Class II antigen (HLA-DR) expression by intestinal epithelial cells in inflammatory diseases of colon

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SUMMARY Eighty-four colonic biopsy specimens were obtained from patients with ulcerative colitis, Crohn's disease, radiation colitis, infectious colitis, and from normal controls. Paired specimens were examined by histological and immunohistochemical methods using monoclonal antibodies to the β chain of HLA-DR antigen. The expression of HLA-DR antigen in mucosal epithelial cells was strongly related to whether the specimens were actively inflamed: epithelial cells from 34 of 37 inflamed specimens (vthree of 42 non-inflamed specimens) were HLA-DR positive (p < 0.0001). Epithelial cells were uniformly HLA-DR negative in specimens from normal control patients despite the presence of HLA-DR positive lymphoid cells and macrophages in the lamina propria. Epithelial cells in specimens from patients with ulcerative colitis, Crohn's disease, and radiation colitis were HLA-DR positive in 30 of 33 inflamed biopsy specimens and in only three of 25 non-inflamed specimens (p < 0.0001). Epithelial cells were HLA-DR positive in nine of 10 biopsy specimens from patients with acute infectious colitis (p < 0.01).

The major histocompatibility complex is a series of genes that participate in the regulation of the immune response. This complex encodes two classes of cell-surface glycoprotein antigens: class I, found on all nucleated cells; and class II antigens, normally found only on a limited number of cells (B-lymphocytes, macrophages, Langerhans' cells, dendritic cells, vascular endothelial cells and some epithelial cells). Class II antigens control cellular interactions between lymphocytes. In man at least three class II antigens (DR, DQ, and DP), each consisting of α and β glycoprotein chains, are encoded by the HLA-D region of chromosome 6.2-4

In several inflammatory states class II antigens may be expressed by cells that normally do not contain these glycoproteins.6-11 This has led to the hypothesis that expression of these antigens is related to the pathogenesis of several autoimmune diseases12-13—that is, cells which are normally HLA-DR negative may act as antigen presenters when they are HLA-DR positive.13 Although normal intestinal mucosa contains lamina propria macrophages and lymphocytes, which are strongly HLA-DR positive, colonic epithelial cells are negative.3-14-17 Epithelial cells from colon biopsy specimens from patients with active inflammatory bowel disease, however, are strongly HLA-DR positive, suggesting continuous presentation of antigen to the immune system.14

This study tests the hypothesis that expression of HLA-DR antigen by colonic epithelial cells is specific for idiopathic inflammatory bowel disease. A previous report had suggested that epithelial cell HLA-DR expression was limited to patients with ulcerative colitis and Crohn's disease, as a single patient with Salmonella colitis had HLA-DR negative epithelial cells.14 We included 10 patients with infectious self-limited colitis as disease controls, as well as patients with both normal colons and idiopathic inflammation of the colon. Paired biopsy specimens were examined by histological and immunoperoxidase techniques using monoclonal antibodies to the β chain of HLA-DR antigen.

Material and methods

Biopsy specimens of colonic mucosa were obtained from five groups of patients: eight patients with irritable bowel syndrome whose colons were normal by endoscopy, barium enema, and histology (A); 34 patients with chronic ulcerative colitis, defined by history, abnormal barium enema, and histology (B); 11 patients with Crohn's disease defined by history, abnormal intestinal x-ray, and histology (C); four patients with radiation injury to the rectal mucosa (one with friable mucosa and three with tel-
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angiectasia) (D); 10 patients with infectious colitis (four Clostridium difficile, three Campylobacter, one Salmonella, and two with acute self-limited colitis of unknown cause) (E). These last two patients met both clinical and histological criteria for the diagnosis of acute self-limited colitis, although neither grew pathogenic organisms on stool culture. Informed consent was obtained from all patients.

