

Alpha/beta intraepithelial lymphocytes, serum gliadin antibodies and allergy test positivity in children

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Aim: To determine whether the amount of $\alpha\beta$ -positive intraepithelial lymphocytes (IELs) is connected to allergy test positivity in children with gastrointestinal symptoms and whether elevated serum gliadin antibodies have any role in the diagnosis. **Methods:** Twenty-seven children suffering from gastrointestinal-symptoms in whom intestinal biopsies had previously been obtained via endoscopy or with capsule biopsy to exclude coeliac disease were included into the study. The other inclusion criteria were increased amounts of CD3 and $\alpha\beta$ -positive IELs with normal amounts of $\gamma\delta$ -positive IELs in duodenal or jejunal biopsy specimens. At the control visit, the children underwent a physical examination and parents filled in a questionnaire concerning gastrointestinal- and atopic symptoms. Skin prick- and patch tests were done and serum gliadin, endomysium, transglutaminase antibodies and specific IgEs were measured. **Results:** Only nine children (33%) had at least one positive result in the allergy tests, the rest remaining test negative. In children with digestive symptoms, IgG-class gliadin antibody titres were higher than those of the non-symptomatic children. A significant correlation was found between IgG-class gliadin antibodies and total amount of $\alpha\beta$ -positive IELs ($p = 0.017$).

Conclusion: No positive skin test or specific IgE positivity for cereals in children with high intestinal T-cell densities was observed. The correlation between IgG-class gliadin antibodies and the total amount of $\alpha\beta$ -TCR positive-cells is likely to be a reflection of local immune response of the gut.

Key words: Allergy test positivity, gliadin antibodies, intraepithelial lymphocytes

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Food allergy (FA) is an adverse immunological reaction that can be classified as IgE-antibody-related acute reactions, subacute non-IgE (mainly T cell)-mediated reactions and chronic IgE-associated or cell-mediated disorders (1). IgE-related atopic (type I) allergy to different foods is the best-understood and scientifically accepted mechanism (2). Acute IgE-related reactions can affect multiple target organs while subacute and chronic reactions typically influence the skin and the gastrointestinal tract (1). IgE-mediated sensitization can be studied using skin-prick tests and by measuring specific IgE in blood (1, 3). Previous studies comparing the results of prick-, patch- and specific IgE-test results with those of elimination-challenge tests in wheat and cow's milk allergy have shown that the specificity of FA is difficult to establish and the only scientifically accepted method is to perform an elimination-challenge test (3).

In food hypersensitivity the normal immune responses to dietary proteins are disturbed (1). In FA, the immune response releases local inflammatory agents of the gut (7), and elevated IgG- and IgA-gliadin antibody levels (IgG- and IgA-AGAs) in the sera are observed along that. Cereal allergy is usually considered as a disease of small children and adult data are scant (8). In adult patients suffering from gastrointestinal (GI) symptoms related to cereal ingestion, increased amounts of IgA AGAs have been found (9).

A small intestinal biopsy and histopathological examination of the duodenal specimens are essential procedures in the investigation of coeliac disease (CD), and when CD is untreated, high duodenal intraepithelial lymphocyte counts are observed (10). In immunohistochemical staining, these consist of CD3-positive cells that are mostly $\alpha\beta$ -TCR- and $\gamma\delta$ -TCR positive. Typically, in CD, the amount of positive $\gamma\delta$ -IELs is high (11). The reason for the elevated $\alpha\beta$ -positive T-cell

Table 1. Gastrointestinal symptoms and gliadin antibodies at the time of the second evaluation. Individual cell counts, routine histology as well as gliadin IgG- and IgA-antibody levels by the time of endoscopy/capsule biopsy are shown in parallel. Mean time interval between the evaluations was 42 mo.

