

Duodenal IgE-positive cells and elimination diet responsiveness in children with atopic dermatitis

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Background: Parameters for identifying eczematous children who could respond to an elimination diet are needed. In children with food allergy, duodenal IgE-containing cells are enhanced.

Objective: To determine the presence of duodenal mucosal IgE-positive cells in atopic dermatitis and to determine whether duodenal IgE-positive cells may identify eczematous children who will benefit from an elimination diet.

Methods: Thirty-one children with severe eczema underwent gastrointestinal endoscopy because of gastrointestinal symptoms and were treated with an elimination diet. A clinical score to skin lesions was given before and after diet. All subjects were skin-prick tested with food antigens and aeroallergens. Serum IgE levels were measured. Duodenal IgE-positive cells were investigated in 18 control subjects and in all eczematous children before diet.

Results: The number of duodenal IgE-positive cells in children with atopic dermatitis was significantly increased compared with that of control group ($P < 0.001$). Nineteen (61%) eczematous children improved on a few food diet. Diet-responsive children had significantly higher IgE-positive cells compared with both nondiet-responsive and controls. Positive predictive accuracy of duodenal IgE-positive cells was poor, whereas negative predictive accuracy was high at the cutoff level of 50 IgE-positive cells/10 visual fields. Diagnostic accuracy both of SPT reactions with foods and of food-specific serum IgE antibodies was poor.

Conclusions: An intestinal IgE-mediated reaction occurred in children with severe atopic dermatitis who underwent intestinal endoscopy because of gastrointestinal symptoms. In these eczematous children, the number of IgE-positive cells in the duodenal mucosa might be helpful for excluding a positive response to the elimination diet.

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INTRODUCTION

Food hypersensitivity is believed to play a role in the pathogenesis of atopic dermatitis (AD) in some children.¹ Skin lesions improve on a elimination diet.^{2,3} Double-blind, placebo-controlled food challenges cause immediate hypersensitivity reactions in most eczematous children.^{4,5}

In children with AD not responsive to conventional treatment, a trial of

elimination diet is considered a therapeutic option.⁶ Elimination diets are annoying and difficult to follow. Unfortunately, neither history nor objective parameters, such as skin prick test (SPT) or food-specific IgE antibodies,³ can predict the response to dietary treatment or indicate which foods should be eliminated.

We demonstrated that the number of duodenal IgE-containing cells was higher in children with documented clinical reactions to foods than in the control group.⁷ We found that there was no difference between number of duodenal IgE-positive cells and results of SPT to foods or food-specific IgE antibodies in identifying food-allergic children. However, we found that IgE-

containing cells were increased in children with delayed reactions to foods and negative immunologic tests. In children with AD, conflicting results have been reported about the enhancement of IgE-positive cells in the intestinal mucosa.^{8–11} There is no study on the relationship between dietary manipulation and duodenal IgE-positive cells in children with AD.

We report the distribution of duodenal mucosal IgE-positive cells in children with AD not controlled by conventional treatment, who underwent intestinal endoscopy because of gastrointestinal symptoms.

The primary hypothesis to be tested was that a IgE-mediated reaction would be present at the duodenal mucosal site of children with AD. Secondary hypothesis, that number of duodenal IgE-positive cells would predict the outcome of a few food diet, was examined. Any association of duodenal IgE-positive cells with SPT results or serum IgE levels has also been evaluated.

METHODS

Subjects

Thirty-one children with AD according to Hanifin and Rajka's criteria¹² were enrolled in the study group (17 male and 14 female subjects, age median 16 months, with an age range of 4 months to 13 years 6 months). All children had skin symptoms which were not adequately controlled by conventional treatment (topical steroids, antihistamines, emollients, bandages, antibiotics, avoidance of triggers), and underwent intestinal endoscopy because of gastrointestinal complaints.

Eighteen healthy subjects consecutively referred to the gastrointestinal unit for gastrointestinal endoscopy

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were included in the control group. The control group comprised 12 male and 6 female children, with an age range of 5 months to 11 years, median 2 years 4 months, without atopic symptoms, such as eczema, asthma, oculorhinitis, or food allergy.

