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Immune complexes in IgA nephropathy: presence of antibodies against diet antigens and delayed clearance of specific polymeric IgA immune complexes

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SUMMARY

Several features suggest that IgA nephropathy is an immune complex (IC)-mediated disease. The source of antigen(s) is unknown but the predominant involvement of IgA suggest that it is associated in some way with the gut or respiratory tract. Taking into account the specific hepatobiliary transport by polymeric IgA of circulating antigens entering through the mucosal surfaces we examined the possible involvement of antibodies against food antigens in the circulating IC and the existence of a defect in their blood clearance in patients with IgA nephropathy. A rise in multimeric IgA-IC (Raji assay) occurred in three of seven control subjects with a peak at 2–4 h after food ingestion. The amount of multimeric IgA-IC present at fasting in four out of six patients, diminished 2–4 h after food challenge, reaching a new peak around 6 h. At fasting, three out of six patients had IC containing antibodies against diet antigens (e.g. ovalbumin). These IC paralleled, both in patients and controls, the levels of multimeric IgA-IC. In patients small multimeric IgA-IC predominated at fasting and 24 h after food ingestion, while larger IC were detected at 2–4 h of food challenge. The specific polymeric IgA-IC showed in controls a maximal peak with similar distribution to that of multimeric IgA-IC, but with a quicker disappearance from the circulation. By contrast, polymeric IgA-IC remained elevated 24 h after food ingestion in most patients. These results suggest that antibodies against common antigens are within circulating IC and that a defect in the hepatic clearance of circulating polymeric IgA-IC exists in patients with IgA nephropathy.

Keywords IgA nephropathy polymeric IgA diet antigens immune complexes

INTRODUCTION

Several reports have recently demonstrated the presence of IgA and IgG immune complexes (IC) in patients with primary IgA nephropathy (reviewed by Egido *et al.*, 1982b) but the antigens implicated are not known. The elevation of serum antibody titres to respiratory pathogens (mycoplasma pneumoniae, herpes virus, influenza), gut flora (*E. Coli* 07) and bovine serum albumin found in these patients suggest that common antigens may be involved (Woodroffe *et al.*, 1977). It is possible that a specific abnormality in the regulation of IgA may have a role (Sakai, Nomoto & Arimori, 1979; Egido, Blasco & Sancho, 1983a, Egido *et al.*, 1983b).

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The existence of high serum IgA levels in a large proportion of patients with IgA nephropathy (reviewed by Egido *et al.*, 1982b) suggests that, together with an increased production of IgA (Egido *et al.*, 1982a), a defect in the hepatic clearance of IgA, similar to that observed in certain liver diseases (Kaartinen, 1980; Sancho *et al.*, 1982), might occur. Taking into account the specific hepatobiliary transport by IgA of circulating antigens entering through mucosal surfaces (Russell, Brown & Mestecky, 1982), the study of the effect of food protein adsorption on circulating IC of patients with IgA nephropathy seems interesting. In fact, circulating soluble IC have been demonstrated after food ingestion in healthy subjects (Delire, Cambiaso & Masson, 1978; Paganelli *et al.*, 1979), in patients with food allergy (Paganelli *et al.*, 1979; Brostoff *et al.*, 1979), IgA deficiency (Cunningham-Rundles *et al.*, 1979) and idiopathic glomerulonephritis (Cairns, London & Mallick, 1981).

In this paper we examined two hypotheses, that in the immune complexes of patients with IgA nephropathy there are antibodies against food antigens, and that the clearance of specific polymeric IgA-IC is delayed in this disease.

MATERIALS AND METHODS

Human sera. Seven healthy non-atopic adults and six patients with IgA nephropathy with normal renal function, moderate proteinuria and microhaematuria were subjected to an oral challenge of 100 g of protein. Sequential blood samples were collected after an overnight fast and 2,4,6 and 24 h after the food intake. The blood was clotted at room temperature for 60–120 min, centrifuged and the sera stored at $-70°C$ in aliquots until analysis. Each aliquot was thawed at room temperature and used only once.