Patient no./GI-symptoms ^a Y: yes; N: no	CD3 cells	$\alpha\beta/\gamma\delta$ -cells	IEL counts	Villus height	Eosinophils/ lymphocytes/ neutrophils	IgG AGA EU/ml	IgG AGA ^a EU/ml	IgA AGA EU/ml	IgA AGA ^a EU/ml
1/Y	32	17/2.2	N	N	Z/N/N	1.48	2.40	<0.10	<0.10
2/N	30	10/3.7	N	N	N/N/N	1.20	1.60	<0.10	<0.10
3/Y	46	35/4.2	I	N	C/N/Z	27.80 ^b	26.30 ^b	0.32 ^b	<0.10
4/Y	34	24/2.5	N	N	N/N/Z	30.10 ^b	41.50 ^b	0.48 ^b	0.80 ^b
5/Y	37	30/3.0	C	F	N/N/N	0.01	2.55	<0.10	<0.10
6/N	45	18/3.0	I	N	N/N/Z	5.35	3.00	<0.10	<0.10
7/ Y	81	73/5.3	N	N	N/N/N	38.00 ^b	43.85 ^b	0.82 ^b	0.31 ^b
8/ Y	33	20/2.1	N	N	N/N/Z	-	0.50	0.50 ^b	1.84 ^b
9/ Y	58	29/0.0	N	N	N/C/Z	20.0 ^b	47.10 ^b	0.62 ^b	0.69 ^b
10/ N	41	30/1.4	N	N	N/N/N	2.43	1.80	<0.10	<0.10
11/N	74	69/2.1	C	N	N/N/N	-	0.70	-	<0.10
12/Y	33	16/2.2	N	N	N/I/Z	-	2.70	-	<0.10
13/Y	32	35/5.5	I	N	I/I/N	0.81	0.50	<0.10	<0.10
14/N	32	12/2.0	N	N	N/C/Z	0.69	0.50	<0.10	<0.10
15/N	34	33/4.6	N	N	N/N/Z	12.80	5.45	0.20 ^b	0.11
16/Y	54	33/2.7	N	N	N/N/Z	-	0.95	0.93 ^b	0.90 ^b
17/Y	75	39/0.8	I	N	I/N/Z	6.70	19.90 ^b	<0.10	0.21 ^b
18/Y	86	49/1.4	I	N	N/I/Z	28.60 ^b	30.60 ^b	0.59 ^b	0.47 ^b
19/Y	35	16/1.5	N	N	N/N/Z	-	0.50	-	<0.10
20/Y	44	32/3.2	N	N	N/N/N	0.88	0.50	<0.10	<0.10
21/N	32	18/2.6	N	N	N/N/Z	2.05	0.70	0.19	<0.10
22/Y	33	33/2.0	N	N	N/N/Z	62.00 ^b	38.30 ^b	0.33 ^b	0.26 ^b
23/Y	34	30/1.5	N	N	N/N/Z	21.34 ^b	26.25 ^b	0.20 ^b	0.27 ^b
24/N	38	26/1.4	N	N	Z/N/Z	5.43	1.55	0.16	0.88 ^b
25/N	33	16/1.7	N	N	N/N/Z	-	0.50	0.62 ^b	0.24 ^b
26/Y	30	10/1.4	N	N	N/N/Z	28.00 ^b	13.80	0.21 ^b	<0.10
27/N	32	19/3.0	N	N	N/N/Z	-	24.45 ^b	0.72 ^b	0.14

N: normal; I: increased; C: clearly increased; F: flat; Z: zero.

^aResults by the time of re-evaluation.

^bValues over normal (IgG AGA ≥ 15 EU/ml, IgA AGA ≥ 0.20 EU/ml).

count is not clearly understood, but it might be driven directly by gluten. Serological screening tests are widely used to detect CD, even among patients with only a slight clinical suspicion of the disease (12). Serum AGAs are predominantly of the IgG and IgA classes (13) of which IgG-class AGAs are poorly correlated with CD (14, 15) and may be related to a non-specific gut inflammatory process (16).

The objective of the present study was to explore the purported association of gut IELs and allergy test positivity for cereals in a selected group of children with previously demonstrated, yet not definitively diagnosed, local immune response of the gut.

Patients and methods

A group of children who had undergone endoscopy with biopsies ($n = 20$) or capsule biopsy ($n = 7$) between September 1993 and November 1998 in Tampere University Hospital because of suspected CD were examined. Based on histological investigation of duodenal of jejunal biopsies CD had initially been excluded according to the acknowledged diagnostic

criteria of the European Society for Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) (17). In the initial histological examination of the small-bowel biopsies, the amount of IELs was quantitated as being normal or slightly- or markedly increased. The presence of eosinophils, lymphocytes and/or neutrophils was also noted (see Table 1).

