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T LYMPHOCYTE SUBSETS IN THE DUODENAL EPITHELIUM IN DERMATITIS HERPETIFORMIS

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Abstract. Monoclonal antibodies to human T lymphocyte subsets and HLA-DR antigens were used to characterize the intra-epithelial T lymphocytes and HLA-DR reactive cells in the duodenal epithelium in dermatitis herpetiformis (DH).

The expression of HLA-DR antigens on the epithelial cells was similar in patients and controls.

There were significantly more Leu 2a (suppressor/cytotoxic phenotype) reactive cells, as related to epithelial length, in 7 patients with DH than in the epithelium of 5 controls. However, the ratio of Leu 2a (suppressor/cytotoxic phenotype) to Leu 3a (helper/inducer phenotype) reactive cells was the same in the patients as in the controls. Thus this study indicates that there is a quantitative rather than a qualitative difference in T lymphocyte subpopulations in duodenal epithelium between patients with DH and normal persons.

Key words: T lymphocytes; Dermatitis herpetiformis; Duodenal mucosa

Lymphocytes are found both in the lamina propria and in the epithelium of the normal human small intestine (3). More than 95% of the intra-epithelial lymphocytes in the proximal gut are T lymphocytes (22) and 67–90% of these are of the suppressor/cytotoxic phenotype (21) as shown by monoclonal antibodies.

The cell membranes of the small intestinal epithelium in man express the gene products of the major histocompatibility complex (MHC) known as HLA-DR antigens (in other species Ia antigens) (19). There is evidence that these antigens are of vital importance in the regulation of immune responses (14). Many HLA-DR expressing cells, such as the Langerhans cells of the epidermis (18), dendritic cells in the spleen (15) and Ia positive macrophages (25), are known to be directly involved in T cell activation by antigens as antigen-presenting cells in vitro. It is not known whether gut epithelial cells have an immune regulating function. However, the occurrence of intra-epithelial T lymphocytes close to HLA-DR expressing epithelial cells in the normal human small intestine (22, 21) suggests such a function.

The spatial relationship between HLA-DR reactive cells and T lymphocyte subsets has recently been defined in the skin in delayed hypersensitivity reactions and in synovial membrane in rheumatoid arthritis (17, 10). Lymphocytes of the T-helper/inducer phenotype seem to predominate in these clinical situations and an increased number of HLA-DR expressing cells are seen. The in situ relationship between T lymphocyte subsets and HLA-DR expressing cells in vivo can thus provide indirect insight into the mechanisms of the immune reactions that take place.

The aim of the present investigation was to define the phenotypes of the T lymphocytes observed in small intestinal mucosa from patients with DH.

MATERIALS AND METHODS

Patients

Six men and one woman aged 35–74 years (mean 57 years) with a well established diagnosis of dermatitis herpetiformis (DH)—all had granular deposits of IgA in the papillary dermis—were included. None was on a strict gluten-free diet; 2 had been on a gluten-restricted diet for several years. Five patients were on dapsone medication. Three had partial villous atrophy of the duodenal mucosa with broad and short villi and some inflammation. Four had a mucosa with normal tall, narrow villi (Table I).

Control subjects

Five men aged 23–70 years (mean 41 years) investigated for abdominal dyspepsia and diagnosed as having functional gastro-intestinal symptoms, served as controls (Table I). They all had a morphologically normal mucosa with tall narrow villi.

Biopsy specimens

Endoscopy of the upper gastrointestinal tract was performed after an overnight fast. Two to three biopsy specimens were obtained from the mucosa in the post-papillary

Table I. *Number of Leu 1 ("pan T"), Leu 2a (suppressor/cytotoxic phenotype) and Leu 3a (helper/inducer phenotype) reactive lymphocytes related to a unit-length of epithelium on the duodenal villi of patients with dermatitis herpetiformis (DH) and controls, and clinical data*

