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Glomerular deposition of food antigens in IgA nephropathy

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SUMMARY

Recently, we reported on the importance of food antigens on the pathogenesis of an experimentally-induced model of, and some patients with, IgA nephropathy. In this paper, the glomerular deposition of food antigens (casein, lactalbumin, peanut protein, soy bean protein, rice protein, ovalbumin) was investigated by an immunofluorescence technique in 28 patients with IgA nephropathy and 32 controls (ten with lupus nephritis, three with Henoch-Schoenlein purpura nephritis and 19 with other glomerulonephritis). Glomerular IgA deposition was demonstrated in all IgA nephropathy and Henoch-Schoenlein purpura nephritis, and in four lupus nephritis. Positive findings of food antigens, observed as mesangial pattern, were obtained in eleven cases (39·3%) with casein, 21 (75·0%) with soy bean protein and one (3·6%) with rice protein in IgA nephropathy, even though no such findings were seen in the control group. Eleven of 28 patients with IgA nephropathy were positive with soy bean protein alone, nine were positive with soy bean protein + casein, one was positive with soy bean protein + casein + rice protein, and one was positive with casein alone. The deposition of food antigens was not observed in six cases only. Furthermore, no correlation was noted between the deposition of food antigens and the deposition of IgA_1, IgA_2 or J chain, *in vitro* binding of the secretory component, or histopathological grades. These results suggest that the exact meanings of glomerular deposition are unclear. Food antigens are postulated, however, as possibly participating strongly in the pathogenesis and as being localized in the glomerular mesangium as an antigen in some patients with IgA nephropathy.

Keywords IgA nephropathy food antigens

INTRODUCTION

Although many clinical and experimental investigations of IgA nephropathy have been reported, the pathogenesis of this disease has not yet been fully elucidated.

Recently, the participation of some food antigens has been reported, and the mucosal immune system of the gastrointestinal tract has been thought to play a part in the development of this disease. Experimentally, the strong relationship between mesangial IgA deposition and oral immunization by food antigens was clarified by Emancipator *et al.* (1983) and by us (Sato *et al.*, 1986; Sato *et al.*, 1987a). Clinically, since Sancho *et al.* (1983) first described the participation of food antigens in the onset of human IgA nephropathy, several reports (Coppo *et al.*, 1986; Laurent *et al.*, 1987; Fornasieri *et al.*, 1987) including that by ourselves (Sato *et al.*, 1987b) have described the importance of food antigens in the pathogenesis of this disease. Russell *et al.* (1986), on the other hand, demonstrated the glomerular deposition of environmental antigens using an immunofluorescence technique.

This study deals with the deposition of food antigens (casein, lactalbumin, peanut protein, soy bean protein, rice protein, ovalbumin) in the glomerular mesangium of patients with IgA nephropathy.

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MATERIALS AND METHODS

Patients

Renal materials were obtained by kidney biopsy from 28 patients with primary IgA nephropathy (13–63 years old, F/M = 12/16), and from ten with lupus nephritis, three with Henoch-Schoenlein purpura nephritis, four with mesangial proliferative glomerulonephritis, four with membranous nephropathy, two with amyloidosis, and two with minimal change nephrotic syndrome. As controls, renal materials were obtained from one patient with each of the following: focal glomerulosclerosis, membranoproliferative glomerulonephritis, diabetic nephropathy, post-streptococcal acute glomerulonephritis, toxaemia of pregnancy, benign nephrosclerosis, and hereditary nephritis.

Table 1. Prevalence of glomerular antigens in patients with various glomerular diseases (number of patients with antigen/number tested)

	Peanut	Rice	Ovalbumin	Soy bean	Casein	Lactalbumin	IgA
IgA nephropathy	0/28	1/28	0/28	21/28	11/28	0/28	28/28
Lupus nephritis	0/10	0/10	0/10	0/10	0/10	0/10	4/10
Henoch-Schoenlein purpura nephritis	0/3	0/3	0/3	0/3	0/3	0/3	3/3
Other glomerulonephritis*	0/19	0/19	0/19	0/19	0/19	0/19	0/19

* mesangial proliferative glomerulonephritis (4), membranous nephropathy (4), minimal change nephrotic syndrome (2), amyloidosis (2), focal glomerulosclerosis (1), membranoproliferative glomerulonephritis (1), diabetic nephropathy (1), post-streptococcal acute glomerulonephritis (1), toxaemia of pregnancy (1), benign nephroscerosis (1), hereditary nephritis (1).

