

Morphology of the Jejunum in Children with Eczema due to Food Allergy

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We report the results of a morphometric study of jejunal biopsy specimens from 18 children with eczema due to food allergy. The measurements were compared with those for 13 age-matched children without gastrointestinal or immunological diseases. The patients' ages ranged from 2 months to 4 years. In 14 the food responsible for the symptoms was cow's milk. During the challenge tests with suspected foods 10 children had skin symptoms only (group I) and eight had gastrointestinal symptoms in addition (group II). Both groups showed a significant decrease in the villus/crypt ratio, mostly due to an increase in crypt depth. The counts of lymphocytes and plasma cells in the lamina propria and of intraepithelial lymphocytes did not differ from those seen in the controls. Four patients had increased counts of jejunal eosinophils, but the mean count for the whole group was not significantly increased. Considering nine measurements only five of the patients had moderate changes in jejunal morphology.

Key words: eczema; food allergy; jejunal morphology.

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When gastrointestinal symptoms predominate in patients with intolerance to cow's milk and soy protein, they have been shown to be associated with severe intestinal damage (1, 7, 8, 9, 13, 14), much like that seen in coeliac disease. Although atopy was increased in a group of patients with intolerance to cow's milk (9), we did not observe skin reactions in connection with gastrointestinal symptoms during food challenge (9, 12). Cutaneous reactions to food allergens are sometimes associated with reaginic antibodies, but in many patients eczematous reactions do not appear until many hours after challenge and no reagins can be demonstrated (10). Nothing is known about the association between skin symptoms and intestinal structure in these patients (5). It has been speculated that the principal cause of food allergy in infancy is increased permeability of the intestine and this

could be based on mucosal damage or immunological immaturity (10).

We therefore studied the intestinal structure, intestinal immunoglobulins, complement activation and antibodies to food proteins in infants with eczema due to food allergy. In this paper we describe the morphological analysis of 18 biopsy specimens taken after positive skin reactions to oral challenge with food and compare these measurements with an age-matched group in whom intestinal and immunological disease had been excluded.

PATIENTS

The series consists of 18 patients admitted to the Children's Hospital and the Hospital for Allergic Diseases, University of Helsinki and Jorvi Hospital, from September 1977 to May

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1978, because of a history of eczematous reactions to a variety of foods. Their ages ranged from 2 months to 4 years (median 9 months) (Table 1).

Challenge. In hospital the patients were placed on an elimination diet containing none of the foods suspected to cause eczema on the basis of the history. When the symptoms had cleared, the patients were challenged on the hospital wards with standard amounts of cow's milk formula, a 10% aqueous gruel of cereals, and boiled egg white. Skin and gastrointestinal symptoms were recorded by the nursing staff, and blood samples were taken before the challenge and as soon as symptoms were noted. Provocations were carried on for 1 to 10 days. When skin symptoms appeared the challenge was stopped and jejunal biopsy was taken as soon as was practically possible (Table 1). During challenge the patients fell into two groups. Ten patients had skin symptoms only (group I), whereas the other eight patients had gastrointestinal symptoms as well (group II).

As controls, biopsy specimens were studied from an age-matched group of 13 children. In these children intestinal pathology and malabsorption had been excluded, they had normal immunoglobulins in their serum and their intestinal immunoglobulins were considered normal (11).

METHODS

Peroral biopsies of the jejunum were performed under fluoroscopic control with a paediatric Crosby capsule. The specimens were taken at or shortly distal to the duodenojejunal junction. The biopsies were studied under the dissecting microscope and graded (3). They were fixed in buffered 10% formalin for 24 h, processed and embedded in paraffin. Sections were stained with haematoxylin-eosin and PAS.

The measurements were made with a Leitz micrometer ocular and included villous height, depth of the crypts of Lieberkühn and height of the epithelial cells in both the villi and

crypts (8). The mass estimations were performed by Weibel's point-counting technique (15), using an integration ocular. Measurements were made at $\times 1,000$ magnification. The percentages of sections occupied by epithelial cells, goblet cells and lamina propria were determined (8). Plasma cells, lymphocytes and eosinophils were calculated as percentages of the lamina propria. The number of intraepithelial lymphocytes was determined according to the method of Ferguson & Murray (6). The method of Dunnill & Whitehead (4) was used to measure an index related to the surface-to-volume ratio of the mucosa. The length of each line cast on the section was 0.07 cm.

