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Lymphocyte subpopulations in the human small intestine. The findings in normal mucosa and in the mucosa of patients with adult coeliac disease

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SUMMARY

Lymphocyte subpopulations in human small intestinal mucosa have been studied using an immunofluorescence technique on tissue sections. In the normal intestine, the majority of intraepithelial lymphocytes (IEL) were of suppressor–cytotoxic phenotype (HuTLA$^+$ UCHTI$^+$ OKT8$^+$ OKT4$^-$; 84%). Only one-third of these OKT8$^+$IEL reacted with anti-Leu-1, and antibody directed towards a 67,000 dalton antigen found on peripheral blood T cells. IEL failed to express the activation antigen, Tac, and also lacked detectable C3b receptor (C3RTO5$^-$). The remaining T IEL, as well as the predominant lamina propria T lymphocytes (LPL), were OKT4$^+$ OKT8$^-$, helper type T cells. Most of the lamina propria OKT8$^+$ cells were also Leu-1$^-$. In patients with adult coeliac disease, the proportions of OKT8$^+$ and OKT4$^+$ lymphocytes in the epithelium were not altered. However, the proportion of OKT8$^+$ Leu-1$^+$ T IEL was significantly increased (56 *vs* 32%; $P < 0{\cdot}02$). IEL were also HLA-DR$^-$, Tac$^-$ and C3RTO5$^-$. The proportion of OKT8$^+$ cells in the lamina propria was slightly, but significantly, increased (40 *vs* 32%; $P < 0{\cdot}005$). Mucosal findings in treated patients did not differ from normal. Lymphocytes with the phenotype of natural killer cells (HNK-1) were rarely found in normal or diseased mucosa. No alterations in the proportions of circulating T lymphocytes or their subsets were found in patients with coeliac disease. These findings illustrate the heterogeneity of lymphocyte subpopulations in normal and in diseased small intestinal mucosa. The changes found in adult coeliac disease may reflect the increased traffic of IEL into the epithelium.

INTRODUCTION

Recent studies, using a panel of heterologous and monoclonal antibodies in a double marker immunofluorescence technique on tissue sections, have shown that lymphocytes within the normal intestinal mucosa represent a heterogeneous population. Almost all intraepithelial lymphocytes (IEL) react with an antiserum to human T lymphocytes, and the majority of these are of suppressor–cytotoxic phenotype (HuTLA$^+$ OKT8$^+$; Selby *et al.*, 1981a; Selby, Janossy & Jewell, 1981b). In contrast, HuTLA$^+$ lymphocytes within the lamina propria are predominantly OKT8$^-$. In order to characterize the lymphocyte populations in the small intestine more fully, sections of histologically normal tissue have been examined using additional combinations of monoclonal antibodies. The small intestinal mucosa of patients with adult coeliac disease has also been studied.

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Findings such as the sensitivity of peripheral blood lymphocytes to gluten fractions, the occurrence in many patients of circulating immune complexes and antibodies to reticulin, and the close association between the development of coeliac disease and the histocompatibility antigens HLA-B8 and DR3, suggest that immunological mechanisms are important in the pathogenesis of this disorder (Asquith & Haeney, 1979).

MATERIALS AND METHODS

Tissues. Jejunal biopsies were obtained perorally, using a Crosby capsule or steerable catheter (Meditech) from one normal volunteer, and from seven patients undergoing investigation of diarrhoea or anaemia (four male and four female; mean age = 40 years; range = 29–66 years). In each case, the histology of formalin fixed and of frozen sections was normal. Six patients with adult coeliac disease were studied whilst on a normal diet (two males and four females; mean age = 41·8 years; range = 16–72 years). Jejunal biopsies were performed on three of these patients after at least 3 months on a gluten free diet. In addition, biopsies were obtained in a further four patients with coeliac disease who were already maintained on the diet (two male and five female; mean age = 37·6 years; range = 19–72 years). In each case the patient had responded clinically and the histological appearances had returned nearly or fully to normal. All patients gave informed consent to the study.

