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The tissue distribution of T lymphocytes expressing different CD45 polypeptides

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SUMMARY

The distribution of T lymphocytes expressing the different polypeptides of the leucocyte common antigen (LCA) family detected by CD45R and UCHL1 antibodies has been studied in normal lymphoid tissues. In the thymus most cortical thymocytes express UCHL1 and co-express CD4 and CD8. The more mature membrane CD3$^+$ (mainly medullary) T cells are heterogeneous and may express both UCHL1 and CD45R weakly or be restricted to display CD45R or UCHL1 alone. In the medulla both the CD45R$^+$ and UCHL1$^+$ subpopulations contain single positive CD4 and CD8 cells. In tonsils, germinal centre T cells are almost exclusively UCHL1$^+$, CD4$^+$ and a proportion also express HNK-1 (Leu 7) antigen. In the paracortical areas approximately equal numbers of CD45R$^+$ and UCHL1$^+$ cells are found but these separately occupy nests of cells containing one or the other type. Again, both CD45R$^+$ and UCHL1$^+$ cells include single CD4$^+$ and CD8$^+$ lymphocytes. A small proportion ($<5\%$) of strongly CD45R$^+$, UCHL1$^+$ double-stained cells are also seen, and these probably represent recently activated lymphocytes. In the gut, small clusters of such strongly double-labelled cells are in the submuscular mucosae while cells of the lamina propria are almost exclusively UCHL1$^+$. Many intra-epithelial lymphocytes are only weakly positive or negative for UCHL1 and appear to be CD45R$^-$. These results are consistent with the view that expression of different CD45 polypeptides identifies successive stages of thymocyte–T-cell maturation and that following their thymic education, unprimed T lymphocytes are CD45R$^+$, while primed memory T cells are UCHL1$^+$. These populations occupy different microenvironments.

INTRODUCTION

Monoclonal antibodies (mAb) to leucocyte cell surface antigens have allowed the separation of distinct subpopulations of leucocytes and have thrown light on the function of the molecules themselves. CD4 and CD8 mAb delineate two major T-cell subsets and direct evidence from binding studies suggests that the CD4 and CD8 molecules play a role in determining one of the most characteristic properties of these subsets, their interaction with class I or II major histocompatibility complex antigens (Doyle & Strominger, 1987). Several mAbs reveal further heterogeneity within the CD4 and CD8 subsets (reviewed by Beverley, 1987). Some authors have suggested that this heterogeneity may represent further functionally divergent subpopulations such as the helper and suppressor inducer subsets of CD4 (Morimoto *et al.*, 1985a, b), while others consider that some of the phenotypic differences among both CD4 and CD8 T cells represent changes occurring during maturation. Thus the antigens defined by the CD45R mAb group are probably associated with unprimed virgin T cells in the rat (Arthur & Mason, 1986; Powrie & Mason, 1988), while mAbs 5/9 and Ta1 have been suggested to identify memory T cells (Corte *et al.*, 1982; Hafler *et al.*, 1986). This view is supported by the separation of T cells at different stages of maturity by mAbs HB10 and HB11 into functionally different subsets which produce distinct cytokines (Tedder, Cooper & Clement, 1985). It has recently become clear that some mAbs, such as 2H4 (Morimoto *et al.*, 1985, a, b) and UCHL1 (Smith *et al.*, 1986), which have been used to identify subsets within CD4, are directed to different components of the leucocyte common antigen (LCA or T200) complex (Beverley, 1987; Thomas & Lefrancois, 1988).

Several of these mAbs, including 2H4, HB10, 4KB5, RFB2 and SN130, etc., are restricted to precipitate only the polypeptides of 220,000 MW and 205,000 MW (Cobbold, Hale & Waldmann, 1987). These reagents show particularly strong reactivity with B lymphocytes both in the mouse (Kincade *et al.*,

Abbreviations: DC, duoCHROME; FITC, fluorescein isothiocyanate; GAM, goat anti-mouse; LCA, leucocyte common antigen; mAb, monoclonal antibody; PBS, phosphate-buffered saline; PE, phycoerythrin; TdT, terminal deoxynucleotidyl transferase.

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1981) and in humans and also react with a proportion of T cells but not with monocytes or granulocytes. In this paper these mAbs are referred to as CD45R (p220, 205). On the other hand, UCHL1 reacts with a LCA polypeptide of 180,000 MW and stains monocytes, granulocytes as well as a proportion of T cells. Furthermore, double-labelling studies on the T-lymphoid populations have clearly established that the CD45R (p220, 205)- and UCHL1-positive populations are reciprocal in the normal peripheral blood (Beverley, 1987; Akbar *et al.*, 1988).

The subset heterogeneity of CD45R$^+$ and UCHL1$^+$ T cells seems to be important because these cells differ in their functions *in vitro*. This concept has been introduced by the original observation of Morimoto *et al.* (1985a) and Tedder *et al.* (1985; see above) and has been substantiated by the demonstration of the UCHL1$^+$ cells' selective response to recall antigens (Smith *et al.*, 1986). In addition, we have shown that when CD45R$^+$, UCHL1$^-$ cells are stimulated they acquire UCHL1 and lose CD45R (Akbar *et al.*, 1988). This has led us to propose that UCHL1, like 5/9 and Ta1 is a marker for primed memory T cells. Thus expression of different CD45 polypeptides appears to be developmentally regulated and changes are closely associated with activation or maturation in T cells. These molecules are derived by alternative splicing from a single gene (Ralph *et al.*, 1987; Thomas & Lefrancois, 1988).

