

Immunohistochemical Study of Intestinal Biopsies from Children with Atopic Eczema due to Food Allergy

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In 18 patients with skin reactions after food challenge and in controls the numbers of immunoglobulin-containing cells in the intestinal mucosa were studied by the direct immunoperoxidase technique; IgE and C₃ were studied by FITC-conjugated sera also. Serum immunoglobulins, complement fractions 3 and 4, and reaginic antibodies to the foods were measured during the provocation. None of the patients had any deficiency in the numbers of immunoglobulin-containing cells in the intestinal mucosa or in the serum immunoglobulin concentrations. The numbers of IgM- and IgA-containing cells were higher in the patients than in the controls. But patients and controls had equally low numbers of IgE-containing cells. No deposits of C₃ were seen in any of the biopsy specimens. Serum IgE was increased in 13 cases. The presence of reaginic antibodies to cow's milk and cereals correlated well with the clinical reaction (12/15 and 5/7 of the cases). There was a negative correlation between the logarithm of serum IgE and the number of IgA-containing cells in the jejunal mucosa ($P < 0.01$). High prechallenge counts of blood eosinophils were seen in seven cases and these showed a close correlation with the eosinophils in the jejunal mucosa ($P < 0.001$).

Key words: food allergy; immunoglobulin-containing cells; reaginic antibodies; serum immunoglobulins.

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The role of IgE and reaginic antibodies is well established in atopic reactions leading to histamine liberation and allergic symptoms (6). However, only some food allergies are known to be mediated by IgE antibodies (2, 9). Even in IgE-mediated food allergy the site of formation of the specific antibodies is unknown.

Other immune mechanisms are connected with the genesis of symptoms due to food: in the case of malabsorption syndrome with cow's milk protein intolerance there are marked increases of IgA- and IgM-containing cells in the intestinal mucosa (14). Complement activation has been noted in one study (8), and cell-mediated immunity may also play a role in cow's milk protein intolerance (15).

In 18 cases with skin reactions after food challenge, studies were made of the local immunological state of the gut mucosa, and also of serum immunoglobulins, complement fractions 3 and 4, and reaginic antibodies to food materials during such provocations.

MATERIAL AND METHODS

Patients

Details of the 18 patients are given in the previous paper. During the challenge the patients fell into two groups, 10 having skin symptoms only (Cases 1-10; group I) and

eight having gastrointestinal symptoms as well (Cases 11–18; group II).

Biopsies. Within 24–72 h after the last feed causing symptoms a jejunal biopsy specimen was taken in all but two cases (Cases 11 and 17), in whom the specimen was taken after 8 days. Simultaneously, 12 rectal biopsies (Cases 2–6, 9, 11–14, 16 and 17) were taken and 11 skin biopsies of the left volar antebrachium (Cases 2–6, 8–10, 13–14 and 17). A prechallenge jejunal biopsy was taken from Case 6 and a prechallenge rectal biopsy from Cases 6 and 11.

Jejunal biopsy specimens were studied from 11 age-matched children as controls. These biopsies were taken because of: growth failure in four cases, recurrent upper respiratory infections and positive cytotoxic test to wheat in four cases, regurgitation in two cases and anemia in one case. None of these children had diarrhoea. They had normal intestinal morphology. Their serum immunoglobulins were normal for the age. Controls for the rectal biopsies were taken from another 11 age-matched children suspected to have Hirschsprung's disease (11). The skin biopsy controls were from six retarded children without any signs or symptoms suggestive of gastrointestinal or cutaneous disease. The skin biopsies were taken to investigate the possibility of storage disease.

Each biopsy specimen was divided, before further handling, into two or three parts. One part was fixed in formalin, embedded in paraffin and processed for normal histology and immunoperoxidase study (11). Another part was processed for electron microscopy, and in the case of 11 jejunal biopsy specimens, five rectal biopsies and nine skin biopsies a third part was used for direct immunofluorescent study (12). The jejunal biopsy specimen from Case 12 was too small to be studied with the immunoperoxidase technique.