	Leu 1 reactive cells *n*	Leu 2a reactive cells *n*	Leu 3a reactive cells *n*	Age of patient (years)	Diet	Duodenal mucosal morphology	Dapsone medication
DH patients							
1	17.5	25.0	2.6	35	Normal	PVA[b]	No
2	4.3	12.7	1.1	44	GRD[a]	Normal	Yes
3	3.4	24.1	0.4	59	Normal	Normal	Yes
4	11.4	23.3	0.5	60	Normal	PVA	Yes
5	20.2	32.4	0.8	62	Normal	PVA	Yes
6	3.8	9.6	1.5	68	Normal	Normal	Yes
7	3.5	6.7	2.4	74	GRD	Normal	No
Mean values	9.2*	19.1	1.3	57*			
Controls							
1	3.2	6.4	0.9	23		Normal	
2	4.0	3.5	1.3	33		Normal	
3	1.3	5.0	0.4	35		Normal	
4	2.6	11.3	0.6	43		Normal	
5	2.7	8.4	0.4	70		Normal	
Mean values	2.7	6.9	0.7	41			

[a] Gluten-reduced diet.
[b] Partial villous atrophy.
* $p<0.05$ versus control value.

part of the descending duodenum. The tissue samples were quickly frozen in liquid isopentane (−70°C) and stored at −70°C.

Antisera and other reagents

Biotinylated horse anti-mouse IgG, Avidin DH and biotinylated horseradish peroxidase ("ABC kit") were purchased from Vector Laboratories (Burlinggame, Calif. USA). Three-amino-9-ethyl carbazol was purchased from Sigma (St Louis, Mo, USA). The monoclonal antibodies denoted Leu 1, Leu 2a, Leu 3a and anti-HLA-DR were all from Becton-Dickinson Corp. (Sunnyvale, Calif., USA). Leu 1 is considered to define all T cells (2), Leu 2a to define a "suppressor/cytotoxic" T-cell subset and Leu 3a a "helper/inducer" T cell subset (1). The anti-HLA-DR antibody is directed against a common framework determinant on HLA-DR antigen molecules (11).

Immunocytochemical staining procedure

Sections from the duodenal biopsy specimens, 6 μm thick, were cut on a cryostat and stored at −70°C until used. After thawing, the sections were fixed in acid acetone for 5 min, washed in phosphate-buffered saline (PBS) and exhausted of endogenous peroxidase by incubation in 0.3% H_2O_2 for 10 min. Fifty-microlitre portions of primary monoclonal antibodies diluted in PBS containing 4% bovine serum albumin (PBS-BSA) were allowed to react with the tissue sections for 30 min at room temperature in a humid atmosphere. After washings in PBS the second step of biotinylated anti-mouse IgG, diluted in PBS-BSA, was allowed to react with the sections for another 30 min. After new washes, a complex of biotinylated peroxidase and Avidin DH (incubated together for 30 min at room temperature) was allowed to react with the sections for 30 min. The peroxidase reaction was now carried out by the use of a carbazol-containing buffer (8) and the sections were counterstained with haemotoxylin and mounted in glycerin–gelatin.

Counting of cells in epithelium and lamina propria

The technique of freezing intestinal mucosal biopsy specimens without first fixing them does not yield regularly well orientated villi in the sections. The intra-epithelial Leu-positive cells were only counted in those parts of the specimen that were suitably orientated, i.e. where the epithelium was sectioned in a plane at right angles to the underlying connective tissue. The Leu-positive cells were counted on the villi, not in the crypts.

A standard length unit, the 10-graded scale in the Zeiss measuring eye-piece, was used for quantification of Leu-positive cells in the duodenal epithelium. A magnification of ×400 was used. The total count was made from different parts of the specimen. Twenty length units (200 grades) in the measuring eye-piece were counted in each patient.

The sections were examined blind without knowledge of the diagnosis. Stained material that could not be positively identified was ignored.

The Leu-staining cells in the lamina propria were not counted but were grossly estimated visually.

