biliar cirrhosis an immune complex disease? *Lancet*, **ii,** 1261.

THORELL, J.I. & JOHANSSON, B.G. (1971) Enzymatic iodination of polypeptides with ^{125}I to high specific activity. *Biochim. Biophys. Acta.* **251,** 363.

UNDERDOWN, B.J., DE ROSE, J., KOCZEKAN, K., SOCKEN, D. & WEICKER, J. (1977) Isolation of human secretory component by affinity chromatography on IgM-Sepharose. *Immunochemistry*, **14,** 111.