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Relation between T cell number and epithelial HLA class II expression quantified by image analysis in normal and inflamed human gastric mucosa

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Abstract

Epithelial expression of HLA class II determinants and the number of lamina propria and intraepithelial T cells were quantified in gastric body mucosa by means of paired immunofluorescence staining which was subjected to computerised image analysis. In normal mucosa, epithelial HLA-DR expression was virtually absent. A significantly increased expression was seen in simple chronic gastritis, most extensively in the isthmus zone, where a positive reaction was seen in 34% of the epithelial area when the gastritis was of low degree and in 85% when it was of moderate severity. The most extensive HLA-DR expression was found in moderate 'stump gastritis' 28 to 32 years after Billroth II resections. In these patients the epithelial area in the foveolar and isthmus zones showed 83% and 92% positive responses, respectively. The HLA subregion products were expressed in a differential manner (DR>DP>DQ). The number of both intraepithelial and lamina propria T cells increased significantly with increasing severity of gastritis, and the fraction of putative memory T cells was also raised. Correlation analyses showed a positive relation between the epithelial expression of HLA-DR and the intraepithelial as well as the lamina propria density of T cells. These results suggest a biological link between T cells, aberrant HLA-DR expression, and gastritis, although the pathogenic importance of this relation is unknown. Enhanced epithelial presentation of autoantigens or luminal antigens, or both leading to increased activation of T cells is one possible explanation.

The major histocompatibility complex class II molecules are crucial restriction elements in the regulation of immune responses to T cell dependent antigens. Several variants of these cell surface glycoproteins have been described in humans, representing different products (HLA-DR, -DP, and -DQ) encoded by gene loci within the class II region on chromosone 6.[1] These molecules are expressed on different cell types including B lymphocytes, activated T lymphocytes, some macrophages,[2–4] and various dendritic antigen-presenting cells such as the epidermal Langerhans' cells.[5 6] Antigen presentation to CD4+ T lymphocytes ('helper/inducer' phenotype) is directly dependent on the class II molecules.[1]

Major histocompatibility complex class II expression can also be seen in cells without any clearly established immunological function, like capillary endothelium[7] and various epithelia.[8] HLA-DR determinants have been shown in normal human intestinal villous epithelium,[9] jejunal crypt epithelium in coeliac disease,[10] and colonic epithelium in ulcerative colitis and Crohn's disease.[11 12] The term 'aberrant' has been used to describe disease associated major histocompatibility complex class II expression by cells beyond the traditional immune system.[13]

In the human stomach enhanced epithelial expression of HLA-DR has been noted in gastritis.[14 15] The biological importance of this observation is obscure. Activated T cells and macrophages probably play an important role in the modulation of major histocompatibility complex class II expression via cytokines such as interferon γ and tumour necrosis factor α.[13 16–19] This immunohistochemical study was performed to evaluate more closely class II expression in human gastric epithelium and its relation to inflammation and T cells. Two colour immunofluorescence was used for co-staining of class II determinants (or T cells) and epithelium, which was delineated with keratin as a marker. The observations were recorded by computerised image analysis. To our knowledge this is the first quantitative study of major histocompatibility complex class II expression in tissue sections.

Methods

SAMPLING, CATEGORIES OF SPECIMENS, AND TISSUE PREPARATION

Selected samples of gastric body mucosa were obtained from kidney donors maintained on artificial respiration (histologically normal specimen group) and from patients subjected to Billroth II resections for duodenal or gastric ulcer (simple gastritis group). The gross mucosal specimens were collected in ice-cold 0·01 M phosphate buffer (pH 7·6) containing 0·15 M saline (PBS), and were brought to the laboratory within one hour. Small slices (about 5×5 mm) were then excised from macroscopically normal mucosa. In addition, biopsy specimens were obtained endoscopically from patients with duodenal ulcer subjected to Billroth II resections 28 to 32 years earlier (Billroth II group). Histopathological grading of gastritis according to Rao *et al*,[20] was used to define the following specimen categories: *normal*, no apparent increase in inflammatory cells; *mild simple gastritis*, grade 1 gastritis with a mild degree of inflammatory cell infiltration, mostly superficial; and *moderate simple gastritis*, grade 2 gastritis with moderate inflammation. All specimens from the Billroth II

