Dietary Antigens and Primary Immunoglobulin A Nephropathy

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ABSTRACT
To investigate the role of dietary components in immunoglobulin A mesangial nephropathy (IgAGN), this study focused on gliadin, based on the reported association between coeliac disease and IgAGN as well as the pilot observation that a gluten-free diet was able to reduce the levels of circulating IgA immune complexes (IgAIC). IgA mesangial deposits in mice were induced by oral immunization with gliadin and in rats by inducing alcoholic liver cirrhosis, which increased the levels of IgA against dietary antigens (Ag). Gliadin was able to bind to cultured mesangial cells by a lectinic bond, which was reversed by competitive sugars. Binding increased mesangial cell tumor necrosis factor synthesis and decreased prostaglandin E2 production. Several gluten lectinic fractions modulate leukocyte oxidative metabolism, cytotoxicity, and chemotaxis. In IgAGN patients, serum IgA to dietary Ag were sporadically positive and IgAIC containing IgA to dietary components were significantly increased. The affinity of serum IgA to various lectins was increased in some patients. Conversely, no substantial deposition in renal tissue of dietary Ags was observed by immunofluorescence. A gluten-free diet, given to IgAGN patients with high levels of circulating IgAIC and positive antigliadin IgA, was followed by a decrease in the mean levels of both IgAIC and IgA to various dietary Ag, parallel to a reduction in proteinuria. These data suggest that dietary components, such as Ag or lectins, may play a role in IgAGN by promoting IgAIC formation and perhaps favoring mesangial localization via lectinic interactions.

Key Words: Immunoglobulin A nephropathy, dietary antigens, lectins

In the pathogenesis of immunoglobulin A nephropathy (IgAGN), a major role is played by the hyper-reactive mucosal immune system, as demonstrated by experimental models and clinical observations (1–6). However, the disease develops only after antigenic exposure to infectious or dietary antigens (7–11).

In the last few years, we investigated the role of dietary components in IgAGN by various studies including experimental models in animals, in vitro tests on cultured mesangial cells, and search of specific antigens (Ag) or antibodies (Ab) in sera and renal tissue from IgAGN patients. Moreover, we evaluated the immunological and clinical effects of different Ag-free dietetic regimens.

THEORETICAL GROUND
Dietary Components as Antigens

Under normal circumstances, ingested dietary proteins do not activate immunological mechanisms because secretory IgA prevents Ag entry (12). Ag escaping the IgA barrier enter the oral tolerance mechanism (13). The switch from IgG to IgA immune response leads to the formation of poorly complement-fixing IgA immune complexes (IgAIC), instead of flogogenic IgGIC.

The breakdown of oral tolerance can be favored by perturbations of epithelial cell function, resulting in abnormal processing of dietary Ag, which renders them immunogenic rather than tolerogenic. The cytokines produced by immune system activation influence epithelial cell surface secretory component and class II Ag expression. These modifications lead to intestinal permeability changes, allowing increased peptide uptake and Ag presentation with stimulation of the mucosal immune system (14,15).

Dietary Components as Toxic Lectins

Food is rich in lectins, carbohydrate-binding proteins from animal or plant origin, which can be also of bacterial and viral nature (16). Cooking and digestion do not completely destroy their ability to interact
with carbohydrates. Some ingested lectins reach the systemic circulation in an undigested form, which retains the lectinic properties (17).

Ingested lectins can alter the physical integrity of the intestinal wall and/or modify its permeability (18,19). Then, lectins can damage lymphocytes of the mucosal immune system, thus making more likely both bacterial overgrowth and eventually food allergy (20). Therefore, lectins abrogate the normal gut tolerance mechanism and encourage systemic immunization (21–23).

Lectins and IgA

Lectins can bind oligosaccharide-containing IgA, particularly IgA1, which have a set of galactose and N-acetyl-galactosamine residues in the hinge region, and polymeric IgA, which are rich in terminal glycop biosides (24,25). This reactions leads to the formation of macromolecular aggregates.

