Immunohistological characterisation of intraepithelial lymphocytes of the human gastrointestinal tract

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SUMMARY Intraepithelial lymphocytes (IEL) of the normal human stomach, small intestine, and large intestine have been characterised in tissue sections by a double marker immunofluorescent technique. A panel of reagents was used in combination, including antisera to T lymphocyte antigen (HuTLA), Ia-like (p28, 33) antigens and immunoglobulin subclasses, as well as a mouse monoclonal antibody to a human leucocyte antigen (HLe-1). In stomach and proximal small intestine over 95% of IEL were T lymphocytes (HLe-1+, HuTLA+). The proportion was slightly lower in the colon and rectum (85–95%). IEL rarely expressed Ia-like antigens. B lymphocytes were not seen within the epithelium of any of the tissues examined. The functions of IEL must be assessed in the light of the finding that they are predominantly T cells.

This population of cells, and the nature of the non-T population was not elucidated.

In view of the conflicting data in man, and the lack of detailed study of the IEL population of the human gastrointestinal tract, we have characterised these cells in tissue sections of normal stomach, small bowel, colon, and rectum. The study uses immunofluorescent techniques with specific antisera to T lymphocytes, immunoglobulin classes, and Ia-like antigens as well as a monoclonal antibody to human leucocytes. The antisera were used in various combinations in double immunofluorescence tests with different fluorochromes (green—FITC, and red—TRITC).

METHODS

SUBJECTS

Gastric and duodenal biopsies were obtained at upper gastrointestinal endoscopy in patients undergoing investigation for abdominal pain or diarrhoea. Jejunal specimens were obtained by Crosby capsule biopsy from normal volunteers or patients with diarrhoea in whom coeliac disease was being excluded. Specimens of ileum and colon were taken at laparotomy for colonic carcinoma or at endoscopic follow-up of colonic polyps or cancer. Rectal biopsies were obtained at sigmoidoscopy in patients with haemorrhoids, rectal polyps, or irritable colon syndrome, in all cases, endoscopic findings were normal. In addition, the histological appearances of

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formalin-fixed and frozen sections of each biopsy were normal by light microscopy.

**TISSUE HANDLING**

As most of the biopsies were endoscopic, they were small, and careful handling was essential to preserve epithelial integrity and morphology. Immediately after the specimen was obtained it was orientated, placed on a piece of cork, and covered with OCT compound (Ames Co.). The tissue was then snap frozen by placing it in a beaker of isopentane suspended over liquid nitrogen. The specimen, mounted on cork and correctly orientated for sectioning, was stored in liquid nitrogen until used.

**IMMUNOFLUORESCENT TECHNIQUES**

Five micron frozen sections were cut, mounted onto glass slides, and dried in air for one hour. They were fixed for 10 minutes in absolute alcohol at 4°C, and then washed in phosphate buffered saline, pH 7.2 (PBS), before incubation with antisera. Both indirect and direct immunofluorescent methods were used. After fixation and washing of the sections, the relevant antisera were added, and incubated at room temperature for 30 minutes. Sections were then washed in PBS for 30 minutes. In the indirect technique, secondary layer antisera were next added, incubated for 30 minutes, and followed by a final wash for the same period. Slides were mounted in glycerol: PBS mixture, covered with a cover slip and sealed with nail varnish.

**ANTISERA**

**Unconjugated reagents**

Mouse monoclonal anti-human leucocyte antibody (2D1, reacting with HLe-1 antigen) was kindly provided by Dr P Beverley. This antibody was produced by the mouse-myeloma hybridisation technique. It reacts with all T and B lymphocytes in peripheral blood and tissues and with over 99% of peripheral blood lymphocytes from normal subjects and from patients with inflammatory bowel disease or coeliac disease (Selby et al., unpublished data). It reacts also with thymocytes, and weakly with some monocytes and mature myeloid cells. Epithelial cells are unreactive. 2D1, a culture supernatant, was used neat. The control was another mouse monoclonal antibody, NA1/34, which is specific for cortical thymocytes. This produced no staining of gut biopsies.