In this paper our working hypothesis has been that if changes of CD45R/UCHL1 expression are indeed relevant to the efficient function of the immune system, then the tissue distribution of the CD45R$^+$ and UCHL1$^+$ populations may also be different and could reveal the sites of the generation, as well as the recirculation patterns, of putative virgin and memory T cells. We have therefore examined the distribution of CD45R and UCHL1 antigen-expressing cells in the thymus and peripheral lymphoid tissues, including the gut, both by immunocytochemistry and two-colour immunofluorescence (IF). In these studies both combinations of the CD45 antibodies, with each other and also with T-cell-associated antibodies such as CD3, CD4 and CD8, have been applied. It is also known that the expression of UCHL1 on T-lineage cells is biphasic: strong UCHL1 positivity first appears in the thymus on cortical thymocytes and is then lost on naïve peripheral T cells prior to the emergence of UCHL1 molecules during the activation of cells by antigens and mitogens (Beverley, 1987). Nevertheless the tissue localization of these sequential changes in relation to other markers such as nuclear TdT and membrane CD3 (mCD3) has not yet been studied.

MATERIALS AND METHODS

Tissues

Thymi were taken from six fetuses at 19–20 weeks of gestational age, from two infants of 1–5 years undergoing open heart surgery, and from three adults who had mild symptoms of myasthenia gravis and unaffected thymus glands as judged by immunohistology. Tonsils and jejunal gut biopsies were obtained at surgery. Tissues were snap-frozen in isopentane. Serial 6-μm sections were cut and were dried for >1 hr at room temperature prior to staining. From selected thymic and tonsil samples suspensions were prepared in order to quantify the cells with double IF labelling on the fluorescence microscope and triple IF labelling on a FACScan (see below).

Biotinylation

MAbs were dialysed into 0·1 M sodium bicarbonate and the concentration adjusted to 1 mg/ml. Three microlitres of Biotin NH-X (Sigma, Poole, Dorset) at 1 mg/ml in DMSO (Sigma) were added per ml of mAb solution and the mixture incubated for 4 hr at room temperature. The conjugate was then dialysed overnight into phosphate-buffered saline 'A' (PBS; Oxid, Basingstoke) containing 0·1% sodium azide and the optimal concentration determined by titration.

Immunocytochemical staining

All incubations were with 25 μl of antibody at room temperature unless stated otherwise. The slides were washed in PBS between layers. The test mAb was followed by rabbit anti-mouse immunoglobulin (Dako, High Wycombe, Bucks) at a concentration of 1/100 (optimal concentration determined by titration) containing 1/25 normal human serum. After washing in PBS the slides were incubated with an alkaline phosphatase anti-alkaline phosphatase conjugate containing 10 mg/ml alkaline phosphatase type I (Sigma) in tissue culture supernatant containing anti-alkaline phosphatase mAb. The slides were washed again in PBS, then incubated with 3% normal mouse serum for 30 min and washed with PBS. Slides were incubated with biotinylated UCHL1, washed, incubated with avidin-biotin-peroxidase complex (Vectastain, Sera Labs, Crawley Down, Sussex) and washed again. The peroxidase staining was developed with diaminobenzidine hydrochloride (Sigma Isopac) diluted to 0·6 mg/ml in 0·2 Tris, pH 7·6, and filtered into imidazole (Sigma) to give a concentration of 1 mg imidazole per ml of diaminobenzidine. Slides were incubated in this solution of 3 min at room temperature, and washed in running tap water for 5 min. Finally, the alkaline phosphatase staining was developed with a solution containing 5 mg napthol AS phosphate (Sigma) in 480 μl dimethyl formamide (BDH, Dagenham) and 6 mg/ml Levamisole (Sigma) in 50 μl distilled water; 100 μl of this were added to 5 ml of a 1 mg/ml solution of fast blue in 0·2 M Tris-HCl, pH 9·1. The solution was filtered onto the slides which were incubated for 15 min. They were then rinsed in tap water and mounted in Apathy's mounting medium.

Immunofluorescence staining

For direct immunofluorescence, CD45R (2H4, directly conjugated with phycoerythrin; 2H4-PE, Coulter, Luton, Beds) and UCHL1 (labelled with fluorescein-isothiocyanate; UCHL1-FITC, locally prepared) were used. Cell suspensions or sections were incubated for 30 min, washed and mounted without drying. For indirect immunofluorescence (IF) studies in cell suspensions, CD45R (SN130, IgG2 class) or UCHL1 (IgG2) were used in combination with CD3 (T10B9μ, a gift of Professor J. S. Thompson and Dr S. Brown, University of Kentucky), CD4 (OKT4Bμ, Ortho, Raritan, U.S.A.) and CD8 (RFT8μ, Royal Free Hospital, London) or Leu 7 (HNK-1μ, ATTC, Rockville, MD) antibodies of IgM class (Janossy, Bofill & Poulter, 1985). The second layers were goat anti-mouse IgG (GAMμ; Seralab) and goat anti-mouse IgM (GAMμ; Seralab) conjugated with FITC or tetramethyl-rhodamine isothiocyanate (TRITC). In suspensions of thymus the observations of 2-colour IF on the microscope were confirmed by the triple-colour IF studies performed on a FACScan (Becton-Dickinson, Mountain View, CA). The third colour was provided by biotinylated mAbs detected by streptavidin-duo CHROME

Table 1. The percentages of cells expressing TdT, CD3, B-cell markers and CD45-associated molecules in the thymus

Samples		TdT^+	$mCD3^{+}$*	$UCHL1^+$ TdT^-	$CD45R^+$ TdT^-	sIgM†	CD20†	RFB7†
Fetal 19 weeks	1	61	24*	5·0	NT	0·1	0·2	0·2
20 weeks	2	57	44*	7·2	11·1	0·4	0·5	0·6
20 weeks	3	60	42*	5·7	NT	0·1	0·2	0·2
Infant	1	72	40*	7·9	9·2	0·3	0·3	0·4
Infant	2	71	37*	14	7·1	0·3	0·35	0·4
Adult	1	57	35‡	27	3·0	1·0	NT	1·5
Adult	2	42	40‡	24	17·0	8·4	12·0	9·1
Adult	3	39	51	19	22	11·5	15	20

* These cells correspond to the + cells in Fig. 2d, e. TdT^+, $mCD3^+$ double-labelled cells are 5, 14, 12, 10 and 16%. mCD3: membrane CD3.

