Increased HLA-DR Expression by Enterocytes in Children With Celiac Disease

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Class II histocompatibility antigens, known to be present on immunocompetent cells, were recently demonstrated on enterocytes. Because of their role in antigen presentation and immune response regulation, HLA-DR antigens were studied in patients with celiac disease. Cryostat sections of jejunal biopsy specimens were stained with several anti-DR monoclonal antibodies using an avidin-biotin peroxidase technique. Thirty patients with celiac disease either active (n = 5), in remission (n = 10), or in relapse (n = 15) were compared with 16 controls, 9 with a normal mucosa and 7 with a flat mucosa but without celiac disease. In celiac patients with active disease or in relapse, enterocytes were heavily stained on the surface epithelium and the crypts. This contrasted with the absence of crypt staining in the biopsy specimens of the other patients. Increase in DR expression was associated with an increase in the number of T8(+) lymphocytes in the crypts. Modulation of DR expression by enterocytes may be involved in the pathogenesis of celiac disease.

HLA-DR antigens are polymorphic transmembrane glycoproteins encoded by genes within class II loci of the major histocompatibility complex. Unlike class I antigens, which are ubiquitous, DR antigens are expressed on some cells of the immune system, B cells, macrophages, activated T cells, and on some epithelial (1) and endothelial cells (2) as recently shown. Whatever the cell type, DR expression is modulated after immunologic stimulations, particularly through γ-interferon secretion. In pathological circumstances, expression also appears on cells that do not normally express DR antigens, such as thyrocytes (3), keratinocytes (4,5), and epithelial cells of the colon (6) or the bile duct (7). Whereas DR expression in the immune system cells plays a major role in antigen presentation and activation of lymphocytes, its function in nonimmune cells has not yet been elucidated. It has been suggested that DR expression by epithelial cells may be involved in the pathogenesis of diseases affecting these epithelia (3).

In celiac disease, the observation of an increase in the number of intraepithelial lymphocytes (IELs) (8), mainly of the cytotoxic/suppressor phenotype (9,10), suggested that these cells might play a role in epithelial lesions. Strober (11) propounded the theory that they might react against epithelial determinants, these determinants possibly being DR antigens that have been modified after exposure to gliadin. As normal enterocytes express DR antigens on their surfaces (4,9,10,12–14), particularly on the basolateral membranes in close contact with IELs, we examined DR expression in children with celiac disease. Staining of small intestinal biopsy specimens using an immunoperoxidase technique and monoclonal antibodies directed against DR antigens demonstrated an increase in DR expression, especially at the crypt level, together with an increase in the number of T8(+) IELs. Modulation of DR expression by enterocytes may be involved in the pathogenesis of celiac disease.

Materials and Methods

Patients

Thirty biopsy specimens from children with celiac disease were examined (Table 1). Five biopsies showing subtotal villous atrophy were performed at the active phase of the disease for diagnostic purpose in children.
Table 1. HLA-DR Antigen Expression on Enterocytes

<table>
<thead>
<tr>
<th>n</th>
<th>Mucosal histology</th>
<th>Age (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celiac patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active</td>
<td>5</td>
<td>STVA</td>
</tr>
<tr>
<td>Remission</td>
<td>10</td>
<td>Normal</td>
</tr>
<tr>
<td>Relapse</td>
<td>15</td>
<td>STVA</td>
</tr>
<tr>
<td>Controls</td>
<td>9</td>
<td>Normal</td>
</tr>
<tr>
<td>Controls</td>
<td>7</td>
<td>STVA</td>
</tr>
</tbody>
</table>

STVA, subtotal villous atrophy.

with a malabsorption syndrome. Subsequently, favorable clinical evolution after gluten exclusion corroborated the diagnosis of celiac disease. Ten biopsy specimens were obtained from children maintained on a gluten-free diet for more than 1 yr to control the effects of gluten withdrawal. In all cases, mucosa was nearly normal or normal. Finally, 15 biopsies were performed in children with confirmed celiac disease, according to ESPGAN criteria (15). These biopsies were done either to prove the relapse, usually after 1 yr on a normal diet (7 cases), or later on during the course of a follow-up program to study mucosal lesions in children left on a normal diet (8 cases). All specimens showed a flat mucosa except two from children on a normal diet for 4 and 10 yr. As already observed (16,17) in these patients, mucosal lesions had improved after a typical relapse despite gluten in the diet. Nine biopsies, performed for diagnostic purposes in children with short stature but without digestive symptoms, turned out to be normal and served as controls. Finally, seven biopsies that were performed in children presenting with diarrhea of nonceliac origin and that showed severe villous atrophy were included in this study. Two patients with probable cow's milk protein intolerance had been on an elimination diet for 2 and 12 days respectively. One patient had microvillous atrophy, and 4 children had untractable diarrhea of unknown origin that was unresponsive to gluten exclusion. None of these children was taking drugs at the time of biopsy.