We observed an in vitro binding of gluten and its lectin fractions gliadin and glyc-gli to human polymeric IgA, which was reversed by the addition of competitive sugars (7) (Figure 1).

Intra-aortically injected IgA-lectin nonimmune complex can induce IgA and C3 mesangial deposits in mice (26).

EXPERIMENTAL MODELS IN ANIMALS

After the demonstration (11) that in mice dietary Ag induce mesangial IgA deposits containing the Ag and the specific Ab, we focused our studies on gliadin, intrigued by the reported association between coeliac disease and IgAGN (27). Furthermore, we considered that gliadin could play a role as a dietary Ag as well as an enterotoxic lectin.

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Binding of various gluten fractions to polymeric IgA (pIgA) and inhibition by competitive sugars. OD, optical density; PBS, phosphate-buffered saline.

![Gliadin-Induced Experimental IgAGN](https://example.com/figure2.png)

**Gliadin-Induced Experimental IgAGN**

We studied different groups of four-wk-old BALB/c mice fed for 14 wk with a basal gluten-free diet, which was made normoproteic by the addition of gelatin and rice to obtain the usual 20% protein content (28).

The first group did not receive any dietary immunogen in the drinking water (control). Another group received standard gluten-containing chow. Two other groups of mice were orally immunized by gliadin or ovalbumin (1 mg/mL in the drinking water).

Mice developed bright IgA deposits corresponding to electron-dense deposits seen by electron microscopy. IgA deposits, semiquantified by immunofluorescence (IF) scores, were found to be significantly greater in mice orally immunized with ovalbumin and gliadin than in those kept on a gluten-free diet (P < 0.001). Mice fed with standard gluten-containing chow had greater deposits than did mice fed the gluten-free diet (P < 0.05) (Figure 2). The presence of evident IF-positive staining (>2 of 6 scores) was significantly more frequent in mice orally immunized by purified gliadin or receiving the common gluten-containing diet than in mice on the gluten-free diet (P < 0.02 and P < 0.04, respectively). In the same groups of mice, total serum IgA and IgA antigliadin Ab were significantly increased in circulating as well as in renal deposit eluates. No IgG or complement deposition was found in any group. Mice displayed no increase in proteinuria and/or microscopic hematuria.

Because gluten is a basal component of the ordinary mouse food, we speculated about its possible role in the formation of the "spontaneous" IgA mesangial deposits very common in control animals after 30 wk of age. In newborn mice, we failed to show any IgA deposits.

Our analysis of kidney tissue eluates of adult mice indicated that common dietary Ag, and particularly gluten, may play a key role in the development of
some of these apparently spontaneous IgA deposits (28).

Soya as Possible Oral Immunogen in Experimental IgAGN

It is of interest that gluten is abundant in the diet of populations with a high prevalence of IgAGN, such as in Italy, France, and Spain, whereas soya, which is, like gluten, rich in lectic components, is a very common food in Far East Asia, the other area with an extremely high frequency of IgAGN (29).

To investigate a possible role for soya, we studied four groups of BALB/c mice after 14 wk on different diets (30). The basal diet consisted of a gluten- and soya-free chow. The control group had the basal diet alone. Other groups were orally immunized with gliadin (1 mg/mL) in the drinking water or soya in amounts accounting for 25% of chow dry weight. A final group was nourished with standard pellet mouse chow containing wheat (3% of gluten) and 12% soya.

Mice on the soya-rich diet displayed only a mild and not significant increase in mesangial deposits, whereas those orally immunized with gliadin showed bright IgA mesangial deposits.

Therefore, the two lectins more commonly eaten in countries with the highest prevalence of IgA nephropathy, gliadin and soya, did not show a similar capability of inducing experimental IgAGN in mice, suggesting that differences in biochemical properties condition the nephritogenic potential of the dietary lectins.

Alcohol-Induced Experimental IgAGN

The role played by dietary Ag may be consequent to increased antigenic exposure or to increased dietary Ag absorption from the intestinal lumen. This last mechanism can be induced not only by enterotox lectins, but also by alcohol consumption. Patients with alcoholic liver cirrhosis frequently develop IgAGN, which is thought to be consequent to both increased dietary Ag absorption and defective clearance of polymeric IgA and IgAIC (31).