† These cells are strongly $CD45R^+$, $UCHL1^-$.

‡ TdT^+, $mCD3^+$ double-labelled cells are 6% and 11%.

(Becton-Dickinson). The thymus samples were run in parallel with the suspensions of peripheral blood mononuclear cells gated on lymphocytes. This was necessary to set the levels of $CD45R^+$ and $UCHL1^+$ cells which in the blood represent >95% separate populations. On the FACScan detector and compensation levels were set using, firstly, CallBRITE beads (Becton-Dickinson) in conjunction with the AutoCOMP software (Becton-Dickinson) and then, secondly, peripheral lymphocytes stained with anti-Leu 12 (CD19), FITC (Becton-Dickinson), anti-Leu 2a (CD8), PE (Becton-Dickinson) and anti-Leu 3a (CD4) Biotin DuoCHROME (Becton Dickinson). Data were acquired in list mode by FACScan Research software for three-colour and Consort 30 software for two-colour analyses. The three-colour observations have been analysed by the Paint-A-Gate software programme. The compensation values for fluorescence channel (FL)1-FL2 and FL2-FL1 were 0·7% and 28·4%, while for FL2-FL3 and FL3-FL2 were 56·9% and 22·9%. In order to complement these studies with the investigation of nuclear antigens, cytocentrifuge preparations were also made. In these studies the total percentage of T-lineage cells was determined by combination staining for nuclear terminal deoxynucleotidyl transferase (TdT) using rabbit anti-TdT and goat anti-rabbit-Ig-TRITC for detecting immature thymocytes together with membrane CD3 (mCD3) staining using T10B9 (CD3, μ) and GAM-FITC (Seralab) for detecting mature thymic cells (Campana *et al.*, 1987, 1989). Once the proportions of these cells were known, the $mCD3^+$ (GAMμ-TRITC) cells were studied with CD45R(γ) or UCHL1(γ) or with the mix of the two mAbs and class-specific second layers (GAMγ-FITC).

RESULTS

Thymus

The main features of thymic staining have been that while CD45R staining was confined to the medulla, except for scattered strongly positive cortical cells, UCHL1 labelled the majority of cortical and a proportion of medullary thymocytes as well (Fig. 1a). Cells in the outermost cortical layer were often only weakly stained by UCHL1 corresponding to some thymic blast cells present in the subcapsular region. Double-label experiments combining UCHL1 with CD4 or CD8 (not shown) have confirmed that most cortical thymocytes expressed all three antigens. These thymocytes are immature TdT^+ cells. Because of the close packing of cortical thymocytes it has been difficult to determine, using double-label immunohistochemistry, whether the few strongly stained cortical $CD45R^+$ cells also expressed CD4 (Fig. 1b) and CD8.

In order to define the features of mature medullary thymocytes two approaches have been used. First, immunohistological studies of the medulla in infant thymus have shown that 30–40% of cells are either $CD45R^+$ or $UCHL1^+$ but a significant proportion of medullary cells are only weakly stained by either mAb if at all (Fig. 1a). Double labelling in sections has also indicated that 30–45% of medullary $CD45R^+$ cells are $CD4^+$ (Fig. 1b) and 20–35% are $CD8^+$ (not shown). The medullary $UCHL1^+$ cells have also been found to be heterogeneous: 24–33% are $CD4^+$ and 10–15% $CD8^+$, while non-T cells such as medullary epithelium and interdigitating cells lack both CD45R and UCHL1. The few B cells present are strongly $CD45R^+$ but $UCHL1^-$.

In the second part of the study cell suspensions have been investigated with both flow cytometry and TdT/membrane CD3 double-IF on cytocentrifuge preparations (Table 1). The flow cytometric studies have been calibrated on normal blood mononuclear cells (Fig. 2a) and lymphoblasts stimulated by phytohaemagglutinin for 64 hr (Fig. 2b) gated on the lymphocytes and the large lymphoid scatter, respectively. The discriminating level between $UCHL1^+$ and $CD45R^+$ cells is at 5×10 (UCHL1 label) and 4×10 (CD45R label), clearly separating the $CD45R^+$, $UCHL1^-$ (47%) and the $UCHL1^+$, $CD45R^-$ lymphocytes (46%; Fig. 2a). After stimulation 25–30% of lymphoblasts remain $UCHL1^+$, $CD45R^-$ and most of the $CD45R^+$ cells 'move up' to the strongly double positive category (62% in Fig. 2b; Akbar, Timms & Janossy, 1989).