The eczematous children and controls were subjected to immunologic studies. Further, esophagogastro-duodenoscopy with an Olympus endoscope (Olympus, Hamburg, Germany) was performed on both patient and control groups because of gastrointestinal symptoms. Duodenal biopsy specimens were taken. Children with AD presented the following abdominal complaints: vomiting in 29 cases, diarrhea in 4, abdominal pain in 3, and failure to thrive in 2. Control subjects were referred for evaluation of suspected malabsorptive disorders. In the control group, upper tract endoscopy gave negative results.

Children with AD went on a 14-day elimination diet^{13,14} based on foods rarely involved in clinical hypersensitivity reactions in our population. During this period, infants <6 months of age were allowed only soy milk, and patients older than 6 months were also permitted rice, rabbit, carrot, lettuce, potato, pear, olive oil, tea, water, and sucrose if none of these foods was suspected of eliciting clinical reactions, and SPT and specific IgE antibodies in the blood were negative.

During the diet, parents recorded on a diary card the children's daily intake of oral antihistamines which were allowed for symptomatic treatment of pruritus. At the beginning and at the end of the diet, a clinical score was given by a blind observer. The skin was divided into 20 separate zones of roughly equal area. A score 0 to 3 was recorded for each of the following: erythema, excoriation, vesiculation, and lichenification. The diet was considered effective if the total score was reduced by at least 60%. This threshold was arbitrarily chosen. We believe that a reduction of the clinical score greater than 60% indicates that child had a useful clinical benefit from the dietary treatment. Such improvement,

therefore, can justify the complexity of an unpleasant and annoying diet. Parents of all patients gave written consent.

Immunologic Studies

All subjects were skin prick-tested with food extracts (1:20 wt:vol; (Bayropharm, Milan, Italy). The size of the skin test wheal was compared with histamine, 1 mg/mL and the diluent. A positive SPT response was defined as a wheal 3 mm larger than the negative (diluent) control and was graded in comparison to the histamine wheal; 4+ if greater than twice the area of histamine standard; 3+ if twice the area of the histamine standard; 2+ if equal to the area of the histamine standard; and 1+ if half the area of the histamine standard.

Serum circulating IgE antibodies to foods were measured by CAP system and graded from 0 to 6 in accordance with manufacturer's recommendations (Pharmacia, Uppsala, Sweden). Grade 1+ or more was considered positive. Total serum IgE was measured by CAP (Pharmacia, Uppsala, Sweden).

Immunofluorescence Study

The study was performed as previously described.⁷ Duodenal biopsies were immediately fixed in periodate-lysine-paraformaldehyde. The periodate-lysine-paraformaldehyde solution was prepared as follows: to 0.2 M lysine-HCl in distilled water; 0.1 M dibasic sodium phosphate was added until the pH was 7.4. The solution was then diluted to 0.1 M lysine with 0.1 M sodium phosphate buffer, pH 7.4 (resulting phosphate concentration was 0.05 M). The paraformaldehyde solution and solid sodium m-periodate was added. After fixation the tissues were washed with three changes of cacodylate 0.1 M buffer solution for 30 minutes each and then stored overnight at 4° C with cacodylate 0.1 M buffer solution to which 7% sucrose had been added. Excess washing solution was removed and the specimens were rinsed with four changes of acetone for 10 minutes each. Dehydrated specimens were treated with xylol for 30

minutes and embedded in paraffin. Thin sections of approximately 2- to 3- μ m were cut on a sliding microtome (Leica, Wetzlar, Germany). The specimens were dried on a plate at 37° C. Tissue sections were stained with hematoxylin and eosin. The sections were treated with xylol for 15 minutes, then with ethanol and washed with water. After treatment with phosphate-buffered saline buffer, the specimens were incubated overnight with commercially prepared fluoresceinated antisera specific for human IgE (Dakopath, Copenhagen, Denmark) at 4° C. The sections were washed with three changes of phosphate-buffered saline buffer.

The specimens were examined under a Leitz Ortholux incident-light fluorescent microscope (Esselte Leitz, Stuttgart, Germany). The stained epithelial and lamina propria cells were counted by an observer unaware of the clinical data. Counting was performed with the 40 $\times$ objective. In each sample, the number of cells in 10 different adjacent areas of the mucosa above the muscularis mucosae were counted giving the number of cells/10 visual fields.

