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Preferential activation of CD4 T lymphocytes in the lamina propria of gluten-sensitive enteropathy

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SUMMARY

The distribution and activation of T-lymphocyte subsets in the small intestinal mucosa of coeliac disease and dermatitis herpetiformis subjects on a normal diet has been studied and compared to normal controls. Double-labelling immunofluorescence techniques with monoclonal antibodies were used on cryostat tissue sections. Intestinal epithelial cells demonstrated staining for HLA-DR, the intensity being proportional to the degree of enteropathy. In both patients and controls nearly all (97%) intra-epithelial lymphocytes were of the CD8 subset and not activated as judged by HLA-DR expression. In the lamina propria there was an approximate 50-fold increase in T cells in the patients as compared with the controls. Whilst the ratio of total CD4 to total CD8 cells was unchanged, the CD4 subset was preferentially activated in the patients. Thus in the normal controls the median ratio of activated CD4 cells to activated CD8 cells was 1·67 whilst for dermatitis herpetiformis and coeliac disease it was 3·42 and 6·07 respectively. These findings suggest that the lamina propria is a site of vigorous T-cell activity in gluten-sensitive individuals and is consistent with the view that the enteropathy of dermatitis herpetiformis and coeliac disease is the result of a delayed-type hypersensitivity against gliadin.

Keywords Dermatitis herpetiformis coeliac disease lamina propria T-lymphocytes immunofluorescence

INTRODUCTION

Both dermatitis herpetiformis (DH) and coeliac disease (CD) are characterized by a gluten-sensitive enteropathy (GSE) and in the case of DH by a characteristic gluten-sensitive rash. In CD the enteropathy is invariably severe with either partial or total villous atrophy, whilst in DH the degree of enteropathy is variable. The study by Fry (Fry *et al.*, 1974) reported 54% of DH cases as having a macroscopically abnormal jejunal biopsy and 93% are abnormal if histological criteria include an intra-epithelial lymphocyte count. The presence of lymphocytes within the jejunal epithelium of DH and CD patients and the reduction of absolute lymphocyte numbers on a gluten-free diet imply that these cells play a part in the induction of the enteropathy.

It has previously been demonstrated that T lymphocytes constitute the major part of the intra-epithelial lymphocyte (IEL) population in both normal and diseased small intestine (Selby *et al.*, 1981a, 1981b, 1983; Ljunghall, Loof & Forsum, 1982; Kelly *et al.*, 1987). The majority of these cells are of the suppressor/cytotoxic (CD8) subset and, on the basis of non-expression of HLA-DR and the lack of receptors for interleukin-2, are not activated (Selby *et al.*, 1983; Kelly *et al.*, 1987). T cells form the greatest proportion of lymphocytes in the lamina propria and the numbers are markedly increased in GSE. Approximately two-thirds of lamina propria T cells are CD4 positive. The only difference so far established in T-lymphocyte subsets between CD and normal intestinal mucosa is an increase in the numbers of OKT8$^+$ Leu-1$^+$ in CD epithelium (Selby *et al.*, 1983). This increase may represent a functionally distinct population of T cells responding to gluten. The failure of previous studies to demonstrate natural killer cells in the small intestinal mucosa indicates that GSE is unlikely to be a natural killer-cell-mediated event.

It is not clear what proportion of T cells in the lamina propria are activated and if the mucosal damage in GSE is indeed T-cell mediated this is an important consideration. In this study we have used a double-labelling immunofluorescence technique employing monoclonal antibodies to T-cell subpopulations and DR antigens for the detection of activated T cells. The aim was to determine the distribution, relationship

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and DR expression of T-cell sub-populations in CD, DH and normal controls. A comparison between CD and DH has not previously been reported.

PATIENTS AND METHODS

Jejunal biopsies were obtained from six control subjects (four male and two female, age range 19–56 years) undergoing investigation for diarrhoea or anaemia, six untreated patients with adult coeliac disease on a normal diet (three male and three female, age range 16–58 years) and 13 patients with DH also on a normal diet (12 male and one female, age range 17–76 years). The diagnosis of DH was confirmed in each case by the presence of granular deposits of IgA in the dermal papillae of uninvolved skin (Fry & Seah, 1974).

In each patient a jejunal biopsy was performed at or distal to the duodeno-jejunal flexure using a Crosby capsule via an Olympus GIFQ endoscope. The biopsies were immediately cut in half, one-half was orientated and embedded in OCT compound (Ames Co.), frozen in liquid nitrogen and then stored at −70°C until use. The other half was orientated on card, examined under a dissecting microscope and the gross morphology recorded prior to fixing in formalin and processing to wax. 7 μm sections were cut, stained with haemotoxylin and eosin and examined by light microscopy.

