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LOSS OF CD45R AND GAIN OF UCHL1 REACTIVITY IS A FEATURE OF PRIMED T CELLS

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From the *Department of Immunology, Royal Free Hospital School of Medicine, and the Imperial Cancer Research Fund Human Tumour Immunology Group, Faculty of Clinical Sciences, University College, London, United Kingdom

A group of mAb recognizing the 200- and/or 220-kDa determinants (CD45R) of the leukocyte common Ag such as 2H4, WR16, MD4.3, and SN130 cross-block each other showing that they recognize a closely related epitope. The antibody UCHL1 reacts with a 180-kDa determinant of the leukocyte common Ag and exhibits a reciprocal T subset distribution pattern to the CD45R group. Peripheral blood T cells were 40% positive for UCHL1 and 58 to 65% positive for the CD45R antibodies; <1% of cells stained for both. On activation of CD45R⁺,UCHL₁⁻ cells by PHA, up to 40% of cells became positive for both CD45R antibodies; <1% of cells stained for both. On activation of CD45R⁺,UCHL₁⁻ T cells by PHA, up to 40% of cells became positive for both CD45R and UCHL1 by day 3. By day 7, CD45R⁺,UCHL₁⁻ cells fell from 90 to <21% whereas UCHL₁⁺,CD45R⁻ cells rose from 2 to 93%. Conversely, PHA-stimulated UCHL₁⁺,CD45R⁻ cells remained UCHL₁⁺,CD45R⁻ during the 7 days in culture showing that phenotypic change was unidirectional from CD45R⁺ to UCHL₁⁺. In primary allogeneic mixed lymphocyte reactions, activated CD45R⁺ T cells also showed a change to UCHL₁⁺. When these cells were rechallenged by the original alloantigen, the UCHL₁⁺ cells showed 7- to 20-fold greater proliferation than the CD45R⁺ cells on day 3 after rechallenge. The recovery of virtually all alloantigen induced secondary proliferative response in the UCHL₁⁺,CD45R⁻ T cell population suggests that UCHL1 identifies a primed population of T cells which may include memory cells.

Heterogeneity of functional T lymphocytes in terms of helper and suppressor/cytotoxic cell types is well documented in man and rodents (1–4). The further distinction between virgin and memory cells within these populations by phenotypic criteria is less clear-cut. In order to approach this second distinction between various T cell populations, antibodies which show a consistent change during activation are required. Transferrin-R (5) and IL-2R (6) are useful as markers of activation but not for discrimination between virgin or memory cells as they are lost when cells return to the resting state (6). More recently, it has been shown that the 220-kDa determinant of the LCA three termed CD45R is lost upon stimulation by PHA (7), Con A (8) and PWM (9). The possibility of a reciprocal gain of a marker on these activated cells which may be of use in identifying memory cells is therefore of importance. We find that upon activation by PHA or alloantigen, T cells which initially express CD45R determinants (205/220 kDa) lose these membrane moieties and concomitantly acquire the 180-kDa component of the LCA. This molecule is recognized by an antibody known as UCHL1 (10). Conversely, T cells which are initially CD45R⁻ but 180-kDa positive (UCHL1⁺) can also be induced to proliferate to a similar extent, but do not switch their phenotype. These findings, together with the observation that 180-kDa positive (CD45R⁻) CD4⁺ cells respond to soluble Ag maximally whereas the reciprocal population does not (10–13), suggest that the acquisition of the 180-kDa LCA surface component may identify primed T lymphocytes. We have studied this further in secondary MLR applied as a model. This experimental system enables the priming of T cells to be investigated (13, 14). When MLR-activated T cells are rechallenged with the priming alloantigen, T cells exhibiting secondary MLR proliferative activity are found to be almost exclusively UCHL1⁺, supporting the concept that UCHL1⁺ T cells are a primed population within which memory cells may reside.

MATERIALS AND METHODS

Preparation of peripheral blood leukocytes. Human PMNC were prepared from heparinized blood obtained from normal donors by Ficoll-Hypaque density centrifugation. T cells were separated from non-T cells by E-rosette formation and from neutrophils by adherence for 30 min at 37°C to petri dishes coated with microcucate from rambler liver or kidney cells (fibroinectin plates) (16). Granulocytes were prepared from the washed Ficoll-Hypaque red cell pellet by diluting with an equal volume of ACK lysis buffer (Fresenius AG, Bad-Homburg, FRG) and allowing the red cells to settle for 45 min. Granulocytes in the upper layer were removed and washed twice before staining. Cell suspensions were prepared from tonsil by teasing. These cell suspensions were then fractionated as described for peripheral blood.

Indirect immunofluorescence and cell sorting. Cells were incubated with saturating amounts of mAb for 10 min at room temperature and then washed three times in RPMI 1640 medium (GIBCO, Uxbridge, UK) containing 2% FBS (Flow Laboratories, Ltd., Rickmansworth, UK).

Abbreviations used in this paper: LCA, leukocyte common Ag; CD45R, a group of antibodies reacting with 220/205 kDa Ag; FALS, forward angle light scatter; MLR, mixed lymphocyte reaction; FBS, fetal bovine serum; PE, phycoerythrin.

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treated E- stimulator cells to generate primary allogeneic MLR. The E- responder cells and E- stimulator cells were mixed in equal numbers (5 x 10^6 each) in 200 μl RPMI 1640 medium supplemented with 10% FBS, glutamine, and antibiotics (GIBCO) in 5% CO_2 in air. In order to establish the time course of MLR proliferative activity, identical triplicate wells of cells were pulsed on subsequent days with methyl _3H_thymidine (Amersham) and harvested 18 h later onto glass fiber paper (Titertek, Flow Labs). Proliferation was determined by liquid scintillation counting.