We recently developed, in collaboration with S. Emancipator (Case Western Reserve University, Cleveland, OH), an experimental model of alcoholic liver cirrhosis in Lewis rats (32) by episodic intragastric delivery of whiskey with or without a lipotrope-deficient diet. Rats were killed after 3 months. Severe fatty changes, fibrosis, and regeneration nodules were found in the liver, mainly in rats receiving whiskey in association with a lipotrope-deficient diet.

Bright granular IgA mesangial deposits were significantly increased in rats receiving alcohol (P < 0.02) or alcohol with a lipotrope-deficient diet (P < 0.001), in comparison to the control group, along with C3 mesangial deposits (P < 0.001 versus controls).

Increased levels of IgA specific to dietary Ag were found in all experimental groups, with prevalence ranging from 33 to 100%, whereas IgA specific for Ag not contained in the chow were within the normal range. The association of alcohol and a lipotrope-deficient diet altered the intestinal barrier permeability, as was documented by the xylose absorption test, which gave increased values in comparison to controls (P < 0.01).

In other experiments, we tried to evaluate the effects of alcohol consumption in amounts unable to induce liver cirrhosis and therefore unlikely to influence the hepatic clearance of circulating IgA or IgAIC (33). We studied three groups of Lewis rats. One group was nourished with standard food and 20% ethanol in the drinking water given by spontaneous administration, without gavage. Another group similarly had 20% ethanol in the drinking water in association with a nutrient-deficient diet. The other group of rats was the control group with regular chow and water.

After 14 wk, the liver occasionally showed a slight degree of steatosis in rats consuming ethanol. No IgA mesangial deposits were detectable in the glomeruli. Serum IgA to dietary Ag were significantly increased in rats drinking ethanol with (P < 0.005) or without (P < 0.05) a nutrient-deficient diet.

Therefore, we concluded that alcohol consumption per se favors, possibly via enhanced intestinal mucosal permeability, the production and circulation of IgA to dietary Ag, leading to a final glomerular deposition, which generally occurs after severe liver damage with resulting defective hepatic clearance.

IN VITRO STUDIES OF CULTURED MESANGIAL CELLS

Lectin-binding sites exist in several glomerular structures (34), and a decrease in glomerular sialic acid/lectin-binding sites has been observed in patients with IgAGN (35). We investigated the functional consequences of the binding of various lectins to cultured rat mesangial cells.

Binding of Lectins to Isolated Glomeruli and Cultured Mesangial Cells

The first series of experiments was performed on isolated glomeruli obtained from Sprague-Dawley rats by sequential sieving (36). After treatment with collagenase, the glomerular cores were transferred onto glass by cytocentrifugation and were tested for binding to various fluoresceinated lectins: gliadin, concanavalin A (conA), Ulex, Arachis Ipogea, and soya bean (Sigma Chemical Co., St. Louis, MO). Among lectins, we observed different binding capacities,
maximal for ConA, gliadin, and soybean and, to a lesser extent, for Ulex.

The binding of gliadin to isolated glomeruli was further refined by investigating its attachment to cultured pure mesangial cells (37). Mesangial cells were cultured from Sprague-Dawley rat glomeruli isolated by sieving. The epithelial component was then removed by digestion with type IV collagenase, and glomerular cores were plated on plastic culture dishes. After 20 days, cells were detached by incubation in trypsin-EDTA, washed, and subcultured. After trypsinization, mesangial cells were plated on glass slides, grown to monolayer, and fixed with paraformaldehyde.

A bright IF was observed after incubation with gliadin and the addition of fluoresceinated antigliadin Ab. This binding in IF was inhibited by preincubation of gliadin with sugars competitive for the lectinic bond.

