References

1. Ansell D, Feehally J, Feest TG *et al*. *UK Renal Registry Report 2007*. UK Renal Registry, Bristol, UK: 2007
2. Tuomilehto J, Borch-Johnsen K, Molarius A *et al*. Incidence of cardiovascular disease in type 1 (insulin-dependent) diabetic subjects with and without diabetic nephropathy in Finland. *Diabetologia* 1998; 41: 784–790
3. Lindsay RS, Little J, Jaap AJ *et al*. Diabetic nephropathy is associated with an increased familial risk of stroke. *Diabetes Care* 1999; 22: 422–425
4. Thorn LM, Forsblom C, Fagerudd J *et al*. Clustering of risk factors in parents of patients with type 1 diabetes and nephropathy. *Diabetes Care* 2007; 30: 1162–1167
5. Department of Health. National Service Frameworks, 2006. http://www.dh.gov.uk
6. Savage DA, Bain SC, McKnight AJ *et al*. Gene discovery in diabetic nephropathy. *Curr Diab Rep* 2007; 7: 139–145
7. McKnight AJ, O'Donoghue D, Maxwell AP. Annotated chromosome maps for renal disease. *Hum Mutat* 2008; 0: 1–8, DOI: 10.1002/humu.20885
8. Wellcome Trust Case Control Consortium. Genome-wide association study of 14 000 cases of seven common diseases and 3000 shared controls. *Nature* 2007; 447: 661–678
9. Samani NJ, Erdmann J, Hall AS *et al*. Genome-wide association analysis of coronary artery disease. *N Engl J Med* 2007; 357: 443–453
10. McPherson R, Pertsemlidis A, Kavaslar N *et al*. A common allele on chromosome 9 associated with coronary heart disease. *Science* 2007; 316: 1488–1491
11. Currie D, Maxwell AP, Sadlier D *et al*. for the Warren 3/UK GoKinD Study Group. Investigation of Adducin 2 (beta) DNA polymorphisms in genetic predisposition to diabetic nephropathy in type 1 diabetes. *Diabet Med* 2008; 25: 1001–1005
12. McKnight AJ, Savage DA, Patterson CC *et al*. Resequencing of genes for transforming growth factor beta1 (TGFB1) type 1 and 2 receptors (TGFBR1, TGFBR2), and association analysis of variants with diabetic nephropathy. *BMC Med Genet* 2007; 8: 5
13. Purcell S, Neale B, Todd-Brown K *et al*. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81: 559–575. http://pngu.mgh.harvard.edu/purcell/plink/
14. Koopmans SJ, Jong MC, Que I *et al*. Hyperlipidaemia is associated with increased insulin-mediated glucose metabolism, reduced fatty acid metabolism and normal blood pressure in transgenic mice over-expressing human apolipoprotein C1. *Diabetologia* 2001; 44: 437–443
15. Hirano T, Sakaue T, Misaki A *et al*. Very low-density lipoprotein–apoprotein CI is increased in diabetic nephropathy: comparison with apoprotein CIII. *Kidney Int* 2003; 63: 2171–2177
16. Li H, Wetten S, Li L *et al*. Candidate single-nucleotide polymorphisms from a genomewide association study of Alzheimer disease. *Arch Neurol* 2008; 65: 45–53

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 endomysium 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

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 transglutaminase 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 hypothesize that such sub-clinical inflammation to gluten might be involved in the pathogenesis of IgAN in a subgroup of patients.

