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Leucocyte common antigen expression on T cells in normal and inflamed human gut

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SUMMARY

The expression of the 220,000 MW (p220) glycoprotein component of the leucocyte common antigen (LCA) family by intestinal mucosal lymphocytes was studied using the CD45R monoclonal antibody WR16. In normal intestine, a proportion of $CD3^+$ mucosal T cells were $WR16^+$ and this population resided predominantly in the mid-villus and crypt region of lamina propria. In the inflammatory infiltrates of both coeliac disease and Crohn's disease the $CD3^+$, $WR16^+$ population was markedly reduced. The monoclonal antibody UCHL1 identifies the 180,000 MW member of the LCA family and is expressed on T cells and in macrophages. $CD3^+$ lymphocytes expressing this marker were widespread in normal lamina propria and epithelium. In contrast with WR16, $UCHL1^+$ cells remained at a high level in coeliac disease and Crohn's disease. Our results support the view that loss of the p220 molecule occurs upon T-cell activation in inflammation.

INTRODUCTION

The leucocyte common antigen (LCA) represents a family of cell surface glycoproteins of which at least four molecular species are known, with molecular weights (MW) of 220,000, 205,000, 190,000 and 180,000 (Woollet *et al.*, 1985). The LCA molecule spans the cell membrane, with a highly conserved cytoplasmic region and considerable N-terminal variation (Dalchau, Flanagan & Fabre, 1986). At the *Third Leucocyte Typing Workshop* (1987) antibodies recognizing epitopes present on the LCA molecule were classified as either CD45 or CD45R (Cobbold, Hale & Waldmann, 1987). CD45 epitopes are common to all four molecular species. CD45R antibodies recognize epitopes within the variable N terminal region of the molecule (Dalchau *et al.*, 1986). Expression of CD45R epitopes is restricted to three or less molecular species of LCA.

$CD4^+$ T lymphocytes may be subdivided according to the species of LCA they express, as either helper-inducer or suppressor-inducer populations: the former express the p180 species of LCA, the latter the p220 species (Smith *et al.*, 1986, Moore & Nesbitt, 1987; Rudd *et al.*, 1987). Activation of peripheral blood p220-positive T lymphocytes with mitogen leads to a loss of the p220 molecule and increased p180 expression at the cell surface (Moore & Nesbitt, 1987, Ledbetter *et al.*, 1985).

Recently, a loss of the p220 high molecular weight species of LCA has been described on $CD4^+$ cells in the inflammatory lesion of rheumatoid arthritis (Moore *et al.*, 1988). It was proposed that the loss of the p220 molecule occurred as a result of $CD4^+$ T-cell activation within the inflammatory infiltrate. Further to this we have examined p220 expression in two intestinal inflammatory conditions, each of distinct pathogenesis, Crohn's disease and coeliac disease. The results show a similar loss of the p220 molecule (identified by the CD45R antibody WR16) on T lymphocytes in both conditions.

Abbreviations: IEL, intra-epithelial lymphocyte; LCA, leucocyte common antigen.

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MATERIALS AND METHODS

Tissue

Samples of small intestine were obtained from jejunal biopsy specimens (five controls and three biopsies from patients with untreated coeliac disease). Samples of control large intestine were obtained from five patients who had undergone resection for carcinoma. The control tissue was taken from a site distant from the tumour. Sections of resected bowel from four patients diagnosed as having Crohn's disease were also collected. Two cases of ulcerative colitis were also included in this study for comparison with Crohn's disease. All samples were snap-frozen in liquid nitrogen and stored until required for immunoperoxidase staining.

Immunoperoxidase labelling of frozen sections

Six micrometre cryostat sections of frozen tissue were mounted onto 0·01% poly-L-lysine (Sigma, Poole, Dorset)-coated slides and stored over silica gel at $-20°$ for up to 14 days. On the day of staining, sections were air-dried at room temperature for 30 min and then fixed in acetone for 10 min.