Functional Effects of Lectinic Binding on Cultured Mesangial Cells

Cultured rat mesangial cells were incubated with various lectins for 1 h and tumor necrosis factor (TNF) produced by mesangial cell activation was measured in the supernatants. The addition of lectins to cultured mesangial cells significantly modulated their TNF production in different ways. Gliadin, wheat germ agglutinin, ConA, Ulex, and Limulus (Sigma) significantly increased TNF synthesis, and the effect was reversed by the addition of sugars competitive for the lectinic binding. Conversely, other lectins like soybean did not affect basal production.

Similarly, we measured the effects of the binding of gliadin to mesangial cell by detecting prostaglandin E2 (PGE2) levels in culture supernatants (38). We considered different conditions beside basal ones: massive stimulation with Ca2+ ionophore and incubation with gliadin and with indomethacin together with gliadin. PGE2 was measured on cultured supernatants by competitive RIA by using a specific polyclonal Ab (kindly provided by Prof. M. Dunn, Case Western Reserve University). After incubation with gliadin, PGE2 production from mesangial cells was significantly inhibited (P < 0.02) and even more so in comparison to values obtained after Ca-ionophore stimulation (P < 0.001), which induced the expected stimulation in PGE2 synthesis. The effect was mediated by a true lectinic binding, because it was blocked by competitive sugars. The coinubcation of gliadin with indomethacin further inhibited PGE2 production, indicating a submaximal effect of gliadin alone (P < 0.01). In conclusion, these data indicate that lectins, particularly gliadin, can bind to mesangial cells and modulate the production of immunological mediators and hemodynamic factors.

It is of interest that these carbohydrate-binding proteins can bridge in vitro polymeric IgA to mesangial cells (37). It is also known that the presence of IgA in an immune latex, together with IgG, inhibits complement fixation by the complex. Interaction of gliadin with the carbohydrate side chains of IgA might attenuate this ability, leading to a greater C3 incorporation in IgA-IgG immune deposits and its unfavorable effects on hematuria and kidney tissue damage (1).

Effects of Lectins on Peripheral Leukocytes

We investigated the ability of two wheat proteins, gliadin and glyc-gli carrying lectinic properties, to modulate cell oxidative metabolism, locomotion, and cytotoxic activity (39).

The chemiluminescence generation of neutrophils was found to be markedly affected by prior incubation with the two wheat fractions. An increase in the chemiluminescence response to gliadin was found to be almost linear by increasing its concentration in the incubation medium (from 1.2 to 20 µg/mL), although a bimodal response was observed by its lectinic fraction glyc-gli with an enhancement up to 50 µg/mL followed by dose-dependent suppressive effects. Coincubation of competitive sugars resulted in a complete abolition of these effects. Of interest, stimulating effects on monocytes were not observed. Incubation of peripheral blood mononuclear cells with gliadin (1.2 to 5 µg/mL) resulted in an increased cytotoxic activity with the greater concentrations, approaching the levels of enhancement achieved by a well-known stimulus, such as the gamma interferon. Also, these effects were completely abolished by competitive sugars.

Moreover, human neutrophils were tested in chemotaxis chambers for responsiveness to the concentration of glyc-gli effective in inducing oxygen radical generation. Data from locomotion assays revealed a predominant chemokinetic effect stimulating the random migration.

From these studies, we concluded that gliadin and glyc-gli are powerful modulators of leukocyte function. By enhancing chemokinesis and generation of oxygen reactive species, these substances could contribute, at least in predisposed subjects, to both changes of intestinal permeability and tissue damage.

STUDIES IN IgAGN PATIENTS

Levels of IgA to Dietary Ag

IgAGN is particularly frequent in Mediterranean Europe, Japan, and Australia, and it is far less common in North America and northern Europe (29).
Beside different biopsy policies, genetic predisposition or environmental factors may favor this non-homogenous geographic distribution.

We evaluated serum samples from Italy, Australia, and Japan, measuring by ELISA (40) IgA to heterologous albumins (ovalbumin and BSA) and to several gluten fractions (ethanol-soluble gliadin, bicarbonate buffer-soluble gliadin, the nonlectinic fraction glutenin, and the lectin fraction glyc-gli).