The severity of the mucosal changes was scored according to the method described by Iyngkaran et al. (7), with modifications based on our own control series (Table 2). Six parameters were graded: 1. villous morphology under the dissecting microscope, 2. villous length/crypt depth ratio, 3. epithelial cell height, 4. intraepithelial lymphocyte infiltration, 5. cellular infiltration of the lamina propria, 6. index c/lh related to surface to volume ratio (4).

Statistical analysis of results was done with Student's t-test when distribution of the results was parametric. In the non-parametric distribution of eosinophils Wilcoxon's test was used.

RESULTS

Villous height (Fig. 1) was lowest in the patients with both intestinal and skin symptoms (group II), mean 297 μm , 314 μm in group I. The difference from the control values (mean 337 μm) was not significant.

Crypts of Lieberkühn (Fig. 2) were significantly deeper in both groups of patients than in controls, in group I $P < 0.005$ and II $P < 0.02$. The mean ratio of villous height to crypt depth in the control subjects (mean 3.0) was significantly higher than in the patients of

Table 1

Clinical features of the 18 patients with eczema due to food allergy

Patient no.	Age		Sex	Foods used in provocations	Signs and symptoms after provocations		Time of biopsy (interval in hours from last feed causing symptoms)
	Years	Months			Time of appearance	Nature	
1		8	F	Oats 100 g Barley 100 g CM 100 ml $\times$ 5 CM 100 ml	2 h 6 h 24 h —	gluteal rash ^b gluteal rash perioral rash	48 h (5) ^a
2		8	M	CM 100 ml, 200 ml $\times$ 5	4 d	perianal rash ^b	24
3		8	F	CM 200 ml $\times$ 5	4 d	perianal rash ^b	24
4		8	M	Rye 50 g, 100 g Oats 50 g, 100 Wheat 50 g CM 1 50 ml	6 h 10 h 4 h 6 h	skin rash ^b facial rash cheek rash cheek rash	24
5		10	F	Rye 100 g Rye 40 g	12 h —	skin rash ^b	72 (1) ^a
6		11	M	CMF 5 ml $\times$ 4	15 min	skin rash ^b	24
7	1		M	CM 20 ml $\times$ 4	1 h	skin rash, eyelid oedema	24
8	1	1	M	CMF 5 ml-200 ml $\times$ 5	4 d	perioral rash	24
9	2	10	M	CM 200 ml $\times$ 3	6 h	skin rash ^b	24
10	4		M	Wheat 100-200 g	2 d	skin rash ^b	24
11		2	M	CMF 10 ml $\times$ 5	12 h	diarrhoea, vomiting, urticaria	8 d
12		4	F	CM 5 ml-5 ml $\times$ 5	2 d	vomiting, skin rash	24
13		5	F	CM 5 ml-200 ml $\times$ 5	8 d	diarrhoea, vomiting, facial rash	24
14		8	F	CMF 10 ml-80 ml $\times$ 6	3 d	diarrhoea, vomiting, perioral rash	24
15		9	M	Rye 100 g Rice 100 g	24 h 6 h	diarrhoea, vomiting, gluteal rash ^b vomiting	24
16		11	M	CMF 5 ml-10 ml $\times$ 4	3 d	diarrhoea, cheek rash	48
17		11	F	Rye 200 g Barley 100 g Rye 100-200 g	12 h 12 h —	diarrhoea, vomiting, skin rash diarrhoea —	8 d (24) ^a
18	1	1	M	CM 5 ml Egg yolk 2.5 g Wheat 20 g Wheat 50 g Rye 50 g	15 min 15 min 20 min 12 h 12 h	skin rash skin rash skin rash vomiting vomiting	48

CM = unadapted cow's milk formula, $\frac{1}{2}$ or $\frac{2}{3}$ diluted pasteurized milk.

CMF = adapted cow's milk formula.

F = female, M = male.

d = day(s), h = hour, min = minute(s).

^ainterval from last feeding with a food which had previously caused symptoms.^bgeneralized eczema in the history.