Staining was carried out by a two-layer indirect immunoperoxidase technique, a modification of the method of Stein *et al.* (1980). Primary mouse monoclonal antibodies employed in this

Table 1. Monoclonal antibodies used in this study

Monoclonal antibody	Specificity	Cluster of differentiation	Reference
UCHT1	Mature T lymphocytes	CD3	Beverley & Callard (1981)
HB2	T lymphocytes	CD7	Haynes, Eisenbarth & Fauci (1979)
UCHL1	$CD4^+$ helper-inducer cells, and $CD8^+$ cells	(p180)*	Smith *et al.* (1986)
WR16	$CD4^+$ suppressor-inducer cells, $CD8^+$ and B lymphocytes	CD45R	Moore & Nesbitt (1986)
WR17	B lymphocytes	CD37	Moore, Cooper & Jones (1987)

* UCHL1 recognizes the p180 molecular species of leucocyte common antigen.

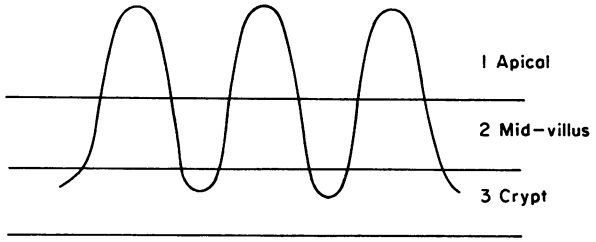

Figure 1. Regions of lamina propria. Ten graticule units were counted in regions 1, 2 and 3 for each case.

study are described in Table 1. Working titres of primary antibodies were determined previously by titration on cryostat sections of human tonsil. Rabbit anti-mouse immunoglobulin peroxidase conjugate (Dakopatts, High Wycombe, Bucks) was used as the second layer, the concentration being batch dependent. The reaction product was developed using diaminobenzidine tetrachloride (Sigma) as described by Graham & Karnovsky (1966). Controls were performed using the second-layer antibody alone to determine the endogenous peroxidase present in the frozen sections.

Following staining, sections were dehydrated into xylene and mounted in DPX (BDH Chemicals, Poole, Dorset).

Evaluation of positively stained cells

Cells stained with the antibodies described in Table 1 were initially assessed using a $\times 25$ eyepiece magnification and scored on a scale of $+++$ (many positive cells in all cases) to $-$ (no positive cells in any cases). Positive cells in both villus epithelium and lamina propria were assessed (Tables 2 & 3). This gave an overall view of staining patterns obtained and highlighted differences between controls and cases of either coeliac or Crohn's disease.

In addition to the above method, positive staining with the two antibodies WR16 (CD45R) and UCHT1 (CD3) (Table 1) was assessed quantitatively to accurately compare the balance of these populations in the different tissues.

Positive cells were counted by light microscopy using an eyepiece graticule on $\times 25$ magnification (eyepiece lens $= \times 10$ magnification). A total of 30 graticule units each containing 20–30 mononuclear cells were counted for each case (giving a count of 600–900 cells per case.) The total number of stained and unstained lamina propria cells (excluding lymphoid follicle cells, epithelial and endothelial cells) and the number of positively stained cells were recorded for each graticule unit. Lymphoid follicles were excluded from the count since follicles were not present in all cases. An attempt was made to position the graticule units equally between three regions of lamina propria, by counting 10 graticule units from each region (Fig. 1). The percentage of positive cells out of the total number of cells in each graticule unit was calculated and results show the mean percentages for each antibody out of 30 graticule units.

Table 2. Distribution of positively stained cells in jejunal biopsies in lamina propria (LP) and epithelium (EP)

		Controls ($n=5$)		Untreated coeliac disease ($n=3$)	
Antibody	CD	EP	LP	EP	LP
UCHT1	3	++	++	+++	+++
WR17	37	−	+	−	+/−
UCHL1	(p180)	+	+++	+++	+++
WR16	45R	+	++	+/−	+
HB2	7	++	++	+++	++

Positive cells scored on a scale of: $-$ = no positive cells in any cases, $+/-$ = positive cells rare, not present in all cases, $+$ = some positive cells in all cases, $++$ = positive cells frequent in all cases, $+++$ = many positive cells in all cases.

