Gluten sensitivity in patients with IgA nephropathy

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Abstract

Background. Coeliac disease is more frequent in IgA nephropathy (IgAN) patients compared to the healthy population. Several hypotheses postulate that food antigens like gluten may be involved in the onset of IgAN.

Methods. In this study, we used a recently developed mucosal patch technique to evaluate the rectal mucosal inflammatory reaction to gluten in patients with IgAN (n = 27) compared to healthy subjects (n = 18). The rectal mucosal production of nitric oxide (NO) and release of myeloperoxidase (MPO) and eosinophil cationic protein (ECP) were measured. Serum samples were analysed for IgA and IgG antigliadin antibodies (AGA), IgA antibodies against tissue transglutaminase and IgA endomyositis antibodies.

Results. Gluten reactivity, defined as increase in MPO and/or NO after gluten exposure, was observed in 8 of 27 IgAN patients. The prevalence of HLA-DQ2 and DQ8 was not increased among gluten-sensitive patients, and the total prevalence among IgAN patients was the same as for the normal population. An elevated serum IgA AGA response was seen in 9 of 27 IgAN patients. The increase in IgA AGA

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Gluten sensitivity in patients with IgA nephropathy
did not correlate with the gluten sensitivity as measured by
NO and/or MPO. A specific serum IgG AGA response was
seen in one patient only. Antibodies against tissue transglu-
taminase and endomysium were not observed.

Conclusion. It is concluded that approximately one-third
of our IgAN patients have a rectal mucosal sensitivity to
 gluten, but without signs of coeliac disease, and we hypothe-
size that such sub-clinical inflammation to gluten might
be involved in the pathogenesis of IgAN in a subgroup of

patients.

Keywords: food antigens; gluten sensitivity; IgA nephropathy;
myeloperoxidase; nitric oxide

Introduction

IgA nephropathy (IgAN) is the most common glomeru-
lonephritis characterized by circulating immune complexes
and deposition of IgA1 and complement C3 in the glomeru-
lar mesangium [1]. An association with infections in the
respiratory or gastrointestinal tract with a triggering mu-
cosal immune reaction is commonly observed [2]. The
exact mechanism or antigens involved are, however, not
known.

In the late 1980s, potential food antigens were suggested
as being involved in the onset of IgAN [3–6], and several
studies have since then been conducted as to which food
antigens are responsible, if any, and by which mechanism.
Gluten has been proposed as one potential antigen [7–10].
Deposition of other food antigens (like soy bean protein,
casein and rice protein) has been found in the mesangium of
patients with IgAN [11]. Coppo et al. [12] have shown that
experimental IgAN can be induced by gliadin in mice, and
an association between IgAN and coeliac disease has been
reported in clinical investigations [8,13]. Approximately
4% of the IgAN patients have coeliac disease, as compared
with 0.5–1% in a healthy population [13]. Still, a gluten
trigging immune response in IgAN is disputable and the
mechanism remains unclear.

Food hypersensitivity reactions are classified into two
groups: food allergy, which is an immune-mediated re-
action, and food intolerance, which has various path-
omenismis [14]. Food allergies may be divided into
IgE-mediated and non-IgE-mediated reactions, with coeliac
disease representing the best characterized non-IgE food
allergy. It is hypothesized that the food antigens possibly
being involved in IgAN also trigger non-IgE reactions, al-
though the mechanisms remain to be investigated.

Whereas IgE-mediated reactions are typically diagnosed
with a skin prick test or measurement of IgE antibody levels
to antigen, non-IgE-mediated reactions have traditionally
been difficult to diagnose, relying primarily on food elimi-
nation and food challenge tests. With a recently developed
mucosal patch technique [15], we are able to evaluate in-
flammatory reaction in the rectal mucosa before and after
food antigen challenge [16]. In this study, we employed this
new technique to test for gluten sensitivity among patients
with IgAN.

Table 1. Baseline demographics

<table>
<thead>
<tr>
<th></th>
<th>IgAN patients</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>27</td>
<td>4.6 ± 0.9</td>
</tr>
<tr>
<td>Disease duration (years since biopsy)</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Urine albumin (mg/day)</td>
<td>&lt;30 mg/day</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>30–300 mg/day</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>&gt;300 mg/g/day</td>
<td>8</td>
</tr>
<tr>
<td>Estimated glomerular filtration rate (eGFR)</td>
<td>9</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>&lt;30 mL/min</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>30–60 mL/min</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>&gt;60 mL/min</td>
<td>11</td>
</tr>
<tr>
<td>Concomitant medication</td>
<td>ACE inhibitor/ARB</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>Other antihypertensive</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>Corticosteroid</td>
<td>4</td>
</tr>
</tbody>
</table>

For the three patients having undergone kidney transplantation, the disease duration is calculated from time of biopsy confirming recurrence of IgAN in kidney transplant.