Nasal polyps were used as positive control tissue, whereas negative controls were carried out by the absence of staining in positive sections after blocking antigenic binding sites with nonconjugated antisera.

Data Analysis

Data were expressed as median unless mentioned otherwise. Kruskal-Wallis ANOVA procedure was applied first to continuous data. When it was significant, each pairing was examined by means of the Mann-Whitney test. Categorical data were compared using either Pearson's χ^2 test, Fisher's exact test, or McNemar's test. Corrections were applied for multiple comparisons. The odds ratios with 95% confidence intervals (CIs) were also calculated. Sensitivity, specificity, and predictive accuracies were calculated using standard formulas. Diagnostic accuracy of the number of duodenal IgE-containing cells was calculated using the ROC

(receiver operating characteristic) curve. ROC curve was constructed to choose the optimal cutoff point, defined as a calculated value that maximizes "true positive" and minimizes "false-positive" (1-specificity). Performance characteristics for duodenal IgE-positive cells were also calculated at the cutoff level defined as the upper limit of duodenal IgE-positive cells in the control group and at the cutoff level that provided a positive predictive accuracy greater than 90%. Correlations were examined by Spearman rank correlation test. $P < 0.05$ was considered statistically significant.

RESULTS

The distribution of the number of duodenal IgE-containing cells in the specimens of the 31 children with AD (median [range] = 85 [2 to 282]) were significantly increased compared with that of the control group (median [range] = 10 [2 to 50]; $P < 0.0001$; difference between medians [95%CI] = 62[44 to 80]; Fig 1).

Nineteen of the 31 (61%) children with AD improved with a few food diet. Age and sex were similar in the children who improved on an elimination diet and in those who did not. (Table 1).

Twenty (64%) of the 31 children with AD had at least one positive SPT to foods. Sixteen children were positive to egg, 14 to cow's milk, 5 to wheat, 4 to lamb, 3 to pork, 3 to potatoes, 3 to chicken, 2 to beef, 2 to oats, 2 to barley, 2 to peanuts, 2 to peas, 2 to tomatoes, 2 to soy, 1 to carrots, 1 to beans, 1 to rabbit, 1 to cod, 1 to salmon, and 1 to tuna. Eight (25%) of the 31 children with AD had at least one positive SPT to aeroallergens. Five children were positive to cat, 3 to grasses, 3 to *Dermatophagoides pteronyssinus*, 2 to *Alternaria*, 1 to *Dermatophagoides farinae*, 1 to dog, 1 to mugwort, and 1 to hazel.

Circulant specific IgE antibodies to foods were found in 21 (67%) of 31 children with AD. Fifteen children were positive to egg, 12 to cow's milk, 6 to wheat, 6 to peanuts, 5 to potatoes, 5 to peas, 5 to tomatoes, 4 to cod, 4 to pork, 4 to soy 3 to rice, 3 to lamb, 2 to

carrot, 2 to corn, 2 to chicken, 2 to kiwi, 1 to beans, 1 to chicken, 1 to pear, 1 to apple, 1 to barley, 1 to oat, 1 to rabbit, 1 to salmon, 1 to beef, 1 to almond, and 1 to banana. Circulant specific IgE antibodies to aeroallergens were found in 8 (25%) of 31 children with AD. Four children were positive to cat, 4 to grasses, 4 to *D. pteronyssinus*, 2 to dog, 1 to *Alternaria*, 1 to mugwort, 1 to hazel, 1 to birch, 1 to pellitory, and 1 to *Cladosporium*. In the control group, all patients had both negative SPT and specific serum IgE antibodies.

Levels of total serum IgE were significantly higher in eczematous children (median [range] = 150 [6 to 7,890]) than in controls (median [range] = 31[2 to 209]), difference between medians [95% CI] = 102[28 to 181]; $P = 0.0009$). Twenty of the 31 eczematous children had total serum IgE levels which were higher than the published mean + 2 SD value for age.¹⁵ In the control group, 2 children had elevated total serum IgE levels (OR [95% CI] = 0.06[0.13 to 0.35]; $P < 0.001$).

