

MAY GLUTEN-FREE DIET REDUCE THE LEVELS OF IgA IMMUNE COMPLEXES IN PRIMARY IgA NEPHROPATHY?

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Summary

The influence of different diets on circulating IgA immune complexes (IgAIC) was investigated in six patients with primary IgA nephropathy selected as having persistently high IgAIC values and heavy microscopic haematuria or nephrotic range proteinuria.

During three test periods of 10 days, these patients received gluten-free, meat-free and egg-free diets. After the gluten-free diet IgAIC levels significantly decreased, whereas they were unaffected by meat- or egg-free diets. IgAIC showed a rebound after one month of unrestricted diet, but again significantly decreased during a subsequent period of six months of a gluten-free diet.

Introduction

Experimental models and clinical observations indicate a pathogenetic role of circulating IgA containing immune complexes (IgAIC) in primary IgA nephropathy [1-3].

Since IgA is the prevalent immunoglobulin class in secretions, a great interest has been directed to the mucosal immune system as a major factor in the pathogenesis of IgA nephropathy.

Moreover, recent experimental models in mice have demonstrated that IgA nephropathy can be induced by alimentary antigen challenge through the production of specific IgA by the mucosal immune system [4].

The aim of our study was to investigate whether in IgA nephropathy one may identify common alimentary antigens able to elicit a specific IgA response leading to the formation of circulating IgAIC.

Materials and methods

Patients Six patients with IgA nephropathy (4 males, mean age 34 years, range 19- 55 years), having had a renal biopsy two to nine years before, were selected

as having, during the previous six month follow-up, signs of constant major urinary activity with persistent heavy microscopic haematuria (>30 red blood cells/x400 microscopic field) or, in one case, nephrotic-range proteinuria. Over the same period these patients had high IgAIC levels in three out of three determinations. Four of them had a slight increase in creatinine (range 1.4–2.7mg/dl). Three were mildly hypertensive. No patients had clinical or laboratory signs of liver, bowel, skin or systemic diseases. In each patient skin biopsy excluded dermatitis herpetiformis and the oral xylose test and the jejunal biopsy, performed in each patient by endoscopy, excluded even a subclinical form of coeliac enteropathy. Of note, three IgA nephropathy patients had first degree villous changes, insufficient for a diagnosis of coeliac disease, but making a slight difference with normal jejunal mucosa.

Dietetic protocols The general design of our study was to evaluate the effect of dietetic protocols with different alimentary antigen challenge or withdrawal.

During an initial study of three test periods of 10 days the patients received gluten-free, meat-free and egg-free diets. In each diet the protein intake was 1–1.2g/kg body weight and the total caloric content was 1600–2000Kcal/day. Gluten-free diet was identical to that commonly prescribed to patients with coeliac disease, strictly forbidding wheat gluten flour and its derivatives, mostly pasta, bread and pastries.

At the end of each 10 day period of gluten, meat or egg withdrawal, a challenge of the same presumed antigen was given. These alimentary antigen challenge meals consisted of 1) 150g of pasta and 250g of bread; 2) 300g of bovine meat; 3) three eggs. Blood samples were sequentially taken each hour up to the eighth hour after the meal.

After this initial period, patients have been allowed to follow a gluten-containing diet for three months and then a six month period of a gluten-free diet was prescribed.

Detection of circulating IgAIC A modified conglutinin solid phase assay, previously described [2,3], employing affinity chromatography-purified anti-human IgA antibodies, conjugated with alkaline phosphatase, was used to detect IgAIC. Day-to-day variations were corrected by comparing results obtained in control sera [5].

Controls IgAIC were measured in 30 healthy subjects from the Nephrology Staff. The upper 90th percentile was the limit chosen to define the normal range.

In six control subjects blood samples were sequentially taken each hour up to the eighth hour after a single meal.

Statistical evaluation Both parametrical (p_1 =Student's test for paired observations) and non-parametrical tests (p_2 =Rank Signed test) were used for statistical analysis.

Results

Each patient entering this study displayed, over the previous six months, abnormal IgAIC values in three out of three determinations (mean values 1.47 OD, range 0.55–2.60 OD versus controls mean values 0.25 OD, range 0.17–0.61 OD, normal values being <0.50 OD).