Tissue Specimens

Small intestinal biopsies were performed with a Carey capsule, then oriented, embedded in Tissue Tek OCT compound (Miles Scientific, Naperville, Ill.), and snap frozen in liquid nitrogen. Cryostat sections, 5 µm thick, were air-dried, fixed in acetone for 10 min, and stored at −80°C.

Immunoperoxidase Staining

Frozen sections of small intestine were stained by an immunoperoxidase technique using the avidin-biotin complex method as previously described (10). Briefly, the sections were incubated with normal horse serum, then with optimal dilutions (determined on tonsils, normal spleen, and gut specimens) of mouse monoclonal antibodies directed against nonpolymorphic determinants of DR molecules (Table 2) and T8 antigens (Ortho-Mune, Ortho-Diagnostic Systems, Raritan, N.J.; Immunotech, Luminy-Marseille, France) present on T cells with the cytotoxic/suppressor phenotype. This was followed by incubation with biotinylated horse antimouse immunoglobulin G and avidin-biotin-peroxidase complex (Vectastain-Vector Laboratories, Burlingame, Calif.). Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in phosphate-buffered saline before incubation in avidin-biotin-peroxidase complex. The sections were stained by incubation in a solution containing 0.05% diaminobenzidine (Sigma Chemical Company, St. Louis, Mo.) and 0.03% hydrogen peroxide in phosphate-buffered saline. They were counterstained with hematoxylin.

Examination of the sections was carried out by two independent investigators, one of whom was not informed of the diagnosis. Table 2 indicates the number of biopsy specimens tested with each anti-DR monoclonal antibody. There was no significant difference in the staining pattern when several antibodies were used. However, labeling was most intense with anti-DR (Becton Dickinson, Mountain View, Calif.) and IoT2a (Immunotech), and much fainter with IoT2b (Immunotech).

Results

Normal Intestinal Mucosa

In the nine control biopsy specimens of normal intestinal mucosa, epithelial staining with anti-DR antibodies was maximum at the top of the villi, decreased toward the base of the villi, and was absent or very faint in the crypts (Figures 1a and 1b). Within the enterocytes, staining was more intense just below the brush border, with a patchy aspect, and was also present on the basolateral membranes (Figure 1c). In the lamina propria, anti-DR antibodies

Table 2. HLA-DR Antigen Expression on Enterocytes

<table>
<thead>
<tr>
<th>Name</th>
<th>Source</th>
<th>Clone</th>
<th>Class</th>
<th>Number of tested biopsy specimens</th>
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<tbody>
<tr>
<td>IOT2a</td>
<td>Immunotech</td>
<td>B8.12.12</td>
<td>IgG2b</td>
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<td>IOT2b</td>
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<td>BL2</td>
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<tr>
<td>Anti-DR</td>
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<td>L 243</td>
<td>IgG2a</td>
<td>30</td>
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<tr>
<td>206</td>
<td>Dr. D. Charron</td>
<td>206</td>
<td>IgG2b</td>
<td>8</td>
</tr>
</tbody>
</table>

a DR-specific monoclonal antibodies used in this study. b Luminy-Marseille, France. c Ortho-Diagnostic Systems, Raritan, N.J. d Mountain View, Calif. e Hôpital de la Pitié, Paris, France.
stained large cells with cytologic features of macrophages that were more numerous at the top of the villi, a few small cells with membrane staining that were most likely B cells, and vessel endothelium. Cells with endogenous peroxidase activity, mainly eosinophils and mast cells, were easily identified.

Intestinal Mucosa of Celiac Patients

Patients Diagnosed With Active Celiac Disease. In the 5 patients just diagnosed with active celiac disease, anti-DR monoclonal antibodies intensely stained the surface epithelium (Figure 2a). In
addition, labeling of the crypt enterocytes was observed (Figure 2b). The number of T8(+) IELs was increased within both surface and crypt epithelium. There were 5–15 T8(+) IELs per crypt section (Figure 3) compared with only 0–2 T8(+) IELs per crypt section of comparable size in normal control biopsy specimens (Figure 4).

Celiac patients in remission. In the 10 celiac patients on a gluten-free diet and with a normal mucosa, distribution of DR antigens and of T8(+) cells was similar to that observed in normal control biopsy specimens.

Celiac patients in relapse. In 13 patients on a normal diet and with a flat mucosa, the staining pattern with anti-DR and anti-T8 monoclonal antibodies was similar to that observed in patients at the time of the diagnosis. Interestingly, in 2 children having proven celiac disease despite following a normal diet, alterations of mucosal histology were minor and the staining pattern was identical to that of normal control biopsy specimens.