ACE inhibitor (angiotensin-converting enzyme inhibitor); ARB (angiotensin II receptor blocker).

Subjects and methods

Study subjects

A total of 28 subjects (20 males) with established IgAN, recruited from the University Hospital of Uppsala, Sweden, and 18 healthy controls (13 males) were included in the study. One of the included patients was ex-
cluded from the study analyses due to an already established coeliac dis-
case. The mean age for the patient group was 42 years (range: 23–70)
whereas the mean age for the control group was 31 years (range: 19–58).
In order to participate, the subjects could not have a history of anaphylaxis
or anorectal inflammation.

The IgAN diagnosis was verified by a biopsy in all patients. Three
patients had recurrent IgAN in renal transplant allografts, of which one
was transplanted twice and experienced recurrent IgAN in both allografts.

The description of renal impairment and concomitant medication is
listed in Table 1. Sixty-seven percent of the patients had albuminuria of
>300 mg/day, and 16 patients (59%) had a significantly reduced renal
function as calculated using the MDRD (Modification of Diet in Renal
Disease) formula. Twenty-four of 27 patients received antihypertensive
medication.

There was no recognized food intolerance in the patients at inclusion,
although two patients indicated that they had some form of food intolerance
without knowing the specific food antigen. Three patients reported family
members (parents and/or children) with milk or gluten intolerance.

All subjects gave their informed consent to participation prior to any
study-specific procedures.

Rectal challenge

The patients and the healthy control subjects were challenged with 6.2–6.5
g wheat gluten suspended in a 25 mL 0.9% NaCl solution (Crude Wheat
Gluten; Sigma Chemical Co., St. Louis, Mo, USA) in the rectum. The
subjects retained their gluten enema for at least 1 h. Rectal challenge was
performed between 4 and 6 p.m., and samplings were made 15 h later,
between 7 and 9 a.m. The subjects were told to fast for 1 h before and
1 h after the challenge and also from midnight until the samplings were
made. All patients and controls were given a rectal enema (Klyx 120 mL;
Ferring, Copenhagen, Denmark) within 1 h before being tested with the
mucosal patch technique.

Mucosal patch technique and analytical measurements

The instrument used was a plastic catheter with a silicon balloon at the
end of the catheter. Three patches made of a highly absorptive cellulose
material (Phadia AB, Uppsala, Sweden) were attached to the balloon. After
the instrument was positioned in the rectum with the subject lying in the
left lateral position, the balloon was inflated with air bringing the patches in
contact with the mucosa for 20 min. The balloon was then deflated and the content of the patches extracted as previously described by Kristjansson et al. [15]. The extraction solutions were frozen at –70°C and analysed, according to the instructions of the manufacturers, for concentrations of granule constituents from neutrophils (myeloperoxidase; MPO) and eosinophils (eosinophil cationic protein; ECP) using the ELISA-MPO kit from Diagnostics Development, Uppsala, Sweden, and ECP ImmunoCap from Phadia Diagnostics, Uppsala, Sweden.

Air samples were collected with glass syringes during deflation of the balloons and analysed for nitric oxide (NO) with a chemiluminescence NO analyser (model Sievers NOA 280, Ionics Instrument Business Group, Boulder, CO, USA) as previously described [16]. Each patient's baseline value was obtained without protein challenge (13 patients) or after another negative protein challenge (soy protein or cow's milk protein; 14 patients), at least one week before or after the gluten challenge.

Serum samples were analysed for IgA and IgG antibodies to gliadin and IgA antibodies against tissue transglutaminase by use of ELISA. IgA endomysium antibodies were measured by use of an indirect fluorescence technique (Kallestad, Diagnostics, Chaska, MN, USA). All analyses were undertaken by the Department of Clinical Immunology, University Hospital, Uppsala, Sweden.

eGFR was calculated from serum creatinine. Twenty-four-hour albuminuria data were routinely collected for all IgAN patients, and the sample date closest to the gluten challenge mucosal patch test was chosen for the evaluation of proteinuria in this study. HLA-DQBI genotype testing was performed by use of PCR-SSOP (polymerase chain reaction sequence-specific oligonucleotide probes) on the Luminex flow bead platform (One Lambda Inc, Canoga Park, CA, USA).

Statistical analyses

The statistical analyses were undertaken using SPSS version 14.0 (Chicago, IL, USA). The results are presented as means ± standard error of the mean (SEM) for data with normal distribution and median and interquartile range within brackets for skewed data. The Mann–Whitney U-test, Fisher's exact test and Spearman's rank correlation test (rho) were used for the statistical analyses.

Ethics and administration

The study was approved by the Ethics Committee of the Medical Faculty, Uppsala University, and performed in accordance with the Declaration of Helsinki.