Relationship among Duodenal IgE-Positive Cell, Elimination Diet, SPT Results, and Serum IgE

The number of mucosal duodenal IgE-positive cells was significantly higher

in children who responded to the restricted diet in comparison to those who did not (Table1). Both diet-responsive and nondiet-responsive children with AD had significantly higher numbers of duodenal IgE-positive cells than the control group (Table1). There was no significant association between clinical AD score or daily use of antihistamine and the number of duodenal IgE-containing cells in the eczematous children. The number of IgE-positive cells in the biopsy specimens from eczematous children as well as from controls showed no correlation with sex or age (< 12 months of age vs > 12 months) or with positive specific serum IgE antibodies to at least one food or positive SPT results to at least one food. Further, no correlation was found between IgE-positive cells and positive specific serum IgE antibodies to at least one inhalant allergen or positive SPT results to at least one inhalant allergen.

Table 2 shows the performance characteristics for the number of duodenal IgE-positive cells at different cutoff values when compared with a few food diet. Cutoff value for a positive result was set at 50 duodenal IgE-positive cells/10 visual fields that was the highest value in the control group; 61 duodenal IgE-positive cells/ 10 vi-

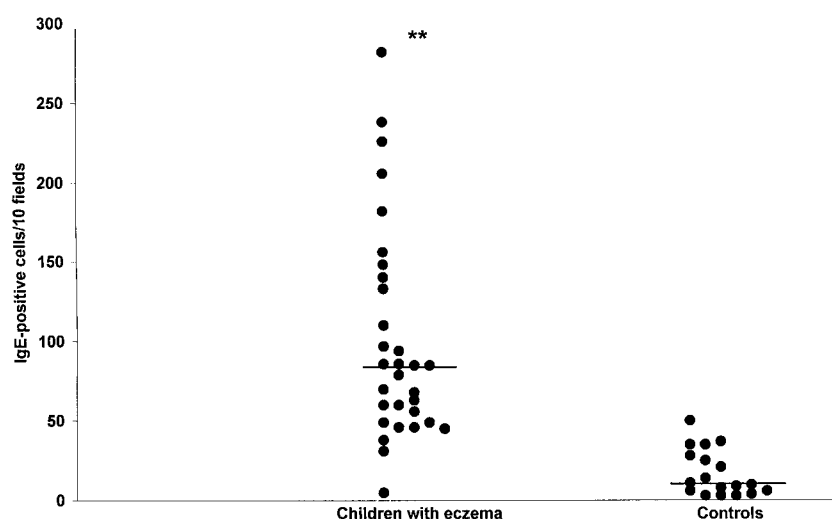


Figure 1. Duodenal IgE-positive cells in children with atopic eczema and in controls. Significance of difference from control children:*, $P < 0.0001$. Bars = median.

Table 1. Characteristics of Children with Atopic Eczema Who Respond to Elimination Diet and Who Did Not Respond and of Control Group

	Children with atopic eczema		Control group (N = 18)	P value
	Diet-responsive (N = 19)	Non-diet-responsive (N = 12)		
Number (%)				
Male	13 (68)	4 (33)	12 (66)	NS†
Any + food-specific IgE	14 (73)	7 (58)		NS
Any + food skin prick test	16 (84)	4 (33)		NS
Median (range)				
Age (mo)	9 (5–56)	26 (4–162)	29 (5–132)	NS†
Duodenal IgE+ cells*	97 (5–282)‡Δ	49 (31–94)§Δ	10 (2–50)§‡	<0.0001†
Total serum IgE (IU/mL)	178 (29–1980)¶	111 (6–7890)	31 (2–209)¶	0.0017†

NS = $P > 0.05$.

* Results are expressed as the number of IgE+ cells per 10 visual fields.

† Comparison across groups Kruskal-Wallis test.

Mann-Whitney test: $\Delta P = 0.0007$, difference between medians (95% CI) = -54 (-107 to -24).

‡ $P < 0.0001$, 95.5 (64 to 140).

§ $P < 0.0001$, 39 (25 to 50).

¶ $P = 0.0001$, 113 (51 to 183).