Isolation of proteins. Human secretory component (SC) was isolated from human whey according to Underdown *et al.* (1977). Human IgG and IgM was obtained from a myeloma by standard methods. Both immunoglobulins were used as markers in the ultracentrifugation studies.

Radiolabelling. Bovine serum albumin (BSA), ovalbumin (OA), the γ-globulin fraction of the rabbit anti-human IgG or anti human IgA serum (Behringwerke AG, Marburg, West Germany) and human IgM were iodinated with ^{125}I according to the procedure of McConahey & Dixon (1966). SC were iodinated by the glucose oxidase–lactoperoxidase method as described by the manufacturers (BioRad Laboratories, California, USA).

Specific IgE antibodies. Specific IgE antibodies to whole egg and cow milk antigens were measured by the RAST technique (Pharmacia, Uppsala, Sweden).

Immune complexes containing antibodies against food antigens. Purified bovine serum albumin (BSA, Fraction V) and ovalbumin (OA, grade V) were obtained from Sigma Chemical Co, St Louis, Missouri, USA. Antibodies to these proteins were quantitated in polyethylene glycol precipitated IC by a radiometric immune assay according to the method of Paganelli, Levinski & Atherton (1981) with only one modification, the antigens were radiolabelled instead of the antisera.

IgA and IgG containing IC. IgG-IC were measured by the Raji cell radioimmunoassay (Raji IgG-IC) (Theofilopoulos, Wilson & Dixon, 1976). IgA-IC were determined by the Raji cell radioimmunoassay modified by Hall *et al.* (1980) (Raji IgA-IC) and by an anti-IgA inhibition binding assay (a-IgA Inh BA) (Kauffmann, Van Es & Daha, 1980). Specific polymeric IgA-IC were detected by the Raji SC binding assay previously described (Sancho, Egido & Gonzalez, 1983). The mean duplicate values of the test sera (Ti) in the different assays was divided by an upper 95% confidence limit (Ui) constructed as described previously by Woodroffe *et al.* (1977) and by Hall *et al.* (1980). The mean of the duplicate values of the test sera (Ti) was divided by Ui. If the value Ti/Ui was $\geqslant 1$, the test serum was judged abnormal.

Sucrose density gradient ultracentrifugation. Sucrose density gradient ultracentrifugation experiments were performed with 5–40% sucrose as published (Lopez-Trascasa *et al.*, 1980). After centrifugation the different fractions were tested for IC by the techniques described above.

RESULTS

IgA- and IgG-IC and antibodies against food antigens within IC prior to the meal

The presence of IgA- and IgG-IC, as well as the amount of specific antibodies to food antigens in circulating IC, was studied in healthy non-atopic adults and in patients with IgA nephropathy. Prior to the food ingestion antibodies to BSA in PEG precipitated IC were detected only in one of the seven controls (case 6, Ti/Ui = 1·87). In the other six individuals we could not detect antibodies to ovalbumin as well as IgA or IgG-IC by the four techniques employed (data not shown). In the same way only one out of six patients with IgA nephropathy had antibodies to BSA prior to the meal (Table 1). However, the presence of antibodies to ovalbumin and IgA-IC measured by the Raji cell assay were closely related. The only exception was case 3, who had no detectable antibodies to ovalbumin and in whom the level of IgA-IC was low. There was no correlation between antibodies to ovalbumin and the IgA or IgG-IC detected by the other techniques.