Results

Inflammatory response upon gluten challenge

Six of 27 patients (22%) had a significant NO production response upon rectal challenge with gluten, as compared to the 97.5 centile (i.e. 73 p.p.b.) in the control group (Figure 1). Six of 27 patients (22%) demonstrated higher MPO values after gluten challenge as compared to the mean level + 2 SD (i.e. 25 µg/L) in healthy controls (Figure 2). The median baseline NO and MPO values were 9 p.p.b. (7 p.p.b., 19 p.p.b.) (without protein challenge) or 13 p.p.b. (11 p.p.b., 24 p.p.b.) (after another negative protein challenge), and 8 µg/L (4 µg/L, 11 µg/L) (without protein challenge) or 15 µg/L (8 µg/L, 25 µg/L) (after another negative protein challenge), respectively. For the healthy controls, the median baseline NO and MPO values were 14 p.p.b. (8 p.p.b., 31 p.p.b.) and 4 µg/L (0.4 µg/L, 11.1 µg/L), respectively.

Individual data for patients with a positive rectal mucosal inflammatory reaction to gluten exposure as documented by increased NO and/or MPO levels are given in Table 2. A positive correlation was found between MPO and NO responses (Spearman’s rho = 0.46, P < 0.05) in the patient group. No differences were seen in ECP values between the two groups (data not shown).

None of the patients had elevated levels of tissue transglutaminase antibodies or endomysial antibodies.

Significantly increased levels of the serum IgA antgliadin antibody (IgA AGA > 40 kU/L, i.e. 97.5 centile of control values) were seen in nine IgAN patients, i.e. 33% of the patients. The increase in IgA AGA did not correlate with the gluten sensitivity as measured by NO and/or MPO (Spearman’s rho = 0.12, P = 0.55 and Spearman’s rho = 0.24, P = 0.24, respectively). Neither did the levels of IgA AGA correlate with age nor with disease duration (data not shown). A specific serum IgG AGA response was seen in only one patient that was gluten sensitive with increased ΔMPO and ΔNO values.

HLA genotyping

Twenty-four patients accepted HLA (human leukocyte antigen) genotyping. Of these, six were HLA-DQ8 positive and one was HLA-DQ2 positive. None had both haplotypes. No
Not related to latent or a sub-clinical form of coeliac disease. We conclude that the gluten reactivity finding is related to tissue transglutaminase antibodies and antiendomysium antibodies, which could indicate predominantly an immune response to gluten. Among patients with coeliac disease, while only two of five had antibodies to tTG, which could indicate predominantly an immune response to gluten [25]. Among patients with cerebellar ataxia supposed to be related to gluten sensitivity (‘gluten ataxia’; ~70% are HLA-DQ2 positive, 56% are serum transglutaminase antibody positive and virtually all are AGA, primarily IgG, positive [26]. Hadjivassiliou et al. suggested that AGA cross-react with epitopes on Purkinje cells in these patients [27], which may succeed a potential blood–brain barrier disruption due to a type 2 transglutaminase autoantibody reaction [28]. Antitissue transglutaminase IgA antibodies have been demonstrated in both the jejunum and within the muscular layer of brain vessels of patients with gluten ataxia [28].

The gluten sensitivity related to other immune-mediated diseases has both characteristics similar to coeliac disease, but also patterns that indicate a unique sensitivity reaction. In a recent study, gluten-sensitive patients with primary Sjögren’s syndrome were all HLA-DQ2 or DQ8 sensitive, and had DQ2 alterations in circulating T cells similar to patients with coeliac disease, while only two of five had antibodies to tTG, which could indicate predominantly an innate immune response to gluten [25]. Among patients with cerebellar ataxia supposed to be related to gluten sensitivity (“gluten ataxia”), ~70% are HLA-DQ2 positive, 56% are serum transglutaminase antibody positive and virtually all are AGA, primarily IgG, positive [26]. Hadjivassiliou et al. suggested that AGA cross-react with epitopes on Purkinje cells in these patients [27], which may succeed a potential blood–brain barrier disruption due to a type 2 transglutaminase autoantibody reaction [28]. Antitissue transglutaminase IgA antibodies have been demonstrated in both the jejunum and within the muscular layer of brain vessels of patients with gluten ataxia [28]. The gluten-sensitivity reaction observed in IgAN patients seems to be of a unique character, different from the classical coeliac disease pattern and diseases like Sjögren’s syndrome and gluten ataxia. However, as we in our study did not analyse kidney biopsies for tissue transglutaminase autoantibodies, a disease pattern similar to gluten ataxia cannot be excluded. As no increase in HLA-DQ2/DQ8 haplotypes is seen in IgAN patients, it correlation was seen between the HLA genotype and gluten sensitivity.