The inclusion criteria for the study were: increased total amount of CD3 (30 or more/high-power field) and $\alpha\beta$ -positive IELs (16 or more/high-power field) without increased $\gamma\delta$ -positive IELs (4 or under/high-power field or less than 15% of total CD3 count) in duodenal or jejunal biopsy. Three children with normal $\alpha\beta$ -positive IEL counts who expressed pathological staining in the lamina propria but not in the crypts (typical for CD) were also included. One patient who initially had been on a gluten-free diet expressed flat duodenal mucosa with similar histology in the control biopsy (patient 5, Table 1). All this patient's other markers for CD (serology and $\gamma\delta$ -cells) were negative, however, and he was later found to have SLE. In total, 27 children (12 boys and 15 girls) were available. By the time of the endoscopic examination or capsule biopsy (first evaluation), these children were at a mean of 8.4 y of age

(range 2.5–16.5 y), and the second evaluation (clinical, skin tests and serology) was performed at the mean age of 11.9 y (range 6.3–19.6 y). The mean time from biopsy to re-investigation was 42 mo, (range 19–68 mo). All the children underwent a physical examination and the parents filled in a questionnaire. We inquired specifically about GI symptoms and symptoms of atopic allergy (respiratory-, skin- and GI symptoms). Other diseases were listed and parents were asked to give information about their own allergies and diseases and those of the siblings. Growth was evaluated by using standardized Finnish growth charts (18).

To stain monoclonal antibodies (MAbs) on duodenal biopsy, specimens were obtained as described earlier (19). All specimens were checked by one of the two experienced investigators.

Skin tests

All skin testing were performed as earlier described (20), and a dermatologist interpreted the patch-test reactions. Prick testing was done using commercially available cow's milk, egg and soy bean (ALK, Allergologisk Laboratorium A/S, Hørsholm, Denmark) and local wheat-, barley-, rye- and oats flours diluted in physiologic saline. Gliadin was prepared from commercially available crude gliadin (1 mg/ml in 20% ethanol; Sigma Chemical Co., St Louis, Mo) (21). Histamine dihydrochloride 10 mg/ml was used as the positive and physiologic saline as the negative control. Reactions were read after 15 min. The reaction was considered positive if the mean diameter of the wheal was at least 3 mm and the negative control 0 mm, simultaneously. Patch testing was performed as described elsewhere (20). The test material was prepared from milk powder, lyophilized egg white, wheat, barley, rye, oats and soy flours mixed with physiologic saline. Gliadin powder was made from wheat gluten of Sigma Chemical, which was diluted to physiologic saline as well. Test material was set on small aluminium test cups (Finn Chamber, Epitest Ltd., Hyrylä, Finland) and applied to healthy skin of the back with the help of Scanpore adhesive. Microcrystalline cellulose served as a negative control. The occlusion time was 24 h, and the reactions were evaluated 48 h later. The reaction was deemed negative in the case of no visual or palpable changes in the skin, irritation in the case of light redness on the contact area of the cup, and positive if there was clearly a visible redness extending beyond the area of the aluminium cup with less (+) or more (++) eczema and the negative contact had to remain negative.

Laboratory analysis

Sera were drawn on the day of the allergy tests. Serum-specific IgEs were measured towards cereals and glutenin by using the immunoluminometry method (Bayer Corporation, Tarrytown, NY 10591-5097 USA) as described earlier (4, 5). Values <0.4 kU/L were con-

sidered normal. Serum gliadin antibodies were measured by solid-phase enzyme-linked immunoabsorbent assay (ELISA), and the cut-off for seropositivity was 15 EU/mL for IgG-class AGAs, and 0.20 EU/ml for IgA-class AGAs (14, 22). Serum endomysium antibodies (EMA) were measured with an indirect immunofluorescence method using tissue from the preterm umbilical cord, and the established selected titre for cut-off was 1:5 (23). Transglutaminase (TGA) antibodies were determined by the tTGA-ELISA method (INOVA diagnostics, Inc., San Diego, Calif.), where the normal range is 0–20 AU (12).