Blood samples were taken before the challenge and within 24 h of the last feed causing symptoms. In Case 16 the second sample was

obtained after 48 h and in Cases 2 and 3 only the prechallenge blood sample was taken.

Methods

The procedures for fluorescent microscopy, peroxidase staining and measurement of cell numbers have been described earlier (11, 12). Cell densities were expressed as cells/mm². Kidney biopsies staining positively with FITC-conjugated anti-C₃ serum served as controls for peroxidase-conjugated anti-C₃ serum. IgE-containing cells and C₃ were studied in both cryostat sections stained with FITC-conjugated serum and in paraffin sections stained with peroxidase-conjugated serum. The monospecificity of these FITC-conjugated antisera was checked by immunoelectrophoresis and by a double diffusion micro-method (12). Staining in positive kidney biopsy specimens was compared with and found similar to that with the FITC-conjugated antisera used in the International Study of Kidney Disease in Children (19).

Serum concentrations of immunoglobulins G, A and M were measured by single radial immunodiffusion (5). C₄ was measured in a similar manner to C₃ (14), the mean value for 30 healthy children being 0.27 g/l (s.d. 0.11 g/l); as a reference a commercial standard serum was used (Behringwerke, Germany). IgE was measured with a commercial radio-immunosorbent kit (Phadebas, Pharmacia, Sweden). Normal values for serum IgE were based on 136 samples from a follow-up study of healthy children aged 2 months to 1 year, from whom atopic children were excluded (4). Specific IgE antibodies to cow's milk, wheat and rye were determined with Pharmacia's radioabsorbent test. Precipitating antibodies to cow's milk and gluten were studied with a double diffusion micromethod (5).

Statistical analysis between the groups was done with Wilcoxon's test. Correlation coefficients between the various measurements were calculated with a Hewlett-Packard 9100 B calculator according to its standard programs.

RESULTS

Immunoglobulin-containing cells in the jejunal mucosa

The number of IgM-containing cells of both groups was significantly higher than that of the controls ($P < 0.02$ and $P < 0.01$, Fig. 1). The number of IgA-containing cells was higher, too, but in group II the difference was not significant ($P < 0.01$ and $P < 0.05$, Fig. 2). There was no difference between groups I and II in the numbers of IgA- or IgM-containing cells. The number of IgG-containing cells was the same in all three groups (Fig. 3). Sparse IgE-containing cells were observed in three specimens in the control group (6, 12 and 36 cells/mm²), in one in group I (12 cells/mm²) and in four in group II (6, 6, 6 and 12 cells/mm²); in the rest no IgE-containing cells were seen in the area examined (density less than 2 cells/mm²). The findings with the immunofluorescent technique were similar when staining was done with anti-IgE serum.

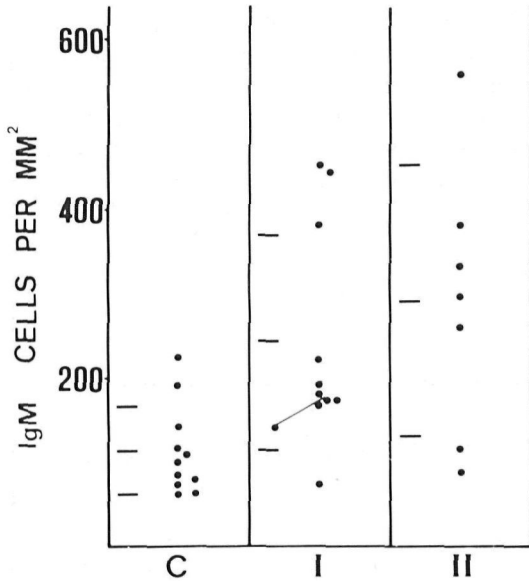


Fig. 1. Numbers of IgM-containing cells in the lamina propria of jejunal biopsy specimens of controls (C), group I (I) and group II (II). The counts of the same patient are connected with a line. Median count is indicated.