Immediately after being taken, biopsies were orientated, embedded in O.C.T. compound (Ames Co.), frozen in isopentane suspended over liquid nitrogen, and then stored in liquid nitrogen until used.

Immunofluorescence. The method used has been outlined previously (Selby *et al.*, 1981b). Briefly, 5 μm sections were cut on a cryostat, air dried, fixed in absolute alcohol at 4°C for 10 min, and then washed in phosphate-buffered saline with 0·1% sodium azide (PBS). When two monoclonal antibodies were to be applied, sections were lyophilized for 2 h prior to staining or storage (Janossy *et al.*, 1982). Antibodies were applied to the sections for 30 min, followed by washing in PBS for 30 min. Second and third layer antisera were applied for a similar period. After a final wash, slides were mounted in 50% glycerol in PBS.

Antibodies. Lymphocytes were identified using 2D1, a monoclonal antibody which reacts with human leucocyte antigen (HLe-1) (Beverley, 1979; Selby *et al.*, 1981b).

T lymphocytes were demonstrated using a specific rabbit antiserum to human T lymphocytes (R anti-HuTLA) (Selby *et al.*, 1981a; Janossy *et al.*, 1981) as well as by UCHT1, an OKT3 like monoclonal antibody which detects peripheral blood T cells (Beverley & Callard, 1981). In addition, anti-Leu-1, an OKT1 like antibody against a 67,000 dalton antigen found on circulating T cells and on thymocytes, was also used (Ledbetter *et al.*, 1981; Martin *et al.*, 1981). Helper type T lymphocytes and suppressor–cytotoxic type T lymphocytes were identified using OKT4 and OKT8, respectively (Ortho, Raritan, New Jersey, USA) (Reinherz & Schlossman, 1980). The latter antibody was conjugated with arsanilic acid (OKT8-ARS) for double labelling studies with OKT4 or anti-Leu-1, both of which were conjugated with biotin (Janossy *et al.*, 1982).

HLA-DR, Ia like, antigens were detected using an antiserum raised in chickens against P28,33 antigens (C anti-Ia) (Janossy *et al.*, 1979). Anti-Tac, reacting with an antigen expressed by T lymphocytes following stimulation in culture, was used to detect activated T cells (Uchiyama, Broder & Waldmann, 1981). Cells with receptors for C3b were detected using C3RTO5 (Gerdes *et al.*, 1982). Finally, cells with the phenotype of natural killer (NK) cells were detected using HNK-1 (anti-Leu-7; Becton-Dickinson), a monoclonal antibody of IgM class, which defines a population of large granular lymphocytes, some of which are $OKT8^+$, and which includes cells capable of NK activity (Abo & Balch, 1981). Controls were performed using NA1/34, a monoclonal antibody which labels thymocytes but does not stain intestinal tissue (McMichael *et al.*, 1979; Selby *et al.*, 1981b), as well as with normal rabbit serum and normal chicken serum in place of first layer antisera. Immunoglobulins were detected using antisera to IgA, labelled with tetramethylrhodamine isothiocyanate (TRITC), to IgM, labelled with fluorescein isothiocyanate (FITC), to IgG (FITC), IgD (FITC), kappa light chains (FITC) or to lambda light chains (TRITC) (Selby *et al.*, 1981b).

Monoclonal antibodies applied alone or in combination with conventional antisera were used in indirect immunofluorescence with goat anti-mouse Ig antiserum conjugated with FITC (Nordic). R anti-HuTLA was used with swine anti-rabbit Ig TRITC (Dako), C anti-Ia with sheep anti-chicken Ig TRITC (Royal Free). Antibodies conjugated with biotin were detected using fluoresceinated avidin as the second layer (avidin FITC). OKT8 ARS was used in a triple layer system, with rabbit anti-arsanilate as the second layer (Becton-Dickinson) and swine anti-rabbit Ig (TRITC) as the third.