Flat Mucosae of Nonceliac Origin

In 6 of 7 nonceliac patients with a flat mucosa, distribution of DR antigens was similar to that observed in normal controls without any staining of the crypt epithelium (Figure 5). In 1 patient, a 2-yr-old boy with untractable diarrhea of unknown origin, resistant to total parenteral nutrition, a strong positive staining of both surface and crypt epithelium was present. It was associated with an increase in the number of T8(+) cells within and around the crypts.
a finding that was not observed in the 6 other patients.

Discussion

Using a sensitive immunoperoxidase technique, DR antigens were localized in cryostat sections of human small intestine. With the five monoclonal antibodies directed to nonpolymorphic determinants of DR antigens used in this study, staining of the crypt epithelium was very faint or absent in controls or in nonactive celiac disease (patients on a gluten-free diet), whereas it increased markedly in active celiac disease. In all patients with active celiac disease, staining of the crypts was intense. This finding differentiated celiac from most nonceliac flat mucosae where no staining of the crypts was observed.

The results of this study are consistent with previous observations using similar techniques concerning normal small intestine in humans (9,10) and in animals (4,13,14). However, different results have been obtained using different methods of fixation. Patchy staining of the apical pole of the enterocytes with no staining of the basolateral membranes and absence of modifications in celiac patients were observed with ethanol fixation and paraffin embedding (18). In addition, DR staining of crypt epithelium in control biopsy specimens and complete disappearance of DR expression by enterocytes of both villi and crypts in celiac patients were described in a recent study of ultrathin sections of paraformaldehyde-fixed biopsy specimens (19,20). Whereas the latter techniques allow demonstration of intracytoplasmic antigens, they may alter antige-
DR expression by enterocytes in celiacs

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Figure 5. Flat mucosa of a patient with intractable diarrhea of unknown origin, stained with an anti-DR monoclonal antibody. It shows positive labeling of villous epithelium and absence of staining in the crypts (×100).

The crypt enterocytes is not a characteristic feature of celiac disease but occurs simultaneously with local lymphocyte infiltration whatever its cause. Using an in vitro model, we have previously shown that DR expression by rat enterocytes can be modulated by supernatants of activated IELs containing a γ-interferon-like activity (13). The present study strongly suggests that, in humans as well as in animals, DR expression by enterocytes can be modulated by activated IELs. This hypothesis is further substantiated by observations in 2 cases of severe combined immunodeficiency. In these 2 patients, no T lymphocytes were detected in the gut mucosa: DR staining of enterocytes was absent or very faint, whereas lamina propria macrophages were normally stained with the anti-DR antibodies (unpublished personal observation). Absence of DR staining of the crypts in 1 celiac patient 3 mo after withdrawal of gluten in spite of a still flat mucosa, suggests that the presence of the antigenic stimulus is necessary to induce DR expression by crypt enterocytes in celiac patients. Absence of lymphocyte infiltration and DR staining in nonceliac patients with a flat mucosa may indicate that activation of IELs was absent in these patients. In this respect, it is noteworthy that the 2 patients with cow's milk protein intolerance were on an elimination diet. It would be interesting to study patients before their withdrawal from cow’s milk protein.

In the 2 celiac patients who had recovered a nearly normal mucosa in spite of several years on a normal diet, distribution of DR antigens and T8(+) cells was normal, suggesting that abnormal lymphocyte reactivity had indeed disappeared in these patients.

Whether modulation of DR expression by enterocytes is a bystander phenomenon due to local inflammation or is an essential step in the appearance of mucosal lesions is not yet known. Further work is needed to determine if increase in DR expression by enterocytes can lead to activation of gut lymphocytes of celiac patients in the presence of gluten. It would likewise carry us a step further if we could determine the functional capabilities of these lymphocytes, particularly their lymphokine secretion.

References

4. Barclay AN, Mason DW. Induction of Ia antigen in rat epider-

nicity of membrane protein antigens much more than the acetone-fixation technique used in the present study (21,22).

In patients with active celiac disease, the increase in DR expression by enterocytes was associated with an increase in the number of T8(+) IELs in both villi and crypt epithelium. Our observation of this increase in the number of T8(+) IELs in the crypts supplements the already known facts that the majority of IELs express the cytotoxic/suppressor phenotype (9,10), and that the number of IELs increases in celiac patients.

DR staining of the crypts, although always present in our celiac patients, is not fully characteristic of gluten intolerance as is the case in some other intestinal diseases. It was observed in animals during the course of parasitic intestinal infestation or graft vs. host disease (4) where an increase in the number of IELs was also demonstrated (8). In our study, it was present in all patients with active celiac disease and in 1 of 7 nonceliac patients with a flat mucosa. Interestingly, it was associated in the latter patient with an increase in the number of T8(+) cells in the crypts. This indicates that DR expression by...
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