No Increase in Antibodies to Six Food Antigens in Japanese Patients with IgA Nephropathy

Takao KURAMOTO, Naohiro YANO, Masanobu MIYAZAKI, Masayuki ENDOH, Yasuo NOMOTO and Hideto SAKAI

Department of Internal Medicine, Tokai University School of Medicine, Isehara, Kanagawa, Japan
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It has been postulated that mucosa-related antigens are involved in the development of IgA nephropathy. The aim of this study was to elucidate whether food antigens have any relation to IgA nephropathy in Japan. Sera from 15 patients with IgA nephropathy and 15 healthy controls were examined using an enzyme linked immunosorbent assay (ELISA). IgG, IgA, and IgM antibody titers were measured against six food-derived antigens, i.e. rice, soy bean paste, soy sauce, egg yolk, egg white and gluten, all of which are frequently found in the ordinary japanese diet.

No significant differences between patients and controls were found.

It was concluded that food antigens appear to have little, if any, relation to IgA nephropathy.

(Key words: IgA nephropathy, food antigens, serum immunoglobulins)

INTRODUCTION

IgA nephropathy is assumed to be an immune complex-mediated glomerular disease caused by the deposition of IgA dominant immune complexes in the glomeruli. However, the origin of the antigenic components of the IgA immune complexes is unknown. The alimentary tract is known as an important IgA producing site because of abundant IgA-producing cells in the submucosa. Cordo et al. (3) suggested that dietary gluten may be responsible for the development of IgA nephropathy in Europe, and recently reported the beneficial effects of a gluten-free diet in the treatment of patients (4). In Japan, Sato et al. (12) reported that soy bean protein antigens were involved in the formation of the IgA immune complexes in patients with IgA nephropathy. We recorded the serum antibody titers for six antigens derived from foods present in the ordinary Japanese diet to determine if any causative relation existed between food antigens and IgA nephropathy.

PATIENTS AND METHODS

Patients

The subjects were 15 patients with IgA nephropathy, including 8 with slight tissue damage, and 7 with severe damage. The diagnosis of glomerulonephritis was made histologically, using light microscopy and immunofluorescence staining of open renal biopsy specimens. Systemic diseases such as lupus erythematosus, liver cirrhosis, Henoch-Schönlein purpura and diabetes mellitus were excluded. None of the patients had serum levels of creatinine exceeding 1.5 mg/dl.

Fifteen healthy adults served as controls.

Serum antibodies to food antigens

IgG, IgA and IgM antibody titers against six food antigens, i.e. rice, soy bean paste, soy sauce, egg yolk, egg white and gluten, were measured by ELISA. Each food was emulsified in phosphate buffered saline (PBS) and, after centrifugation, the supernatant was recovered and dialyzed against PBS. Protein concentrations of these supernatants were measured by
Tonein® TP assay (Otsuka Assay Lab., Japan), and adjusted to 10 µg/ml with PBS. Each supernatant was coated onto ELISA plates (Limbro-Titertek, Flow Lab., Mclean, VA), incubated at 4°C overnight, and blocked with 1% bovine serum albumin (BSA) in PBS. Fifty microliters of serum samples, diluted ten-fold in PBS, were applied in duplicate and incubated at 4°C overnight. The 15 control and 15 patients’ sera were applied on the same plate to minimize different plate effects. After washing with 0.1% Tween in PBS (PBS-Tween), 50 µl of 1:200 diluted anti IgG, IgA, IgM antibodies labeled with alkaline phosphatase (Sigma, St Louis, Mo), were added to each well, and reacted for 2 hours at room temperature. After three washings with PBS-Tween, phosphatase substrate dissolved in diethanolamine buffer (pH 9.8) was added to each well and the optical absorbance, measured by an ELISA spectrophotometer, was used as the serum antibody titer.

**Immunofluorescence study for soy bean protein in the glomeruli**

Kidney biopsy specimens from 18 patients with IgA nephropathy and 15 patients with non-IgA proliferative glomerulonephritis were examined by immunofluorescence microscopy to determine if there was any soy bean protein in the glomeruli. Antiserum to soy bean protein was purchased from Calbiochem, Tokyo and conjugated with fluorescein isothiocyanate (FITC, Sigma) according to the method of Kawamura (9). (F/P ratio 2.1). Renal biopsy specimens, embedded in a gelatin matrix (Tissue-Tek®, Lab-Tek Products, USA), were frozen with dry ice and acetone, sectioned at 4 µm in a cryostat (Ames Cryostat II, No. 4559-1141, Miles Sankyo Co, Ltd, Tokyo, Japan) at about -25°C, and air dried. Direct immunofluorescence was performed using the FITC-conjugated soy bean antiserum diluted 1:10 in PBS. Renal cryostat sections were stained with the FITC-conjugated antiserum in a moist chamber at 4°C overnight. The sections were washed with PBS and then examined with a Zeiss Ortholux microscope (Model 9902, Carl Zeiss, Inc, New York, USA).