The effect of protein ingestion on circulating IC

IgG IC. Fig. 1 shows the IgG-IC levels in patients with IgA nephropathy before and after food ingestion. In five out of six patients there was a fall in the levels of IgG complexes to below fasting

Table 1. Circulating IC in patients with IgA nephropathy before food ingestion

	Anti-BSA-IC*	Anti-OA-IC	IgA Raji-IC	IgG Raji-IC	a-IgA-Ih-IC	Polymeric IgA-IC
Case 1	2·08	0·82	0·9	0·94	1·0	0·75
Case 2	0·81	1·04	1·19	1·0	1·11	0·83
Case 3	0·44	0·78	1·07	0·85	0·94	1·02
Case 4	0·41	1·10	1·21	0·68	0·84	0·73
Case 5	0·77	0·68	0·74	0·70	0·93	0·87
Case 6	0·35	1·28	1·20	0·71	0·97	0·76

* Levels of IC with a Ti/Ui ratio lower than 1 are considered negative.

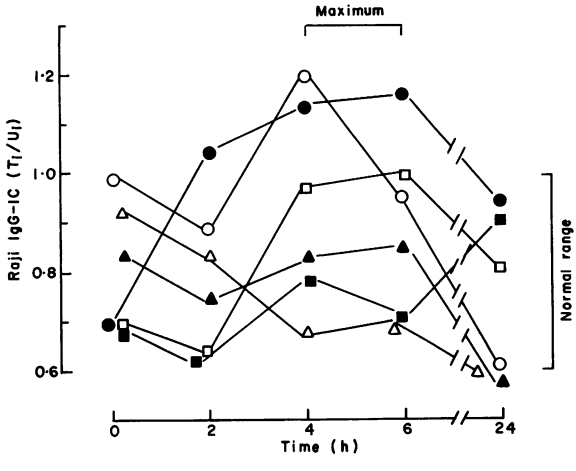

Fig. 1. Levels of Raji IgG-IC in six patients with IgA nephropathy after food ingestion. Patient 1 (△), patient 2 (○), patient 3 (▲), patient 4 (●), patient 5 (□), patient 6 (■). 0 hours represents the timing of finishing the meal.

values at 2 h after protein challenge. After this, a rise in the levels of IgG complexes was seen in five patients 4–6 h after the meal. However, at this time only three of them were above the normal range previously established for the assay (Ti/Ui $\geqslant$ 1) and returned to fasting or even lower levels, 24 h after food. The seven normal individuals were within normal values before and after the meal. Only in patient 1 was seen a similar distribution of IgG and BSA containing IC (results not shown).

IgA-IC and antibodies against food antigens within IC. A rise in IgA Raji IC occurred in five out of seven controls but only in three of these subjects were found levels of Ti/Ui $\geqslant$ 1 (Fig. 2a) with a peak at 2–4 h after the meal. Interestingly a similar peak distribution of IC containing antibodies against ovalbumin (OA-IC) was observed with a maximum at 2–6 h after eating (Fig. 2b).

In all patients with IgA Raji-IC at the start of the food challenge (Table 1, patients 2,3,4 and 6) the IC fell to normal levels (patient 4 to a nearly normal value at 2 h after food) at 2–4 h interval (Fig. 3a). After this, the levels of IgA-IC in all these patients rose again to reach a new peak, which occurred at 6 h following the meal. The other two patients (Table 1, patients 1 & 5) had no IgA-IC at any time. A similar distribution of IC containing antibodies against ovalbumin was obtained, that is, a fall at 2–4 h and a rise at 6–24 h after protein challenge (Fig. 3b). These results suggest that the anti-OA antibodies found in the IC of patients with IgA nephropathy might be of IgA class. However there was no correlation between IgA-IC detected by Raji cells and anti-BSA containing IC in all six patients studied. In contrast to the results observed with IgA-IC determined by Raji cell assay, only three controls and two patients showed a rise in the IgA-IC detected by the anti-IgA inhibition binding assay with no correlation with the anti-ovalbumin and BSA containing IC.