Rabbit antiserum reacting with human T cells and thymocytes (R-anti-HuTLA) was made against monkey thymocytes and absorbed with human liver, red cells, leukaemic B and myeloid cell lines, and bone marrow. This antiserum reacts with peripheral T lymphocytes, cortical and medullary thymocytes, and stains the thymus-dependent areas of human tonsil. It does not react with B lymphocytes, non-T, non-B (null) lymphocytes, myeloid cells, monocytes, or epithelial tissues. R-anti-HuTLA was used in a dilution of 1 in 5. Normal rabbit gamma-globulin used as a control gave no T cell staining.

Chicken antiserum to Ia-like antigens was made against purified human p28, 33 antigens. It reacts strongly with B lymphocytes, myeloblasts, some monocytes, tissue macrophages, and with a small population of peripheral T lymphocytes. It is unreactive with thymocytes, mature myeloid cells, platelets, or erythrocytes. It was used at 1 in 40 dilution. Specificity in tissue sections was confirmed by absorption of activity with B-CLL cells. A rat monoclonal antibody to Ia-like antigens gave an identical pattern of fluorescence to the chicken anti-Ia in tissue sections. Normal chicken serum was used as a negative control.

**CONJUGATED REAGENTS**

Goat antiserum to human IgA, coupled with tetramethylrhodamine isothiocyanate (TRITC) was obtained from Kallestad, Minnesota, and used at 1 in 20 dilution. Sheep anti-human IgM conjugated with fluorescein isothiocyanate (FITC) and sheep anti-human IgG-FITC (Wellcome Research Laboratories) were used at 1 in 20 and 1 in 40 respectively. Several sections were also stained with goat anti-human IgD-FITC (Kallestad) and with anti-human Kappa light chain FITC and anti-human Lambda light chain TRITC (Dako Labs). In addition, some sections were stained to detect IgE using sheep anti-human IgE (Cappell) and rabbit anti-sheep FITC (Wellcome). Controls for the anti-immunoglobulin antisera were performed as outlined by Nairn.

Swine anti-rabbit TRITC (Dako) was used at a dilution of 1 in 20 as a second layer for R anti-HuTLA. Goat anti-mouse IgG FITC (Nordic, 1 in 10) was used with mouse monoclonal 2D1 antibody. Sheep anti-chicken TRITC, (Royal Free; 1 in 20) or sheep anti-chicken FITC, (Royal Free; 1 in 10) was the second layer for chicken anti-Ia. These four conjugated antisera were absorbed with human liver homogenate before use.

**COMBINATIONS OF REAGENTS**

Selective filters for fluorescein (green) and rhodamine (red) enabled combinations of antisera to be used on the same section. This facilitated recognition of staining patterns and quantitation of antigen-positive cells. The main combinations used were: (1) 2D1 and R anti-HuTLA to ascertain the proportion of T cells in the intraepithelial lymphocyte population; (2) chicken anti-Ia and R anti-HuTLA to determine
Table  Antigenic determinants on intraepithelial lymphocytes (IEL) along the normal human gastrointestinal tract

<table>
<thead>
<tr>
<th>Tissue</th>
<th>No.*</th>
<th>% HLe-1+ IEL expressing HuTLA</th>
<th>% HuTLA+ IEL expressing Ia-like antigens</th>
<th>% HLe-1+ IEL expressing Ia-like antigens</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>4</td>
<td>99% (97-100)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Duodenum</td>
<td>3</td>
<td>97% (92-96)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Jejunum</td>
<td>4</td>
<td>99% (95-100)</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ileum</td>
<td>2</td>
<td>93% (89-100)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Total small intestine</td>
<td>9</td>
<td>97%</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>3</td>
<td>92% (82-96)</td>
<td>4 (1-9)</td>
<td>4 (1-13)</td>
</tr>
<tr>
<td>Descending colon</td>
<td>2</td>
<td>87% (84-94)</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Rectum</td>
<td>6</td>
<td>95% (85-98)</td>
<td>&lt;1</td>
<td>2</td>
</tr>
<tr>
<td>Total colon</td>
<td>11</td>
<td>92%</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

*Number of specimens examined.
†Mean, with range in parentheses. Results for surface and glandular intraepithelial lymphocytes are combined as findings were similar in these areas in all tissues.

whether intraepithelial T lymphocytes express Ia-like antigens; (3) 2D1 and chicken anti-Ia to identify the proportion of total IEL bearing Ia-like antigens.