Immunofluorescence

Monoclonal antibodies. T-lymphocyte subsets were determined using biotinylated Leu 2a (CD8 cells), Leu 3a (CD4 cells) and Leu 4 (CD3 cells) antibodies (Beckton Dickinson, Sunnyvale, California, CA). A further monoclonal antibody YE2/36 HLK, reactive with a non-polymorphic region of HLA-DR antigens was provided by Dr McConnell (Mechanisms in Tumour Immunity unit, MRC centre Cambridge, UK). Cells reactive with this antibody were designated DR^+.

Fluorescent labelling. On a cryostat (Slee TE/HRM) 5 μm sections were cut, air dried for at least 30 min and either stained immediately or stored at −70°C. After drying at room termperature the sections were fixed in a chloroform/acetone mixture (1:1 v/v) for 10 min at 4°C and washed in phosphate buffered saline (PBS: pH 7·2). Staining for T-cell subsets was carried out using the biotin-avidin-fluorochrome technique. Sections were given 20 min incubation with Leu 2a, Leu 3a or Leu 4 diluted 1:5 followed by biotin conjugated goat anti-mouse antibody (Tago, Burlingame, California, CA) diluted 1:50 and finally rhodamine-labelled avidin (Vector laboratories, Burlingame, California, CA) diluted 1:100. The sections were washed in PBS between each incubation. Immediately following this technique sections were stained for HLA-DR antigens using the indirect immunofluorescence method. After a 20 min incubation with anti-HLA-DR the sections were washed in PBS and incubated for a further 20 min with a 1:100 dilution of fluoroscein-labelled rabbit anti-rat antibody and given a final wash in PBS.

All sections were mounted in 10% PBS in glycerol under coverslips, sealed with nail varnish and examined under a Leitz fluorescent microscope (Wetzlar, Germany) equipped with selective filters for fluoroscein isothiocyanate (FITC: green) and tetraethylrhodamine isothiocyanate (TRIC: red).

Counting. Epithelium: DR^+ epithelial cells were noted and the percentage of Leu $2a^+$ and Leu $3a^+$ cells per section recorded.

Table 1. Activated CD4 and CD8 T lymphocytes in the lamina propria of the small intestine in patients with coeliac disease and dermatitis herpetiformis and in controls

	n	CD4 activated* (%)	CD8 activated* (%)	CD4/CD8 activated*
Controls	6	20 (10–40)	12·5 (7–50)	1·67 (1·43–2·66)
Coeliac disease	6	33 (18–66)	8 (0–21)	6·07§† (2·77–26)
Dermatitis herpetiformis	13	21 (8·48)	9·5 (0–52)	3·42‡ (1·30–22)

* Median and range; *n* = number of subjects studied.
† $P<0·01$ *vs* controls.
‡ $P<0·05$ *vs* controls.
§ Not significant *vs* dermatitis herpetiformis.

Lamina Propria: A count of 100 Leu $2a^+$ and 100 Leu $3a^+$ cells (using a × 50 objective) was made on each biopsy and the percentage of those which were also DR^+ recorded. The presence of large DR^+Leu $2a^-$ Leu $3a^-$ dendritic cells was also noted. It was not possible to perform absolute counts in the lamina propria due to the abundance of T cells within this compartment in both controls and GSE and thus ratios alone are given for Leu $2a^+$ and Leu $3a^+$ cells.

Statistical analysis. Wilcoxon's rank sum test was used to compare the results of the patient group with those of the disease groups and the disease groups with each other.

RESULTS

Epithelium

In normal controls, DH and CD 97% of intra-epithelial lymphocytes were of the CD8 phenotype and only 3% CD4. These CD8 cells were mainly distributed along the basal and perinuclear zone although aggregates of up to six of these cells were occasionally seen in the immediate suprabasement membrane area in both normals and DH. In only one normal and in neither of the disease groups was a DR^+ CD8 cell observed and this appeared to be straddling the basement membrane.

HLA-DR staining of epithelial cells was faint in normals and confined to the tips of the villi and there was no correlation between the clumps of CD8 cells in the epithelium and DR expression of adjacent epithelial cells. In DH the HLA-DR staining was bright on the villous tips but was also evident in a granular pattern midway along the villi, the crypts did not express HLA-DR. In the CD group HLA-DR was more strongly expressed and extended into the crypt region.