**RESULTS**

Figure 1 shows the antibody titers against rice. There was no significant difference between controls and patients.

Figures 2 and 3 show the antibody titers against soy bean paste and soy sauce. There were no significant differences between controls and patients concerning soy proteins.

Figures 4 and 5 show the antibody titers against egg yolk and egg white. There were no significant differences to the egg-derived proteins between controls and patients.

Figure 6 shows the antibody titers against gluten. Once again, no significant difference between controls and patients was seen.

Patients with either slight or severe tissue damage likewise did not demonstrate any significant differences in serum titers to the spectrum of food antigens.

Fluorescence microscopy did not reveal soy bean protein in the glomeruli from patients with IgA nephropathy or proliferative glomerulonephritis.

**DISCUSSION**

First described by Berger and Henglais in 1968 (1), IgA nephropathy is now known to be the most common primary glomerulonephritis in many countries. The diagnostic feature of primary IgA nephropathy are mesangial deposits of IgA, usually accompanied by complement components and sometimes by IgG, IgM and fibrinogen (2). Many reports have indicated that IgA immune complexes may play a major pathogenic role in IgA nephropathy. Patients with macroscopic hematuria often show unspecified infections in the upper respiratory or gastrointestinal tract although significant rises in antibody titers to specific infectious agents are relatively rare (14). Increased antibody titers against gut flora and food antigens have been found in some IgA nephropathy patients, and the role of food antigens have been investigated in vivo and in vitro (5–8, 10, 11, 13).

Emancipator et al. (6) found that orally administered antigen induced a specific IgA mucosal immune response that resulted in glomerular mesangial deposits of IgA antibody and antigens. Sato et al (13) produced IgA nephropathy in mice by administration of lac-
Fig. 1  ELISA values of three classes of immunoglobulins (Ig) against rice antigens. The absorbance readings are plotted on the ordinate.

Fig. 2  See legend of Fig. 1. ELISA values against soy paste.
Fig. 3  See legend of Fig. 1. ELISA values against soy sauce.

Fig. 4  See legend of Fig. 1. ELISA values against egg yolk.
Fig. 5  See legend of Fig. 1. ELISA values against egg white.

Fig. 6  See legend of Fig. 1. ELISA values against gluten. Note the reduction in the number of controls, from 15 to 11.
talalbumin. Laurent et al. (10) indicated that anti-gliadin IgA was of diagnostic value in distinguishing IgA glomerulonephritis from other types of glomerular disorders. Coppo et al. (5) indicated that gliadin induced IgA immune deposits in mice, and later discussed the therapeutic significance of a gluten-free diet in patients with IgA nephropathy (4). In a related study in Japan, Sato et al. (12) showed that soy protein might be a participating antigen in the pathogenesis of this disease. In all these studies, food antigens were considered to be strong candidates for inducing the development of IgA immune complexes in IgA nephropathy.

We determined the serum antibody titers against the antigens of six foods commonly found as part of the typical Japanese diet. The six foods were rice protein, soy bean protein (soy paste and soy sauce), egg protein (egg yolk and egg white) and gluten, and extractable preparations from these foods were used as antigens in our assay. ELISA reactions of sera with high titers were blocked by the addition of excess antigen, indicating the specificity of this assay system. The patients and the healthy adults had comparable levels of IgM antibody to the rice protein antigen, with low levels of IgG and IgA. A slight increase in IgG and IgM antibodies to soy products was observed both in controls and patients, with a low level of IgA antibody to these products. However, there were no significant differences between controls and patients. Concerning egg antigens, we observed IgG, IgA and IgM antibodies although the levels of IgM anti-egg yolk antibodies were relatively low. IgM anti-gluten antibody was found both in patients and controls, with undetectable amounts of the other isotypes. These data indicate many people have some kind of antibody to a variety of food antigens in their serum without, however, any pathogenic manifestations such as gastrointestinal symptoms or immune complex disease. We observed no significant increases in serum antibodies to any food antigens in patients with IgA nephropathy. We subsequently attempted to stain kidney specimens with a fluorescein-labeled antiserum to soy bean protein, but positive results were not obtained in patients with IgA nephropathy. This observation denies the involvement of soy protein in the development of IgA nephropathy.

It was concluded from this study that the six food antigens investigated are not responsible for the development of IgA nephropathy in Japan.

REFERENCES