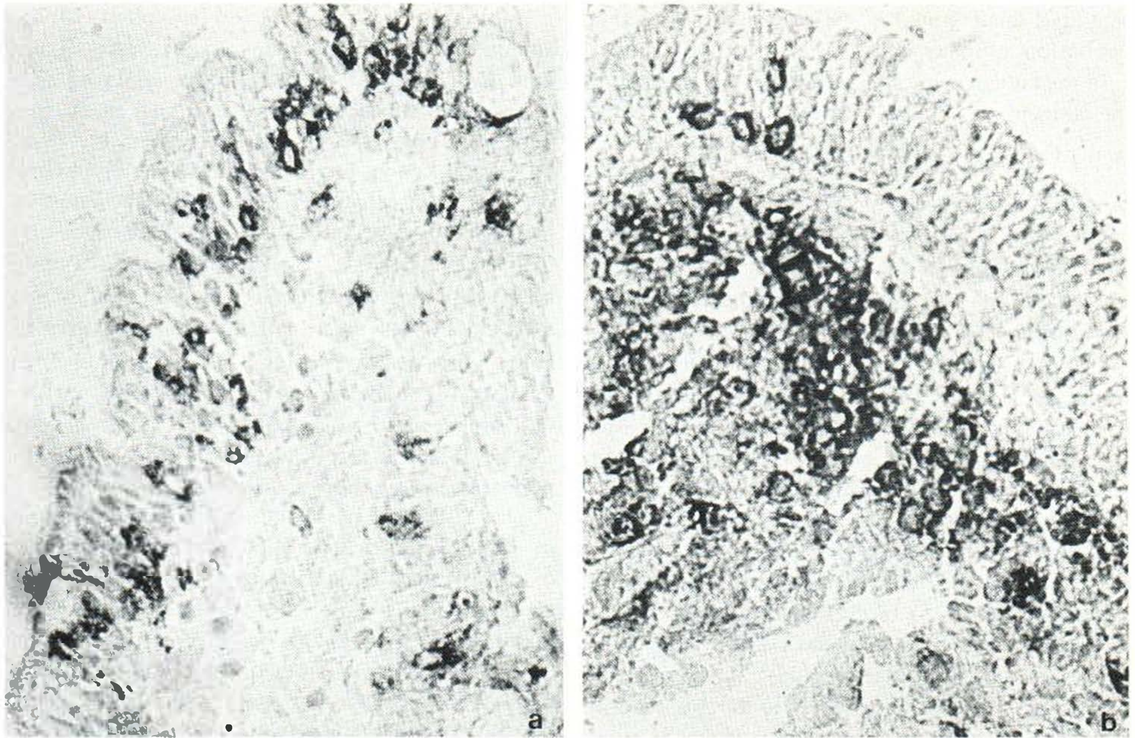

Fig. 1. Immunohistochemically stained cryostat sections of human duodenal epithelium in a patient with dermatitis herpetiformis. Cells reacting with the monoclonal anti-Leu 2a (suppressor/cytotoxic phenotype T lymphocytes) (*a*), and cells reacting with monoclonal anti-Leu 3a (helper/inducer phenotype T lymphocytes) (*b*), are visualized by peroxidase catalysed staining.

RESULTS

T lymphocyte subsets in duodenal mucosa

Leu 1, Leu 2a and Leu 3a reactive cells were found in the epithelium (Fig. 1). Leu 2a reactive cells were much more frequent than the Leu 3a reactive cells both in DH patients and in controls (Table I). Significantly more Leu 2a reactive cells were seen in the epithelium of the patients than in the controls (Table I). However, the relation between the numbers of the Leu 2a and Leu 3a reactive cells did not differ between the patient and the control group.

In the lamina propria the Leu 3a reactive cells dominated over the Leu 2a reactive cells in all subjects, patients as well as controls. In most control specimens few Leu 2a positive cells were seen in the lamina propria. The overall impression was that there were more Leu antigen reactive cells in the lamina propria of most DH patients than in the controls.