Introduction

IgA nephropathy (IgAN) is the most common glomerulonephritis characterized by circulating immune complexes and deposition of IgA1 and complement C3 in the glomerular mesangium [1]. An association with infections in the respiratory or gastrointestinal tract with a triggering mucosal 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 triggering 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 reaction, and food intolerance, which has various pathomechanisms [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, although 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 elimination and food challenge tests. With a recently developed mucosal patch technique [15], we are able to evaluate inflammatory 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

		IgAN patients
N		27
Disease duration (years since biopsy)[a]		4.6 ± 0.9
Urine albumin (mg/day)	<30 mg/day	3
	30–300 mg/day	6
	300 mg to 1 g/day	8
	>1 g/day	10
Estimated glomerular filtration rate (eGFR)	<30 mL/min	7
	30–60 mL/min	9
	>60 mL/min	11
Concomitant medication	ACE inhibitor/ARB[b]	20
	Other antihypertensive	19
	Corticosteroid	4

[a]For the three patients having undergone kidney transplantation, the disease duration is calculated from time of biopsy confirming recurrence of IgAN in kidney transplant.
[b]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 excluded from the study analyses due to an already established coeliac disease. 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-DQB1 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
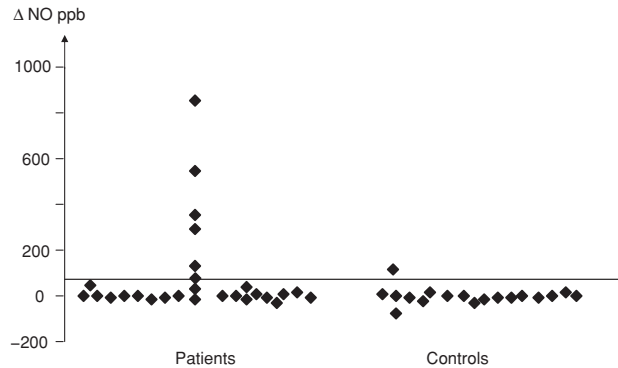

Fig. 1. Increase in rectal nitric oxide (ΔNO) after gluten challenge in patients with IgAN and in healthy controls. The horizontal reference line represents the response cut-off level, defined as two standard deviations (SD) above the mean of control subjects (ΔNO = 73 p.p.b.).
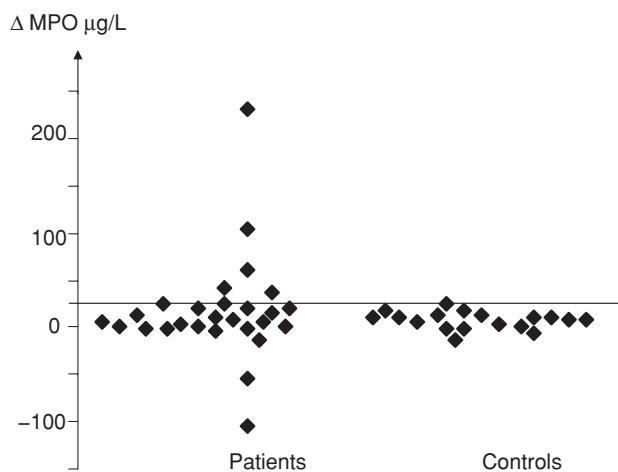

Fig. 2. Increase in rectal myeloperoxidase (ΔMPO) after gluten challenge in patients with IgAN and in healthy controls. The horizontal reference line represents the response cut-off level, defined as two standard deviations (SD) above the mean of control subjects (ΔMPO = 25 μg/L).

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 antigliadin 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

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

Subject	ΔNO (p.p.b.)	ΔMPO (µg/mL)	IgA AGA (kU/L)	Urine albumin (mg/day)	eGFR (mL/min)	HLA typing
# 28	853	34	70	20	15	Negative
# 12	550	60	39	1224	93	Negative
# 9	354	231	55	50	53	ND
# 4	295	9	39	1800	74	Negative
# 11	131	26	33	29	58	DQ8
# 18	78	10	47	26	81	DQ8
# 33	47	41	14	106	20	Negative
# 19	0,5	103	138	219	78	ND
Ref.	<73	<25	<40	<30	>60	NA

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).

correlation was seen between the HLA genotype and gluten sensitivity.

Albuminuria and renal function

As expected, most of the IgAN patients experienced some degree of albuminuria, ranging from 50 mg to 4.5 g/day (Table 1). The degree of albuminuria did not seem to correlate with the degree of inflammatory response upon rectal challenge nor with eGFR, using the MDRD (Modification of Diet in Renal Disease) formula for estimation. Furthermore, there was no correlation between eGFR and inflammatory response.