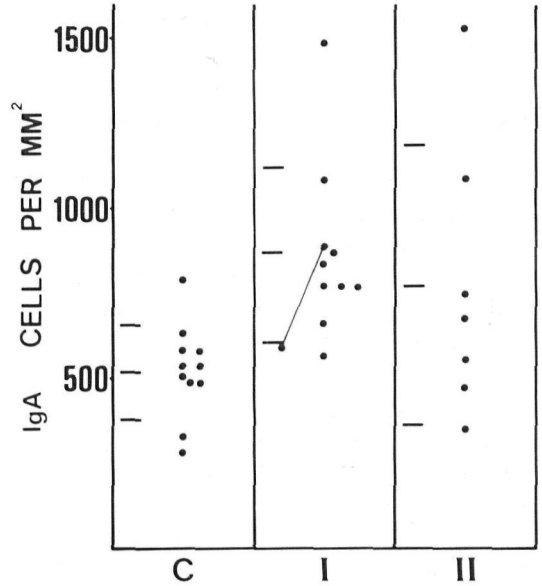


Fig. 2. Numbers of IgA-containing cells in the lamina propria of jejunal biopsy specimens. Symbols as in Fig. 1.

In the specimens from Case 6, in which a biopsy was also taken before the challenge, there was a 1.3-fold increase in the numbers of IgA- and IgM-containing cells and a decrease

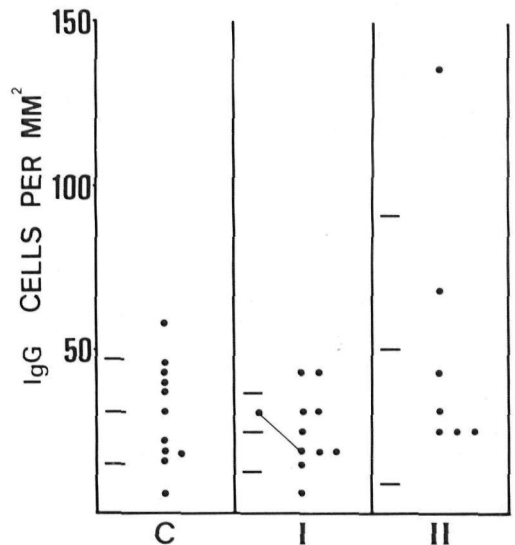


Fig. 3. Numbers of IgG-containing cells in the lamina propria of jejunal biopsy specimens. Symbols as in Fig. 1.

in the number of IgG-containing cells (Figs. 1-3).

Positive staining with anti-C₃ serum was seen in the capillary endothelium and in the intercellular spaces in the lamina propria with both the immunofluorescent and immunoperoxidase techniques. No deposits of C₃ were seen in the basement membrane zone.

No correlation was observed between the numbers of IgA- and IgM-containing cells. The numbers of immunoglobulin-containing cells in the jejunal mucosa did not correlate with the degree of damage estimated as the villus/crypt ratio. There was a positive correlation between the number of IgM-containing cells and the serum level of IgM ($r=0.6$, $n=14$, $P<0.05$), but the numbers of IgA- and IgG-containing cells did not show a corresponding correlation with the serum levels of the respective immunoglobulins.

Immunoglobulin-containing cells in the rectal mucosa (Table 1)

In the numbers of IgA-, IgM- or IgE-containing cells between the three groups no significant differences were observed, although the number of IgA-containing cells was slightly higher in group I than in the controls ($0.05 P<0.10$). In group I the number of IgG-containing cells was higher than in the controls ($P<0.05$) but about the same as in group II. Staining with anti-C₃ serum in the capillary

endothelium and the lamina propria was as in the jejunal specimens.