Glomerular IgA deposition was demonstrated in all IgA nephropathy and Henoch-Schoenlein purpura nephritis, and in four lupus nephritis (Table 1).

Antisera

Antisera were obtained commercially. FITC-conjugated anti-human-IgG, IgA, IgM and C3c (Behringwerke AG, Marburg-Lahn, West Germany). Anti-human-IgA_1 and IgA_2 (Nordic Immunol. Lab., Tilberg, Netherlands, and Becton Dickinson Lab., Mountain View, CA, USA). Anti-J chain (Nordic). Secretory component (SC) (Behringwerke AG). Anti-human-SC: (Cappel, Cochraville, PA, USA). FITC-conjugated anti-rabbit IgG and anti-mouse IgG (Cappel). Anti-peanut protein (Lot No. 003261), anti-rice protein (Lot No. 506818), anti-ovalbumin (Lot No. 607683), anti-soy bean protein (Lot No. 606838) and anti-casein (Lot No. 606838) rabbit serum (Calbiochem-Behring, La Jolla, LA. USA). Anti-lactalbumin rabbit serum (produced in our laboratory).

The specificity of these antisera was demonstrated by immunodiffusion and immunoelectrophoresis.

Immunofluorescence studies

Direct or indirect immunofluorescence procedure was performed as previously reported (Sato *et al.*, 1981), on 4-μm-thick sections of frozen renal samples. FITC-labelled anti-rabbit IgG and anti-mouse IgG were utilized after absorption by fresh human serum. The intensity of the fluorescence was graded as $-$, $+$, $++$ or $+++$. The deposition of J chain was observed after treatment with 6 M urea in glycine-HCl buffer overnight at 4°C as previously described (Nagura *et al.*, 1979). The SC binding test was as follows. Renal sections were incubated with free SC for 60 min at 37°C. After washing with PBS, an indirect immunofluorescence procedure was performed by using anti-SC serum and FITC-conjugated mouse IgG (Béné *et al.*, 1982).

Acid elution study

Renal sections were incubated with 0·02 M citrate buffer at pH 3·2 for 60 min (Woodroffe & Wilson, 1977), and the deposition of IgA or food antigens following acid buffer treatment was observed.

Absorption test

Anti-soy bean protein and anti-casein antisera were absorbed by specific antigen, either casein (Wako Pure Chemical Ind. Ltd, Osaka, Japan) or soy bean protein (International Biological INC, Piedmont, Oklahoma, USA) respectively. The positiv specimens of casein or soy bean protein were re-examined b absorbed antisera. Anti-food antigen antisera with human Ig, (Cappel) were used also.

RESULTS

Glomerular deposition of food antigens

The glomerular deposition of food antigens, such as peanı protein, rice protein, ovalbumin, soy bean protein, casein c lactalbumin, was observed by indirect immunofluorescence te: (Table 1). As shown in Fig. 1, the deposition of soy bean protei or casein was demonstrated in the glomerular mesangium lik that of IgA in the renal sections from a patient with Ig, nephropathy. Casein was indicated not only in the mesangiur but also along the glomerular capillary walls, but the depositio of soy bean protein was principally a purely mesangial patter Positive results were obtained in one case (3·5%) with ric protein, 21 cases (75·0%) with soy bean protein and 11 case (39·3%) with casein, despite not finding any with peanı protein, ovalbumin or lactalbumin in patients with IgA nephrc pathy. Glomerular deposition of food antigens was nc observed in any controls.

The results of acid elution (Fig. 2) show that the depositio of IgA was greatly diminished after the treatment with citrat buffer, but that the soy bean protein remains in the mesangiur

Mesangial deposition was not demonstrated using antiser absorbed with a specific antigen, such as soy bean protein c casein. Anti-food antisera (casein, soy bean protein) absorbe by human IgA stained with the same pattern as did the ur absorbed antisera.