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group showed moderate gastritis. The specimens were fixed directly in cold 96% ethanol for 20 hours at 4°C before being embedded in paraffin.[21]

STAINING PROCEDURES AND IMMUNOLOGICAL REAGENTS

Serial tissue sections were cut at 6 μm perpendicular to the mucosal surface. One section was used for conventional histological staining and two were subjected to two colour immunohistochemistry. A primary rabbit antiserum to keratin was used to visualise the gastric epithelium.[22] This antiserum (1:100) was mixed with a murine monoclonal antibody to the pan-T cell Cluster of Differentiation No 3 or CD3 (1:20, anti-Leu-4) or to a non-polymorphic HLA-DR determinant (1:20; both from Becton-Dickinson, Mountain View, CA, USA). Three serial sections of selected specimens were in addition stained for HLA-DP (1:20; Becton-Dickinson), HLA-DQ (anti-Leu-10, 1:20; Becton-Dickinson), and the UCHL1 (CD45R0) determinant (1:160; supernatant was kindly provided by Professor P G Isaacson, University College, London School of Medicine, London, UK), which is expressed on putative antigen-primed CD4+ and CD8+ T cells.[23] Each monoclonal antibody was combined with the antiserum to keratin as above.

After the primary antibody incubation (20 hours), biotinylated horse antimouse IgG (Vector Laboratories, Burlingane, CA, USA) extensively absorbed with insolubilised human serum, was applied (0·05 g IgG/l; three hours) followed by a mixture of fluorescein isothiocyanate (FITC)-labelled avidin (Vector; 0·025 g IgG/l) and tetramethylrhodamine isothiocyanate (TRITC)-labelled swine antirabbit IgG for 30 minutes.[24] All incubations took place at room temperature. The sections were finally mounted in buffered (pH 8) polyvinyl alcohol.[25]

Standard indirect immunofluorescence test on cryostat sections of rat tissue was used for detection of serum autoantibodies to gastric parietal cell antigen.

MICROSCOPY, COMPUTERISED IMAGE ANALYSIS, AND EVALUATION OF RESULTS

Immunofluorescent sections were examined in a Nikon Microphot microscope equipped with an epi-illuminator and a 100 W mercury lamp. A B2E filter block was used for evaluation of FITC emission, and a G2 filter block was used for TRITC. The microscope was attached to a high sensitivity videocamera (JAI 733 SIT, JAI, Copenhagen, Denmark). Computerised image analysis was performed with a Magiscan image analysing system (Joyce-Loebl, Gateshead, UK) employing the Genias general image analysis program. Grey images of TRITC and FITC emission from the same area were sequentially digitised. The epithelial region was defined by the keratin staining and interactively partitioned into foveolar and isthmus zones. Using this image as an overlay, the percentage positive area and fluorescence intensity of HLA-DR staining were determined in the relevant grey image. The keratin image was also used as an overlay to align the next serial section, which was co-stained for keratin and CD3+ T lymphocytes. After digitising the two corresponding grey images, the epithelium and lamina propria were interactively segregated and fluorescent T cells counted within these areas. In every section, five random fields, each 25 600 μm^2, were measured at ×1250 magnification. The HLA-DR positive areas were expressed as fractions of total epithelium – areas less than 20 μm^2 being excluded by the computer. Fluorescence intensities were expressed as weighed mean intensities of positive areas. The number of T cells was given per mm^2 epithelium or lamina propria. Results were calculated as median and 95% confidence interval or observed range for each specimen.

In selected serial sections from 12 mucosal specimens, HLA-DP and HLA-DQ were recorded in addition to HLA-DR; two corresponding epithelial areas were evaluated for percentage positive staining of each of the three markers.

Reproducibility for calculation of total area and fluorescent fraction was tested: (a) by measuring the same epithelial area (keratin positive) 15 times (coefficient of variation, 1·9%) and (b) by calculating the FITC fluorescent fraction of the same area 15 times (coefficient of variation, 7·9%). For the latter test some of the variation was due to fading on repeated photography.