Specific polymeric IgA-IC. After food ingestion the three normal controls having simultaneously IgA Raji-IC and OA-IC (Fig. 2a & b) presented a similar time distribution of specific polymeric IgA-IC (Fig. 2c), which is in favor of a good clearance of these IC. Repeated assays of

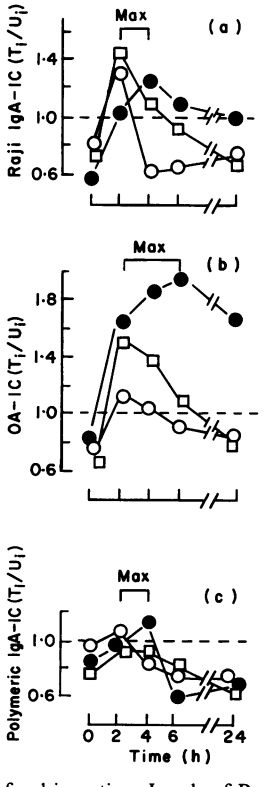

Fig. 2. IC in three control subjects after food ingestion. Levels of Raji IgA-IC (a), IC containing antibodies against ovalbumin (b) and polymeric IgA-IC (c), control 2 (○), control 4 (●), control 5 (□).

these samples consistently showed the same values. By contrast, a continuing rise in specific polymeric IgA-IC levels was observed in five out of six patients after protein ingestion (Fig. 4). Three of them still persisted above the normal range at 24 h. The mean values of these complexes were significantly higher ($P < 0{\cdot}005$) in patients than in controls at 6 h ($0{\cdot}96 \pm 0{\cdot}11$ *vs* $0{\cdot}71 \pm 0{\cdot}11$) and 24 h ($0{\cdot}97 \pm 0{\cdot}08$ *vs* $0{\cdot}65 \pm 0{\cdot}05$) after feeding. These data suggest the existence of a specific defect in the clearance of polymeric IgA-IC in patients with IgA nephropathy.

Study of the IC size

Experiments using serum samples of patients 2,3,4 and 6 before food ingestion showed that IgA Raji-IC were predominantly between 7–13S ($82{\cdot}4 \pm 11{\cdot}4\%$). In patient 4 (Fig. 5b), at 4 h after food

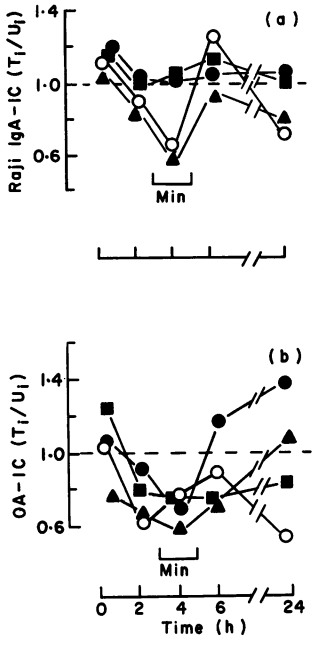

Fig. 3. Levels of Raji IgA-IC (a) and IC containing antibodies against OA (b) in four patients after food ingestion. Patient 2 (○), patient 3 (▲), patient 4 (●) and patient 6 (■).

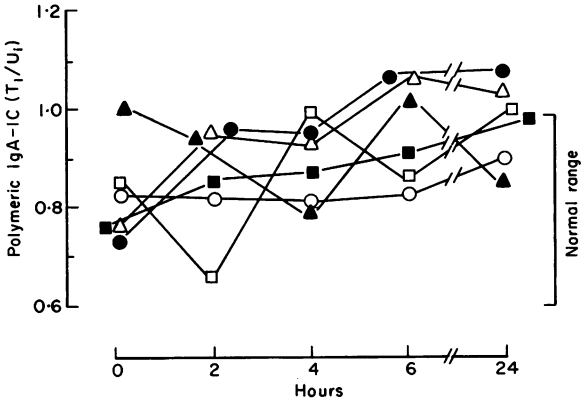

Fig. 4. Levels of polymeric IgA-IC in six patients with IgA nephropathy after food ingestion. Patient 1 (△), patient 2 (○), patient 3 (▲), patient 4 (●) patient 5 (□), patient 6 (■).