Lamina propria

In normals, DH and CD the ratio of CD4 to CD8 cells was approximately 2:1, however in both DH and CD the number of CD3 cells present was increased approximately 50 times that seen in normals. In the normal controls the median of the individual Leu $3a^+DR^+$: Leu $2a^+DR^+$ ratios was 1:67 differing significantly (see Table 1 and Fig. 1) from that observed for the

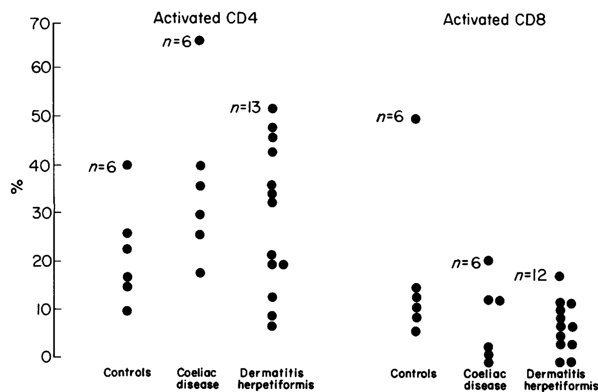

Fig. 1. Activated CD4 and CD8 T lymphocytes in the lamina propria of the small intestine in patients with coeliac disease and dermatitis herpetiformis and in controls. Showing individual values.

disease groups, the value being 3·42 and 6·07 for DH and CD respectively. This increase in the ratio of Leu $3a^+DR^+$ to Leu $2a^+DR^+$ cells in DH and CD is mostly due to an increase in the Leu $3a^+$ cells but is also in part, but to a lesser extent, due to a decrease in the Leu $2a^+$ cells.

In both DH and CD aggregations of Leu $3a^+DR^+$ cells were observed around large Leu $2a^-3a^-DR^+$ cells which were most probably macrophages or other antigen-presenting cells, this phenomenon was not observed in normals. In DH clumping of CD4 cells was seen immediately below the basement membrane.

DISCUSSION

Our study confirms that nearly all of the IELS are of the CD8 phenotype and the absence of HLA-DR antigen expression indicates that these cells are not activated. No dendritic or $DR^+3a^-2a^-$ cells were observed in the epithelium indicating that unless the epithelial cells themselves are presenting antigen no antigen-processing takes place within this compartment. The epithelial cells express HLA-DR antigen with differing patterns between the three groups under study. The more severe the GSE the more extensive is the epithelial expression of DR. However there is no relationship between the proximity of IELs and that part of the epithelium which is DR^+.

In this study the lamina propria revealed the most striking differences between control and disease groups with an approximate 50-fold increase in CD3 T cells in CD and DH compared to normal controls. The double-labelling technique was able to further differentiate GSE from normal as regards preferential activation of CD4 T cells within the lamina propria. Moreover a slightly lower proportion of CD8 T cells are activated within the lamina propria of the patients. When the ratio of activated CD4 cells to activated CD8 cells is calculated a significant difference between DH, CD and normal controls is observed. Taking into account the much larger numbers of T cells present in the lamina propria of both CD and DH these differences become even more impressive. However, the difference between DH and CD ratios is not significant and thus it is not possible to distinguish the disease groups on this basis.

In the disease groups clustering of activated CD4 cells is seen around large dendritic $DR^+3a^-2a^-$ cells which are probably antigen-presenting cells.

The Tac antibody has been used as a marker of activation in CD and normals (Selby *et al.*, 1983; Kelly *et al.*, 1987) but was found to be expressed infrequently, perhaps due to the Tac receptor being expressed for only a small part of the cell cycle. Unlike the present investigation, previous studies have not included double-labelling techniques to investigate the state of T-cell activation in DH and CD. The finding of an increase in activated CD4 cells in both CD and DH coupled with the observations of clustering of these cells around probable antigen-presenting cells and the absence of activated IELs implies that the lamina propria is a site of primary activation in GSE. Although there are activated T cells in normal lamina propria the difference in absolute numbers of activated CD4 cells between normal and the disease state is very marked. In the normal lamina propria there may be a low-grade background activation as dietary antigens are continually processed. However, we postulate that in DH and CD sensitization to gliadin has induced the marked increase in activated CD4 cells observed. It has been argued that the enteropathy of CD is a specific interaction within the lamina propria involving DR-compatible macrophages, gluten, T helper (CD4) cells and mast cells (Marsh, 1983). Immune complexes (Shiner, 1981) and anti-gliadin antibodies (Anand *et al.*, 1981) have not been demonstrated in coeliac intestinal mucosa and this argues against a type-3 hypersensitivity reaction. The findings of this study would concur with this view and we postulate that the enteropathy in DH and CD is the result of a delayed hypersensitivity reaction against gliadin within the lamina propria.

In conclusion, it has been demonstrated that the small intestinal mucosa of both CD and DH is distinguished from normal by a marked increase in activated CD4 T cells in the lamina propria. There is no evidence that the immunological features of the GSE in both DH and CD are intrinsically different other than that the enteropathy in DH is of a lesser degree than that associated with CD. The findings do, however, imply that the lamina propria is the site of primary activation in GSE, probably in the form of a delayed hypersensitivity reaction with gliadin as the antigen.

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