Among thymocytes 35–62% are TdT^+, $mCD3^-$ (Table 1, Fig. 2d). On the basis of their strong TdT positivity, CD3 negativity (Fig. 2d, e) and their subcapsular location (see above), a population of immature thymic blasts can be identi-

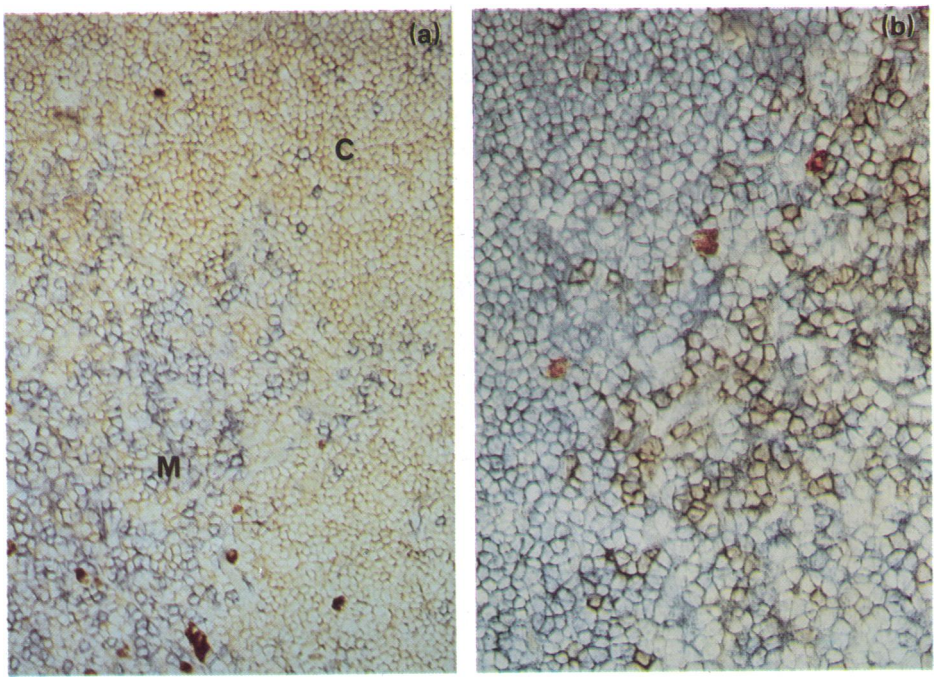

Figure 1. Double immunohistochemical staining of the human thymus with UCHL1 (brown in a), CD45R (blue in a and brown in b) and CD4 (blue in b). In (a) the cortex (C) is mainly UCHL1 positive, and the medulla (M) contains mainly CD45R-positive cells with some doubles. In (b) the cortex is stained predominantly with CD4 (blue) with a few isolated cells which may be brown or double stained. The medulla is a mixture of CD45R alone, CD4 alone and double positives or negatives.

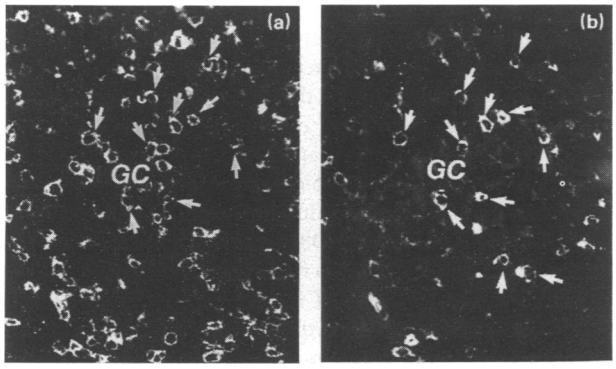

Figure 3. T cells in the germinal centres (GC) of tonsil express UCHL1 (a) and a subset of these cells (arrows) are also Leu 7-positive (b).

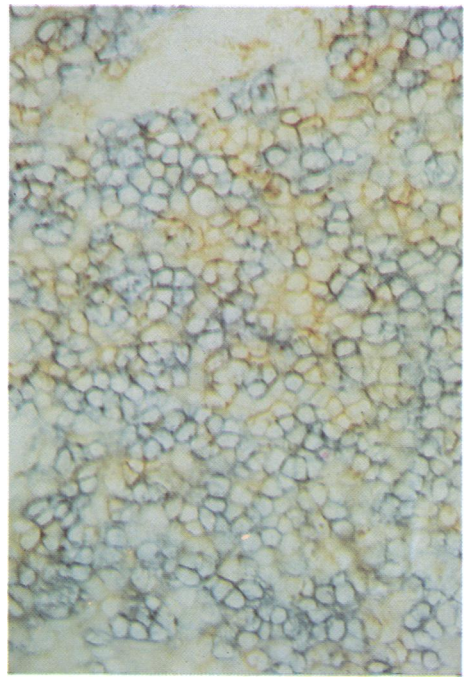

Figure 4. T cells in the paracortical area of tonsil are arranged in nests of $UCHL1^+$ cells (brown) and $CD45R^+$ cells (blue).

fied which appear to be weakly $UCHL1^+$ while expressing some or minimal amounts of CD45R (see arrows in Fig. 2d, e). The typical cortical thymocytes, as expected from histology, are strongly $UCHL1^+$, $CD45R^-$. The weakly $mCD3^\pm$ cells are still TdT^+, albeit weakly (Campana *et al.*, 1989), and show a gradually decreasing expression of UCHL1 but remain $CD45R^-$. These cells correspond to the weakly $UCHL1^+$, $CD45R^-$ population depicted in Fig. 2c. Finally, among the $mCD3^+$ cells, $CD45R^+$ (Fig. 2e), $UCHL1^-$ (Fig. 2d) cells appear. $UCHL1^+$ T lymphocytes are also present in roughly similar proportions to the $CD45R^+$ population (25–45% and 12–50% of $mCD3^+$ cells, respectively), and the observations tend to indicate increasing proportions of $UCHL1^+$ T cells with age (Table 1). These $UCHL1^+$, $CD45R^-$ T cells are more abundant (14–27% of all thymocytes) in the thymi taken from adult patients with mild myasthenia gravis (Table 1). In addition, a few $CD45R^+$, $mCD3^-$ B lymphocytes can also be seen in fetal (0·1–0·4%) and infant (0·3%) thymic samples.