RESULTS

Immunoperoxidase staining of jejunal biopsy specimens

Controls. The results of immunoperoxidase staining of jejunal biopsies are summarized in Table 2. The antibody UCHT1 (CD3) identified a major population of lymphocytes diffusely distributed throughout the epithelium and lamina propria. HB2 (CD7) identified a similarly distributed popula-

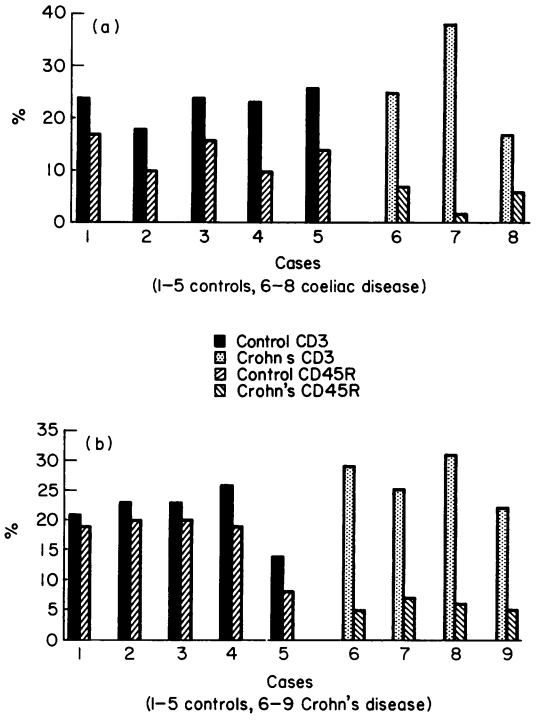

Figure 2. Percentages of UCHT1$^+$ (CD3) and WR16$^+$ (CD45R) cells in the lamina propria of (a) jejunal biopsies and (b) large bowel resections.

tion; however, CD7$^+$ intra-epithelial lymphocytes (IEL), which occurred at the extreme tips of villi, did not appear to be CD3$^+$. WR17$^+$ B cells were rare in control jejunal biopsies, occurring mainly in the mid-villus and crypt regions of the lamina propria.

The number of WR16$^+$ (CD45R) cells clearly outnumbered WR17$^+$ B cells, showing that the majority of WR16$^+$ cells stained were in fact T cells. WR16$^+$ T lymphocytes were also predominantly present in the mid-villus and crypt region of the lamina propria. UCHLI$^+$ (p180) cells consisted of a dense population of both macrophages and T cells; the latter were clearly identifiable morphologically by their discrete membrane staining (Fig. 3). The distribution of UCHL1$^+$ IEL on sequential sections was notably similar to that of CD3$^+$ IEL. Thus the majority of CD3$^+$ IEL were also UCHL1$^+$. Both WR16 and UCHL1 showed background staining of the mucus lining the villus epithelium and also lamina propria granulocytes, in particular neutrophils. UCHL1 also stains neutrophils specifically.

Biopsies from patients with coeliac disease. A small increase in the local density of T cells occurred in the mucosa of patients with coeliac disease when compared with controls. Within this population the ratio of UCHT1$^+$ (CD3) to WR16$^+$ (CD45R) T cells in the lamina propria was increased (Fig. 2a). The population of WR16$^+$ lamina propria cells was visibly diminished, particularly in the mid-villus region of lamina propria. UCHL1$^+$ T cells remained dense in both lamina propria and epithelium (Fig. 3). The number of HB2$^+$ IEL appeared to exceed that identified by either UCHL1 or UCHT1.

Immunoperoxidase staining of large bowel resections

Controls. Although fewer IEL were observed in the colon when compared with control jejunal biopsies, the overall distribution of the markers in both epithelium and lamina propria was remarkably similar (Table 3). However, UCHL1$^+$ macrophages and T cells showed a high density immediately beneath the villus epithelium. This feature was not noted in jejunal biopsy specimens.