The following combinations of antisera were used: (1) 2D1 and R anti-HuTLA to determine the proportion of IEL that were T cells; (2) R anti-HuTLA and OKT8 to ascertain the proportion of T IEL and LPL that were of suppressor–cytotoxic phenotype; (3) OKT4 and OKT8; (4) OKT8 and anti-Leu-1 to determine the heterogeneity of $OKT8^+$ cells with respect to Leu-1 expression and (5) R anti- HuTLA or UCHT1 in combination with C anti-Ia to determine the expression of HLA-DR antigens by IEL.

The epithelium and lamina propria of several sections (10–20 high power fields) were examined using a Zeiss Universal fluorescence microscope with epifluorescence and selective filters for FITC and TRITC. This enabled simultaneous detection of two antigens on the one tissue section.

Peripheral blood mononuclear cells. Nine patients with biopsy proven coeliac disease were studied whilst they were taking a normal diet (two male and seven female; mean age 47·1 years; range 29–72 years). Eight patients (including three of the untreated group) were studied after treatment with a gluten free diet (one male and seven female; mean age 39·9 years; range 19–72 years). No patient had hyposplenism, as determined by the absence of Howell–Jolly bodies on a peripheral blood film. Two groups of controls were studied: (1) 13 normal volunteers with no known gastrointestinal disease (five male and eight female; mean age 37·4 years; range 21–65 years) and (2) 13 patients (five male and eight female) undergoing investigation of gastrointestinal symptoms or anaemia (three with iron deficiency anaemia, two with hypolactasia, two with unexplained abdominal pain, two with colonic polyps and one each with pancreatic carcinoma and steatorrhoea, ischaemic colitis, radiation proctitis and Hirschprung's disease). The mean age of these patients was 46·5 years (range 19–70 years).

Peripheral blood mononuclear cells were isolated on Ficoll-Hypaque (Pharmacia, Sweden). Immunofluorescence was performed on the cell suspensions as described previously (Selby & Jewell, 1982).

Statistical analysis. Wilcoxon's rank sum test was used to compare the results of the patient groups with those of the control groups. Results are expressed as mean value $\pm$ standard deviation.

RESULTS

Tissues

Normal jejunum. The results have been reported in part previously (Selby *et al.*, 1981a & 1981b). Ninety-nine per cent of IEL ($HLe\text{-}1^+$) were $HuTLA^+$ (Table 1). Of these T IEL, 80% were of suppressor–cytotoxic phenotype ($HuTLA^+$ $OKT8^+$). Double labelling using OKT8 and OKT4 revealed a similar proportion of $OKT8^+$ $OKT4^-$ IEL (84%), with a small proportion of $OKT8^-$ $OKT4^+$, helper type, T lymphocytes (16%) within the epithelium. No $OKT8^+$ $OKT4^+$ double labelled cells were seen. The similar proportions of $OKT8^+$ IEL detected using both methods suggest that all T IEL are either $OKT8^+$ or $OKT4^+$. IEL were also $UCHT1^+$ ($OKT3^+$). However, when OKT8 was used in combination with anti-Leu-1, it was found that only about one-third of $OKT8^+$ IEL were $Leu\text{-}1^+$ with varying intensity of expression (Table 1, Fig. 1). This was true of IEL in the villi as well as of those in the crypts. $OKT8^-Leu\text{-}1^+$ IEL were also identified, in a similar proportion to $OKT4^+$ IEL. The expression of Leu-1 on these cells was bright. Normal IEL rarely expressed HLA-DR antigens (see also Selby *et al.*, 1981a). In addition, IEL were Ig^-, activation marker $(Tac)^-$ and C3b receptor $(C3RTO5)^-$. In only one normal biopsy (from a 29 year old male with irritable colon syndrome) were rare $HNK\text{-}1^+$ cells seen in the epithelium ($< 1\%$).