Tissue Processing

Rectal biopsy specimens obtained with grasp forceps at sigmoidoscopy were bisected with scalpel blades; one half was fixed in buffered formalin, and the other half mounted in OCT compound (Lab-Tek, Naperville, Illinois) to allow sections to be cut perpendicular to the plane of the mucosa. Specimens mounted in OCT were then frozen in liquid nitrogen. Multiple colonoscopic biopsy specimens were obtained from two patients with active left sided ulcerative colitis and from two patients with normal colon mucosa; paired specimens were taken from each site sampled—one was formalin fixed and the other frozen after mounting in OCT compound.

Formalin fixed biopsy specimens were imbedded in paraffin, sectioned at 4 μm, stained with hamatoxylin and eosin, and reviewed without knowledge of immunohistological results. Biopsy specimens were judged to be inflamed when increased numbers of mononuclear cells or polymorphonuclear leukocytes were found in the lamina propria.

Immunoperoxidase Staining

Cryostat sections (5 μm) were placed on slides coated with gelatin, air dried, and fixed in petroleum ether:acetone (40:60) for 10 minutes. The sections were then stained by a two stage immunoperoxidase procedure, in which the following first layer monoclonal antibodies were incubated on the sections in a humid covered chamber for 40 minutes: CR3/43 and Tü 35, which react with the β chain of human HLA-DR antigens, and anti-IgD, which reacts with human immunoglobulin D. Tü 35 was kindly supplied by A. Ziegler. Antibodies were used undiluted. One section from each specimen was incubated with Tris buffered saline (TBS) as a negative control.

After incubation with monoclonal antibodies sections were washed in TBS and then incubated for 30 minutes using peroxidase conjugated rabbit antimouse immunoglobulin (Dakopatts, Denmark) as a 2% solution in TBS:normal human serum (2:1). After another wash the peroxidase reaction was developed by incubating sections with 3, 4, 3', 4' tetraaminobenzidine (0-6 mg/ml) and hydrogen peroxide (0-01%) for eight minutes at room temperature. Sections were washed in tap water, counterstained with hamatoxylin, and mounted for light microscopy. Coded sections were examined without knowledge of clinical or histological diagnoses.

Statistical Analysis

Two by two tables relating expression of HLA-DR antigen on colonic epithelial cells to the presence or absence of lamina propria inflammation were prepared (table 1). Pearson χ² tests were applied to the hypothesis that expression of HLA-DR in epithelial cells was related to underlying inflammation.

Results

Normal Controls (fig 1a)

Light microscopy showed normal crypt architecture, epithelial cells containing mucus, and lamina propria mononuclear cells. Immunoperoxidase stains showed HLA-DR positive macrophages, other mononuclear cells, and endothelial cells in the lamina propria, as well as HLA-DR positive cells at the periphery of lymphoid follicles. Negative controls showed absence of stain precipitate in these areas. Epithelial cells were uniformly HLA-DR negative in biopsy specimens from all areas of the colon.

Table 1 Correlation between expression of HLA-DR antigen and presence of inflammation in colon biopsy specimens from normal patients and those with inflammatory colitis

<table>
<thead>
<tr>
<th>HLA-DR</th>
<th>Inflamed</th>
<th>Not inflamed</th>
<th>Total</th>
<th>p value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DR plus</td>
<td>39</td>
<td>3</td>
<td>42</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>HLA-DR minus</td>
<td>3</td>
<td>39</td>
<td>42</td>
<td></td>
</tr>
<tr>
<td>Normal patients:</td>
<td>16</td>
<td>16</td>
<td>32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Inflamed</td>
<td>0</td>
<td>16</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Not inflamed</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ulcerative colitis:</td>
<td>14</td>
<td>14</td>
<td>28</td>
<td>&lt;0.0001</td>
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<tr>
<td>Inflamed</td>
<td>2</td>
<td>12</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Not inflamed</td>
<td>26</td>
<td>14</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Crohn's disease:</td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>NS</td>
</tr>
<tr>
<td>Inflamed</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Not inflamed</td>
<td>1</td>
<td>7</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Radiation injury:</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>NS</td>
</tr>
<tr>
<td>Inflamed</td>
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<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Not inflamed</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td></td>
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<tr>
<td>Infectious colitis:</td>
<td>&lt;0.01</td>
<td>&lt;0.01</td>
<td>9</td>
<td></td>
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<tr>
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</tr>
<tr>
<td>Not inflamed</td>
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<td>1</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Tests the hypothesis that expression of HLA-DR in colonic epithelial cells was related to the presence of inflammation.
Fig. 1  Photomicrographs of colonic mucosa: immunoperoxidase stains of frozen sections using monoclonal antibodies to β chain of HLA-DR antigen (haematoxylin counterstain.)