Discussion

By use of the mucosal patch technique, in this study we have showed that approximately one-third of IgAN patients (8/27) experience gluten reactivity, as demonstrated by increased local mucosal NO production and/or MPO release after gluten challenge. The enhanced NO production is probably a result of the major inducible isoform of NO synthase NOS IIa, which produces NO in high concentrations for as long as it is activated due to inflammatory principles [17,18]. The cellular source of induced luminal NO synthesis after gluten challenge has not been identified, but epithelial cells and macrophages are probably major sources based on previous studies. However, other inflammatory cells may also be contributors to enhanced NO synthesis since the synthase NOS IIa is constitutively present in such cells [19–22]. The increased MPO release, which should reflect degranulation of activated neutrophils accumulating in the gut mucosa, precedes NO production in patients with coeliac disease challenged with gluten, but 15 h after challenge there is both an MPO and NO response present [16]. No increase in ECP was seen, indicating an absence of eosinophil activation at least within 15 h after challenge.

As none of the patients had high serum levels of tissue transglutaminase antibodies and antiendomysium antibodies, we conclude that the gluten reactivity finding is not related to latent or a sub-clinical form of coeliac dis-

ease. Our results are thus in line with previous findings by Sategna-Guidetti *et al.* [23], demonstrating no antiendomysium antibodies and indicating no latent coeliac disease in IgAN patients as such. The prevalence of HLA-DQ2/DQ8 haplotypes in our patients was similar to the general population [24]. Also Collin *et al.* have previously shown that there is no increase in HLA-DQ2/DQ8 haplotypes in patients with IgAN [13], although the incidence of coeliac disease is higher in IgAN patients compared to the healthy population [13].

The magnitude of NO and MPO responses among the IgAN patient responders is 10-fold lower than what is seen among patients with coeliac disease [16], including one patient with IgAN and coeliac disease demonstrating an increase in NO and MPO of 6682 p.p.b. and 1155 µg/L, respectively.

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

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 of the patients experience this adverse reaction upon exposure. The significant correlation between an MPO and NO response was weak (rho = 0.46, $P < 0.05$), but in line with previous findings in coeliac disease patients after gluten exposure (rho = 0.64, $P < 0.05$) [16]. This could indicate that the immunological reaction pattern is highly individual, but could also simply be due to individual test sensitivity as it is likely that e.g. some gaseous NO will escape the system and that MPO released from the patches will depend on mucosal sites of collection.

Three of the IgAN patients in our study had undergone renal transplantation, and thus received immunosuppressive therapy. Further, seven patients had advanced renal failure, which is associated with immunity dysfunction due to uraemia [35]. Alterations in neutrophils have been documented in uraemic patients [36]. These are all factors that might complicate the interpretation of our results, as the mechanism for the mucosal immune response to gluten is not clear. It is worth noticing, however, that only two of the patients with advanced renal failure, of which one was

also transplanted, were sensitive to gluten. This is approximately the same proportion as seen for the other IgAN patients.

Reflecting clinical practice, three of four patients received ACE inhibitors and/or angiotensin II receptor blockers in our study. ACE inhibitors might under certain circumstances exacerbate hypersensitivity reactions [37,38]. As the proportion of patients being sensitive to gluten was approximately the same or slightly smaller among patients receiving ACE inhibitors and/or angiotensin II receptor blockers (5/20) compared to patients not receiving such treatment (3/7), we consider that this effect is minimal and does not affect the results of the study.

The potential relationship between gluten sensitivity and IgAN has led researchers to prescribe such patients gluten-free diets. Coppo *et al.* [39] demonstrated a reduction in circulating IgA-containing immune complexes and a transient reduction of proteinuria in IgAN patients on a gluten-free diet. However, progression of the disease was not halted. Taking into consideration our finding that only 1/3 of the IgAN patients show a mucosal hyperreactivity to gluten, we hypothesize that a more consistent response to a gluten-free diet may be obtained if the patients are screened for sensitivity prior to study inclusion. By use of the mucosal patch technique also sensitivities to other food antigens may be revealed and excluded from the diet.