In the lamina propria approximately two-thirds of T lymphocytes were of helper phenotype ($HuTLA^+$ $OKT4^+$ $OKT8^-$; Table 2). These were $Leu\text{-}1^+$ (bright). Within the smaller $OKT8^+$

Table 1. Intraepithelial lymphocytes (IEL) in the jejunum of patients with coeliac disease and in controls

	HLe-1$^+$ IEL % HuTLA$^+$	HuTLA$^+$ IEL % OKT8$^+$	T IEL				OKT8$^+$ IEL % Leu-1
			n	% OKT8$^+$	% OKT4$^+$	*n*	
Controls	99 (99,99,99,100)*	80 (67,83,90)	8	84±12·1†	16±12·1	7	32±17·9
Coeliac disease—normal diet	95 (91,94,97,97)	90 (88,89,94)	6	93±2·6	7±2·6	6	56±7·3‡
Coeliac disease—gluten free diet	92 (88,88,97)	89 (87,89,92)	7	91±2·4	9±2·4	7	33±15·8§

* Individual values in parentheses.
† mean ±s.d.; *n* is the number of specimens studied.
‡ $P<0·05$ *vs* controls.
§ $P<0·02$ *vs* untreated coeliac disease.

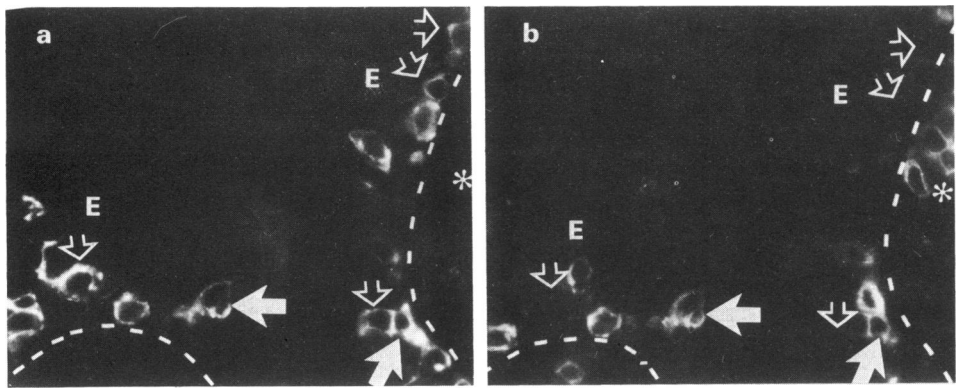

Fig. 1. Section of normal jejunum, labelled for (a) OKT8 (TRITC) and (b) anti-Leu-1 (FITC). The epithelium (E) contains OKT8$^+$ cells, some of which are Leu-1$^+$ (closed arrows) but most of which are Leu-1$^-$ (open arrows). OKT8$^-$ Leu-1$^+$ (OKT4$^+$) cells are also present within the epithelium and in the lamina propria (asterisk). In all figures, the broken line represents the basement membrane.

Table 2. Lamina propria lymphocytes (LPL) in the jejunum of patients with adult coeliac disease and in controls

	HuTLA$^+$ LPL % OKT8$^+$	T LPL				OKT8$^+$ LPL % Leu-1$^+$
		n	% OKT8$^+$	% OKT4$^+$	*n*	
Controls	31 (27,33,33)*	8	31±4·0†	69±4·0	5	33±7·9
Coeliac disease—normal diet	42 (37,44,46)	6	40±4·8‡	60±4·8‡	6	42±8·4
Coeliac disease—gluten free diet	46 (45,46,48)	7	33±7·0	67±7·0	7	33±13·6

* Individual values in parentheses
† mean ±s.d.; *n* is the number of specimens studied.
‡ $P<0·01$ *vs* controls.