Relationship between the deposition of food antigens and that c other antisera

No correlations were noted between the deposition of foo antigens and that of IgG, IgM or C3c. The deposition of IgA IgA_2 and J chain, and SC binding were examined in 22 cases wit IgA nephropathy. The results are shown in Table 2. All patien had IgA_1 but none had IgA_2. In one case J chain was observe and in five cases SC binding was observed. The staining of thes food antigens and others, however, was not always coincidenta

Positive cases of food antigens and the histopathological grade

Eleven of 28 patients with IgA nephropathy were positive wit soy bean protein alone, nine with soy bean protein + casein, or

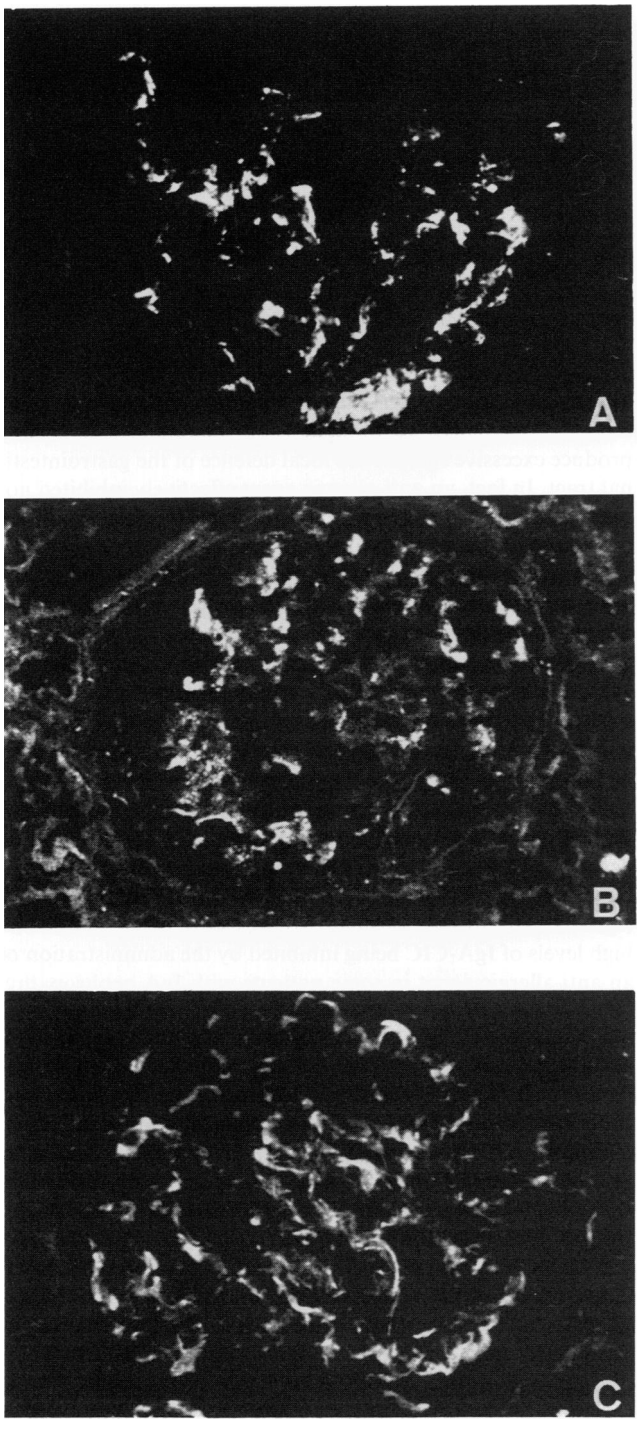

Fig. 1. Renal sections from a patient with IgA nephropathy were stained for anti-human-IgA (a), anti-soy bean protein (b) or anti-casein (c) as a mesangial pattern. (× 200)