In conclusion, approximately one-third of our IgAN patients have a rectal mucosal sensitivity to gluten, without manifest coeliac disease, and we hypothesize that such subclinical inflammation to gluten might be involved in the pathogenesis of the disease in a subgroup of patients with IgAN. The hypothesis and our results need to be confirmed, however, in a larger study.

References

1. Emancipator SN. IgA nephropathy: morphologic expression and pathogenesis. *Am J Kidney Dis* 1994; 23: 451–462
2. Emancipator SN, Gallo GR, Lamm ME. IgA nephropathy: perspectives on pathogenesis and classification. *Clin Nephrol* 1985; 24: 161–179
3. Emancipator SN, Gallo GR, Lamm ME. Experimental IgA nephropathy induced by oral immunization. *J Exp Med* 1983; 157: 572–582
4. Nagy J, Scott H, Brandtzaeg P. Antibodies to dietary antigens in IgA nephropathy. *Clin Nephrol* 1988; 29: 275–279
5. Sancho J, Egido J, Rivera F *et al*. Immune complexes in IgA nephropathy: presence of antibodies against diet antigens and delayed clearance of specific polymeric IgA immune complexes. *Clin Exp Immunol* 1983; 54: 194–202
6. Sato M, Ideura T, Koshikawa S. Experimental IgA nephropathy in mice. *Lab Invest* 1986; 54: 377–384
7. Coppo R, Basolo B, Rollino C *et al*. Dietary gluten and primary IgA nephropathy. *N Engl J Med* 1986; 315: 1167–1168

8. Fornasieri A, Sinico RA, Maldifassi P *et al*. IgA-antigliadin antibodies in IgA mesangial nephropathy (Berger's disease). *Br Med J (Clin Res Ed)* 1987; 295: 78–80

9. Laurent J, Branellec A, Heslan JM *et al*. An increase in circulating IgA antibodies to gliadin in IgA mesangial glomerulonephritis. *Am J Nephrol* 1987; 7: 178–183

10. Coppo R, Amore A, Roccatello D *et al*. Role of food antigens and alcohol in idiopathic nephritis with IgA deposits. *Minerva Urol Nefrol* 1991; 43: 171–174

11. Sato M, Kojima H, Takayama K *et al*. Glomerular deposition of food antigens in IgA nephropathy. *Clin Exp Immunol* 1988; 73: 295–299

12. Coppo R, Mazzucco G, Martina G *et al*. Gluten-induced experimental IgA glomerulopathy. *Lab Invest* 1989; 60: 499–506

13. Collin P, Syrjanen J, Partanen J *et al*. Celiac disease and HLA DQ in patients with IgA nephropathy. *Am J Gastroenterol* 2002; 97: 2572–2576

14. Johansson SG, Hourihane JO, Bousquet J *et al*. A revised nomenclature for allergy. An EAACI position statement from the EAACI nomenclature task force. *Allergy* 2001; 56: 813–824

15. Kristjansson G, Venge P, Wanders A *et al*. Clinical and subclinical intestinal inflammation assessed by the mucosal patch technique: studies of mucosal neutrophil and eosinophil activation in inflammatory bowel diseases and irritable bowel syndrome. *Gut* 2004; 53: 1806–1812

16. Kristjansson G, Hogman M, Venge P *et al*. Gut mucosal granulocyte activation precedes nitric oxide production: studies in coeliac patients challenged with gluten and corn. *Gut* 2005; 54: 769–774

17. Forsythe P, Gilchrist M, Kulka M *et al*. Mast cells and nitric oxide: control of production, mechanisms of response. *Int Immunopharmacol* 2001; 1: 1525–1541