Differences between groups were evaluated by Wilcoxon's test for unpaired samples (two tailed). The relation between T cells and HLA-DR expression was evaluated by (Pearson's) correlation analysis.

Results

GENERAL OBSERVATIONS

Measurements of epithelial HLA class II expression and enumeration of T cells were performed by the same investigator without knowledge of the category of the actual mucosal specimen. Co-staining for keratin delineated clearly the epithelium in the foveolar and isthmus zones. The basal parts of the gastric glands were visualised somewhat more weakly but their identification was nevertheless easy. The evaluated epithelial area (median) per field was 3229 μm^2 and 5521 μm^2 for the foveolar and isthmus zones respectively (ranges, 1052–7624 μm^2 and 2177–9694 μm^2). The evaluated lamina propria area per field was 6238 μm^2 (range, 1824–18 010 μm^2). When HLA-DR, HLA-DP, and HLA-DQ expression were quantified in corresponding fields from serial sections, the median epithelial area (isthmus zone) was 9930 μm^2; the median coefficient of variation was ±6·6% (range, 1·7–18·4%), which attested to a satisfactory method reproducibility. Lymphocytes, histocytes, and capillary endothelium were stained strongly for HLA-DR and more variably for HLA-DP and HLA-DQ in the lamina propria. These elements were not evaluated.

Staining of mononuclear cells was generally stronger with anti-UCHL1 than with anti-CD3.