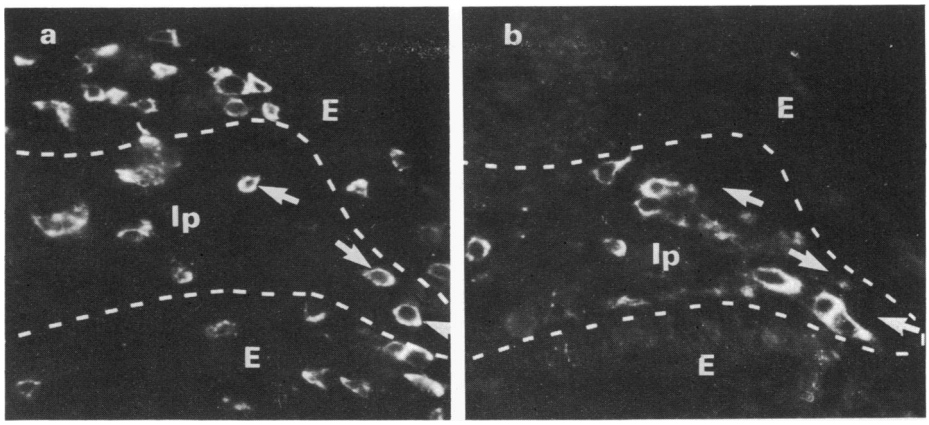

Fig. 2. Untreated coeliac disease. (a) OKT8; (b) OKT4. Within the epithelium (E) there are numerous OKT8$^+$ lymphocytes. No OKT4$^+$ IEL are present. The lamina propria (lp) contains OKT8$^+$ cells (arrows) and OKT4$^+$ cells.

population, only about one-third of cells had detectable, and variable, reactivity with anti-Leu-1. Scattered Tac$^+$ and C3RTO5$^+$ lymphocytes were seen in the lamina propria. Several HNK-1$^+$ cells (< 1 per high power field) were found in the same subject who had HNK-1$^+$ cells in the epithelium.

Coeliac disease. As in the control subjects, almost all IEL were HuTLA$^+$ and UCHT1$^+$. The proportion of HuTLA$^+$ OKT8$^+$ IEL was also similar to that found in controls, although values were at the upper end of the normal range (Table 1, Fig. 2). The proportion of OKT8$^+$ IEL which were Leu-1$^+$ was almost twice that found in the tissues of histologically normal controls, (56 *vs* 32%; $P < 0{\cdot}05$) (Fig. 3). The staining of Leu-1 on OKT8$^+$ IEL was also brighter than in controls. After treatment, the proportion returned to normal values ($P < 0{\cdot}02$, *vs* untreated). This finding was not specific for coeliac disease, as in one control subject the proportion of OKT8$^+$ Leu-1$^+$ IEL was as high as in the coeliac patients (63%; a male of 33 years, with sarcoidosis on prednisolone 5 mg/day orally).

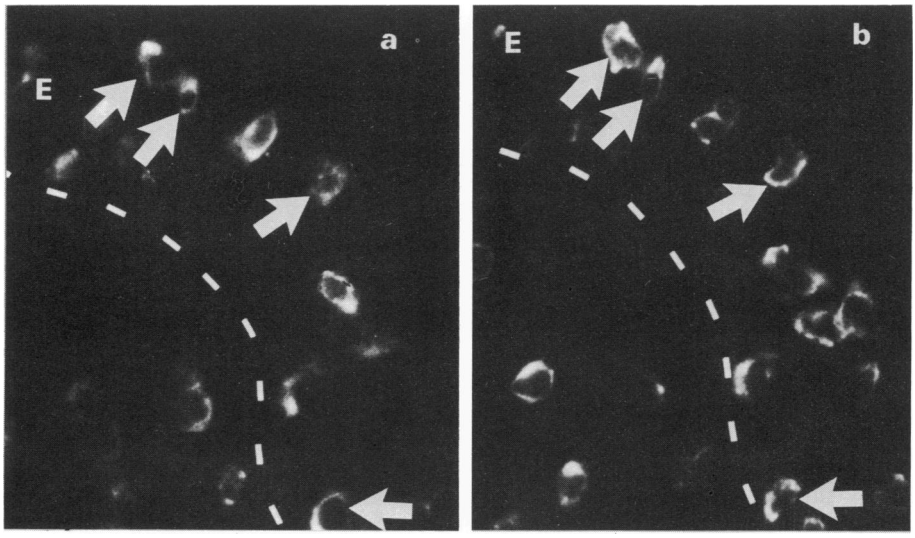

Fig. 3. Untreated coeliac disease. (a) OKT8$^+$ cells are seen within the epithelium (E). (b) Most of these are Leu-1$^+$ (arrows).