Table 2
Histological score of jejunal biopsies^a

A. Dissecting microscope appearance:	Score
1. Villi finger or leaf-like	0
2. Villi ridged or convoluted	1
3. Mucosa flat	2
B. Villus/crypt ratio	
1. 2.8 or more	0
2. 2.3-2.7	1
3. 1.8-2.2	2
4. 1.3-1.7	3
5. 0.8-1.2	4
6. 0.3-0.7	5
7. Less than 0.3	6
C. Villous epithelial cell/crypt cell ratio	
1. Villous cells taller than crypt cells	0
2. Ratio reversed	1
D. Cellular infiltration of lamina propria	
1. Lymphocytes: Normal ^b (<8.5%)	0
Increased (>8.5%)	1
2. Plasma cells: Normal (<1.5%)	0
Increased (>1.5%)	1
3. Eosinophils: Normal (<0.9%)	0
Increased (>0.9%)	1
E. Intraepithelial lymphocyte infiltration	
1. Normal (15-40)	0
2. Moderate (41-50)	1
3. Severe (>50)	2
F. Surface to volume ratio	
1. Normal (>27 cm) ^c	0
2. Less than 27	1
Total score	0-15

^aAdapted from Iyngkaran et al.

^bUpper limit of our own control series.

^cMean for the controls - s.d.

group I (mean 2.3) and group II (mean 2.2) ($P < 0.01$ and 0.05 , respectively).

Epithelial cell height was similar in controls and patients in both groups (Fig. 3).

Crypt epithelial cell height was significantly higher in patients in group I than in controls ($P < 0.005$), while patients in group II did not differ from the controls (Fig. 4). In all cases studied the surface epithelial cells were taller than the crypt epithelial cells (Table 2).

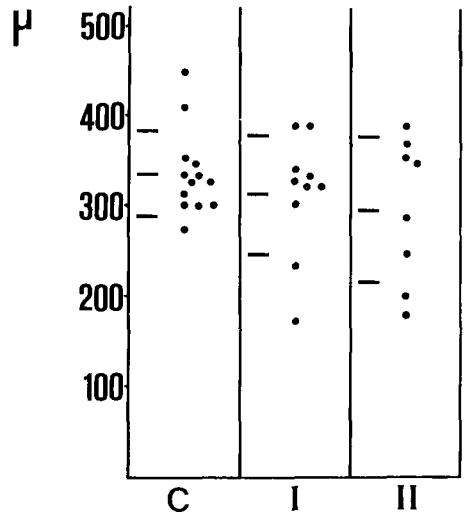


Fig. 1. Villous height (μm) in 13 controls (C), in patients reacting to provocation with cutaneous symptoms only (I) and in patients who had gastrointestinal symptoms also (II). Mean $\pm$ 1 s.d. is indicated.

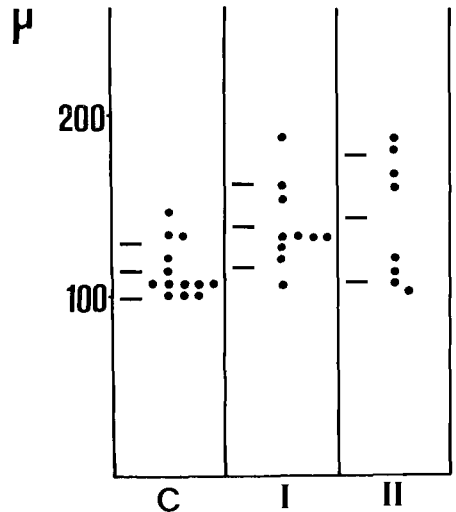


Fig. 2. Crypt depth in μm . Symbols as in Fig. 1.

The index related to surface-to-volume ratio (c/lh) was highest in the controls (mean 42); in group I it was 38 and in group II 36; however, these differences are not significant. In three patients an abnormal ratio below 27 (mean - 2 s.d.:s of the controls) was seen (Table 3 and Fig. 5).

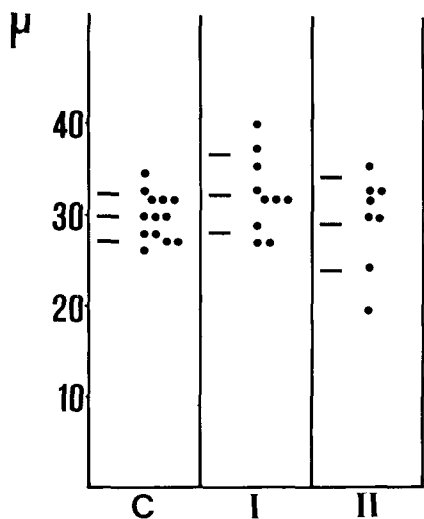


Fig. 3. Villous epithelial cell height in μm .