Table 2. Diagnostic Accuracy

	Duodenal IgE+ cells*			Any + food SPT	Any + food-specific IgE	Total serum IgE
	<50	<61	>87			
No. of patients positive/negative	23/8	20/11	12/19	20/11	21/10	20/11
Sensitivity (%)	94	89	52	84	73	78
Specificity (%)	58	75	90	66	41	58
Predictive value (%)						
Positive	78	85	91	80	66	75
Negative	87	81	47	72	50	63

Data shown are at different cutoff levels of number of duodenal IgE-containing cells, any positive (+) food skin prick test (SPT), any + food-specific IgE and levels of total serum IgE $>$ mean + 2 SD for age for the presence of positive response to diet in eczematous children (prevalence = 61%).

* Results are expressed as the number of IgE+ cells per 10 visual fields.

sual fields was the optimal cutoff point calculated using the ROC curve; and 87 duodenal IgE-positive cells/10 visual fields provided a positive predictive accuracy greater than 90%. The predictive values depend on the prevalence of the condition in the general population. The prevalence of improvement on a few food diet in a population of children with AD is unknown. In a large population of children with AD not controlled by conventional treatment, the prevalence of diet-responsive children has been reported to be 30%,¹² which is lower than in our study.

We may hypothesize that in a general eczematous population, 30% of children with severe AD refractory to conventional treatment can benefit from an elimination diet. In our study

population, therefore, the positive predictive value is higher than in the general population whereas the negative predictive value is lower. Negative predictive accuracy calculated for a general eczematous population was 95% when the cutoff value for a positive result was set at 50 duodenal IgE-positive cells/10 visual fields, 94% at 61 and 80% at 87, while positive predictive accuracy was 49% at 50, 60% at 61, and 69% at 87. Our results showed that sensitivity and negative predictive accuracy were high only when we used the lower cutoff level. Specificity was high only when we used the higher cutoff level. Positive predictive accuracies were poor at all cutoff levels.

No significant difference was found between number of duodenal IgE-pos-

itive cells and result of SPTs, food-specific IgE antibodies, or levels of total serum IgE in predicting the elimination diet responsiveness. Total serum IgE levels in the AD children correlated significantly with the number of duodenal IgE-positive cells ($r_s = 0.32$; $P = 0.036$).

Comparison of SPT, Serum IgE, and Elimination Diet

There was no association between positive food SPT reactions and response to restrict diet. Effective elimination diet was not associated with food-specific serum IgE antibody results. In children who improved after elimination diet, levels of total IgE in the blood did not differ from those of children who did not improve (Table 1).

Diagnostic indices of SPT with food antigens and of food-specific serum IgE antibodies compared with results of restricted diet are listed in Table 2. Results of SPTs to food, food-specific serum IgE antibodies, and levels of total serum IgE were found to have poor diagnostic accuracy (Table 2). In a hypothetical eczematous population in which the prevalence of children who improved on a diet is 30%, the negative predictive accuracy is 90% for any positive SPT, 77% for any positive food-specific IgE, and 86% for total IgE. There was no significant difference between results of SPT and food-specific IgE antibodies or total serum IgE levels in identifying diet-responsive children.

DISCUSSION

The results of this study show that children with AD who underwent intestinal endoscopy because of gastrointestinal symptoms have increased numbers of mucosal duodenal IgE-positive cells as judged by the immunofluorescent technique.

They corroborate and extend findings of Kalimo et al.¹⁰ who studied eczematous adults with positive SPTs or food-specific IgE antibodies. They are also in agreement with the results of a smaller study by Braathen et al.¹¹ Our data contradict those of an uncontrolled study by Perkkio et al.,⁹ who observed IgE-bearing cells only in a few children with AD attributable to food allergy. However, it is not easy to make a comparison with our findings because of the use of different immunohistochemical techniques¹⁶ and because of the heterogeneity in the trial design.