MICROSCOPY
Sections were examined under a standard 14 Zeiss microscope equipped with a 40 phase oil objective and IV Fl epifluorescence condenser containing selective filters for FITC and TRITC. The entire epithelium of several sections was examined for IEL—usually 10 to 20 or more high-power fields. Photomicrographs were taken with Ilford HP5, ASA 400.

Serial frozen sections were cut for examination by light microscopy. They were air-dried as indicated above, fixed in Lillie's AAF for 30 seconds, and stained with haematoxylin and eosin (H and E). All sections were histologically normal.

Results
TISSUE MORPHOLOGY
Examination by light microscopy of frozen sections stained with H and E revealed satisfactory preservation of tissue morphology, in particular that of the epithelium. Using phase microscopy, the basement membrane of the epithelium was identified easily and the relationship of cells to the basement membrane could be determined.

INTRAEPITHELIAL LYMPHOCYTES
IEL were identified by their morphology and site under phase microscopy and by the expression of HLe-1 on the surface membrane. IEL were seen in stomach, small intestine, and colon, both in the surface epithelium and the epithelium of mucosal glands and crypts (Table).

Fig. 1  Jejunal villus stained for (a) HLe-1 and (b) HuTLA. Intraepithelial lymphocytes are indicated by arrows. They are predominantly HLe-1+ HuTLA+ T lymphocytes. (Basement membrane is indicated by the broken line). ×400.
Fig. 2  Section of ascending colon. (a) HLe-1: Intraepithelial lymphocytes (arrows) are present both in surface and glandular epithelium. (b) HuTLA: the IEL are T lymphocytes. (Basement membrane is indicated by the broken line). ×400.

*T lymphocytes*
Sections were double-labelled for HLe-1 (FITC) and HuTLA (TRITC). In the gastric body and antrum only small numbers of IEL were seen, but over 97% were HuTLA* (intraepithelial T lymphocytes—T IEL). In the small intestine IEL were more numerous and, on average, 97% were T lymphocytes (Fig. 1). The proportion of T IEL was slightly higher in the proximal small bowel (97–99%) than in the ileum (93%). T lymphocytes also constituted the major proportion of IEL in the colon (87–92%) and rectum (95% Fig. 2).

In each tissue studied, the proportion of T cells in the IEL population was similar in the epithelium of the villi or surface as in that of the crypts or glands (Fig. 2).

*Immunoglobulins (Ig)*
All sections were incubated with antisera to IgA, IgM, and IgG. Neither surface nor cytoplasmic Ig

Fig. 3  (a) Jejunal biopsy stained for HLe-1. IEL are shown by the small arrows. (b) The same section labelled for IgA. Abundant IgA-containing plasma cells are present in the lamina propria (large arrows). IgA is found on epithelial cells but the IEL are IgA-negative.
staining could be detected on IEL in any of the tissues. Immunoglobulin staining, particularly for IgA, seen on the surface of columnar epithelial cells sometimes made difficult the exclusion of surface immunoglobulin on IEL. However, this epithelial fluorescence was always much fainter than the staining of extracellular immunoglobulin or the labelling of B-cells or plasma cells in the lamina propria (Fig. 3). In addition, two gastric, three jejunal, and three large bowel specimens were examined with anti-HLe-1 and anti-IgA in double combination. All IEL were HLe-1+ IgA-. Several sections were stained with antibodies to IgD and IgE. No IEL were positive for these immunoglobulins and only occasional positive lamina propria cells were seen. IEL did not react with antibodies to human light chains.