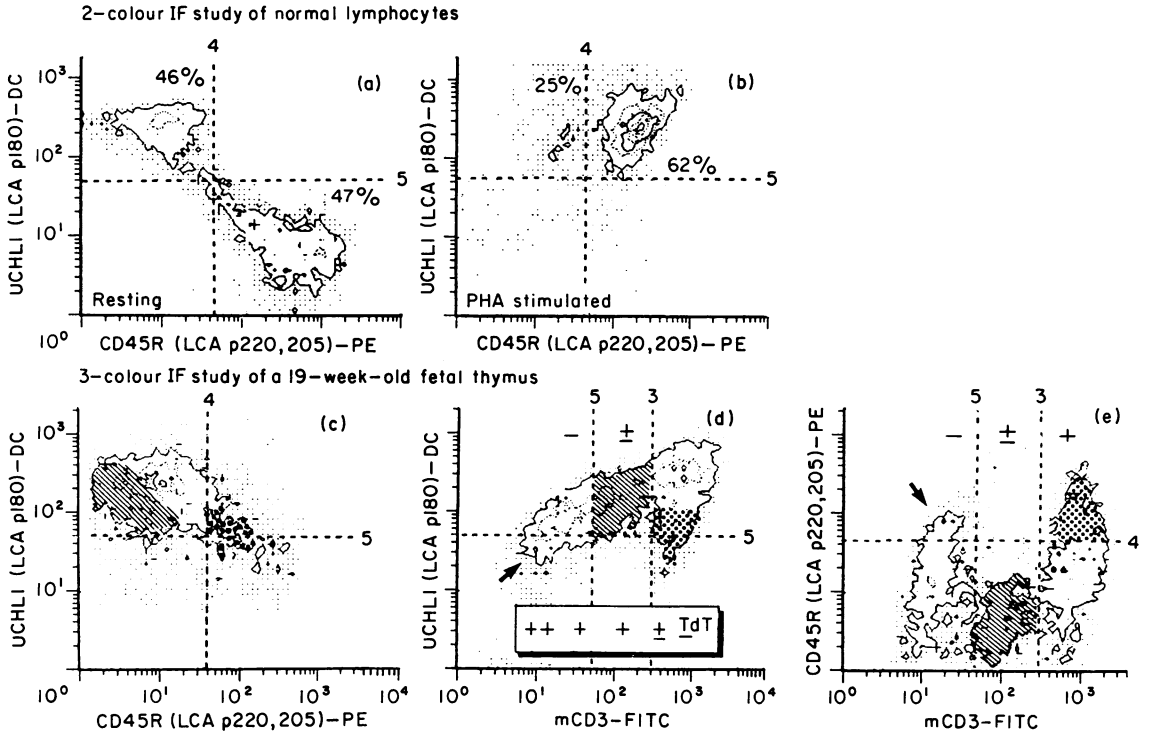

Figure 2. The analysis of resting (a) and stimulated (b) blood lymphocytes as controls and the thymic cell suspensions (c–e) for the expression of restricted LCA antigens. A triple labelling has been performed using CD45R-phycoerythrin (PE; a–c, e), UCHL1-biotin detected by streptavidin-duoCHROME (DC; a–c, d) and mCD3 using T10B9 (IgM class) with GAM-Ig(μ)-FITC second layer (d, e). The levels for positivity have been selected in (a) and (b) and for mCD3 the categories of −, ± and + are distinguished. The mCD3$^+$ (■) and CD45R$^+$ cells (▣) seen in (e) have been identified in (c) and (d) by the 'Paint-a-gate' program. Insert in (d) shows the intensity of nuclear TdT staining as seen in samples double labelled for membrane CD3. Small arrows point to mCD3$^-$, UCHL1$^+$, CD45R$^+$ thymic blasts.

These Ig$^+$ B cells also increase to 3–22% with age, and to higher values in myasthenic disease (Bofill *et al.*, 1985). Finally, the occasional macrophages are weakly UCHL1$^+$.

Tonsils

In tonsils single staining with UCHL1 has confirmed published results showing that UCHL1$^+$ cells are largely confined to the paracortex (Smith *et al.*, 1986), and additional UCHL1$^+$ cells are scattered in the germinal centres and at the junction of the follicle centre and mantle zone. Double staining with CD4 and CD8 has nevertheless revealed a different population structure of UCHL1$^+$ cells at these two sites. In the paracortex, the UCHL1$^+$ cells are a mixture of both CD4$^+$ and CD8$^+$ populations. Only a proportion of CD4$^+$ cells (35–60%) and CD8$^+$ cells (10–33%) are UCHL1$^+$. As a marked contrast, in the germinal centre T cells are uniformly UCHL1$^+$, CD4$^+$ (>90%) and <10% CD8$^+$ and/or UCHL1$^-$. It is known that 30–50% of CD4$^+$ cells in germinal centres also express Leu 7; these are also UCHL1$^+$ (Fig. 3).

It has also been shown previously that high numbers of CD45R$^+$ cells are present in the follicles and germinal centres, but these are almost exclusively B-lineage cells (Ling, MacLennan & Mason, 1987) while at this site the CD3$^+$ T-lymphocyte populations, a minority, appear to be CD45R$^-$. Double staining of CD3, CD4 and CD8 with CD45R has nevertheless shown that among the T cells in the paracortical areas 20–35% of CD4$^+$ and the majority (60–80%) of CD8$^+$ cells are CD45R$^+$.