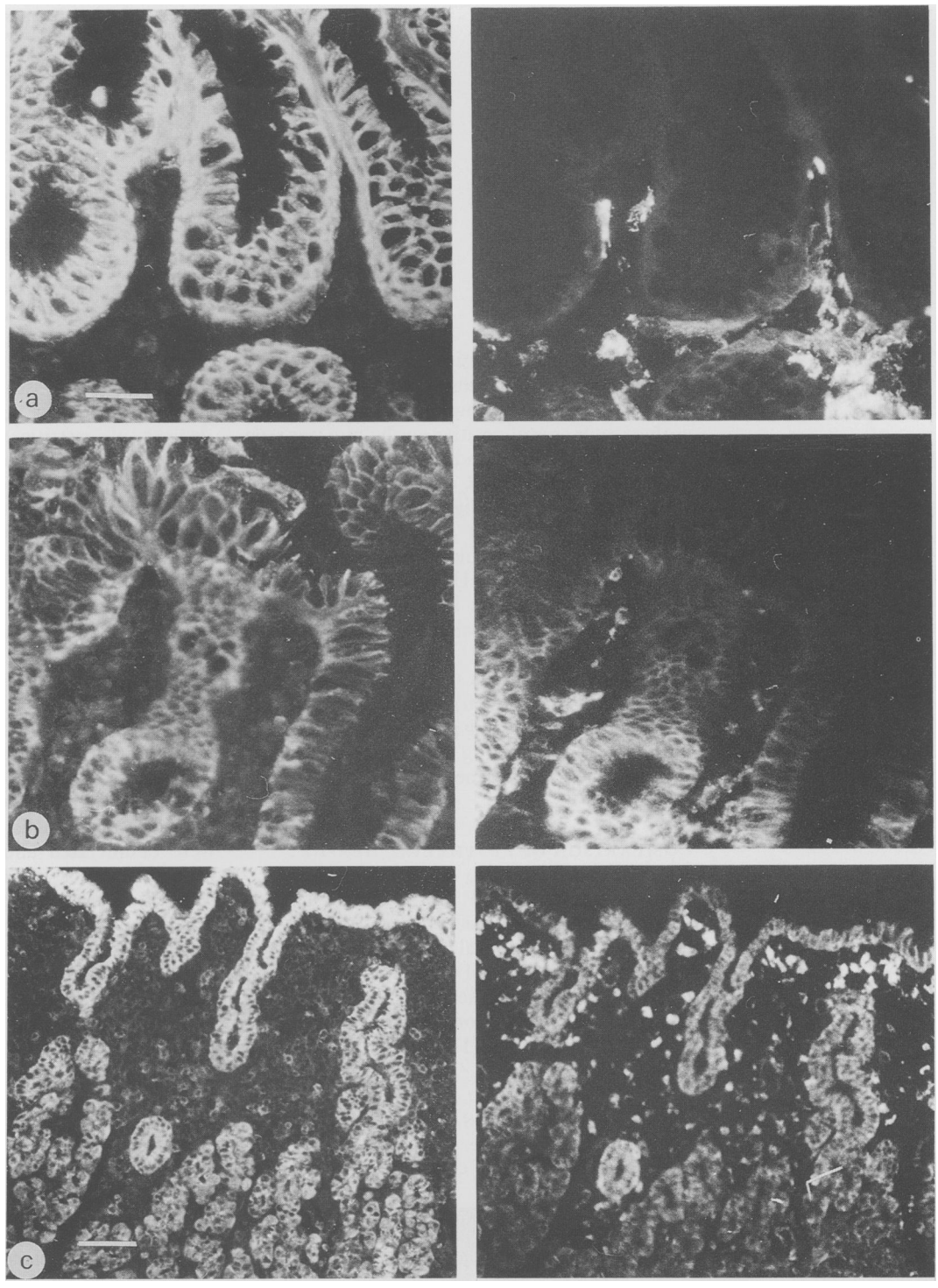

Figure 1: Immunofluorescence staining for keratin (rhodamine, left panels) and HLA-DR (fluorescein, right panels) in the same field. (a) Normal gastric mucosa shows HLA-DR negative epithelium but there are various positive tissue elements in the lamina propria. (b) Typical HLA-DR staining in mild gastritis with most intense expression in the isthmus zone, whereas the foveolar epithelium is negative. (c) Moderate gastritis showing HLA-DR positivity also in foveolar and surface epithelium. Various types of HLA-DR positive cells and surface vascular epithelium are present in lamina propria, and there is some faint non-specific background staining with the antiserum to keratin. Bars=30 μm (a, b) and 70 μm (c).

However, as the former antibody detects only a fraction of T cells – presumably an antigen-primed memory population[23] – and in addition decorates cells of the myelomonocytic series in the lamina propria, enumeration subjected to image analysis was based on the CD3 market. Its staining intensity varied somewhat but the low non-specific background made evaluation simple. Intraepithelial T cells were, in addition, directly counted in the microscope after staining with the UCHL1 antibody for comparison with CD3 in adjacent sections. Antibodies to the CD4 and CD8 could not be used because these markers are destroyed by the applied tissue preparation method.

Serum antibodies to gastric parietal cells were not detected in any of the patients.

EPITHELIAL HLA-DR EXPRESSION

In normal gastric body mucosa, epithelial HLA-DR staining was generally absent (Fig 1a),

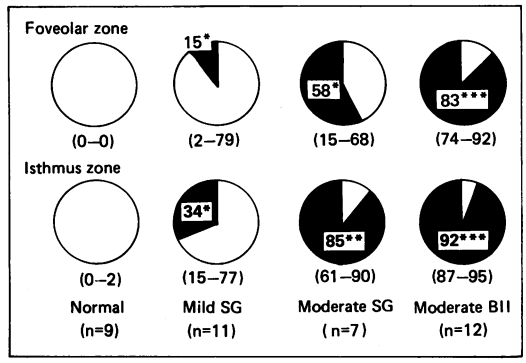

Figure 2: Pie charts of epithelial HLA-DR expression (median and 95% confidence interval of percentage positive area) in normal gastric mucosa; in mild and moderate simple gastritis (SG); and in moderate gastritis after Billroth II resection (BII). Significantly increased values compared with one, two, or three of the other specimen groups are indicated (, **, ***), n=number of specimens.*

although quite faint positivity was occasionally seen in the isthmus zone (Fig 2). With the increasing degree of simple gastritis, a significantly greater area of positive epithelium was seen – both in the foveolar and isthmus zones (Fig 2). The most extensive DR expression occurred in the Billroth II group (Fig 2); the mean fluorescence intensity was, in addition, significantly ($p<0.05$) increased in this group.

Intense epithelial HLA-DR staining was generally seen apically in a granular pattern. With increased expression, staining was also seen basolaterally and throughout the cytoplasm. Expression was usually pronounced in the isthmus zone (Fig 1b), but with an increased degree of gastritis strong positivity also occurred in the foveolar zone (Fig 1c). The basal parts of the glands were always negative.

