IgA Class Antibody Against Human Jejunum in Sera of Children With Dermatitis Herpetiformis

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Sera of 44 children with dermatitis herpetiformis with granular IgA deposits in the papillary dermis were investigated on cryostat sections of normal jejunum of three children aged 2 months, 1 year, and 10 years by indirect immunofluorescence. Eighteen of 25 patients on a normal diet had an IgA class antibody showing the following staining patterns on substrate jejunums: (1) tubular positivity in the lamina propria—around the crypts, beneath the villous epithelial basement membrane, and in some instances in the middle of the villous also, following the capillary system of villi; (2) coalescence of tubular positivity at the muscularis mucosae; and (3) positive blood vessels and smooth muscle endomysium. Eleven of 18 children with positive sera were put on a gluten-free diet (GFD) and their sera became negative. One of these 11 patients was challenged with gluten and the antibody reappeared. Nineteen patients examined only on a GFD and 30 healthy blood donors did not have this antibody. There was no strict correlation between the titer of antibody and the severity of jejunal mucosal damage. *J Invest Dermatol 87:703-706, 1986*

**MATERIALS AND METHODS**

**Patients** Forty-four Hungarian children (19 boys, 25 girls) aged 1.5–12 years with DH and granular IgA deposits in the skin [16] were studied.

**Jejunal Biopsy of Patients** Jejunal biopsy was taken by a double-port Crosby-Kugler capsule of pediatric size, in each case before GFD, in 30 cases after GFD for a year or more, and in 1 case under gluten challenge for 6 months.

In a majority of the cases both of the specimens, and in a few cases only one biopsy specimen, were stained with hematoxylin-eosin and investigated on light microscopy. The villous height and the number of intraepithelial lymphocytes were studied, and the following grades of jejunal damage were given: subtotal villous atrophy, partial villous atrophy, and slight changes [17].

**Serum Samples of Patients and Controls** All but 4 serum samples were taken at the time of jejunal biopsy. In 1 child the jejunal biopsy was not controlled under GFD. In 14 cases sera were collected only before GFD, in 19 cases only during GFD, in 11 cases sera were collected while the patients were on a normal diet and under gluten challenge. In 1 case sera were examined on normal diet, during GFD, and under gluten challenge. Sera of 30 healthy blood donors were used as controls. Serum samples were stored at −35°C in small portions.

**Normal Human Jejunum for Immunofluorescence Studies** In 3 children, whole jejunum (substrate A) or jejunal mucosa (substrates B and C) from the level of the ligand of Treitz were found to be normal on light microscopic histology and were used for immunofluorescence (IF).

**Substrate A:** A 2-month-old girl died of pneumonia and diphtheria. She had not received gluten. A jejunal specimen was taken 2 h after death.

**Substrates B and C:** Two children, aged 1 year and 10 years, underwent small-bowel biopsy to disclose jejunal changes. One
specimen obtained by a double-port Crosby-Kugler capsule of pediatric size was used for the present study, the other for light microscopic examination.

**Immunofluorescence Studies** All 3 jejumens were washed for 20 min in phosphate-buffered saline (PBS) pH 7.4, embedded in OCT (Lab Tek Ames Co., Indiana), quick-frozen in liquid nitrogen, and stored at −35°C for less than a week. Direct and indirect IF was performed on 5-μm cryostat sections of jejumens A, B, and C [18]. Normal human skin was investigated only by indirect IF [18]. For direct IF, fluorescein isothiocyanate (FITC)-conjugated antihuman IgA, IgG, IgM, and complement (Hyland) were used. Indirect IF was carried out with patients’ and controls’ sera at a 1:10 starting dilution and with FITC-labeled antihuman IgA (Hyland and Behring preparations) in dilutions of 1:15 and 1:20. The F/P molar ratio was 2.3 and 2.5. Blocking studies were negative. Because of their relatively small amount, B and C jejumens were tested only in 5 of 30 control sera.

**RESULTS**

**Direct IF** Jejunum A, B, and C showed IgA, IgM, IgG, and complement positive plasma cells in the lamina propria, while jejunum C alone showed slight IgA positivity in the lamina propria and also in the upper third of epithelial cells (more in the crypts).

**Indirect IF** Normal Human Skin: None of our patients had IgA type antibody against the BM.

Normal Human Jejunum A, B, C: Control sections preincubated with PBS or normal human sera showed the picture of direct IF of substrates A, B, or C (Figs 1, 3a) as mentioned above. Sera of 18 of 25 patients on a normal diet reacted with jejunum A, B, C as follows. Beneath the epithelium, along the BM of villi and crypts, and in some places in the middle of the villus also, a band-like, in many places tubular, positivity was observed (Figs 2, 3b) following the capillary structure of villous lamina propria.
(jejnum A, B, C). Coalescence of tubular positivity in the lamina propria, mainly at the muscularis mucosae (Figs 2, 4) was detected (jejnum A). Positive blood vessels and endomysium positivity in smooth muscle layers (jejnum A) were observed as well (Fig 2). (Substrates B and C did not contain muscularis mucosae or smooth muscle layers.)