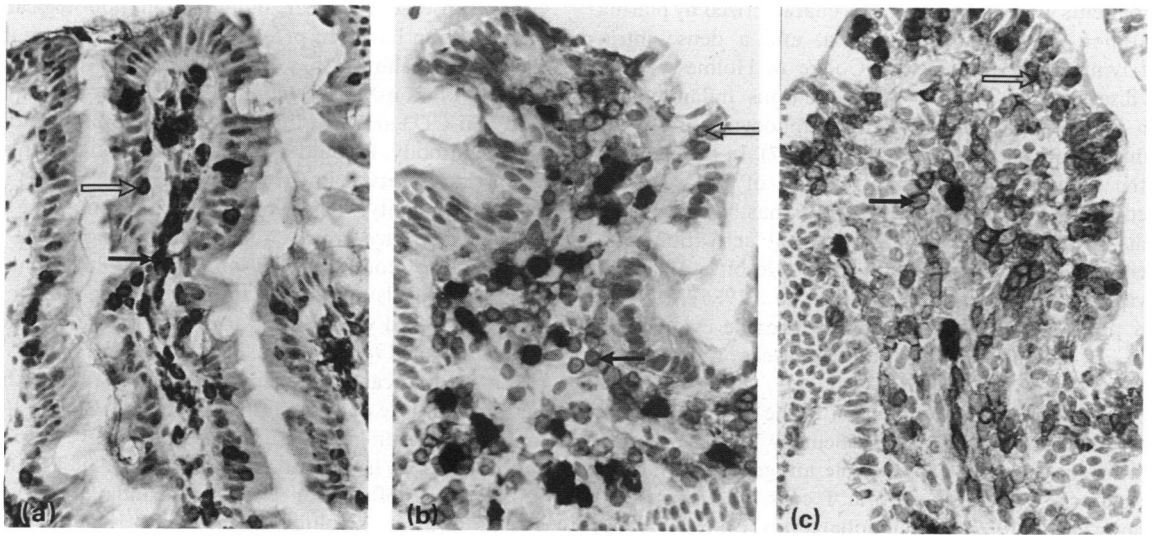

Figure 3. Reactivity of the antibody, UCHL1 (recognizing the p180 species of LCA). (a) Jejunal biopsy showing widespread positive lamina propria T cells (black arrows), IEL (white arrows) and macrophages. T cells are identifiable by their discrete membrane staining. The very dense areas of staining are neutrophils which have endogenous peroxidase activity and are also specifically stained by UCHL1. (b) Jejunal biopsy from a patient with coeliac disease with numerous positive T cells, macrophages and neutrophils. (c) Resection from a patient with Crohn's disease showing dense staining of T cells within the inflammatory infiltrate. (Peroxidase technique, ×240 magnification.)

Table 3. Distribution of positively stained cells in large bowel resections in lamina propria (LP) and epithelium (EP)

		Controls ($n=5$)		Crohn's disease ($n=4$)	
Antibody	CD	EP	LP	EP	LP
UCHT1	3	+	++	+	+++
WR17	7	−	+	−	−
UCHL1	(p180)	+	+++	+	+++
WR16	45r	+	++	+	+
HB2	7	+	++	+	++

Positive cells scored as in Table 2.

Resections from patients with Crohn's disease. As with coeliac disease, a marked loss of WR16$^+$ (CD45R) cells relative to UCHT1$^+$ (CD3) cells occurred in the lamina propria of Crohn's patients (Fig. 2b). UCHL1$^+$ cells were present in high frequency with the greatest density immediately beneath the villus epithelium (Fig. 3). UCHT1$^+$ T cells were also clustered within the apical region of lamina propria.

Resections from patients with ulcerative colitis. The inflammatory infiltrate in ulcerative colitis consisted of large numbers of WR17$^+$ B cells and UCHL1$^+$ T cells and macrophages. Numerous WR16$^+$ cells were present with the same distribution as the WR17$^+$ B cells.

DISCUSSION

The mucosal inflammation occurring in response to dietary gluten in patients with coeliac disease is characterized by plasma cell infiltration of the lamina propria, and a dense intraepithelial lymphocyte population (Cooke & Holmes, 1984). Crohn's disease is a chronic, granulomatous inflammatory condition of unknown cause, where lymphocytic infiltration extends throughout the bowel wall (Lee, 1987). In this study, a loss of the high molecular weight species of LCA (p220), recognized by the CD45R antibody WR16, has been observed on T cells in these disorders. Since T-cell activation with phytohaemagglutinin (PHA) (Moore & Nesbitt, 1987) or concanavalin A (Con A) (Morimoto *et al.*, 1986) leads to decreased p220 expression, our findings provide evidence of mucosal T-cell activation in both coeliac disease and Crohn's disease.