In two cases a biopsy was also taken before challenge; in one of these, the second specimen showed a 1.7-fold increase in the number of IgA-containing cells; the other cell numbers were unchanged.

There was a close correlation between the numbers of IgA-containing cells in the rectal and jejunal mucosae ($r=0.9$, $n=11$, $P<0.001$). No such correlation between the numbers in the rectal and jejunal mucosae existed for the IgM- or IgG-containing cells.

The histology of all the rectal biopsies was normal.

Skin biopsies. No staining with anti-IgA, -IgM, -IgE, or -C₃ serum was seen. Similar intradermal staining with anti-IgG serum was observed in all specimens from controls and patients.

Serum immunoglobulins and complement fractions (Table 2)

Before provocation the serum IgA value was within the normal range for the age in all but Case 11, in which it was elevated. In group I serum IgA was $126 \pm 46\%$ (mean $\pm$ 1 s.d.) of the mean for the age and in group II $109 \pm 76\%$. Serum IgM was low in Case 18; the mean values were $98 \pm 38\%$ in group I and $72 \pm 27\%$ in group II. Serum IgG was low in

Table 1

Immunoglobulin-containing cells in the rectal mucosa (cells/mm²)

Patient groups	<i>n</i>	IgA-containing cells	IgM-containing cells	IgG-containing cells	IgE-containing cells
		Median (range)	Median (range)	Median (range)	Median (range)
Controls	11	125 (56-524)	80 (<2-196)	6 (<2-20)	<2 (<2-11)
Group I	6	305 (148-611)	96 (24-247)	19 (<2-25)	<2 (<2-12)
Group II	6	199 (74-648)	46 (31-142)	9 (<2-37)	<2 (<2-6)

Table 2

Serum concentration of immunoglobulins and complement fractions 3 and 4, specific IgE antibodies to cow's milk and wheat and blood eosinophils before and after challenge

Case	IgA g/l	IgM g/l	IgG g/l	IgE IU/ml	Cow's milk RAST	Wheat RAST	C ₃ g/l	C ₄ g/l	Blood eosinophils × 10 ⁶ /l
1	0.25 0.30	0.85 0.89	8.9 8.5	1200↑ 850↑	3 3	4 4	0.80 0.72	0.52 0.36	90 590
2	0.07	0.43	4.1	50	0		0.74	0.12	40
3	0.18	0.80	3.0	50	0		0.54	0.08	90
4	0.20 0.52↑	0.55 0.70	6.7 9.7↑	302↑ 770↑	0 0	2 2	0.98 1.12	0.42 0.41	200
5	0.24 0.97↑	0.47 0.63	6.0 9.8↑	1800↑ 4180↑	4 4	3 3	0.58 0.48	0.26 0.28	420
6	0.12 0.09	0.35 0.44	5.3 4.0	60 198↑	4 4		0.82 0.74	0.22 0.22	1369
7	0.34 0.35	na 0.71	na 7.1	304↑ 217↑	na 3		na 0.82	na 0.24	1433
8	0.30 0.25	0.25 0.26	7.0 6.0	89 78	2 2		0.76 0.76	0.32 0.20	100 418
9	0.34 0.32	0.41 0.35	7.8 7.1	122 27	1 1		0.58 0.86	0.20 0.31	370
10	0.57 0.57	0.45 0.53	10.8 9.9	310↑ 310↑	0 0	2 2	1.14 0.70	0.28 0.21	240
11	0.17↑ 0.11	0.34 0.40	6.7↑ 6.5↑	400↑ 370↑	4 3		0.64 0.70	0.20 0.22	1800
12	0.08 0.12	0.46 0.34	3.4 3.3	388↑ 89↑	3 na		0.80 0.90	0.32 0.30	5500 2281
13	0.11 0.11	0.27 0.22↓	3.7 2.6	85↑ 64↑	2 3		0.78 0.62	0.24 0.24	1325 231
14	0.07 0.08	na 0.27	na 5.5	na 412↑	na 3		na 0.58	na na	188 68
15	0.06 0.16	0.38 0.60	3.0 7.0	260↑ 120↑	0 0	2 na	0.84 0.60	0.20 0.22	1800
16	0.19 0.15	0.30 0.32	3.7 2.8↓	94 120↑	3 3		0.78 0.44	0.36 0.46	1180
17	0.19 0.38	0.66 0.67	2.8↓ 4.4	20 19	1 1	0 0	0.78 0.62	0.10 0.24	150
18	0.14 0.18	0.24↓ 0.17↓	2.4↓ 2.8↓	160↑ na	3 na	0 na	0.98 1.03	0.62 0.34	40 178