(a) Normal colonic mucosa showing DR positive lamina propria cells and DR negative epithelial cells in both crypt and surface areas. × 90.

(b) Mucosa from patient with active ulcerative colitis showing strong stain reaction (DR positive) in epithelial cells. Mucus is DR negative, as are epithelial cell nuclei (not well seen in black and white photographs). Lamina propria cells are DR positive. × 90.

(c) Mucosa from patient with Salmonella colitis showing DR positive epithelial cells both in crypts and surface areas. × 90.
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INFLAMMATORY BOWEL DISEASE (fig 1b)
Fifty four biopsy specimens were obtained from patients with ulcerative colitis (n = 43) and Crohn's disease (n = 11). Thirty two were from inflamed mucosa, and 22 were from non-inflamed mucosa. There was a strong correlation between expression of HLA-DR antigen in epithelial cells and the presence of inflammation in the lamina propria (p < 0.001) (table 1). The immunoprecipitate in HLA-DR positive cells was diffusely distributed in the cytoplasm of crypt and surface cells, as well as in mononuclear cells, macrophages, and endothelium. The staining reaction was more intense when the first layer monoclonal antibody CR3/43 was used, compared with the staining of the Tu 35 antibody.

Among the 32 inflamed biopsy specimens were 25 with both mononuclear and polymorphonuclear cell infiltrates in the lamina propria; epithelial cells were HLA-DR positive in 24 of 25. Seven inflamed specimens had only a mononuclear cell infiltrate; epithelial cells were HLA-DR positive in six of seven (table 2).

Among the 22 non-inflamed biopsy specimens from patients with ulcerative colitis and Crohn's disease were 14 with crypt atrophy or distortion, and eight with histologically normal epithelium; epithelial cells in 19 of 22 were HLA-DR negative. Two patients with ulcerative colitis who were colonoscoped had similar patterns: HLA-DR positive epithelial cells in the inflamed left colon but HLA-DR negative epithelial cells in the non-inflamed mucosa proximally.

RADIATION INJURY
One patient with active inflammation had HLA-DR negative epithelial cells, as did three patients with telangiectasia of the rectal mucosa.

INFECTION COLITIS (fig 1c)
Nine patients with infection causing inflammation of the rectal mucosa had HLA-DR positive epithelial cells. One patient with Clostridium difficile diarrhoea had shedding of faecal polymorphonuclear leucocytes but normal rectal mucosa: her epithelial cells were HLA-DR negative and the rectal biopsy specimen was histologically normal.

Discussion
This study confirms the observation that colonic epithelial cells are normally HLA-DR negative but become HLA-DR positive in patients with active inflammatory bowel disease. The synthesis of these glycoproteins, however, is a non-specific response to inflammation: epithelial cells were HLA-DR positive in the inflamed colon, independent of the underlying disease process (ulcerative colitis, Crohn's disease, or infectious colitis). Epithelial cells from patients with long standing ulcerative colitis were HLA-DR negative in atrophic but non-inflamed mucosa, even in patients with active inflammation elsewhere in the colon.