The double labelling for UCHL1 and CD45R has further demonstrated two additional findings. Firstly, only a few double-labelled cells (1–5% of all T cells) can be detected in these areas, and the majority of cells express either UCHL1 or CD45R alone. Secondly, these two types of cells have not been intermingled randomly but appear to be grouped in small clusters of cells with either UCHL1$^+$ or CD45R$^+$ phenotype (Fig. 4). Additional combinations with CD11c mAb, a marker associated with macrophages or with mAbs to class II antigens, which are expressed on interdigitating cells, have not revealed a consistent relationship of these accessory cells to clusters of the strongly UCHL1$^+$ or CD45R$^+$ T cells. The CD11c$^+$ macrophages themselves are weakly UCHL1$^+$, CD45R$^-$, as was also demonstrated in the thymic medulla (see above).

Gut-associated lymphoid tissue

The lymphocyte populations of the bowel wall differ in several respects from those of peripheral lymphoid tissue. We have examined the staining of lymphoid cells in the lamina propria (LP) where 50–60% of T lymphocytes are CD4$^+$ and 20–30% CD8$^+$. Among the CD4$^+$ cells >95% are UCHL1$^+$ (Fig. 5a) and very few (<5%) are CD45R$^+$ (Fig. 5b). Among the CD8$^+$ cells 85–90% of lymphocytes are UCHL1$^+$ and the rest have

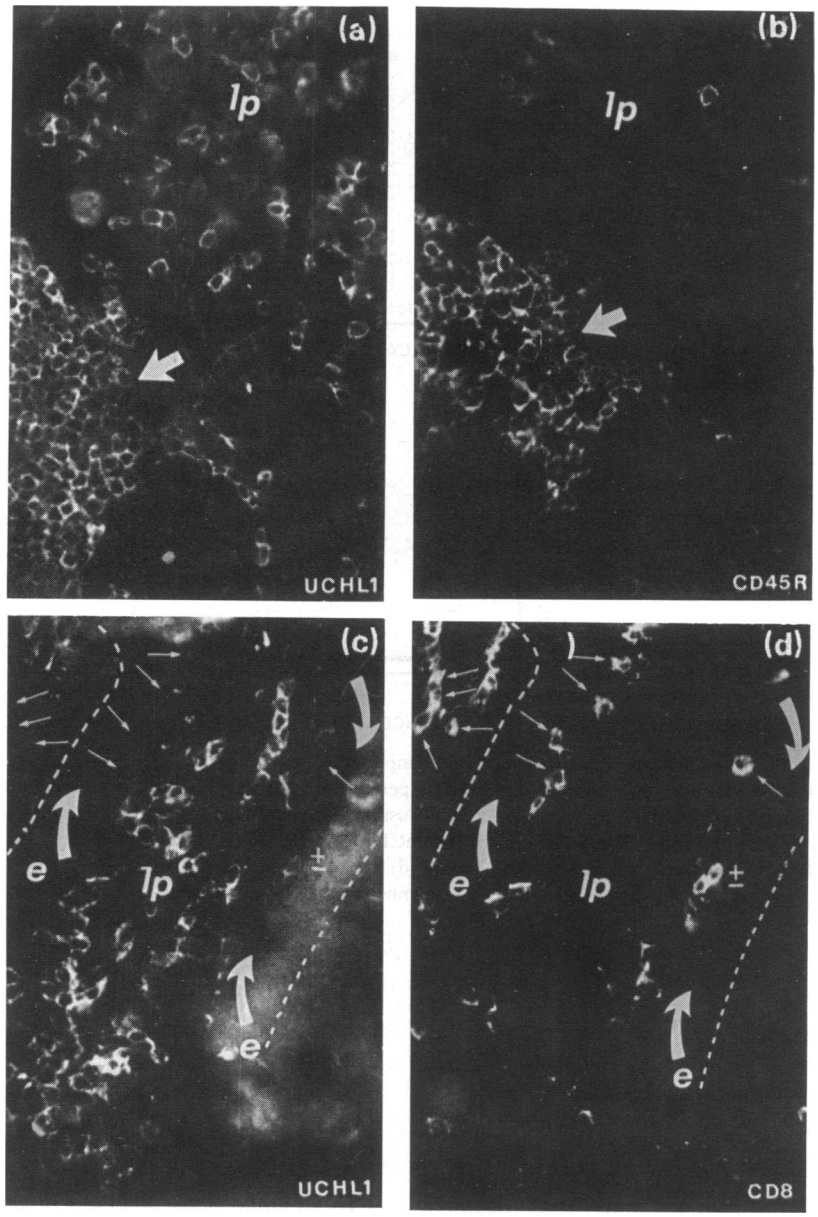

Figure 5. Distribution of UCHL1$^+$ (a, c), CD45R$^+$ (b) and CD8$^+$ (d) cells in the normal jejunum. Cells in the lamina propria (lp) are almost exclusively UCHL1$^+$ but the majority of intra-epithelial lymphocytes (small arrows in c, d) are UCHL1$^-$. These cells are also CD45R$^-$. As a marked contrast, adjacent sections show that T-lymphocyte cell aggregates in the submuscular mucosae (large arrow) are UCHL1$^+$, CD45R$^+$. The gut lumen is shown by a broken line. The row of epithelial cells is indicated by arrow and in (c) and (d).

appeared to be CD45R$^-$, UCHL1$^-$ or expressed these antigens very weakly (double 'negatives'; $<2\%$ CD45R$^+$, CD8$^+$ cells).