In each tissue studied there was a small population of non-T, non-B IEL (HuTLA-, Ig-) ranging from 1% in the upper small bowel to 14% in the descending colon (mean 3% in the small bowel; 8% in the colon).

Ia-like antigens
Intraepithelial T lymphocytes along the gastrointestinal tract were found to express Ia-like antigens only rarely (Fig. 4). Only two cases had more than 5% Ia+ T IEL (values being 7 and 9% of the HuTLA+ cells). These specimens, both from ascending colon were obtained at colonoscopy undertaken for a suspected caecal polyp in one case and for follow-up after colectomy for carcinoma in the other. In both patients, colonoscopic and histological findings were normal. Ileal sections taken at the same time in each patient contained less than 5% HuTLA+, Ia+ IEL.

Within each section of stomach and small bowel the proportions of HuTLA+, Ia+, and HLe-1+ Ia+ IEL were similar, and very low (0–3%). In colonic biopsies, however, particularly in the surface epithelium, there was a small proportion (less than 5%) of weakly HLe-1+, strongly Ia+ cells which could not be accounted for by HuTLA+ Ia+ cells. This was more evident in the two specimens of ascending colon with detectable numbers of HuTLA+, Ia+ cells, as discussed above.

Occasional cells (less than 0.1%) which bore Ia-like but not HLe-1 antigens were seen in both small and large bowel epithelium. The staining of Ia appeared to be cytoplasmic, and the cells were large.

Other cells
Rare cells within the epithelium had the appearance of granulocytes under phase microscopy. These cells demonstrated both green and red autofluorescence when studied without the addition of fluorescent antiserum. Similar cells were seen much more frequently scattered around the lamina propria of all sections of the gut.

Fig. 4 Rectal biopsy stained for HuTLA (a) and Ia-like antigens (b). (a) Intraepithelial T lymphocytes are indicated by the small arrows. (b) Ia-like antigens are not found on the IEL nor on the colonic epithelial cells. The lamina propria contains numerous Ia-rich macrophages (large arrows). × 400.
Discussion

Observations in mice and rats have shown that when B and T blasts from mesenteric lymph nodes and thoracic duct lymph are injected into syngeneic recipients, they migrate to the small and large intestine. Only the T blasts reach the epithelium. Using immunofluorescence on tissue sections, the same authors confirmed that all IEL seen were T cells. Examination of isolated mouse small intestinal IEL demonstrated that 80–90% reacted with anti-mouse-T lymphocyte antigen. Surface Ig-bearing cells were rare, and may have represented contamination from the lamina propria.

The present study is the first to concentrate specifically on human IEL in tissue sections using conventional antisera in combination with mouse monoclonal antibody, and extends the data in rodents to man. Intraepithelial lymphocytes were identified in sections of normal stomach, small bowel, colon, and rectum by the expression of HLe-1, a leucocyte antigen. In all sites of the gut examined, the majority of IEL reacted with a specific anti-T cell serum. These findings are in agreement with those of Meuwissen et al. who studied T lymphocytes in ileal and colonic biopsies.

An interesting finding was the demonstration of IEL within the gastric epithelium. These were, again, T-cells. Pfeiffer and Weibel described three possible IEL in ferret antral mucosa, and Toner and Ferguson confirmed their presence in human gastric biopsies. Other than this, little attention has been paid previously to gastric IEL.

Neither B lymphocytes (HLe-1+ Smlg+) nor plasma cells (Cylg+) could be detected in the gut epithelium in our study. Immunoglobulin, particularly IgA, was found on the membranes of, and between, columnar epithelial cells, as described previously. In all cases, however, the epithelial staining was uniform, and no circumscribed increases were seen basally in the region of the IEL. Epithelial fluorescence was always considerably less than that of extracellular immunoglobulin or the staining of B cells and plasma cells in the lamina propria. Thus, surface immunoglobulin-positive IEL would have been evident if present.

Observations in mice and rats after neonatal thymectomy have been interpreted as indicating the presence of a population of B cells within the epithelium. As the IEL after this procedure were not studied directly, and the presence of non-T, non-B IEL was not excluded, these findings are not necessarily at variance with our observations or those of Guy-Grand et al.