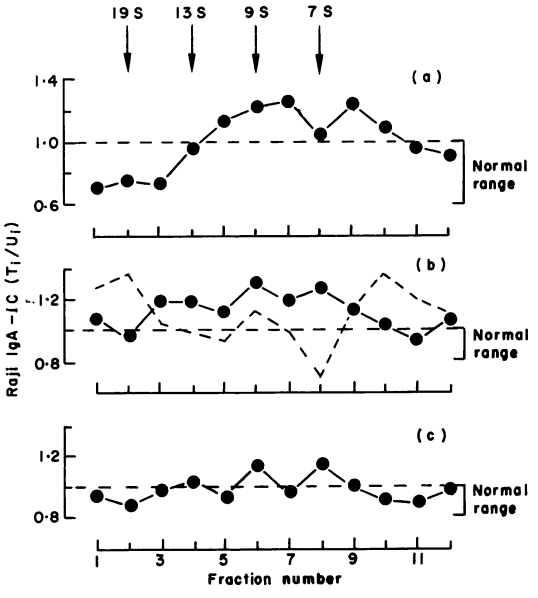

Fig. 5. Sucrose density gradient ultracentrifugation of sera from patient No. 4, at 0 h (a), 4 h (b) and 24 h (c) after food ingestion. The continuous line represent the Raji IgA-IC levels present in each gradient fraction. The dotted line in (b) express the Raji IgA-IC in control 5 at the same hour after meal (no IC were detected at 0 and 24 h). Molecular weight markers are represented at the top of the figure.

ingestion, some complexes larger than 19S and intermediate size (13–19S) appeared, while small complexes (13–17S) persisted. At 24 h the larger complexes had disappeared while small complexes remained at similar levels (Fig. 5c).

In controls 2–4 h after ingestion there was a peak of complexes larger than 19S, a second peak between 13–19S and small complexes between 7–13S (Fig. 5b). By 24 h all complexes disappeared.

Search for specific IgE antibodies

In neither controls nor patients studied, specific IgE antibodies to whole egg and cows milk antigens could be detected by the RAST technique.

DISCUSSION

The detection of circulating IC in most normal subjects following food ingestion is in accordance with previous findings of other authors (Delire *et al.*, 1978; Paganelli *et al.*, 1979). From the different IC studied those detected by IgA Raji assay had an isolated peak at 2 h with disappearance from the circulation between 4 and 24 h, probably through the hepatocytes or the mononuclear phagocytic system. By contrast, those composed of IgG or IgA (these latter detected by the inhibition binding assay) presented two peaks at different time intervals, appeared less often, and had a trend to persist longer in the circulation. Furthermore, only Raji IgA-IC presented a peak distribution similar to that of IC containing antibodies against the proteins ingested (e.g. OA). Taking into account that different assays detect different populations of IC, as the WHO study showed (Lambert *et al.*, 1979) and the data commented above, one must assume that Raji IgA-IC are more specific for those complexes generated by food than the others we have used.

The effect of an acute ingestion of a large amount of protein on the IgA-IC chronically present in patients with IgA nephropathy is surprising and in some way not expected. In short, at fasting, four out of six patients studied had Raji IgA-IC; three out of these four patients had also a high antibody

titre to OA in such complexes. Two to four hours after feeding there was a decrease in both Raji IgA-IC and IC containing antibodies against OA, with a subsequent rise to fasting levels at 6 h. These data suggest that antibodies against food antigens could be involved in these antigen–antibody complexes.