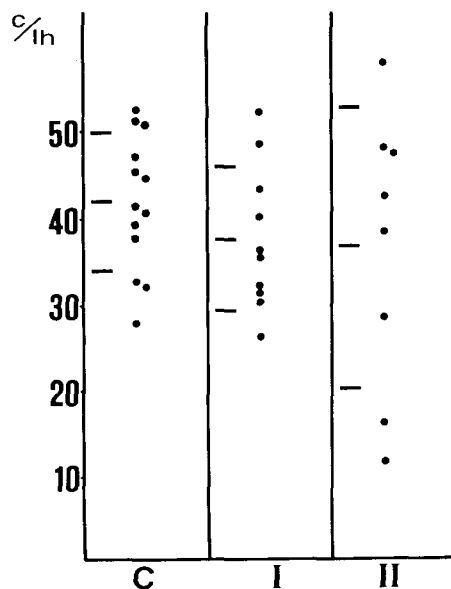


Fig. 5. An index c/lh related to surface-to-volume ratio. 1 was 0.07 cm.

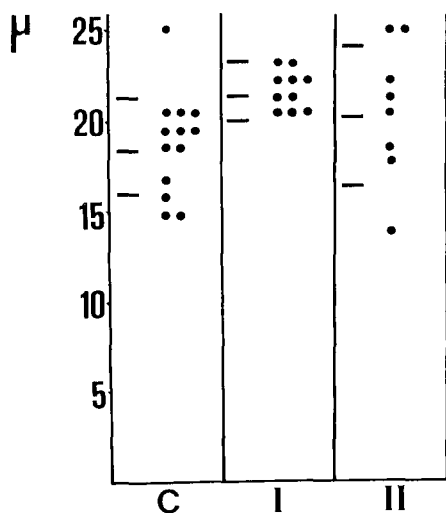


Fig. 4. Crypt epithelial cell height in μm .

Epithelial cell mass. The average epithelial cell mass of the patients with skin symptoms (31%) and that of the patients with skin and gastrointestinal symptoms (29%) were less than that of the control subjects (35%) (Fig. 6); the differences are not significant.

Goblet cell mass. No significant difference between the various groups studied was observed in regard to the goblet cell mass (Fig. 7).

Lymphocytes and plasma cells. The percentages of the lamina propria occupied by lymphocytes and plasma cells were similar in controls and patients (Figs. 8 and 9).

Eosinophils. The median values for eosinophils were higher in both patient groups (Fig. 10), but the difference was not significant. Two cases in both groups had an eosinophil infiltration outside the range observed in the controls (Fig. 10).

Intraepithelial lymphocytes. The numbers were very similar in patients and controls (Fig. 11).

Scoring of the jejunal biopsies. In the two controls with the highest scores these were due to villus/crypt ratios between 1.8–2.2 (Table 2). In five patients a higher total score was observed and the four with the highest scores (Cases 6, 7, 13 and 16) had been challenged with cow's milk formula.

Table 3
Histological findings in patients and controls

Controls	A	B	C	D	E	F	Total score
1- 7	0	0	0	0	0	0	0
8-11	0	1	0	0	0	0	1
12-13	0	2	0	0	0	0	2
Patients							
Group I							
1	0	2	0	0	0	0	2
2	0	1	0	0	0	0	1
3	0	2	0	0	0	0	2
4	0	2	0	0	0	0	2
5	0	0	0	0	0	0	0
6	0	3	0	1	0	1	5
7	0	2	0	2	1	0	5
8	0	2	0	0	0	0	2
9	0	0	0	0	0	0	0
10	0	1	0	0	0	0	1
Group II							
11	0	1	0	0	0	0	1
12	0	0	0	1	0	0	1
13	1	4	0	0	0	1	6
14	0	2	0	0	0	0	2
15	0	0	0	0	0	0	0
16	1	4	0	1	1	1	8
17	0	1	0	2	0	0	3
18	0	2	0	0	0	0	2