COMPARISON OF EPITHELIAL HLA-DR, HLA-DP, AND HLA-DQ EXPRESSION

The distribution of DP and DQ determinants was principally the same as that of DR. When corresponding epithelial areas (isthmus zone) were evaluated in serial sections, however, the expression was significantly ($p<0.01$) more extensive for DR than for DP, and DP was more widely distributed than DQ (Table I).

INTRAEPITHELIAL AND LAMINA PROPRIA T CELLS

Intraepithelial CD3+ T cells were rare in normal gastric mucosa and were usually seen basally. Concurrent with the increased epithelial HLA-DR expression seen in gastritis, there was a greater number of T cells in the lamina propria, particularly in the isthmus zone (Fig 3). Lamina propria T cells were significantly increased in all degrees of gastritis with no difference seen between the three groups (Fig 4). The number of intraepithelial T cells was significantly raised both in moderate simple gastritis and Billroth II gastritis compared with mild simple gastritis and normal mucosa (Fig 4). The T cells tended to be located throughout the epithelial lining in gastritis.

When all mucosal specimens were considered together, there was a positive correlation between the density of lamina propria T cells and epithelial HLA-DR expression in the isthmus zone (Fig 5). A similar significant ($p<0.001$) relation was found between the intraepithelial T cell density and HLA-DR staining, both in the foveolar ($r=0.51$) and the isthmus (Fig 5) zones.

When intrapithelial UCHL1+ lymphocytes were enumerated and compared with CD3+ cells in the corresponding epithelial areas of adjacent sections, the results suggested that the fraction of antigen-primed T cells depended on the degree of gastritis; thus, the percentage UCHL1+ cells was 41% in mild simple gastritis and 60% in moderate simple gastritis and Billroth II gastritis (Table II).

TABLE I *Epithelial expression of HLA-DR, -DP, and -DQ in the gastric body isthmus zone**

Percentage epithelial area showing positive staining (median and 95% confidence interval)

HLA-DR	*HLA-DP*	*HLA-DQ*
90 (76–94)	39 (27–69)**	2·5 (0–22)***

*In two comparable fields from serial sections of 12 selected mucosal specimens of mild (n=2) and moderate (n=10) gastritis.
**Significantly less than HLA-DR.
***Significantly less than both other HLA determinants.

Discussion

Epithelial HLA class II expression was virtually absent in normal gastric body mucosa but was strikingly upregulated in a differential manner