Table 3. Peripheral blood lymphocyte subpopulations

	WBC	Lymphocytes		OKT3+		OKT4+		OKT8+		OKT4+	OKT3+
	No.*	%	No.	%	No.	%	No.	%	NO.	OKT8+	%HLA-DR+
Normal subjects ($n=13$)	7·3±2·4†	34±8·9	2·41±0·81	71±6·4	1·72±0·66	47±5·8	1·15±0·44	20±5·1	0·49±0·21	2·49±0·70	1·4 (0–5·6)‡
Disease controls ($n=12$)	7·2±2·2	29±8·5	2·04±0·87	69±7·7	1·41±0·64	46±8·6	0·96±0·55	20±7·1	0·41±0·23	2·71±1·30	2·2 (0–6·8)
Coeliac disease—normal diet ($n=9$)	7·0±2·2	24±7·4¶	1·64±0·81§	69±7·0	1·14±0·59§	42±10·4	0·74±0·56§	25±8·0	0·37±0·12	2·00±1·34	2·1 (0–6·3)
Coeliac disease—gluten free diet ($n=8$)	5·0±1·6	30±8·6§	1·60±0·56§	68±9·2	1·13±0·51§	41±14·0	0·70±0·45§	22±7·2	0·36±0·17	2·13±1·43	1·9 (0–3·2)

* Absolute number $\times 10^{-9}$/l.
† Mean ±s.d.
‡ Range in parentheses.
§ $P<0·05$ *vs* normal.
¶ $P\leqslant 0·01$ *vs* normal.

IEL in coeliac mucosa did not show increased expression of 'activation' antigens such as HLA-DR or Tac. No surface or cytoplasmic Ig of any class was detected on IEL. Furthermore, staining for C3b receptor (C3RTO5), thymocyte antigen (NA1/34) or NK type cells (HNK-1) was again negative.

Within the lamina propria, the proportion of $OKT8^+$ lymphocytes was slightly, but significantly, increased compared with normal tissue (40 *vs* 32%; $P<0{\cdot}01$) (Table 2). This value, again, fell to normal after treatment. The proportion of $OKT8^+$ $Leu\text{-}1^+$ T cells in the lamina propria tended to be higher in untreated patients, but the difference did not reach the level of significance. Only very rare $HNK\text{-}1^+$ cells were found.

Peripheral blood

The total white blood cell count in patients with untreated coeliac disease was normal. However, the proportion of circulating lymphocytes was reduced when compared to the values seen in normal subjects ($P<0{\cdot}01$; Table 3). The proportions of $OKT3^+$, $OKT4^+$ or $OKT8^+$ lymphocytes in the peripheral blood did not differ significantly from those found in controls. Following treatment, the white cell count tended to fall but the proportions of lymphocytes, T lymphocytes and their subsets remained similar to the control values. The absolute number of circulating T lymphocytes ($OKT3^+$) was significantly below normal in both groups of coeliac patients ($P<0{\cdot}05$). The number of $OKT4^+$ cells was also reduced in patients both before and after treatment ($P<0{\cdot}05$). None of these values differed significantly from those found in the disease control group. In six untreated and four treated coeliacs, and in four controls, the Leu-1 antigen was seen on $>75\%$ of circulating $OKT8^+$ lymphocytes, and on all $OKT4^+$ lymphocytes.

The proportion of $OKT3^+$ lymphocytes expressing HLA-DR or Tac antigens was very low in coeliac patients, and not different from normal (Table 3).