18. Grisham MB, Pavlick KP, Laroux FS *et al*. Nitric oxide and chronic gut inflammation: controversies in inflammatory bowel disease. *J Investig Med* 2002; 50: 272–283

19. Thomassen MJ, Kavuru MS. Human alveolar macrophages and monocytes as a source and target for nitric oxide. *Int Immunopharmacol* 2001; 1: 1479–1490

20. Armstrong R. The physiological role and pharmacological potential of nitric oxide in neutrophil activation. *Int Immunopharmacol* 2001; 1: 1501–1512

21. Barnes PJ, Belvisi MG. Nitric oxide and lung disease. *Thorax* 1993; 48: 1034–1043

22. Payne DN, Adcock IM, Wilson NM *et al*. Relationship between exhaled nitric oxide and mucosal eosinophilic inflammation in children with difficult asthma, after treatment with oral prednisolone. *Am J Respir Crit Care Med* 2001; 164: 1376–1381

23. Sategna-Guidetti C, Ferfoglia G, Bruno M *et al*. Do IgA antigliadin and IgA antiendomysium antibodies show there is latent coeliac disease in primary IgA nephropathy? *Gut* 1992; 33: 476–478

24. Sollid LM, Markussen G, Ek J *et al*. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. *J Exp Med* 1989; 169: 345–350

25. Liden M, Kristjansson G, Valtysdottir S *et al*. Gluten sensitivity in patients with primary Sjogren's syndrome. *Scand J Gastroenterol* 2007; 42: 962–967

26. Hadjivassiliou M, Williamson CA, Woodroofe N. The immunology of gluten sensitivity: beyond the gut. *Trends Immunol* 2004; 25: 578–582

27. Hadjivassiliou M, Grunewald R, Sharrack B *et al*. Gluten ataxia in perspective: epidemiology, genetic susceptibility and clinical characteristics. *Brain* 2003; 126: 685–691

28. Hadjivassiliou M, Maki M, Sanders DS *et al*. Autoantibody targeting of brain and intestinal transglutaminase in gluten ataxia. *Neurology* 2006; 66: 373–377

29. Nikulina M, Habich C, Flohe SB *et al*. Wheat gluten causes dendritic cell maturation and chemokine secretion. *J Immunol* 2004; 173: 1925–1933

30. Tuckova L, Novotna J, Novak P *et al*. Activation of macrophages by gliadin fragments: isolation and characterization of active peptide. *J Leukoc Biol* 2002; 71: 625–631

31. Coppo R. The pathogenetic potential of environmental antigens in IgA nephropathy. *Am J Kidney Dis* 1988; 12: 420–424

32. Kovacs T, Kun L, Schmelczer M *et al*. Do intestinal hyperpermeability and the related food antigens play a role in the progression of IgA nephropathy? I. Study of intestinal permeability. *Am J Nephrol* 1996; 16: 500–505

33. Ots M, Uibo O, Metskula K *et al*. IgA-antigliadin antibodies in patients with IgA nephropathy: the secondary phenomenon? *Am J Nephrol* 1999; 19: 453–458

34. Coppo R, Amore A. Aberrant glycosylation in IgA nephropathy (IgAN). *Kidney Int* 2004; 65: 1544–1547

35. Kato S, Chmielewski M, Honda H *et al*. Aspects of immune dysfunction in end-stage renal disease. *Clin J Am Soc Nephrol* 2008; 3: 1526–1533

36. Haag-Weber M, Horl WH. The immune system in uremia and during its treatment. *N Horiz* 1995; 3: 669–679

37. Scholzen TE, Stander S, Riemann H *et al*. Modulation of cutaneous inflammation by angiotensin-converting enzyme. *J Immunol* 2003; 170: 3866–3873

38. Joseph K, Kaplan AP. Formation of bradykinin: a major contributor to the innate inflammatory response. *Adv Immunol* 2005; 86: 159–208

39. Coppo R, Roccatello D, Amore A *et al*. Effects of a gluten-free diet in primary IgA nephropathy. *Clin Nephrol* 1990; 33: 72–86