Statistical methods

The data were analysed using SPSS 9.0 for windows 1998 and the statistics calculated with the Mann–Whitney U-test and Spearman's rho. A *p*-value <0.05 was considered significant.

Ethical considerations

Patients were included in the study after written consent had been obtained from their parents and the Ethics Committee of Tampere University Hospital approved the study protocol

Results

Seventeen out of 27 children (63%) had had GI symptoms within the previous month before the re-examination (Table 1) and 12/27 (44%) had current atopic eczema (24). Eight out of 27 (30%) children suffered from rhino-conjunctivitis and 5/27 (19%) had diagnoses of asthma. Atopy in the family was common, 67% of children having at least one atopic parent and 56% atopic siblings. Twenty-one children (78%) had normal growth, 3 (11%) had diagnosed reasons for their growth retardation (SLE, FAs), one child had mental and growth retardation for unknown reason, and 3 had mild growth retardation of unknown origin.

Serum IgG-class AGAs were normal in 18 patients and elevated in 9, while serum IgA-class AGAs were normal in 16 patients and elevated in 11. One child had had seroconversion from negative to positive in IgA-class AGAs between the two evaluations (patient 24, Table 1). Both AGAs were elevated in seven patients, whereas serum EMAs and TGAs were normal in all patients. A statistically significant correlation was found between IgG-class AGAs and the total amount of positive IELs (*p* = 0.017). Children with digestive symptoms had higher, though not statistically different IgG-class AGA levels (mean 17.5 EU/ml) than those without GI symptoms (mean 4.1 EU/ml) (*p* = 0.107 Spearman's rho) (Fig. 1A). When comparing IgG-class AGAs and symptoms of eczema, no correlation was found (*p* = 0.905). Children with rhino-conjunctivitis seemed to have lower IgG-class AGAs (mean 8.2 EU/

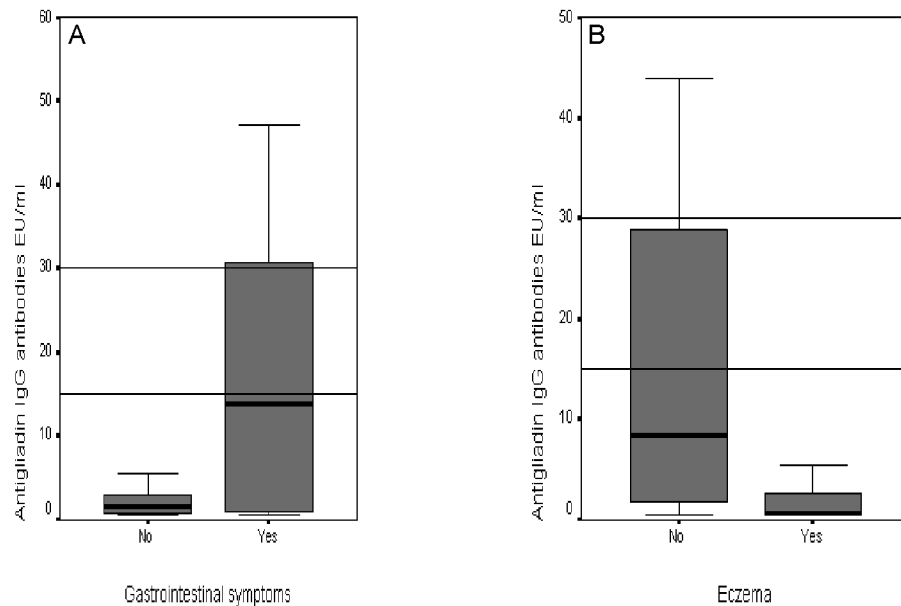


Fig. 1. Comparison of IgG AGAs in children with ($n = 17$) or without ($n = 10$) gastrointestinal symptoms (A). Children with at least one positive allergy test result are shown (B). The group was divided according to the eczema status (with eczema $n = 5$, no eczema $n = 4$) and IgG gliadin antibodies were compared between the groups. The cut-off for seropositivity (15 EU/ml) is marked, as well as the cut-off for strong seropositivity (30 EU/ml) used in some studies. Notice the different scales on the y axis.

ml) than the rest of the group (mean 14.3 EU/ml), but again this finding was not statistically significant.