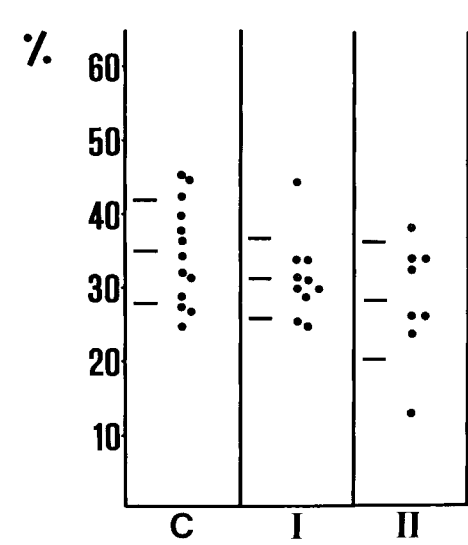


Fig. 6. Epithelial cell mass as a percentage of the total count of the lamina propria and the epithelial tissue.

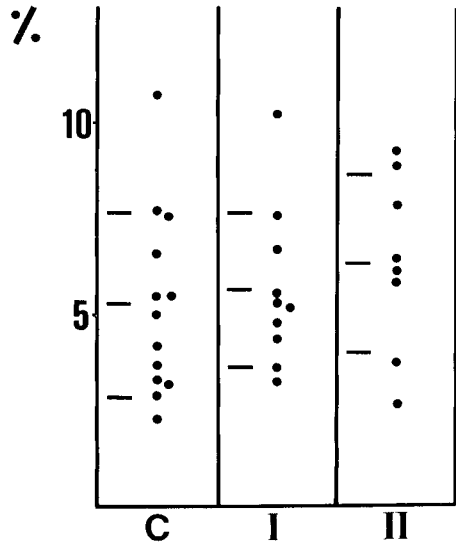


Fig. 7. Goblet cell mass as a percentage of the epithelial tissue.

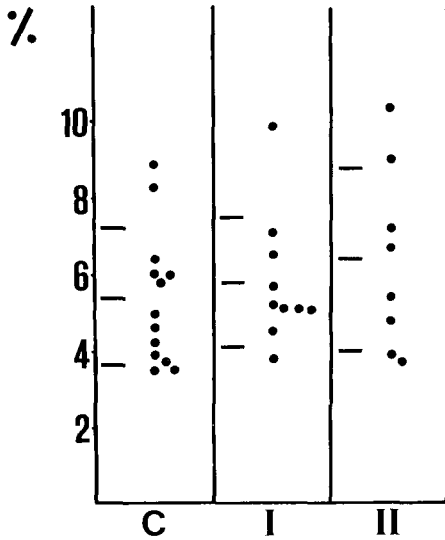


Fig. 8. Lymphocyte cell mass as a percentage of the lamina propria.

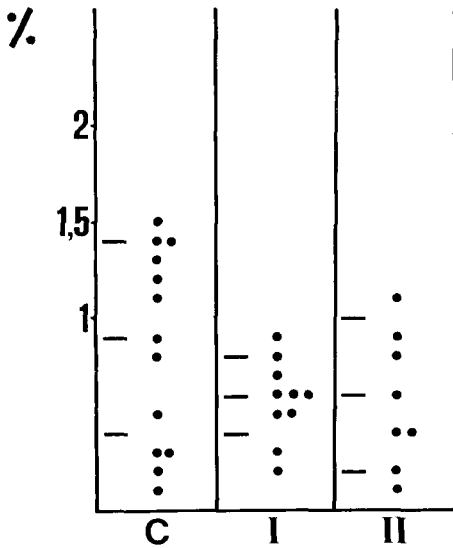


Fig. 9. Plasma cell mass as a percentage of the lamina propria.

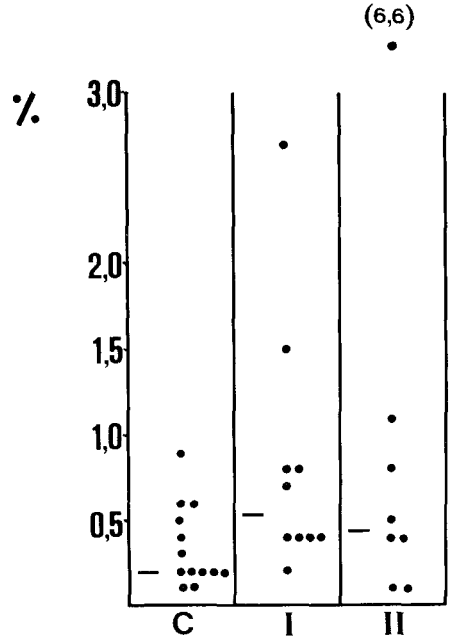


Fig. 10. Eosinophilic cell mass as a percentage of the lamina propria.