DISCUSSION

The results of this study illustrate the heterogeneity of mucosal lymphocyte subpopulations in normal and in diseased human intestine, and extend previous findings (Selby *et al.*, 1981a). Within the epithelium $HuTLA^+$ $UCHT1^+$ $OKT8^+$ $OKT4^-$ T cells predominate, the remaining intraepithelial T cells (and the majority of lamina propria T cells) being $OKT4^+$ $OKT8^-$. Of considerable interest is the observation that only a minority of $OKT8^+$ IEL in histologically normal small intestine (except in one case) react with anti-Leu-1, a monoclonal antibody which, like OKT1, detects a 67,000 dalton antigen on nearly all peripheral blood T cells and on thymocytes (Martin *et al.*, 1981; Janossy *et al.*, 1981). Combination of OKT8 and OKT1 on tissue sections in two subjects gave similar results to those using OKT8 and anti-Leu-1 (data not shown). Although no Leu-1 reactivity was detected on these $OKT8^+$ cells, it is possible that the expression of the antigen is too weak to be detected by immunofluorescence. Nevertheless, distinction between two subclasses of $OKT8^+$ lymphocytes is evident within the mucosa.

Study of IEL isolated from the small intestinal mucosa of rats has demonstrated that most IEL are labelled with MRC OX8 (which detects suppressor–cytotoxic T cells) but not with W3/13, an antibody which is thought to detect all peripheral blood T cells (Lyscom & Brueton, 1982). If the latter antibody reacts with an antigen similar to Leu-1, the reported discrepancy between rat and human IEL may be explained. Further studies of rat IEL using other anti-T reagents, e.g. OKT3 like, may be necessary.

The significance of the $OKT3^+$ $Leu\text{-}1^-$ population in the intestine is unknown. Leu-1 antigen expression is gained during maturation in the thymus, being weak on cortical thymocytes (Janossy *et al.*, 1981; Ledbetter *et al.*, 1981). Thus $OKT8^+$ $Leu\text{-}1^-$ intestinal cells may be relatively immature forms. However, these T cells neither express thymocyte antigen (NA1/34) nor are they $OKT8^+$ $OKT4^+$, and are thus unlike cortical thymocytes (Reinherz & Schlossman, 1980). The expression of Leu-1 may alternatively be weaker on $OKT8^+$ cells at their late stages of differentiation. In the mouse, it has been demonstrated with the equivalent Lyt antigens (Ledbetter *et al.*, 1981) that precursors of cytotoxic T cells are $Lyt\ 1^+23^+$ while cytotoxic effector cells are $Lyt\ 1^-23^+$ (Cantor &

Boyse, 1977). However, this may not apply for all strains (Beverley *et al.*, 1976). In humans, the 67,000 dalton antigen is also found on precursor cytotoxic T cells, whereas its expression on effector cells is variable (Hansen *et al.*, 1981). Similar heterogeneity has been demonstrated within suppressor T cell populations in the mouse (Germain & Benacerraf, 1981). There is no evidence as yet that the $OKT8^+$ $Leu\text{-}1^-$ population in man is committed to either a suppressor or cytotoxic role.

The lack of HLA-DR or Tac antigen expression argues against the loss of Leu-1 expression being a result of activation. Mucosal $OKT8^+$ T cells also fail to express HNK-1 antigen, found on a population of natural killer cells (Abo & Balch, 1981).

In contrast to intraepithelial $OKT8^+$ cells, in the peripheral blood as well as in the tonsil, lymph node and bone marrow, $OKT8^+$ T cells are predominantly $OKT1^+$ ($Leu\text{-}1^+$) (Janossy *et al.*, 1982), the $OKT8^+$ $OKT1^-$ population being only a small subset. However, $OKT8^+$ $OKT1^-$ T cells have been found in the circulation of patients with lymphocytosis and bone marrow dysfunction (Callard *et al.*, 1981) as well as in the involved skin of patients with graft versus host disease, and in liver biopsies from patients with chronic hepatitis B or primary biliary cirrhosis (Janossy *et al.*, 1982).

The functions of human IEL are unknown. IEL isolated from the intestine of animals are capable of cytotoxicity (Arnaud-Battandier *et al.*, 1978; Davies & Parrott, 1981). Mitogen-induced cytotoxicity, a non-specific T cell function, has been shown by human IEL (Chiba *et al.*, 1981). They may also be important in processing intraluminal antigens (Selby *et al.*, 1981b). The intestinal microenvironment is ideally suited to the function of suppressor–cytotoxic T cells in the epithelium since the epithelium exhibits strong expression of HLA-A,B,C antigens, but only weak expression of HLA-DR antigens (Selby *et al.*, 1981a). The small population of helper type T cells within the epithelium may add further control over the functions of $OKT8^+$ IEL.