After the test periods of 10 days a significant decrease in IgAIC was observed only with the dietetic protocol excluding gluten derivatives (Table I) ($p_1 < 0.03$, $p_2 < 0.02$). The decrease was particularly impressive in the patients presenting the higher IgAIC values before any dietetic restriction

TABLE I. Levels of IgAIC in six patients with IgA nephropathy following an initial 10 day gluten-free diet

	IgAIC levels
before diet	400nm OD mean 1.10 range 0.30–1.67
after diet	† ↓ $p_1 < 0.03$ $p_2 < 0.02$ mean 0.48 range 0.29–0.84

p_1 =levels of significance with parametrical analysis (Student's test for paired observations)
 p_2 =levels of significance with non-parametrical analysis (Rank Signed test)

Conversely, the levels of IgAIC were unaffected by meat-free ($p_1 > 0.1$, $p_2 > 0.1$) and egg-free diet ($p_1 > 0.1$, $p_2 > 0.1$).

Gluten flour challenge, given in a single meal, failed to show any significant increase in baseline IgAIC levels over eight hours. Neither were IgAIC modified by bovine meat and egg challenge meals. IgAIC levels were unmodified in six healthy controls in blood samples obtained one to eight hours after a single meal.

The analysis of blood creatinine, serum IgA, urinary protein loss and microscopic haematuria did not show any significant modification coincident with alimentary antigen challenge or restriction.

Then, over three months, patients had diets personalized on the basis of their renal function: four of them had a completely free diet, whereas the other two, having creatinine values of 2.4 and 2.5mg/dl respectively, had a partial restriction in protein intake (45g/day) with half of the usual pasta and bread intake made by gluten-free products.

The mean IgAIC values significantly increased after gluten-containing diet ($p_1 < 0.02$, $p_2 < 0.04$). At the end of the six month period of gluten-free diet a new gradual decrease in IgAIC mean values was observed ($p_1 < 0.05$, $p_2 < 0.02$) (Table II). Interestingly, at that time all the six patients showed IgAIC levels within the normal range.

TABLE II. Levels of IgAIC in six patients with IgA nephropathy following a six month period of gluten-free diet

	IgAIC levels
before diet	400nm OD mean 0.53 range 0.23–0.75
after diet	† ↓ p1 <0.05 p2 <0.02 mean 0.30 range 0.12–0.46

p1=levels of significance with parametrical analysis (Student's test for paired observations)

p2=levels of significance with non-parametrical analysis (Rank Signed test)

Even more impressive was the comparison between the IgAIC levels, detected over the previous year in this group of patients before entering the dietetic programme, and those observed at the end of our study, after six months of gluten-free diet ($p_1 < 0.01$, $p_2 < 0.02$).

From the clinical point of view no significant improvement was evidenced, but it might be worth mentioning that no further deterioration of renal function was observed in these patients who had most evident clinical and immunological data of fully active renal disease.

Discussion

As IgA glomerular deposits can be experimentally induced in animals by oral antigen immunization [4], there is growing evidence that in humans also alimentary antigens can play some role in IgA nephropathy.

Antibodies to intestinal flora (*E Coli*) and to bovine serum albumin have been detected in patients with IgA nephropathy and IgAIC containing anti-albumin antibodies have been found in some cases [6].

Therefore it has been supposed that the levels of circulating IgAIC may be influenced by common alimentary antigens and our study indicates that gluten might be one of the most important.

We failed to show any significant increase in IgAIC levels, both in control subjects after a usual meal, and in IgA nephropathy patients by giving a specific alimentary antigen challenge in a single meal. Conversely, a two week gluten-free diet period was able to induce a significant decrease in IgAIC levels, which was confirmed in a further six month experience. Neither meat-free nor egg-free diets were able to modify the levels of IgAIC.

Interestingly, the gluten-free diet producing the decrease in IgAIC levels in our patients was identical to that commonly known to be able to improve the clinical features of two diseases closely related: coeliac disease and dermatitis herpetiformis, both characterized by high levels of circulating IgAIC [7].

The association between these two diseases and clinically overt nephritis has been reported, albeit remaining quite rare; of interest in these cases is the histological features which are indistinguishable from those of IgA nephropathy [8,9]. Nevertheless, we excluded, by skin and jejunal tissue examination, the presence of subclinical forms of coeliac disease or of dermatitis herpetiformis in our IgA nephropathy patients entered in the study.

Therefore, we must conclude from our preliminary data that gluten-associated antigens are likely to play some role in IgA nephropathy, being significantly combined with high levels of circulating IgAIC.

One might speculate that an abnormal intestinal immune system response to gluten challenge favours the formation of high amounts of circulating IgAIC, which can overcome the physiological mechanism of immune clearance, found to be impaired in IgA nephropathy patients [10], thus inducing the persistent presence in serum of abnormal levels of IgAIC. The total amount of circulating IgAIC might further increase precipitating a gross haematuria when an additional antigen challenge, such as a respiratory mucosal infection, is superimposed [2,3].

How this hypothetical mechanism can be operating in IgA nephropathy and what can be the clinical benefit of a gluten-free diet in these patients, only further investigation will possibly define.

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