**Table 2.** Individual data for patients with a positive rectal mucosal inflammatory reaction to gluten exposure as documented by increased NO and/or MPO levels

<table>
<thead>
<tr>
<th>Subject</th>
<th>ΔNO (p.p.b.)</th>
<th>ΔMPO (µg/mL)</th>
<th>IgA AGA (kU/L)</th>
<th>Urine albumin (mg/day)</th>
<th>eGFR (mL/min)</th>
<th>HLA typing</th>
</tr>
</thead>
<tbody>
<tr>
<td># 28</td>
<td>853</td>
<td>34</td>
<td>70</td>
<td>20</td>
<td>15</td>
<td>Negative</td>
</tr>
<tr>
<td># 12</td>
<td>550</td>
<td>60</td>
<td>39</td>
<td>1224</td>
<td>93</td>
<td>Negative</td>
</tr>
<tr>
<td># 9</td>
<td>354</td>
<td>231</td>
<td>55</td>
<td>50</td>
<td>53</td>
<td>ND</td>
</tr>
<tr>
<td># 4</td>
<td>295</td>
<td>9</td>
<td>39</td>
<td>1800</td>
<td>74</td>
<td>Negative</td>
</tr>
<tr>
<td># 11</td>
<td>131</td>
<td>26</td>
<td>33</td>
<td>29</td>
<td>58</td>
<td>DQ8</td>
</tr>
<tr>
<td># 18</td>
<td>78</td>
<td>10</td>
<td>47</td>
<td>26</td>
<td>81</td>
<td>DQ8</td>
</tr>
<tr>
<td># 33</td>
<td>47</td>
<td>41</td>
<td>14</td>
<td>106</td>
<td>20</td>
<td>Negative</td>
</tr>
<tr>
<td># 19</td>
<td>0.5</td>
<td>103</td>
<td>138</td>
<td>219</td>
<td>78</td>
<td>ND</td>
</tr>
<tr>
<td>Ref.</td>
<td>&lt;73</td>
<td>&lt;25</td>
<td>&lt;40</td>
<td>&lt;30</td>
<td>&gt;60</td>
<td>NA</td>
</tr>
</tbody>
</table>

NO (nitric oxide); MPO (myeloperoxidase); IgA AGA (IgA antigliadin antibody); eGFR (estimated glomerular filtration rate); HLA (human leukocyte antigen), ND (not done), NA (not applicable).

Ref.: Reference values used in project (calculated as 97.5 centile of control values for NO and IgA AGA, mean + 2 SD of control values for MPO, and by using normal range for urine albumin and eGFR).
is possible that another yet unknown HLA haplotype or non-HLA genes could be involved in the immune reaction or it could be that the gluten reaction seen in a subgroup of IgAN patients is unspecific.

Gluten and its proteolytic fragments have been shown to activate macrophages and dendritic cells and induce secretion of selected cytokines and chemokines [29,30]. Furthermore, a significant NO production by peritoneal macrophages has been demonstrated [30]. These general immune responses combined with the gluten-specific T-cell reactions in coeliac disease patients indicate that gluten may act as an immunogenic protein in susceptible individuals. In IgAN patients, it has been hypothesized that gluten may act as a toxic lectin, which modifies the intestinal permeability [31]. The pathogenic sequence remains unclear, though. Kovacs et al. [32] have shown that the intestinal permeability is increased in IgAN patients and that deterioration of the renal function is larger in patients with increased intestinal permeability. However, Ots et al. speculate that abnormal IgA molecules or defective IgA production is the primary part of the pathogenesis of IgAN and that intestinal lesions and increased IgA AGA are secondary phenomena [33].

Increased intestinal permeability, possibly as a direct result of intestinal inflammation, could lead to transfer of immune complexes to the circulation with subsequent deposits in the glomeruli. Due to conflicting results, the theory of circulating immune complexes and renal deposits has received less attention during recent years, with the qualitative properties, in particular the glycosylation pattern, of polymeric IgA1 receiving more attention [34]. However, although inconclusive, antibodies to dietary antigens, including gluten, have been found in circulating IgA immune complexes and in renal eluates [31], meaning that dietary antigens may be directly or indirectly involved in the pathogenesis of IgAN.

The induced intestinal inflammation observed in our study is compatible with an activation of the innate immune system with MPO production by neutrophils and NO production by macrophages—still requiring some kind of individual propensity to react to gluten (and possibly other food antigens), e.g. genetic disposition, as only one-third individual propensity to react to gluten (and possibly other food antigens, including gluten, may be directly or indirectly involved in the pathogenesis of IgAN.

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Conflict of interest statement. GK, RH, PV and BF are stockholders in Alimenta Medical AB, Uppsala, Sweden. PV is a stockholder in P&M Venge AB, Diagnostics Development, Uppsala, Sweden.

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