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IMMUNOLOGICAL, HISTOLOGICAL AND ELECTRON-MICROSCOPICAL INVESTIGATIONS OF THE GUT IN ATOPIC DERMATITIS

Lasse R. Braathen,[1] Kåre Baklien,[2] Torstein Hovig,[3] Olav Fausa[4] and Per Brandtzaeg[5]

[1]*Department of Dermatology,* [2]*Institute of Immunology and Rheumatology,* [3]*Electronmicroscopical Laboratory,* [4]*Gastroenterological Laboratory, Medical Department A,* [5]*Histochemical Laboratory, Institute of Pathology, Rikshospitalet, The National Hospital, Oslo, Norway*

Abstract. Jejunal biopsy specimens were obtained from ten patients with severe atopic dermatitis and 15 controls. Light microscopical examination of hematoxylin-eosin stained sections showed normal conditions in all atopic patients and intra-epithelial lymphocyte counts did not differ significantly from counts in controls. Scanning electron-microscopical examination demonstrated minor mucosal changes in five of the atopic patients. Immunohistochemical studies did not reveal any significant changes in the class proportions and numbers of jejunal immunoglobulin-producing cells.

Food proteins and intestinal bacterial antigens constitute the major gut-derived antigenic challenge to the body. Recent studies have demonstrated that proteins can penetrate the mature intestinal mucosal barrier under physiological conditions and reach both the submucosa and the systemic circulation as intact, undigested macromolecules (18, 19, 20, 21). Such proteins may function as antigens in the host.

The induction of tolerance is complex and depends among other things on activation of suppressor T cells (2), the filtering role of the liver (17), and the effect of small doses of absorbed antigen which induce a state of "low zone tolerance" (15). One of the most important components of specific host defence is the production of intestinal antibodies; thus secretory IgA plays a major role in the immunological inhibition of absorption of undegraded macromolecular material of food or bacterial origin (1, 10).

Low serum levels of IgA have been reported in healthy infants at the age of 3 months who later became atopic (16), and in children with acute gastroenteritis who later became hypersensitive to cow's milk (9). Furthermore the frequency of IgA deficiency has been reported to be high in patients with atopy (12).

In many cases of atopic dermatitis, allergy to food antigens occurs, and exacerbation of the dermatitis after oral provocation of antigen has been reported (8). In a controlled study, antigen-avoidance diet afforded clinical improvement in children with atopic dermatitis (3), and the beneficial effect of such a diet is known to many clinicians.

To investigate whether patients with atopic dermatitis show intestinal abnormalities, we have performed histological, immunohistochemical and electron-microscopical investigations of the jejunal mucosa obtained from 10 patients with severe atopic dermatitis (AD).

MATERIAL AND METHODS

Patient material. Three females and 7 males, mean age 26.4 years, range 16–38 years, were investigated. All had suffered from dermatitis since early childhood and most of them had a history of allergic rhinitis, asthma or urticaria, in addition to the dermatitis. Mean serum IgE was elevated and all patients demonstrated immediate-type hypersensitivity reactions to various allergens. They received local treatment and antihistamines, but no internal steroids.

Seven men and 8 women, mean age 41 (range 12–77) years served as controls. Most of them had abdominal complaints, but their jejunal mucosa was histologically normal, and extensive investigation did not reveal any organic disease.

Tissue preparations

Several specimens were obtained under fluoroscopic control from the proximal jejunum of each patient by means of a multiple biopsy capsule.

One specimen was fixed in formaldehyde and stained with hematoxylin-eosin for light-microscopical examination. Another specimen was fixed in glutaraldehyde and processed for scanning electron microscopy. A third specimen was fixed in cold 96% ethanol to precipitate diffusible proteins, and the fourth specimen was extracted in cold phosphate-buffered saline for 48 h to facilitate registration of Ig-containing cells (5).

Table I. *Intra-epithelial lymphocyte counts in sections of jejunal mucosa from patients with atopic dermatitis and from controls*

	Number of subjects	Number of lymphocytes per 100 epithelial cells		
		Mean	S.D.	Range
Atopic dermatitis	10	9.5	3.7	5.0–15.6
Controls	10	10.9	2.5	7.7–15.0

Immunohistochemical procedures

Serial paraffin sections were cut at 6 μm and incubated with "green" (fluorescein) and "red" (rhodamine) rabbit IgG conjugates, showing specificity for the five human Ig classes. The characteristics of these reagents, the conjugate combinations used in paired staining, and the fluorescence microscopy conditions have been described previously (5).