Antijejunal Antibody and the Activity of the Intestinal Damage (Table 1) Presence of Antibody: All patients with circulating antibody had jejunal villous atrophy. Neither of the 2 patients with normal intestinal histology had this antibody. There were 3 patients with mild and 2 with severe jejunal damage without any detectable antibody. Under GFD the antibody disappeared before the normalization of jejunal histology.

Titer of Antibody: Despite the fact that the majority of patients with strong positive sera had severe intestinal villous atrophy, there was no strict correlation between the serum activity and the degree of jejunal damage.

Antijejunal Antibody and Gluten Intake: The antibody was present in 18 of 25 patients on a normal diet. All seropositive patients became seronegative under GFD. The antibody appeared again after gluten challenge in the 1 case examined. On permanent GFD the children had no antibody.

**DISCUSSION**

In untreated CD, where the small-bowel changes are similar to those of DH, a secretory IgA fluorescence has been found along the BM zone and connective tissues of jejunal mucosa, returning to normal on GFD [19]. After gluten challenge, an IgA-positive staining appeared along the BM of villous and crypt epithelia in patients with CD [15]. Also in CD the most noticeable electron microscopic changes after gluten challenge occurred in the endothelium of capillaries in close proximity to the absorbing cells [20]. Swelling and hypertrophy of endothelial cells, early thickening and "fibrin formation" in the periendothelial and in the epithelial BM were also observed [20,21]. IgA type antibody registered by us was deposited beneath the epithelial BM to a tubular structure, resembling the capillary system of villi [22] (Fig 3b). It was not clear how far the BM region was involved in this positivity, but none of the patients has antibody against the BM of normal human skin. In gluten-sensitive enteropathies 5 types of antireticulin antibodies were detected on different tissue substrates by indirect IF [8], but usually the so-called R3 type is used for screening. Staining patterns of sera of our DH patients on human jejunum show similarities to that of so-called R3 antibody of the above-mentioned classification, though R3 was not demonstrable on human organs. Human jejunum A of the present study, perhaps because it was never exposed to gluten and because of age specificities, was especially suitable for our investigations; except for plasma cells there was no detectable IgA positivity (Fig 1).

The reaction observed at the smooth muscle layers of this human jejunum corresponded to that of endomysial antibody also, described by Chorzelski et al [9-11] on monkey esophagus. The fact that the distribution of some smooth muscle elements in the small intestine is similar to that of our antibody [23] may be significant. Antijejunal antibody described here occurred parallel with the gluten intake, which is an important factor in the pathogenesis of DH [24]. It was present in 72% of patients on normal diet, completely absent on GFD, appearing again under gluten challenge. There was no strict correlation between the titer of antibody and the severity of jejunal mucosal damage. They can be related to each other, but not necessarily.

**Table 1. Distribution of Titer of IgA Type Antibody Against Normal Human Jejunum in Sera of 44 Children with Dermatitis Herpetiformis According to Their Jejunal Morphology**

<table>
<thead>
<tr>
<th>Jejunal Morphology of Patients</th>
<th>Titer of Jejunal Antibody in Sera of Patients&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1280</td>
</tr>
<tr>
<td>Subtotal villous atrophy</td>
<td>□</td>
</tr>
<tr>
<td>Partial villous atrophy</td>
<td>□</td>
</tr>
<tr>
<td>Slight changes</td>
<td>□</td>
</tr>
<tr>
<td>Normal</td>
<td>□</td>
</tr>
<tr>
<td>Not investigated&lt;sup&gt;b&lt;/sup&gt;</td>
<td>□</td>
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<sup>a</sup>Jejunal biopsy and serum samples were taken at the same time.

<sup>b</sup>Patients were labeled according to their gluten intake as follows: □, 1 patient before gluten-free-diet (GFD); □, 1 patient under GFD for a year or more, also investigated before GFD; X, 1 patient investigated only under GFD for a year or more; □□, data of 1 patient before GFD □, under GFD for 1.5 years □, and under gluten challenge for 6 months □.

<sup>c</sup>Titer of antibody was studied on human jejunum A, and positive reactions at the last serum dilutions were detectable along a tubular structure of lamina propria (Fig 4).

<sup>d</sup>In 4 patients no jejunal biopsy was performed, only serum samples were investigated under GFD.
REFERENCES