↓ = less than - 2 s.d. of the mean value for the age.

↑ = more than + 2 s.d. of the mean value for the age.

na = not analysed, the serum sample was too small.

Cases 17 and 18, and elevated in Case 11; the percentage of the mean for the age was $112 \pm 32\%$ in group I and $82 \pm 52\%$ in group II.

During provocation serum IgA showed an increase of at least two-fold in four cases (Table 2, Cases 4, 5, 15 and 17) while in the rest the level remained about the same. In all these four cases the IgG level increased at the same time, and in three (Cases 4, 5 and 15) there was also a rise in IgM. These changes in serum immunoglobulins were all seen in the patients who reacted to cereals.

C_3 fell during provocation in two cases (10 and 16); the level in Case 10 was still within the normal range. In both of them C_4 remained normal; in the others, too, C_4 was normal at the end of the provocation test (Table 2).

Serum IgE (Table 2) exceeded 2 s.d. of the normal in six cases in group I and in seven cases in group II. During provocation it decreased in three cases, but increased in another three cases (all in group I). There was an inverse correlation ($P < 0.01$) between the logarithm of the serum IgE, expressed as a percentage of the mean value for the age, and the number of IgA-containing cells in the jejunal mucosa (Fig. 4). Specific IgE antibodies to cow's milk (Table 2) were detected in five of the eight patients who reacted to cow's milk during provocation in group I and in every one of the seven patients in group II. Specific IgE antibodies to wheat and rye were detected in all the four patients who reacted to wheat and/or rye in group I and in one of the three patients in group II. These specific IgE antibodies showed no changes during provocation.

No precipitins to gluten were seen, whereas precipitins to cow's milk were seen in four cases.

A high count of prechallenge blood eosinophils (Table 2) was observed in two cases (6 and 7) in group I and in five cases (11–13 and 15–16) in group II. During provocation blood eosinophils were recounted in only six cases. There was a close correlation

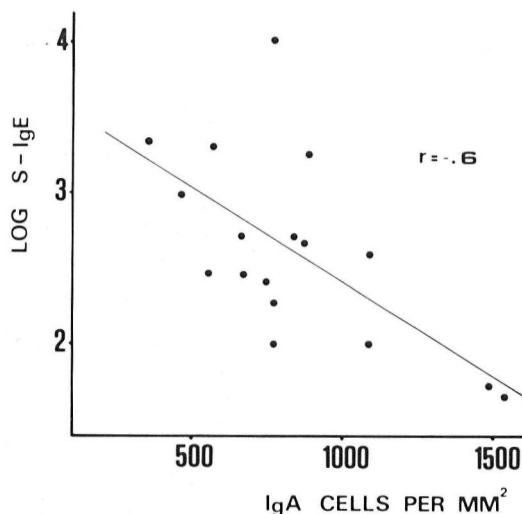


Fig. 4. Correlation between the logarithm of the serum IgE, expressed as a percentage of the mean value for the age, and the number of IgA-containing cells in the jejunal mucosa.

between the prechallenge blood eosinophils and the eosinophil cell mass percentage in the jejunal mucosa ($r = 0.8$, $n = 18$, $P < 0.001$), but no correlation was observed with the serum IgE level.