The extensive UCHL1$^+$ population in the normal jejunum and colon indicates large numbers of memory T cells (Beverley, 1987), consistent with the considerable antigenic load at this site. The majority of lamina propria T cells are also resting. (Trejdosiewicz *et al.*, 1987). In functional terms, UCHL1 defines the CD4$^+$ helper-inducer T-cell population in the lamina propria (Cerf-Bensussan, Scheeberger & Bhan, 1983; Smith *et al.*, 1986), whereas WR16 defines the minority CD4$^+$ suppressor-inducer population. (Moore & Nesbitt, 1986; James *et al.*, 1986). There are at least two possible explanations for the loss of the p220 epitope identified by WR16 in the inflammatory infiltrates of coeliac disease and Crohn's disease. Selective extravasation of the CD4$^+$,WR16$^+$ suppressor-inducer population may have occurred. Alternatively, T-cell activation, either of the resting lamina propria population or of a novel infiltrating population, has taken place. The functional properties of this active population at the site of inflammation remain to be described.

The majority of IEL in both control biopsies and biopsies from patients with coeliac disease were UCHL1$^+$/WR16$^-$. This phenotype is again suggestive of an activated population. The presence of CD7 on CD3$^-$ cells at the tips of villi suggests activation of these cells in particular, since CD7 is present on blast cells (Lazarovits *et al.*, 1987). Furthermore, the absence of the CD6 marker on IEL described by Trejdosiewicz *et al.* (1987) is also consistent with an active phenotype. These workers suggest IEL in the jejunum are normally involved in the maintainance of tolerance to dietary antigens, but their role in the pathogenesis of coeliac disease remains unclear.

A cell-mediated immune response to gluten has long been considered a likely cause of the mucosal damage occurring in coeliac patients (Ferguson *et al.*, 1975). Several workers have looked for evidence of T-cell activation, particularly in the dense IEL population in support of this hypothesis. Early morphometric studies by Marsh (1980) suggested increased activation of IEL in coeliac disease. However, Leigh *et al.* (1985) despite demonstrating gluten-induced lymphoid infiltration of the epithelium in coeliac patients, failed to find evidence of activation. The immunohistochemical studies of Selby *et al.* (1983) and Kelly *et al.* (1987) showed a lack of an activation marker (CD25) on IEL in coeliac disease. Kelly *et al.* (1987) did show, however, expression of a different activation marker (Tal) on T cells in the lamina propria of controls and patients with coeliac disease. In the face of this conflicting evidence, our findings favour the view that mucosal T-cell activation, possibly mediated by dietary gluten, does occur in coeliac disease.

The functional significance of mucosal T-cell activation in Crohn's disease is unclear, though an immunological basis for the condition has been proposed (Lee, 1987). Shanahan *et al.* (1988) have shown that isolated mucosal lymphocytes are capable of non-MHC restricted cytotoxicity when triggered with anti-CD3 and that this cytotoxicity could be blocked by a CD45 antibody. It could be speculated that the change in LCA expression observed in Crohn's disease is related to abnormal cytotoxic activity. This would be important since cytotoxic mechanisms may have a role in mediating tissue injury in inflammatory conditions (Elson *et al.*, 1986). In the mouse, cytotoxic T cells are known to show altered LCA expression when compared with resting or non-cytotoxic T cells (Lefrancois & Bevan, 1985).

In the two cases of ulcerative colitis examined the inflammatory infiltrate consisted almost entirely of B cells (WR17$^+$), and this infiltrating population was also positive for p220. Thus, the loss of p220 in inflammation is a feature of T rather than B cells. The loss of p220 on T cells in the inflammatory lesions of coeliac disease, Crohn's disease and rheumatoid arthritis (Moore *et al.*, 1988) suggests that this may be a general feature of T cells in inflammation.

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