Mean values of IgA to dietary Ag in patients with IgAGN from Italy, Australia, and Japan were not significantly increased in comparison to those of the corresponding healthy control groups. The prevalence of positive data ranged from 19 to 42.8% among Italians, from 0 to 36% in Australians, and from 0 to 16% in Japanese patients versus 10% in each healthy control group.

Some relationships exist between levels of IgAIC and IgA to dietary Ag, because patients with high levels of IgA to dietary Ag components statistically also presented the highest values of IgAIC (41). Moreover, the patients with positive data tended to have a cluster of increased levels of IgA against several dietary Ag at the same time.

Prevalence of positive data for IgG against dietary Ag were always superimposable to controls apart from Ab against gliadin, which were increased in 20% of the patients (41).

IgA and IgG to dietary Ag were found to be increased in 2.5% polyethylene-glycol (PEG)-precipitated large-size immune complexes. IgAIC containing IgA to gliadin and ovalbumin were significantly increased in comparison with controls ($P < 0.005$ and $P < 0.002$, respectively) (Figure 3).

Search of Specific Dietary Ag or IgA Directed to Dietary Ag in Renal Biopsy Tissue

In spite of these data suggesting a role played by dietary Ag, IF studies on kidney biopsies failed to obtain any substantial proof of deposition of dietary Ag or specific Ab in renal tissue (41).

Lectin-Binding Serum IgA Activity

In the same group of IgAGN patients and controls from Italy, Australia, and Japan, we investigated the serum IgA capability to bind lectins (40). We measured by micro-ELISA the binding of IgA to lectins with and without the addition of competitive sugars. The difference between the two values was considered an index of the IgA lectin-binding activity.

An increase in the various lectin-binding IgA activity in comparison to that in healthy controls was found in 0 to 72% of serum samples from Japanese patients, 0 to 56% of Australian patients, and 6 to 33% of Italian patients. Serum samples from Italian and Australian patients did not display an increase in mean values of lectin-binding IgA activity when compared with those from healthy controls. Conversely, serum samples from Japanese IgAGN patients showed a significantly increased lectin-binding activity of IgA versus some of the lectins tested.

These data are consistent with the hypothesis that some patients with IgAGN have increased levels of naturally occurring or aberrantly glycosylated IgA molecules, bearing more or different sugar residues, thus increasing the binding to circulating lectins. A great intake with food or high intestinal permeability could favor lectin-IgA aggregate formation in circulation or in the mesangial area.

Intestinal Permeability in IgAGN Patients

Despite the expected hypothesis, we failed to find an increased intestinal permeability in IgAGN patients versus healthy or diseased controls (membranous nephropathy and focal sclerosing glomerular nephropathy) by testing the gut absorption of $^{51}$Cr-EDTA.

![Figure 3. Ab specific to dietary Ag in serum fractions rich in immune complexes (2.5% polyethylene-glycol precipitates).](image-url)
Relationship Between IgAGN and Coeliac Disease

High titers of IgA to gliadin in patients with IgAGN have drawn much attention on a possible relationship between IgAGN and coeliac disease. The reported prevalences of positive antigliadin IgA in IgAGN patients ranged from 0 to 70–100% (42–45), leading some authors to hypothesize a close relationship between IgAGN and latent coeliac disease (42), whereas others found such an association in only 3% of IgAGN patients in whom a true coeliac disease was then diagnosed (44). On the other hand, the presence of IgAGN in coeliac patients is not the rule. We observed antigliadin IgA in about 37% of our IgAGN patients (40).

To get a further insight into this problem, we measured anti-smooth muscle endomysium Ab, which have a high degree of specificity and sensitivity for gluten-sensitive enteropathy (46). IgG and IgA antigliadin were always negative in IgAGN patients, although they had a 100% specificity for gluten-sensitive enteropathy. Our data therefore suggest that IgAGN cannot be strictly considered a dermatitis herpetiformis of the kidney.