Cell registration

Double-exposed colour slides covering a defined "mucosal tissue unit" (4) constituting a 6 μm thick and 500 μm wide block of tissue including the mucosa at full depth from the muscularis mucosae to the surface, were projected on a paper screen. From such registration of paired staining, differential counts of the three major immunocyte classes (IgA-, IgM- and IgG-producing cells) were recorded for each mucosal unit. Data were obtained in absolute figures and as immunocyte class ratios.

Intra-epithelial lymphocytes (6) were counted in hematoxylin-eosin-stained sections and compared with the counts in 10 controls. Data were obtained as number of lymphocytes per 100 epithelial cells; a total of 1 000 epithelial cells were included in each case. Wilcoxon's two-sample test was used for statistical comparisons.

A scanning electron-microscopical study was performed with the glutaraldehyde-fixed specimens.

RESULTS

Examination of hematoxylin-eosin stained sections from the proximal jejunum of 10 patients with severe atopic dermatitis showed normal histological conditions in all patients. Intra-epithelial lymphocyte counts did not differ significantly from the counts in 10 controls (Table I).

Scanning electron-microscopical examination showed some minor changes in 5 of the 10 investigated patients, i.e. occurrence of ridges in a higher number than normal. The height of the villi varied but they were of normal thickness. The surface appeared normal, with characteristic foldings and epithelial pattern.

Immunochistochemical investigation was performed with jejunal specimens from 7 atopic patients and 15 controls. The results are presented in Table II. The mean total number of Ig-producing cells tended to be decreased (but not significantly so) in the atopic patients. No differences were found in the percentage class distribution of the immunocytes. IgD- and IgE-producing cells were rare, but in atopy, cells with a speckled staining for IgE were regularly observed in the lamina propria. These cells were easily distinguished from IgE-producing cells, and apparently represented mucosal mast cells with bound IgE.

DISCUSSION

To our knowledge, this is the first immunohistochemical study of the gut mucosa in atopic dermatitis. A preliminary study of 7 of our atopic patients indicated a reduced number of jejunal Ig-producing cells, although the immunocyte class proportions were found to be unaltered compared with controls. As we felt that some of the mucosal biopsy specimens were of unsatisfactory quality, the study was repeated in a new series of 7 patients (Table 2); in only one case did the total number of Ig-producing cells fall below the normal lower range (52 vs. 86 cells), and the atopic group as a whole showed only a slight trend towards reduced cell number. Moreover, the immunocyte class proportions were again quite normal. This study, therefore, gives no good support for the idea that a defect of the secretory IgA system may give rise to an increased absorption of intestinal antigens as part of the pathogenesis of AD. However, it does not preclude that such a defect may be present temporarily during the sensibilization period, and individual variations may well persist. The possibility of a qualitative defect in the secretory IgA system should also be con-

Table II. *Distribution of immunoglobulin-producing cells in an average "mucosal tissue unit" of jejunal mucosa from patients with atopic dermatitis and from controls*

	AD patients ($n=7$)	Controls ($n=15$)
Mean percentages (observed range)		
IgA	80 (64–89)	79 (67–88)
IgM	17 (10–25)	18 (10–31)
IgG	3.0 (0.3–14)	2.6 (0.6–6)
Number of cells (Observed range)	122 (52–210)	131 (86–199)

sidered. The presence of IgE-bearing mast cells in the gut mucosa indicates that type I hypersentitivity reactions may occur locally and contribute towards increased intestinal permeability; this observation is consistent with that of Feltkamp-Vroom et al. (11) in other tissues of atopic patients.

In 5 of 10 patients, scanning electron-microscopical examination revealed some minor morphological changes, i.e. occurrence of ridges in a higher number than normal. In our preliminary series, minor changes were found in 4 of 7 patients. The significance of this observation is not clear. The light microscopical examination showed normal conditions, and the intra-epithelial lymphocyte counts did not differ from the control counts.

Matuchansky et al. (14) found in 5 of 10 cases with constitutional eczema a parietal villous atrophy localized most frequently to the proximal small intestine, without surface enterocyte anomalies. Conversely, Fry et al. (7) did not find any significant abnormality of the gross appearance of the mucosa in 4 cases of atopic eczema, and Marks & Shuster (13) concluded that the frequency distribution of both predominant and individual small intestinal mucosal features in patients with eczema differs very little from a local control population, in agreement with our observations.

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DISCUSSION

Barnetson (Edinburgh). Q: Might I suggest in future studies that you try to provoke changes in the small intestine by giving the patient an allergen?

A: That is of course a possibility but these patients were in-patients with a severe dermatitis, so you might say that they were already provoked.