Cells stained for Leu 1 antigen were seen more sparsely than the Leu 2a reactive cells in the epithelium (Table I). In the lamina propria, Leu 1 positive cells seemed to be much more frequent than Leu 2a positive cells and at least as frequent as the Leu 3a reactive cells.

Mostly faint, but in some specimens more pronounced, staining of the goblet cells was observed for all Leu antibodies.

Staining for HLA-DR antigens in duodenal epithelium

The staining for HLA-DR antigens seemed to be similar in the patients and the controls. It was observed as fine "granulae" with a scattered distribution at the luminal end of the enterocytes on the villi and in the upper part of the crypt epithelium. Often the stained "granulae" were seen to be extended over the cytoplasm of the enterocytes, but they were always most dense nearest the lumen. In some specimens the staining appeared as a continuous band or as a pearl necklace in the luminal end of the enterocytes, in both cases surrounded by the

scattered small 'granulae'. No staining was noted in the bottom of the crypts.

In the lamina propria many cells were stained for the antigen.

DISCUSSION

In this study duodenal mucosa was used. This has been shown to be just as representative for gluten-induced changes in the gut of DH patients as is jejunal mucosa (6). The results indicate that the population of T lymphocytes in duodenal epithelium from patients with DH, as in normal subjects, is composed mainly of suppressor/cytotoxic cells (Leu 2a reactive). The number of these cells was significantly increased in the DH patients. The relevance of the findings obtained with various morphometric methods used for the quantification of intra-epithelial gut lymphocytes in gluten-sensitive enteropathy have been debated in several papers and needs to be further evaluated (7, 13). However, when related to the number of enterocytes, as was done in this study, intra-epithelial lymphocyte counts have been found by several groups to be increased in jejunal specimens from patients with coeliac disease and DH (3, 5). The present observations are consistent with these latter studies. Moreover, our results indicate that the increased cell population consists mainly of suppressor/cytotoxic T lymphocytes. Thus with respect to the intra-epithelial lymphocytes the difference between DH patients and controls, diagnosed as functional dyspepsia, seems to be a quantitative rather than a qualitative one.

The Leu 1 antigen was expressed on fewer intra-epithelial lymphocytes than the Leu 2a and Leu 3a antigens together (Table I). The same discrepancy, but less pronounced, was found with respect to the lymphocytes in the lamina propria. These observations are in agreement with the finding of Ledbetter et al. (12) that Leu 1 is expressed in larger amounts on the helper/inducer subset of T lymphocytes than on the suppressor/cytotoxic subset. Thus, our study, including control stainings with various Leu 1 antibody dilutions, indicates that the Leu 1 antibody does not define all the peripheral T lymphocytes in the gut and especially not all the Leu 2a reactive ones.

It should also be noted that staining with the Leu 2a and Leu 3a antibodies does not represent definitive states of the T cells with respect to 'suppressor/cytotoxic' or 'helper/inducer' functions (23).

Both in the present study and in another (20) the amount of HLA-DR antigens, as measured by immunohistochemical methods, on the epithelial cells of small intestine seemed to be similar in DH patients and controls. To our knowledge, however, nothing is known about the MHC haplotypes expressed in gut cells. It is tempting to speculate that in coeliac disease and DH the small intestine epithelial cells express HLA-DR antigens of haplotypes regulating the immune response to gluten in a way that makes these patients more sensitive to gluten than healthy persons. An HLA antigen in linkage disequilibrium with HLA-B8 and HLA-DR3, suggested by many authors to be a reality in DH (9), might be responsible. Data from animal experiments (24) support this concept.

There is a close correlation in DH between gluten in the diet, on the one hand, and the occurrence of disturbances of the villous architecture and increased numbers of intra-epithelial lymphocyte counts in the gut (16) and skin symptoms (4) on the other hand. The spatial relationship of the Leu antibody reactive cells in the epithelium of the small bowel and the HLA-DR antigen of the enterocytes might reflect MHC-mediated interactions taking place in the epithelium that could be of importance in the pathogenesis of the mucosal and immunological changes in gluten-sensitive enteropathy in DH.

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