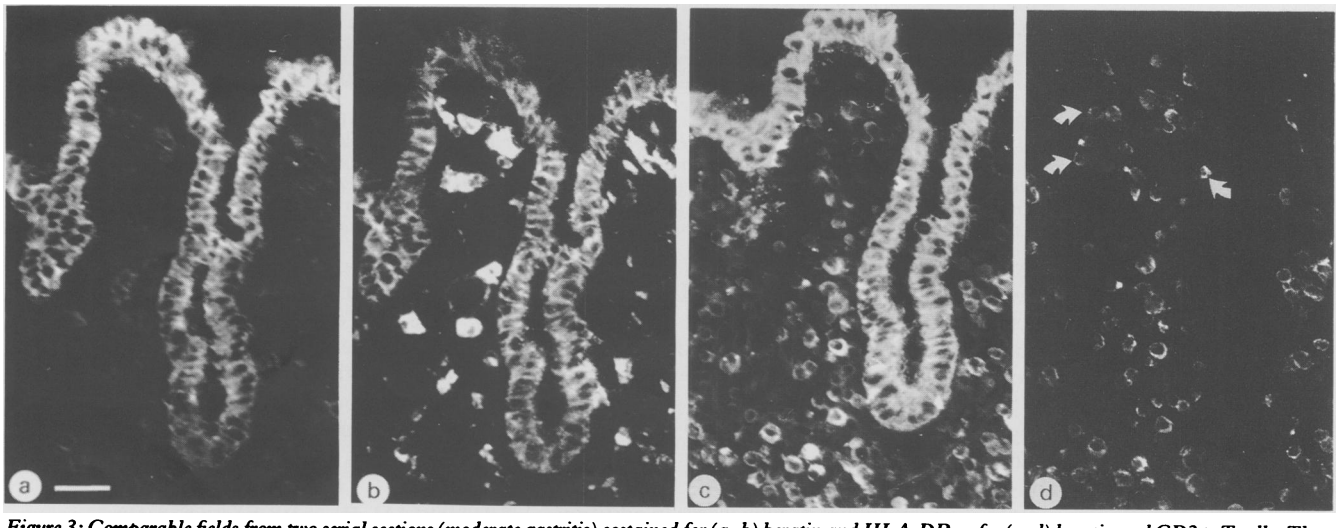

Figure 3: Comparable fields from two serial sections (moderate gastritis) costained for (a, b) keratin and HLA-DR or for (c, d) keratin and CD3+ T cells. The gastric epithelium (decorated by rhodamine fluorescence for keratin in a) shows intense staining for HLA-DR (fluorescein in b). A dense infiltrate of T cells (fluorescein) is found adjacent to the epithelium (rhodamine) (c; double exposed). Intraepithelial T cells (fluorescein) (arrows) are seen in d. Bar=70 μm.

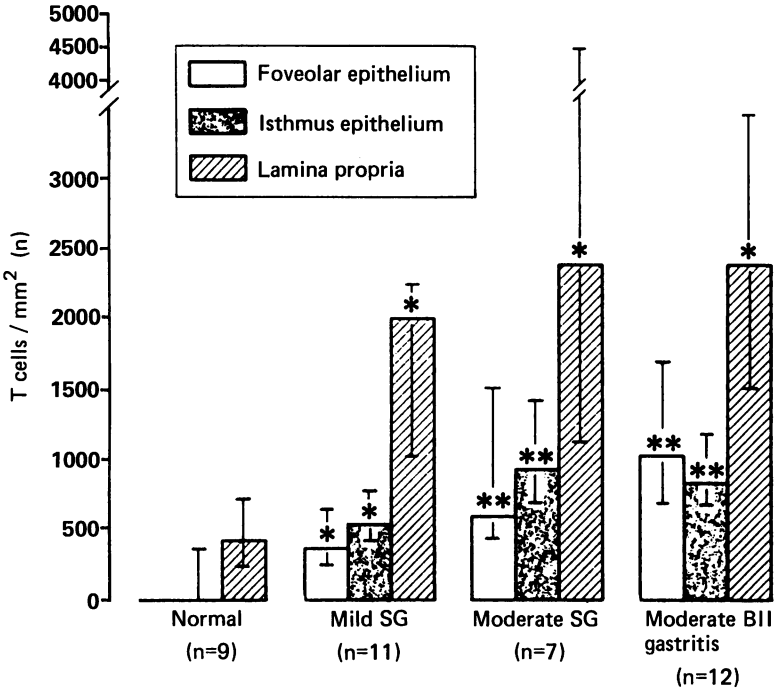

*Figure 4: Number of intraepithelially (foveolar and isthmus zones) and lamina propria CD3+ T cells (median and 95% confidence interval) in normal body mucosa; in mild and moderate simple gastritis (SG); and in moderate gastritis after Billoth II resections. Significantly increased values compared with one * or two ** of the other groups are indicated.*

(DR>DP>DQ) with increasing degrees of gastritis. This result is in agreement with our preliminary observations[14] and with the report of Spencer *et al.*[15] Similar upregulation has also been reported in inflammatory bowel disease[11 12 26 27] and in untreated coeliac disease.[10 28-30] Intestinal villous epithelial cells in newborn rats[31] and human fetuses[32] are major histocompatibility complex class II negative, or only slightly positive.[33] Immunological stimuli to which the gastrointestinal mucosa is exposed therefore seem to induce epithelial class II expression, most likely via various cytokines.[16-19 34]

When HLA class II expression is elicited by interferon γ in the colonic carcinoma cell line HT-29,there is concomitantly increased synthesis of secretory component,[35] which is the epithelial receptor protein for polymeric immunoglobulins.[14] Also tumour necrosis factor α can upregulate both HLA-DR and secretory component in the same cell line.[19 34] Another