Most of the children had no positive allergy test results in this study and only 9 out of 27 (33%) showed one or several positive results. Seven children had a positive result in one or several cereal tests (one prick, others patch tests). Two children had a reaction to cow's milk protein, one of them also testing positive against soy. When a group with at least one positive allergy test was selected, children with current eczema had clearly, though not statistically significantly, lower serum IgG-class AGAs than those without eczema (Fig. 1B).

Discussion

In clinical practice there exists a group of children suffering from GI symptoms and having increased IgG-class AGA-levels without CD. It has been speculated that this antibody positivity might be connected to damage of the intestinal mucosa caused by, for example, infections and some medications (25). In this study our aim was to find out whether allergy-test positivity was connected to the elevation of IgG-class AGAs in sera. A retrospective data collection was carried out among children who had been previously undergone biopsies in order to exclude CD.

The atopic progression usually evolves through GI symptoms and eczema, seen mostly in infants, towards a more mature reaction such as asthma and rhinoconjunctivitis (1). Chronic digestive symptoms seem not to be typically connected to the IgE-mediated

allergic status. In contrast to children having an atopic background and therefore able to produce IgE-antibodies, these non-atopic persons continue to have symptoms localized to the gastrointestinal tract. Children expressing delayed (T-cell mediated) reactions are usually patch-test positive (5), though they are not considered very specific in allergic disorders.

It is quite common for atopic patients and subjects with cereal or other food allergies to present with elevated AGA values in their sera compared with those in the population in general (16). Our results suggest that increased IgG-class AGA-levels are not connected to positive skin-test reactions against cereals, but that GI symptoms per se might be associated with the serological response. In the allergic process, food may cause inflammation of the gut the permeability of which increases allowing the gliadin-protein into the bloodstream and further causing antibodies to appear in the sera (26). In the current material, this theory was not supported, as the amount of inflammatory cells in the small-bowel mucosa was initially normal in most children, only one of them expressing an increased eosinophil count and two clearly elevated lymphocyte counts (Table 1).

In CD, the amount of $\gamma\delta$ -TCR positive cells increases, but this rise is not specific for CD. Neither is AGA positivity associated with coeliac-type genetics (9). In the study by Kokkonen et al. both children with CD and those with cow's milk allergy had elevated values of $\alpha\beta$ - and $\gamma\delta$ -TCR positive cells when compared with the controls (27). We observed a correlation between IgG-class AGA elevation and the total amount of $\alpha\beta$ -TCR

positive cells, assuming this to be a sign of immune-response of the gut. Since the original study group was limited in number, information on 71 children under 18 y of age who were also previously biopsied and whose AGA values had been determined was collected. Only children with histologically manifest CD were excluded. In this larger group the correlation between small-bowel IELs and AGA-values could not be seen, however. During the study one child seroconverted in IgA class AGAs, but none of the children had positive EMAs or TGAs known to be specific to CD (12).

The results of our study can be interpreted in more than one way. First, the $\alpha\beta$ -T-cell reaction that had previously been observed in the biopsies of these children is not associated with any IgE-mediated sensitization. This seems to be demonstrated by the frequencies of positive skin-prick testing in this group of children compared to previous population-based data (28). However, the current study is not adequately powered definitively to draw this conclusion, which therefore remains speculative. Secondly, the interval between the biopsies and the allergological assessments could have resulted in spontaneous normalization of food sensitization had it ever been present. The children studied had only mild symptoms, if any, and were all growing adequately. As no food elimination challenges were performed, being considered too cumbersome for the population studied, accurate information about true clinical food allergy was not obtained. It was interesting that no correlation could be demonstrated between the patch-test results and the immunological reactions in the gut since patch testing has been proposed to be a novel way to assess non-IgE-mediated sensitization (29).

In conclusion, in the current study we could not demonstrate any excess allergic sensitization in children with previous pathological local immunological response in the small-bowel mucosa. The origin of this reaction remains unresolved and warrants further prospective studies.

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