Indeed, coeliac disease has been reported in association with many immune-mediated diseases, in particular dermatitis herpetiformis and less frequently rheumatoid arthritis, sarcoidosis, and other autoimmune diseases beside IgAGN (47). Moreover, in several patients with these diseases, some of the features of coeliac disease including increased intestinal permeability and raised levels of Ab to wheat proteins have been found without signs of frank coeliac disease. Even though these abnormalities do not represent overt coeliac disease, it is possible that these abnormalities indicate lesser degrees of gluten sensitivity, which may well be indicative of wheat protein-specific abnormal process.

Studies with Different Ag-Free Diet: Effects of Gluten Withdrawal

To study the clinical effects of dietary Ag in IgAGN, we evaluated in a pilot study on a small number of patients the effects of short-term diets free of BSA, ovalbumin, and gliadin (48). We observed a significant decrease in IgAIC levels detected by a specific IgA conglutinin assay, only after a gluten-free diet. These effects were repeated in different subsequent periods of gluten-free and gluten-containing diet (49). After our preliminary report, we verified on a larger number of IgAGN patients the effects of a gluten-free diet (50).

IgAIC levels significantly decreased after 6 months on a gluten-free diet (P < 0.01) (Figure 4) and remained low for 4 yr. Among the patients with baseline positive IgAIC values, who accounted for 48% of the total patients, a great majority showed a decrease by more than 50% of the initial value and 64% showed a complete disappearance of detectable IgAIC.

Similar to other groups of IgAGN we had previously investigated, on an unrestricted diet, the mean values of IgA to dietary Ag were not significantly different than those in controls. However, patients with basal IgAIC values greater than controls had significantly increased IgA to dietary Ag (both heterologous albumins and gluten fractions). A decrease in mean levels of IgA to dietary Ag was found after the gluten-free diet: it was significant after 6 months for antiovalbumin IgA and antigliadin IgA, and for all but IgA to glutenin (a nonlectinic gluten fraction) after 1 yr. Of interest, a gluten-free diet was able to induce a significant decrease not only in IgA antigliadin fractions, but also in IgA directed towards heterologous albumins, suggesting possible toxic effects of gliadin on the intestinal mucosa, modifying the intestinal permeability.

Basal positive antigliadin IgA were detectable in 38% of the cases and a decrease by more than 50% was evident in 82% with normalization in 63%. Among the patients with basal positive IgAIC associated with two or more positive data for IgA to dietary Ag, 75% showed a parallel disappearance of IgAIC and IgA to dietary components after a gluten-free diet.

After 6 months of a gluten-free diet, mean proteinuria values significantly decreased and remained lower thereafter. A baseline proteinuria of >0.8 g/day was detectable in 65% of IgAGN patients. After 6 months, it decreased in 47% by more than 50%. Clinically evident microscopic hematuria decreased in 22 patients after 6 months and in 78% after 1 yr.
A relentless progression of renal failure was observed in IgAGN patients even on a gluten-free diet. Because this was not a controlled study, no comparison could be made with patients on a gluten-containing diet.

Some 48% of the IgAGN patients entering the study had high basal levels of IgAIC and/or antigliadin IgA associated with clinically evident abnormalities. After the gluten-free diet, 71% had a decrease in proteinuria and/or hematuria. Each responsive patient had a parallel disappearance of abnormal immunological data. Conversely, only 25% of IgAGN patients without circulating IgAIC and/or antigliadin IgA had some urinary improvement.

From this study, we concluded that gliadin acts as a factor enhancing the process of dietary Ag absorption and mucosal immune system response, because a gluten-free diet was followed by a significant improvement in the immunological parameters monitored. However, the progression of renal disease may indicate that these immunological factors may perhaps play an early role in the development of mesangial deposits but that the disease progresses by different mechanisms.

CONCLUSIONS

In conclusion, our research suggests a role in primary IgAGN of dietary components as antigenic proteins or glycosylated-binding lectins, by promoting the mesangial delivery of circulating IgAIC or in situ deposit formation. Moreover, the dietary components endowed with carbohydrate-binding capacity may directly modify the mesangial reactivity, thus directly influencing the full development of histological lesions.

REFERENCES