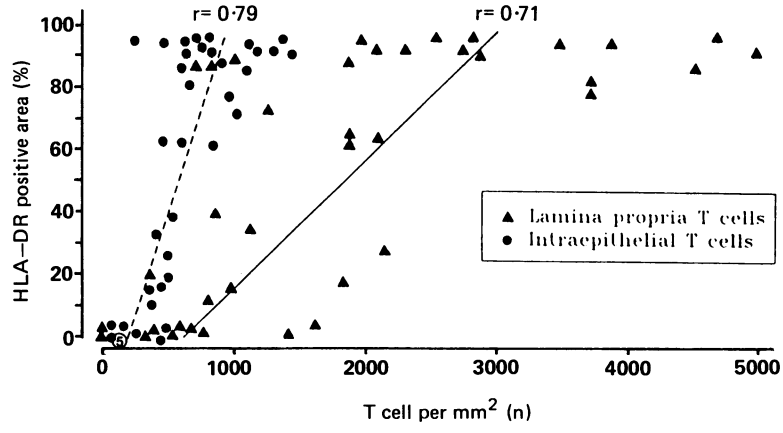

Figure 5: Relation between epithelial HLA-DR expression and the number of intraepithelial and lamina propria CD3+ T cells in the isthmus zone of gastric body mucosa (n=39), including both normal and inflamed specimens (five negative points encircled).

TABLE II *Fraction of putative antigen primed (UCHL1+) intraepithelial CD3+ T cells in gastric body mucosa**

Specimen category	*Percentage UCHL1+ T cells*
Mild simple gastritis (n=10)	41 (31-59)†
Moderate simple and BII gastritis (n=15)	60 (43-86)‡

*Counted in random comparable fields (×400) of adjacent sections (median 85 observed T cells).
†Median and observed range.
‡Significantly increased ($p<0.01$).

observation of increased co-expression was recently made in inflammatory bowel disease. Epithelial staining for HLA-DR in colonic lesions was often accompanied by positivity for the leucocyte marker 4F2 and the transferrin receptor.[36] We have previously reported gastritis-related increase of epithelial secretory component, lactoferrin, and lysozyme.[37] Taken together, our observations suggest that cytokine-induced epithelial class II expression is not a selective biological phenomenon.

Intraepithelial lymphocytes isolated from the rat gut have been shown to enhance epithelial class II expression.[38] Moreover, we have previously noted a positive relation between differential expression of epithelial HLA class II molecules (DR>DP>DQ) in coeliac disease and the number of intraepithelial UHCL1+ T lymphocytes.[30] In a recent study, isolated T lymphocytes from the lamina propria of inflammatory bowel disease lesions showed increased expression of activation markers (transferrin receptor and interleukin 2 receptor or CD25) when the epithelium was positive for HLA-DR.[36] This agrees with our present finding of a significant relation between the number of both lamina propria and intraepithelial T cells in gastric mucosa and epithelial HLA-DR expression. Also, we found that a substantial fraction of the intraepithelial T lymphocytes were UCHL1+, suggesting that they were memory cells with enhanced interferon γ secretion.[23]

It seems justified to propose that epithelial HLA class II expression in gastritis is induced by cytokines mainly derived from antigen primed T cells. It may be too simplistic to consider this aberrant expression merely an epiphenomenon of no pathogenic importance. As reviewed elsewhere,[39] considerable indirect evidence suggests that lymphoepithelial interactions normally contribute to downregulation of systemic types of mucosal hypersensitivity reactions. When HLA class II expression is intensified, however, antigen presentation by gut epithelium may preferentially lead to stimulation of CD4+ helper cells.[40] In addition to luminal antigens, autoantigens may be presented by HLA class II positive epithelial cells.[13] The result may be local overstimulation of the B cell system, particularly induction of exaggerated mucosal IgG responses which are seen both in coeliac disease, inflammatory bowel disease,[14] and chronic gastritis.[41] Because IgG antibodies can activate the complement system and arm cytotoxic cells, aberrant local immune reactions may contribute appreciably to mucosal damage and perpetuation of inflammation.

Although further work is needed to document the biological relevance of class II positive

epithelium in the development of chronic gastritis, our results support the notion that immunological mechanisms play a part in the initiation and perpetuation of this disease.

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