Intra-epithelial lymphocytes are another unique population in the gut. These cells are mostly CD8$^+$ and also express conventional CD45 (Selby *et al.*, 1983) but very few express detectable CD45R and only 35–60% stain with UCHL1. These findings indicate the existence of 40–65% CD8$^+$ cells which are either negative for both CD45R and UCHL1 or express these antigens very weakly (Fig. 5c, d).

Finally, in two gut samples lymphoid nodules have been seen beneath the lamina propria. These small clusters have consisted of T lymphocytes (CD3$^+$) which strongly exhibited both UCHL1 and CD45R on $>65\%$ of cells (Fig. 5a, b) and some of them appeared to be lymphoblasts. These small clusters were present through three to four sections only, so further studies will be required to assess the proliferative activity and CD25(Tac) expression of these double-stained cells.

DISCUSSION

In recent years mAbs have been described which identify further heterogeneity within the major CD4 and CD8 subsets of T lymphocytes. Some authors have taken the view that this heterogeneity indicates the existence of further functionally

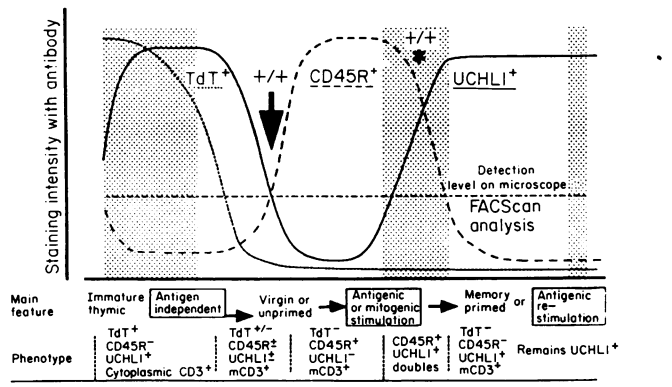

Figure 6. T-cell development in man as defined by mAb to LCA such as CD45R and UCHL1 (hypothesis). Immature cortical thymocytes appear to lose TdT gradually and decrease their UCHL1 expression while acquiring membrane CD3 (mCD3$^+$). At this transitional stage immature T cells are weakly UCHL1$^\pm$/CD45R$^\pm$. The recently produced unprimed T lymphocytes are CD45R$^+$, UCHL1$^-$. These cells, when stimulated by antigens/mitogens, acquire UCHL1 and develop into primed cells through a CD45R$^+$, UCHL1$^+$ stage. Stippled areas indicate the proliferative stages of T lymphoid development (Campana *et al.*, 1989).

distinct, stable subpopulations within CD4 and CD8 (Morimoto *et al.*, 1985a, b) while others consider that phenotypically distinct subpopulations represent stages of maturation of peripheral T cells (Corte *et al.*, 1982; Tedder *et al.*, 1985; Hafler *et al.*, 1986; Beverley, 1987). We have argued elsewhere (Akbar *et al.*, 1988) that CD45R mAbs and UCHL1 identify successive stages of T-cell development. This argument is based principally on three observations: (i) all responses to recall antigens are found in the CD45R$^-$ or UCHL1$^+$ subset (Smith *et al.*, 1986; Morimoto *et al.*, 1985b); (ii) CD45R$^+$ cells become UCHL1$^+$ on stimulation with mitogen or alloantigen (Terry, Pickford & Beverley, 1987); and (iii) when alloantigen-primed CD45R$^+$ cells are restimulated with the same allogeneic cells, the recall response is found in the UCHL1$^+$ subset (Akbar *et al.*, 1988, 1989). Thus CD45R$^+$ cells represent virgin or immature cells which during antigenic (or mitogenic) stimulation rapidly develop UCHL1 positivity (CD45R$^+$, UCHL1$^+$ doubles). These activated cells then lose CD45R and become UCHL1$^+$ memory or primed peripheral T cells (Fig. 6).

Both CD45R and UCHL1 mAbs react with components of the CD45 (leucocyte common or T200) antigen. Although at least four polypeptides can be identified in immunoprecipitates with conventional CD45 mAbs, it is now thought that these are derived by alternative splicing from a single gene (Ralph *et al.*, 1987). CD45R mAbs react with the higher 220,000 and 205,000 MW polypeptides of CD45, while UCHL1 precipitates the lowest 180,000 MW polypeptide (Terry, Brown & Beverley, 1988). Thus in peripheral T cells expression of different CD45 polypeptides appears to be linked to activation and or maturation.

In the present report we have examined the distribution of CD45R and UCHL1 cells in the lymphoid organs and the gut-associated lymphoid tissue. In order to obtain meaningful observations it was essential to apply the antibodies to these two antigens in combinations with each other, and also in combination with other reagents such as TdT, CD3, CD4 and CD8. With such techniques both the thymic development and minority populations of T cells present in peripheral tissues could be investigated.