The fate of these circulating IgA-IC in patients with IgA nephropathy after feeding is unknown. A two peak distribution of immune complexes after food challenge in both normal and atopic subjects was also observed by Paganelli *et al.*, (1979). These authors attributed to a change from complexes in antibody excess to antigen excess in relation to the timing of delivery of the antigen to the circulation. This does not seem to be our case because the antibody titre to OA in our patients ran in parallel to the Raji IgA-IC levels, while the peak of circulation OA antibodies in the atopic patients, studied by Paganelli *et al.* (1979), was in the through between the IC peaks. Our results are unlikely to be an artefact since, as depicted in Fig. 5, the Raji cells assay detect presumably complexes of different sizes. The appearance of larger complexes at 4 h, and their later disappearance at 24 h, is in agreement with their easier phagocytosis when compared with smaller ones (Mannik, 1982), and therefore the longer persistance of the latter in the circulation (Fig. 5).

The similarity of our findings with those observed in patients with selective IgA deficiency is striking. Most of these individuals have high levels of circulating IC in their sera, anti-bovine antibodies are present in the complexes and, about 50% of them also have high antibody titres to bovine serum proteins, β-lactoglobulin and OA, presumably because of the continuous excess absorption of dietary antigens from the gastrointestinal tract (Buckley & Dees, 1969; Cunningham-Rundles *et al.*, 1978). The ingestion of bovine milk produced a sharp fall in these circulating IC similar to that seen in our patients (Cunningham-Rundles *et al.*, 1979). Recently, the isolation of anti-idiotypic antibodies to the $F(ab)'_2$ of anti-casein in the sera of two such IgA deficient patients has been described (Cunningham-Rundles, 1982). The author suggests that anti-idiotype antibodies participate in the *in vivo* IC formation, competing with dietary casein antigen for binding sites on the anti-casein antibody. This could probably explain the diminution of IC after the addition of increasing amounts of the relevant antigen in IgA deficient subjects. We are at the moment exploring this possibility in patients with IgA nephropathy.

Why patients with IgA nephropathy, who frequently have high serum levels of IgA, might have, in some way, an immunological behaviour similar to IgA deficient subjects is not clear. In the last few years, however, a lot of evidence has been accumulated on the properties of IgA as regulatory molecules of complement-dependent or -independent immune effector mechanism (Griffis, 1982). In fact, in pathological states such as cirrhosis that result in an increase of polymeric serum IgA (Sancho *et al.*, 1982), this immunoglobulin inhibits chemotaxis (Van Epps & Williams, 1976) and phagocytosis (Wilton, 1978). A similar inhibition of neutrophil migration by serum IgA was also demonstrated in patients with IgA nephropathy (Egido *et al.*, 1982c). It is conceivable that these facts, together with the IgA specific immune regulatory abnormalities found in these patients (Sakai *et al.*, 1979; Egido *et al.*, 1983a, 1983b) and the slower clearance of IgA complexes in relation to those of IgG class (Egido *et al.*, 1982d), might favour the prolonged circulation of IC and hence the best conditions for producing anti-idiotypic antibodies (Klaus, 1978).

Another interesting finding from our studies was the different behaviour of polymeric IgA-IC in normal subjects and in patients with IgA nephropathy after food ingestion. The three control subjects who had Raji IgA complexes after feeding and IC containing antibodies against OA, also showed an increase in the amount of polymeric IgA-IC. These complexes disappeared quicker from the circulation that the multimeric complexes detected by the Raji IgA assay in agreement with previous data from animals (Peppard *et al.*, 1981). The continuing rise in polymeric IgA IC, throughout the 24 h period after food ingestion observed in patients, is in favour of a defect in the selective transport of polymeric IgA IC. These data, together with the high production of polymeric IgA by peripheral blood lymphocytes after polyclonal stimulation *in vitro* (Egido *et al.*, 1982b) could explain the high serum levels of polymeric IgA often found in patients with IgA nephropathy (Lopez-Trascasa *et al.*, 1980). Since a certain number of healthy relatives of the patient (submitted) also have an increased *in vitro* lymphocyte production of polymeric IgA, usually with normal serum levels of polymeric IgA, hepatic clearance defect could be an important step in the development of the nephropathy.

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