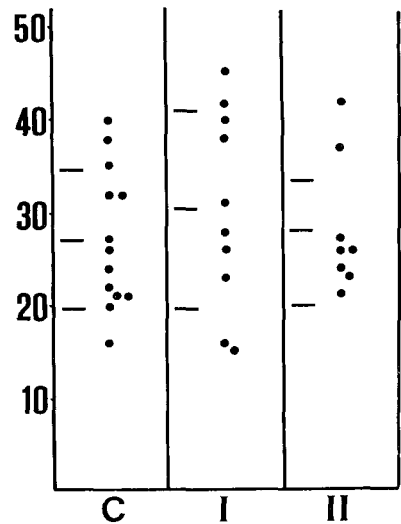


Fig. 11. Intraepithelial lymphocytes/100 epithelial cells.

DISCUSSION

The genesis of food allergy has been the subject of much speculation. Increased absorption of foreign antigenic material due to an altered mucosal barrier or to a local immunological

response has been blamed (5, 10). In this study we set out to ascertain whether, in children with eczema due to food allergy, the intestines show morphological, immunohistochemical or electron-microscopical changes. In the course

of challenge some of the patients were noted to have gastrointestinal symptoms also, and the patients were therefore divided into two groups. Presumably, previous intestinal symptoms had been mild, as they were not indicated in the history and no child had growth failure, which we always saw in the malabsorption syndrome with cow's milk intolerance (9).

Morphometric study of the intestinal biopsies disclosed changes only in the minority of patients, five out of 18, and these changes were moderate. There was a statistically significant increase in crypt depth and this caused a decrease in the mean villus/crypt ratio in both groups. In most cases the villous height remained unchanged. Slight mucosal damage in man as well as in experimental animals has been shown to begin with crypt hypertrophy (16) and this slight change was usually present in patients of this study.

In addition, in the children with eczema only, the crypt epithelial cells were enlarged as compared with those of the controls. In altogether five patients the lamina propria or epithelium showed some inflammatory reaction; in four of these the number of eosinophils was increased. Clearly in the children of this series the gastrointestinal changes like the symptoms were much milder than in children with the malabsorption syndrome with cow's milk intolerance, in whom we nearly always saw at least slight changes in the jejunal mucosa and in half of whom the damage was as severe as in coeliac disease (9).

The estimation of surface-to-volume ratio has been claimed to be a sensitive measure of intestinal damage (4). In our hands it was in good agreement with the villus/crypt ratio and was abnormal in cases with a ratio of less than 1.8. We prefer the more meaningful estimation of villous and crypt lengths and their ratios, and this ratio seems to be the most sensitive indicator of mucosal damage. The scoring of many parameters in this group did not add to the classification of biopsies; in only one case was there a significant increase in eosinophils without any change in the villus/crypt ratio.

In 14 cases out of 18 the skin symptoms were attributable to feeding with cow's milk, in seven the symptoms were caused by cereals and in one egg white was one of many foods provoking allergic symptoms. This is in agreement with many reports on the importance of cow's milk in food allergy in early childhood (2, 5, 10). The most severe mucosal changes in this study (4 cases) were associated with cow's milk allergy. On the other hand, in 10 cases with eczema due to cow's milk the intestinal structure was practically normal.

Morphological analysis of the biopsies did not indicate any damage that could cause the immunization with foreign proteins, as most specimens were quite normal. Apparently food allergy may cause intestinal damage of all grades, which coexists with other symptoms of allergy, such as eczema, especially in patients allergic to cow's milk. The intestinal damage and the intensity of gastrointestinal symptoms are in close association. When such symptoms are weak or absent, the intestinal changes are minimal, and we do not recommend the taking of intestinal biopsy specimens in these cases; other diagnostic criteria must be used.

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