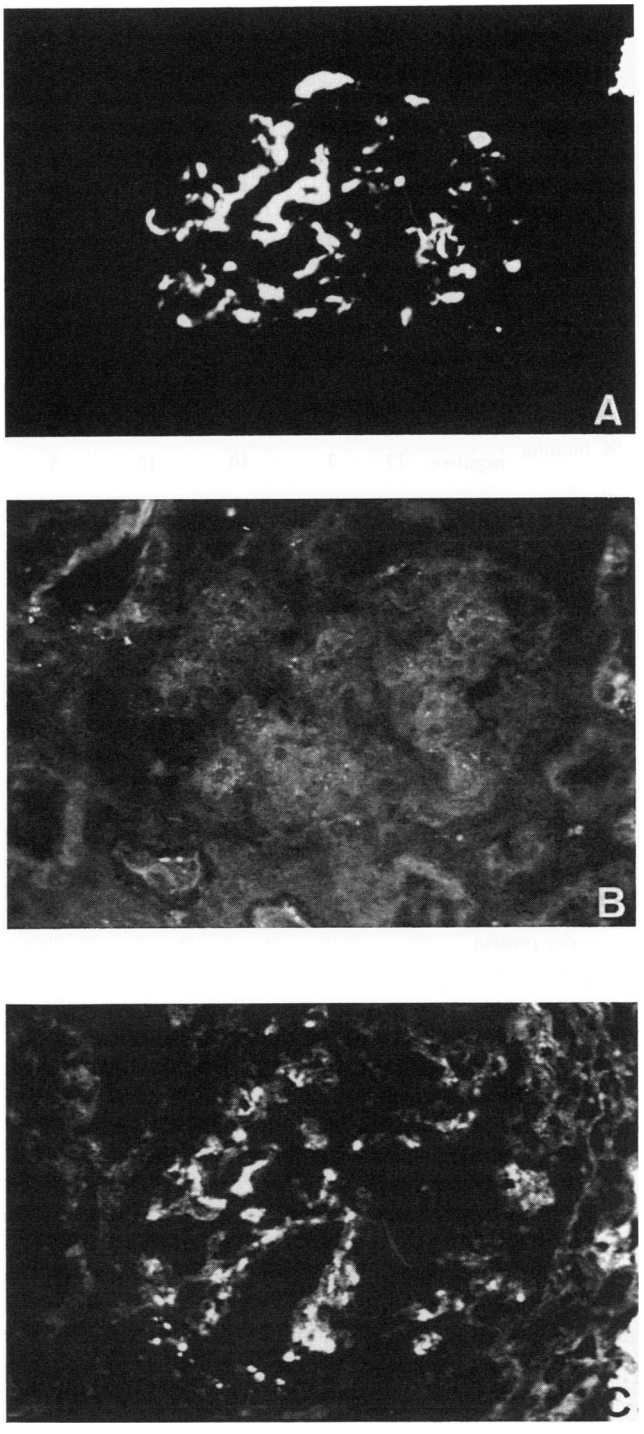

Fig. 2. Renal sections were treated with citrate buffer. Mesangial deposits of IgA (a) disappeared after acid treatment (b). But, deposition of soy bean protein remained (c). (× 200)

Table 2. The deposition of casein or soy bean protein and that of other antibodies in patients with IgA nephropathy

		n	Casein		Soy bean protein	
			Positive	Negative	Positive	Negative
IgG	positive	7	5	2	7	0
	negative	21	6	15	15	6
IgM	positive	10	6	5	9	1
	negative	18	6	12	12	6
C3c	positive	22	8	14	16	6
	negative	6	3	3	5	1
IgA_1	positive	22	8	14	15	7
	negative	0	0	0	0	0
IgA_2	positive	0	0	0	0	0
	negative	22	8	14	15	7
J chain	positive	1	0	1	1	0
	negative	21	8	13	14	7
SC binding	positive	5	1	4	3	2
	negative	17	7	10	12	5

Table 3. Positive cases of food antigens and the histopathological grades in patients with IgA nephropathy

		W.H.O classification*		
	n	A	B	C
positive cases	22	5	5	12
soy bean protein alone	11	1	3	7
soy bean protein + casein	9	4	1	4
soy bean protein + casein + rice protein	1	0	0	1
casein alone	1	0	1	0
negative cases	6	2	1	3

* A: minor glomerular abnormalies B: focal or segmental lesions C: diffuse proliferative changes.

with soy bean protein + casein + rice protein, and one was positive with casein alone (Table 3). The deposition of food antigens was not found in six cases. The glomerular histopathological changes were classified according to the WHO classification (Churg & Sobin, 1982) as minor glomerular abnormalities (A), focal or segmental lesions (B) and diffuse proliferative changes (C). No close relation was noted to exist, however, between the deposition of food antigens and the histopathological grades.

DISCUSSION

Although a great many investigations have surfaced concerning IgA nephropathy, the pathogenesis of this disease remains controversial (Clarkson *et al.*, 1984). Clinically, the disease is characterized by episodes of gross haematuria following upper respiratory tract infection or gastrointestinal tract infection. The antigen components of immune complexes are hypothe sized as being some types of virus (Nagy *et al.*, 1984), bacteria c food antigens.