DISCUSSION

The morphometric study of the intestinal biopsies from children with atopic eczema due to food allergy showed no severe mucosal damage such as could cause the immunization with foreign proteins. This study shows that there is no deficiency in the numbers of immunoglobulin-containing cells in the intestinal mucosa or in the serum immunoglobulins.

In the jejunal mucosa the mean numbers of IgM- and IgA-containing cells in both groups were higher than in the control group. No differences were seen in the numbers of IgG-containing cells, and the numbers of IgE-containing cells were low in all the groups. The numbers of immunoglobulin-containing cells were not correlated with the degree of the

mucosal damage, but there was an inverse logarithmic correlation between serum IgE and the number of IgA-containing cells in the jejunal mucosa. IgA-containing cells were evenly distributed along the gastrointestinal tract in these patients, as in children without intestinal or immunological disease (12). No consistent changes in the serum concentration of immunoglobulins or complement fractions were caused by the challenge. No marked differences were observed in any of the immunological measurements between patients with only cutaneous symptoms and those who also had gastrointestinal symptoms.

The immune reaction observed in the intestine shares features with those reported in malabsorption syndrome with cow's milk protein intolerance (14). There were increases in the numbers of IgM- and IgA-containing cells; however, these increases were less marked than in the malabsorption syndrome with cow's milk protein intolerance. Similar increases in numbers of immunoglobulin-containing cells have been reported in coeliac disease (13) and, for example, in viral gastroenteritis (17), and such increases are thus a common response to various antigens. According to this study, immune reactions involving increases in these cells are a common feature in food allergy presenting as eczema; in contrast to the malabsorption syndrome with cow's milk protein intolerance and coeliac disease with similar increases, most patients in this study have normal morphology of the intestine.

In cow's milk allergy there are reports of elevated numbers of IgE-containing cells in the jejunal mucosa (7, 16), but our findings, together with those on the malabsorption syndrome with cow's milk (14) and on animal studies (10), suggest that IgE is not synthesized by the plasma cells in the intestinal mucosa.

Along with the less pronounced increase in intestinal IgA, the serum IgA rose in only four of the patients, whereas in most cases of the malabsorption syndrome with cow's milk protein intolerance it has been observed to increase during milk provocation (14).

Precipitating antibodies were also infrequent in patients with eczema as the presenting sign of food allergy. As the immune reaction involving IgA subsided, there was a more pronounced increase in the reaginic reaction.

Most patients had an increased serum IgE level, as has been reported earlier (1). There was a close association between the presence of reaginic antibodies to cow's milk and a positive challenge test. Of the 15 patients who reacted with symptoms to cow's milk, 12 had these antibodies, which is a higher percentage than in earlier reports (1, 3). Most of the patients who reacted to cereals had reaginic antibodies to them as well. In the malabsorption syndrome with cow's milk protein intolerance, serum IgE is mostly within normal range (14) and these patients do not have reaginic antibodies to cow's milk.

In this study there was a fall in C_3 in two patients, but no staining with anti- C_3 or $-C_4$ sera conjugated with either fluorescein or peroxidase was present in the basement membranes of the epithelial cells of the jejunum in these or in any other patients. Nor was complement fixation seen in the skin biopsies. Thus at least in the majority of patients complement activation and fixation to tissues is not the mode of reaction by which symptoms are caused in food allergy with eczema as the presenting sign, as has been suggested earlier in cow's milk allergy (16).

In food allergy with cutaneous symptoms there was no quantitative deficiency of secretory immunoglobulins locally in the intestinal mucosa, as has been speculated on grounds that a low serum IgA has been connected with later developing allergy (18). On the contrary, there were increases in the numbers of IgA- and IgM-containing cells, indicating a local immune reaction similar to, though weaker than, that in the malabsorption syndrome with cow's milk protein intolerance (14) and in coeliac disease (13).

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