The biphasic appearance of the p180 protein detected by UCHL1 during thymocyte/T lymphocyte development (Smith *et al.*, 1986) has been confirmed and further documented in this study (Fig. 6). In the thymus most cortical cells are UCHL1$^+$ and represent a functionally immature TdT$^+$ cell population. Expression of UCHL1 in the cortex may be related to cell division since in peripheral T cells UCHL1 is expressed within a few days following activation (Terry *et al.*, 1987; Akbar *et al.*, 1989). From the microscopic and flow cytometric studies shown above it appears that during thymocyte development both TdT and UCHL1 decrease together (Fig. 6). With increasing mCD3 expression, TdT drops to undetectable levels (Campana *et al.*, 1989) but on mCD3$^+$, CD45R$^+$ thymic lymphocytes low amounts of UCHL1 remain detectable by flow cytometry. These observations extend the observations of Serra *et al.* (1988) who studied CD45R on thymic cells in combination with 4B4 (CDw29), an antibody similar, but not identical, to UCHL1. These authors show that during a short concanavalin A stimulation double-positive 4B4^{+s}, CD45R$^+$ thymocytes develop. Finally, in the thymus the CD45R$^+$ cells are likely to represent potential emigrant unprimed T cells (Fig. 6) since among the fetal and infant thymic CD45R$^+$ cells mCD3$^+$ T cells are the majority and medullary B cells a very small minority (0·2–4%; see below).

There are two further comments which are relevant to observation seen in the thymus. Firstly, the weak double expression on thymocytes may be one sign of functional immaturity. A variable proportion (40–60%) of CD8$^+$ intraepithelial T lymphocytes (IEL) in the gut also shows low or undetectable levels of both UCHL1 and CD45R antigens (although expresses common leucocyte antigens seen by a CD45 mAb anti-HLe-1$^+$); the IEL might also be immature cells developing in the gut epithelium (Selby *et al.*, 1983). The low level of double UCHL1-CD45R expression on cortical thymocytes contrasts the strong UCHL1 expression on recently activated mature peripheral CD45R$^+$ T blasts (see below). Secondly, a subpopulation of mCD3$^+$ thymic lymphocytes carries the UCHL1 marker. As these cells become more numerous with age, they might be part of a recirculating peripheral T-cell pool which seed back to the thymic medulla in the same way as they reach the other lymphoid tissues. A similar trend is seen with surface membrane (Sm)Ig$^+$, CD45R$^+$ B lymphocytes, which are present in very small proportions ($<0·5\%$) during fetal and infant thymic development (Table 1 and Neiburger *et al.*, 1976; but see Isaacson, Norton & Addis, 1987) and increase their number with age (Table 1). The extreme example of this phenomenon is the lymph node metaplasia seen in the thymic medulla during myasthenia gravis (Bofill *et al.*, 1985).

The findings above confirm our working hypothesis that the CD45R$^+$, UCHL1$^-$ and UCHL1$^+$, CD45R$^-$ populations show different tissue distributions. Such findings underline the likelihood of their divergent recirculatory pattern and function *in vivo*. Three striking examples of such differential migration are as follows. Firstly, T lymphocytes with strong double CD45R$^+$, UCHL1$^+$ expression could be demonstrated in the paracortical area of tonsils, and also among the lymphoid aggregates in the gut-associated lymphoid tissue (Fig. 5a, b).

These T-cell areas appear to be the sites where virgin lymphocytes respond to antigens, an event which is likely to correspond to the generation of CD45R$^+$, UCHL1$^+$ cells in cultures *in vitro* (see Fig. 2d and asterisk in Fig. 6). Secondly, a virtually homogeneous CD4$^+$, UCHL1$^+$ subset of T cells, some of which are also Leu 7$^+$, accumulates in the germinal centres of lymph nodes at a site where the primed helper T cells can effectively function. Thirdly, an almost exclusively UCHL1$^+$ population, a mix of CD4$^+$ and CD8$^+$ cells, is found in the lamina propria of the gut. It has been shown recently that the UCHL1$^+$ cells express more CD2, CD11b, Pgp1 and LFA-3 (Sanders *et al.*, 1988) and also adhere more readily to umbilical vein endothelial monolayers (Cavender *et al.*, 1987). The preferential migration of UCHL1$^+$ cells may be the result of such adhesive properties. In addition, it is also likely that these CD4$^+$ cells are particularly efficient helper T cells, as has already been demonstrated for the corresponding 4B4$^+$ subset (Morimoto *et al.*, 1985a). UCHL1$^+$ cells may also have less stringent requirements for activation (Sanders *et al.*, 1988).

As an interesting contrast, in the paracortical areas of lymphoid tissue both CD45R$^+$, UCHL1$^-$ and UCHL1$^+$, CD45R$^-$ cells are present but form separate nests of cellular aggregates. This reticular pattern of CD45R$^+$ and UCHL1$^+$ cells (Fig. 4) is intriguing but the groups of CD45R$^+$ or UCHL1$^+$ cells did not show any consistent association to CD11c$^+$ accessory cells (Hogg *et al.*, 1985) or MHC class II$^+$ cells, as has previously been demonstrated for CD4$^+$ cells (Janossy, 1983). Whether these clusters of CD45R$^+$ or UCHL1$^+$ cells respond to the same antigen or are associated by virtue of common adhesive properties remains to be determined. These are, however, the sites where the most efficient presentation of extrinsic antigens, on the surface of interdigitating cells, may take place, and CD45R$^+$ virgin CD4$^+$ cells at these sites may indeed initiate primary immune responses, as suggested by the presence of a few CD45R$^+$, UCHL1$^+$ activated cells in this area of lymph nodes (see above, Fig. 6).

In this paper we have examined the distribution of cells expressing different polypeptides of CD45 detected by UCHL1 and CD45R mAbs. Functional data *in vitro* indicate that CD45R$^+$ and UCHL1$^+$ peripheral T cells may be at different stages of post-thymic differentiation. The tissue distribution of CD45R and UCHL1 reveals that cells of different stages of differentiation occupy particular microenvironments, for example UCHL1$^+$ cells in germinal centres and lamina propria, but there is as yet little firm evidence regarding the real function of CD45 polypeptides.

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