These observations are consistent with findings in inflammatory conditions in other organs. In normal skin only Langerhans' cells are HLA-DR positive, but inflammatory diseases such as contact hypersensitivity and graft versus host disease are associated with expression of DR antigens in keratinocytes in the epidermis. Normal human endocrine cells do not express DR antigens on their surface but can be stimulated by mitogens to synthesise these glycoproteins. Expression of HLA-DR by thyroid endocrine cells is one of the earlier manifestations of autoimmune lymphocytic thyroiditis. Brain cells (oligodendrocytes, astrocytes, and some neurones) can be induced to express class II antigens by exposure to T lymphocytes or to the lymphokine γ-interferon. Renal tubule epithelial cells synthesise class II antigens in response to a nearby graft versus host reaction. Bile duct epithelial cells are HLA-DR positive in primary biliary cirrhosis. In experimental animals intestinal epithelial cells express immune associated antigens in response to graft versus host disease and parasite infection. In man HLA-DR positive epithelial cells have been found in crypt cells adjacent to adenocarcinomas of the colon but not elsewhere in the colon. Epithelial cells seem to synthesise class II antigen glycoprotein, rather than acquire it from neighbouring antigen positive macrophages.

Experiments in a chimaeric rat model of graft versus host disease have shown that immune associated antigens in host epithelial cells were of the host phenotype, rather than being derived from donor mononuclear cells that had repopulated the lamina propria. Normal human colon epithelial cells are HLA-DR negative despite their proximity to HLA-DR positive cells in the lamina propria, suggesting that mere proximity to macrophages does not trigger
DR glycoprotein synthesis.

Class II antigen synthesis by other cells, however, can be induced by both lymphocyte suspensions and by lymphokinins. Activated T cells show enhanced expression of class II antigens, an effect mediated by γ-interferon.9-11 Gamma-interferon has also been shown to induce class II antigen synthesis by peripheral blood monocytes,12 macrophages,9,30,33 epidermal Langerhans' cells,34 and brain cells.10 In the present study expression of HLA-DR by epithelial cells was possibly mediated by lamina propria lymphocytes, as increased lymphoid cell infiltrates were common to all biopsy specimens containing DR positive epithelial cells (table 2). This hypothesis is consistent with the findings of recent studies showing that intraepithelial lymphocytes induce class II antigen synthesis by cultured intestinal epithelial cells.35,36

The following sequence has been proposed to explain continued T lymphocyte activity in autoimmune diseases: a local stimulus causes lymphokine release which induces HLA-DR expression—which presents autoantigen to T lymphocytes—which activate effector B and T lymphocytes.13 Autoimmune disease would then develop if the response of suppressor T lymphocytes were inappropriate.12 The initial steps of this sequence seem to occur both in idiopathic and infectious colitis, but the self-limited nature of infectious colitis suggests that either the DR antigen in epithelial cells is not biologically active or available to lamina propria cells, or that there is an appropriate suppressor T cell response that lessens any stimulatory reaction. Conversely, a defective suppressor T cell response could permit an amplified immune response in which HLA-DR presenting epithelial cells play a key part. Against this hypothesis are observations that DR antigen is diffusely present in the cytoplasm of epithelial cells, rather than on the surface, and that the normal small bowel has HLA-DR positive epithelial cells in the absence of chronic inflammatory "disease."3,14,36 Furthermore, there is little evidence that suppressor activity is defective within the mucosa. Studies using isolated mucosal mononuclear cells have shown normal Con-A-induced suppressor activity.37 The same authors also found that the addition of intestinal to peripheral blood mononuclear cells did not suppress the response of these peripheral blood cells to phytohaemagglutinin; in fact, the response was enhanced.38 A contrasting study found that intestinal cells impaired the response of peripheral blood cells to phytohaemagglutinin; a mechanical method, however, was used for isolating cells.39 The "covert" suppressor cells reported to be present in peripheral blood40,41 are apparently absent in the mucosal population of mononuclear cells.42

There is evidence of polymorphism among cells that express class II antigens,36,42 suggesting that not all class II antigens are equally capable of stimulating an immune response. Recent experiments have shown successful transplantation of epithelial tissue bearing class II antigens across major histocompatibility barriers.43 This suggests that immunogenicity is not solely a function of antigen expression but is influenced by the type of cell in which antigens are expressed.44

In summary, colonic epithelial expression of class II antigens is a non-specific response to inflammation, the biological importance of which is unknown.

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References
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