There is some support for the view that the gastrointestinal tract is abnormal in children with AD. Gastrointestinal symptoms are more common in eczematous children.¹⁷ Previous reports showed morphologic changes of gastrointestinal mucosa associated with impaired maximal gastric acid secretion¹⁸; mild degrees of jejunal villous atrophy with eosinophil infiltration and edema of the lamina propria¹⁹; and increased gastrointestinal perme-

ability to inert molecules^{13,20} or protein antigens,^{21,22} such as egg and milk. Our results extend these previous observations by showing an increased number of duodenal IgE-positive cells.

Some explanations may be offered for the increased number of duodenal IgE immunoreactive cells in AD. First, it has been hypothesized that swallowed inhalant allergens may provoke an intestinal IgE-mediated reaction.¹⁰ In contrast, we observed that the number of duodenal IgE-positive cells was not associated with positive SPT reactions to aeroallergens or inhalant-specific IgE antibodies in eczematous children. Second, in children with AD, an increase of intestinal IgE-positive cells may be the consequence of an hypersensitivity reaction to food. In the current study, a role for dietary antigens is supported by the significant higher number of duodenal IgE-positive cells in diet-responsive children than in nondiet-responsive children. This is in agreement with our previous observation that food-allergic reactions are associated with enhanced duodenal IgE-positive cells.⁷ The intestinal inflammation in food-allergic children is confirmed by findings of increased eosinophil cationic protein and tumor necrosis factor- α in the feces.²³ Further, reactions to food antigens may play a role in the pathogenesis of gastrointestinal symptoms in children with AD.¹⁷ Another possible explanation which does not exclude the former, is that augmentation of duodenal IgE-positive cells may be the result of a systemic primary abnormality associated with AD. Along this line, it may be relevant that we found more elevated IgE-positive cells in children who did not respond to a few food diet in comparison to controls. Further, we found that the number of IgE-positive cells were correlated with IgE levels in the blood, suggesting a systemic IgE production.

Elimination diets may be beneficial in children with AD.^{3,4} However, they are difficult to follow and can cause discomfort to patients. Unfortunately, there are no reliable means for identifying children who will respond to dietary treatment.¹⁴ Our results confirm

that the response to a few food diet could not be predicted by SPT results or total serum IgE level or food-specific IgE antibodies in the blood.³

No comparative data are available on the relationship between the benefit of a low allergen diet and duodenal IgE-containing cells in eczematous children. In this study, we evaluated preliminary the role of the number of duodenal IgE-positive cells in predicting the outcome of an elimination diet in children with AD. We found that the number of duodenal IgE-containing cells was significantly higher in the diet-responsive eczematous children than in both nondiet-responsive eczematous children and in controls. The predictive accuracy values are influenced by the prevalence of the disorder in the population being studied. Higher prevalence rates increase positive predictive accuracy and decrease negative predictive accuracy.

The exact prevalence of children with AD who improve on an elimination diet is not known. The prevalence of diet-responsive eczematous children was 61% in the current study but only 30% in a larger population of children with severe AD not adequately controlled by conventional treatment.³ A number of variables may account for such a difference. We studied younger patients who underwent intestinal endoscopy because of gastrointestinal complaints. Although the exact prevalence of children with AD who had an improvement on a few food diet is unknown, we may hypothesize that in a general eczematous population, 30% of children with severe AD may respond to the diet. We corrected the predictive accuracy to reflect the prevalence of a positive response to a restricted diet in a hypothetical, general eczematous population. Our results showed that positive predictive accuracy of the number of IgE-positive cells was poor in a hypothetical patient population. Therefore, duodenal IgE-positive cells are of little help in identifying the children who will benefit from an elimination diet. In contrast, the number of IgE-positive cells has both high sensitivity and excellent cor-

rected negative predictive accuracy at a cutoff level of 50 IgE-positive cells/10 visual fields is the upper limit of the control group. Therefore, if a patient has < 50 IgE-positive cells/10 visual fields, it is extremely likely that this subject will not benefit from a restricted diet.

CONCLUSION

Our study showed that an intestinal hypersensitivity reaction occurred in children with severe AD who underwent intestinal endoscopy because of gastrointestinal complaints. Our results suggest that in such children, the presence of < 50 duodenal IgE-positive cells/10 visual fields might be a useful parameter to rule out a positive response to an elimination diet. In accordance with our data, this would eliminate the need to perform elimination diet in 25% of children.

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