Recently, a strong relationship has been reported to exi between food antigens and the pathogenesis of IgA nephrc pathy. Emancipator *et al.* (1983) have clarified the participatio of oral immunization, such as ovalbumin, bovine gamm globulin or ferritin, in the induction of IgA nephropathy in mic We subsequently presented a model for IgA nephropath induced by the long-term oral administration of lactalbumin a a food antigen under reticuloendothelial dysfunction (Sato *al.*, 1986). In these experimental models, although Emancipatc *et al.* described the mesangial deposition of antigen adminis tered by mouth we were unable to observe that of lactalbumir We thus assumed that aggregated IgA was predominan quantitatively, over lactalbumin-IgA immune complexes in Ig deposits, and that food antigens were the most importar factors for inducing IgA deposits in the mesangium in ou model. Principally, we thought food allergic reactions ma produce excessive IgA for the local defence of the gastrointesti nal tract. In fact, an anti-allergic agent effectively inhibited nc only the onset but also the progression of IgA nephropathy i our original model (Sato *et al.*, 1987a).

Clinically, Sancho *et al.* (1983) first described the role of foo antigens in the onset of IgA nephropathy. Recently, a Europea centered relationship between a gluten-containing diet and thi disease has been described (Coppo *et al.*, 1986; Laurent *et al* 1987; Fornasieri *et al.*, 1987). An interesting point is whethe gliadin is a specific antigen or not. Serum IgA antibodies t gliadin were not elevated, nor was the glomerular deposition c gliadin observed in patients with IgA nephropathy in the U (Russel *et al.*, 1986). In addition, Fornasieri *et al.* (1987) showe that IgA-anti-gliadin antibodies do not play a large part in th development of IgA nephropathy. We also reported (1987b) o the elevation of circulating immune complexes containing Ig (IgA-CIC) following oral challenge with cow's milk, and on th high levels of IgA-CIC being inhibited by the administration c an anti-allergic agent in some patients with IgA nephropathy Coppo *et al.* (1986) described that the difference in geographica distribution of this nephropathy is related to a factor in the die *e.g.* gluten. However, we are now hypothesizing that numerou dietary proteins may constitute the antigens to produce exces sive IgA for food allergic reactions in the gastrointestinal tract and the gliadin, like soy bean protein or cow's milk, is one of th non-specific antigens associated with this disease.

In the present study, we investigated the glomerular deposi tion of various food antigens using an indirect immunofluores cence procedure. The staining of food antigens demonstrated i a mesangial pattern like IgA in 22 patients (75·9%) with Ig nephropathy, with soy bean protein in particular being observe in high frequency. No deposition was observed in any of th controls, even lupus and Henoch-Schoenlein purpura nephriti patients showing glomerular IgA deposition. The specificity c antisera was confirmed before use, and the positive result eliminated through the use of antisera absorbed with specifi antigen. Moreover, the deposition of food antigens remained i spite of IgA disappearing by acid buffer treatment. Finally, th glomerular deposition of lectins (peanut agglutinin: Vector Lat Burlingame, CA, USA, and soy bean agglutinin: Honen O Company Ltd, Tokyo, Japan) was not demonstrated in Ig nephropathy (unpublished observation).

Russell *et al.* (1986) have already reported the glomerular deposition of environmental antigens, such as soy protein, bovine milk whey, gliadin, protein antigen I/II *Steptococcus mutans* and ovalbumin, with positive findings being obtained in 16 of 37 cases, three of 19 cases, none of 12 cases, none of five cases, and none of two cases respectively. However, the staining of these antigens and IgA_1 was not always coincidental. In this respect similar results were obtained in the present study.

Our observations clarified that food antigens bind specially to the glomerular mesangium from many patients with IgA nephropathy, and that these stainings were never non-specific findings. The exact meaning of glomerular deposition remains unclear, however, indicating that food antigens may in fact participate strongly in the pathogenesis of IgA nephropathy. Moreover, the possibility exists of localization in the glomerular mesangium as 'an antigen' in some patients with IgA nephropathy.

Our intention at this point is to conduct further studies into the mechanism underlying the glomerular binding of these food antigens.

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