Our findings differ from those of Strickland et al., who found both T and B lymphocytes in the epithelium of two controls and two patients with Crohn's disease. The IEL population afforded only brief mention and only small numbers of cells were seen. Bartnik et al. have recently attempted to isolate colonic IEL. They found a high proportion of B cells of each immunoglobulin class, including IgD and IgE, in the isolated cell population. However, using the double marker system we were unable to detect Ig-bearing IEL in tissue sections, nor to identify kappa or lambda light chains on these cells. Previous studies on the distribution of immunoglobulin-positive cells in the human gastrointestinal tract, although concentrating on lamina propria cells, have also failed to report immunoglobulin bearing IEL.

Ia-like antigens are thought to be governed by the HLA-D/DR locus of the major histocompatibility complex. They are expressed strongly on B lymphocytes, on some tissue macrophages, and are present on a small population of T lymphocytes from peripheral blood. The rarity of HUTL- HLe-1+, Ia+ cells in the epithelium is additional evidence against the presence of intraepithelial B lymphocytes.

A small proportion of normal peripheral blood T lymphocytes has recently been shown to bear Ia-like antigens. After activation by mixed leucocyte culture, the proportion of Ia-positive T cells increases. In the present study of normal tissues, T IEL rarely expressed Ia-like antigens, suggesting that they are not activated. The reason for the slightly increased values (5–10%) in the two ascending colon biopsies is not apparent. The patients were well, and endoscopic and histological findings were normal. Yu et al. have demonstrated increased proportion of Ia-positive T cells in peripheral blood of patients with certain diseases, including inflammatory bowel disease. Whether the proportion of T IEL bearing Ia-like antigens is raised in intestinal diseases such as ulcerative colitis, Crohn's disease, or coeliac disease is under study.

In each tissue studied there was a small population of non-T, non-B IEL. In normal blood, non-T, non-B (null) lymphocytes are a heterogeneous population, and the majority bear Ia-like antigens and Fc-receptors for IgG. These Ia+ cells might represent either a B cell or monocyte (non-T) lineage and could include killer (K) and natural killer (NK) cells. In contrast, in gut epithelium the majority of non-T, non-B IEL fail to express Ia-like antigens; therefore a B-cell origin seems unlikely. Guy-Grand et al., in their study on isolated mouse IEL, observed that cells with abundant intracellular metachromatic granules expressed only weak or negative staining with anti-mouse-T cell antiserum. Thus the HuTLa− IEL population may be a
differentiated T cell subset which has lost the expression of HuTLA. Additional studies are essential to clarify whether any of the IEL populations in man can perform cytotoxic functions as K cells or NK cells as described in guinea-pigs.

Eosinophils were identified in the epithelium and lamina propria. They could easily be differentiated from IEL by their autofluorescence and the granular appearance under phase microscopy.

The functions of human IEL must be assessed in the light of the finding that they are predominantly T cells. We have recently reported also that up to 90% of T IEL express a surface marker of suppressor/cytotoxic T cells, as identified by a monoclonal antibody, OKT8.

IEL may be important in processing intraluminal antigens. Intracellular pinocytotic vesicles have been described in human IEL, and are thought to represent material ingested from the intestinal environment. It is possible, therefore, that IEL are the first cells of the immune system to come into contact with luminal antigen, which may then be taken up by the cell or carried on its surface. The IEL are capable of crossing the basement membrane to enter the lamina propria,11 and there they may modulate the immune response to the antigen by interacting with macrophages or other lymphocyte populations. The demonstration of IEL processes crossing the basement membrane and lying in contact with lamina propria macrophages1 as well as the presence of macrophage processes in the epithelium8 support this possibility. Whether the T-suppressor subset of IEL contributes to the development of immunological tolerance to orally-ingested antigens2 remains to be established. The HuTLA−, OKT8+ IEL population may also represent a cytotoxic cell or its precursor with a role in controlling virus infection, tumour development, or autoimmune.

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References