Recent observations indicate that in infant mice IEL function as NK cells, but that this function is lost by 17 weeks of age (Tagliabue *et al.*, 1981). Although $HNK\text{-}1^+$ IEL are rarely seen in normal intestinal mucosa, it is possible that this cell population is lost by adulthood in man.

Within the lamina propria, $OKT4^+$ helper type T cells are predominant. The $OKT8^+$ population resembles that found in the epithelium, i.e. containing mostly $HuTLA^+$ $UCHT1^+$ $OKT8^+$ $Leu\text{-}1^-$ $OKT4^-$ cells. This suggests that they may, at least in part, be in transit to (and from) the epithelium.

In patients with coeliac disease, as in normal controls, almost all IEL are $Hle\text{-}1^+$ $UCHT1^+$ $OKT8^+$ $OKT4^-$, with a small population of $OKT4^+$ $OKT8^-$ cells. However, the proportion of $OKT8^+$ cells that express Leu-1 is approximately twice that seen in control tissues. This increase is not specific to coeliac disease, being seen also in one control subject. The significance of this finding is unknown. IEL in coeliac disease show evidence of activation, and of increased passage across the epithelial basement membrane (Marsh, 1980). The greater proportion of $OKT8^+$ $Leu\text{-}1^+$ IEL in coeliac mucosa may reflect this increased cell traffic, with the entry into the epithelium of a cell population at an earlier stage of differentiation. Although it is not known how the functions of IEL in coeliac disease differ from those in normal subjects, it has been postulated that they participate in an immune response to dietary gluten (Ferguson & Murray, 1971). Thus, the $OKT8^+$ $Leu\text{-}1^+$ cells may indicate the arrival of a functionally distinct cell population responding to gluten. Any alteration in the function of IEL is not reflected in an imbalance between $OKT8^+$ and $OKT4^+$ cells as compared with normal intestine.

Within the lamina propria, the proportion of $OKT8^+$ cells is increased, albeit slightly. This finding may, again, reflect the increased traffic of $OKT8^+$ T cells through the lamina propria into, and out of, the epithelium. Consistent with this is the observation that the proportion of $OKT8^+$ $Leu\text{-}1^+$ lymphocytes in the lamina propria also tends to be increased.

The changes seen in patients with untreated coeliac disease all return to normal when patients are on a gluten free diet, indicating that the alterations are not primary to the disease process.

The importance of analysing local mucosal T cell populations is emphasized by the finding that lymphocytes in the peripheral blood of patients with coeliac disease are not representative of those seen in the jejunum. Although the proportions of T lymphocytes and their subsets in the circulation of patients with coeliac disease do not differ from controls, the absolute numbers of these cells are reduced when compared with normal volunteers, but not when compared with disease control subjects. These changes in the peripheral blood are not specific for patients with coeliac disease, as

similar findings have been shown in patients with inflammatory bowel disease (Selby & Jewell, 1982).

The proportion of peripheral blood T cells which express HLA-DR antigens is not altered in patients with coeliac disease. This is in contrast to the findings in another HLA-B8, DR-3 associated disorder, type 1 diabetes, where the proportion of HLA-DR$^+$ T cells in the circulation is increased (Jackson *et al.*, 1982). This indicates that HLA-DR$^+$ T cells are not essential in the pathogenesis of all disorders associated with these histocompatibility antigens.

At the present time, isolation of small intestinal mucosal populations, particularly IEL, is difficult. This is especially so in patients with coeliac disease because of the limited amount of tissue available. Thus, further advances in our knowledge of the nature of the normal gut immune system, and its role in the pathogenesis of disorders such as coeliac disease, may depend to a large extent on additional studies using immunohistological techniques with antibodies of increasing specificity for functionally distinct cell populations.

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