To investigate the kinetics of MLR proliferation on rechallenge with the primary alloantigen (secondary MLR), bulk primary MLC were first established, consisting of 12 x 10^6 responding cells (E-) and 6 x 10^6 mitomycin C-treated stimulator cells in 18 ml of supplemented RPMI 1640 in 25-cm^2 flasks (Nunc-GIBCO). After 6 days at 37°C in 5% CO_2 in air, the cells were washed twice in RPMI 1640, then stained with antibody for sorting into SN130 or UCHL1 positive or negative populations. Controls consisted of unstimulated or MLR-activated but unfraccionated T cells. After sorting, the cells were washed twice in RPMI 1640 and viable cells counted by trypan blue exclusion. After bulk MLR, the cells were >94% viable whereas after sorting, the viability in both the positive and negative sorted populations was >90%. These T cells were then rechallenged with the original priming E- cells which were obtained fresh at the time of sorting and mitomycin C treated. In certain experiments non-related E- or autologous E- cells were used for the rechallenge. Secondary MLR were performed in round-bottomed plates identical to the primary MLR.

RESULTS

Cross-blocking of binding by CD45R mAb. Among the different mAb to LCA, three groups were distinguished (Table II). 2H4 (positive control), WR16, SN130, and MD4.3 equally strongly inhibited 2H4 binding on PBL. In this system 76% inhibition represents the blocking by the homologous (2H4) antibody. After incubation with the cold 2H4, the cells were washed free of excess antibody, allowing for some replacement by the radioactive 2H4. As the inhibition by all four reagents is >70%, these antibodies bind to the same immunogenic epitopes of the 220/205-kDa molecules. These antibodies, referred to below as the CD45R group, also showed the same tissue distribution when tested in histology (data not shown). One single antibody, MB1, weakly inhibited 2H4 binding in spite of the fact that it reacts with another set of molecules (200/110/100 kDa) (27). The third group including UCHL1, MT1, MT2, 4KB5, 2A1, and 2D1 did not interfere with the binding of 2H4.

The phenotype of resting T cells. One striking difference between UCHL1 and CD45R distribution on leukocyte subsets was the divergent staining of polymorphonuclear leukocytes. 95% of which were UCHL1^+ and 4 to 15% being reactive with the CD45R antibodies 2H4, WR16, SN130, and MD4.3 (Table III). Both UCHL1 and

<table>
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<tr>
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<th>Subclass</th>
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<td>46</td>
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</tr>
<tr>
<td>SN130</td>
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<td>88</td>
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<td>2A1</td>
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*The inhibition of binding of _3H_labeled 2H4 to normal PBL or two cell lines (US37, WMPT) by various mAb. Representative results of one of two experiments are shown.
the antibodies of the CD45R panel were positive with 40 to 50% of T cells (E+). Nevertheless, these represented largely reciprocal populations. CD45R+ T cells, >97% positive after sorting, were <4% positive for UCHL1, whereas UCHL1+ T cells prepared similarly were >98% positive for UCHL1 but <3% for CD45R+ (Fig. 1). Double color immunofluorescence confirmed that <1% of resting T cells stained for both CD45R and UCHL1.

The phenotype of PHA-activated cells. UCHL1-CD45R+ cells were stimulated with PHA for 7 days and the percentage of cells positive with either UCHL1 or CD45R antibodies was determined (Table IV). The proportion of UCHL1+ cells increased from 2% on day 0 to 93% on day 7, indicating that the 160-kDa molecule is acquired during T cell activation. At the same time, positivity for CD45R-like antibodies was reduced from >90% to <21% demonstrating that upon activation CD45R determinants are lost. Unstimulated UCHL1+ T cells did not increase UCHL1 staining and remained positive with all four CD45R antibodies (data not shown).

PHA stimulation of sorted populations. Before stimulation purified T cells (>95% E+), were sorted into UCHL1 negative (Fig. 1A) or positive (Fig. 1B) fractions, or into SN130 (CD45R-like) negative (Fig. 1C) or positive (Fig. 1D) fractions and studied at various times after PHA stimulation by double labeling for SN130/UCHL1. The overlap between the two subsets was minimal. Thus UCHL1+ and SN130- or SN130+ and UCHL1+ populations were essentially the same subsets which were, however, obtained by positive or negative selection, respectively. On PHA stimulation, both SN130+ and UCHL1+ T cells proliferated as measured by [3H]thymidine uptake (data not shown). First, SN130+,UCHL1- cells positively (Fig. 1D) or negatively (Fig. 1A) selected were analyzed. On day 0, the majority (>95%) of cells were SN130+, whereas about 3% stained weakly for UCHL1 and no double staining was observed. By day 3, however, the number of UCHL1+ cells in the positively selected SN130+ population had increased to 73%, whereas SN130+ cells decreased to 78 and 40% of the cells double stained (Fig. 1D). Similar findings were obtained with the negatively selected SN130+ population (Fig. 1A). The double staining observed on negatively selected SN130+ cells indicated that the SN130 positivity was not due to the presence of residual antibody bound to the cells at day 0 (Fig. 1A). By days 5 to 7, 75 to 80% of the originally SN130+,UCHL1- cells were UCHL1+ with <9 to 15% doubles. This indicates that following a transitional phase of dual SN130/UCHL1 phenotype, a complete switch of SN130+ to UCHL1+ phenotype occurs on the majority of cells during the 7-day culture period.

Separated UCHL1+,SN130- cells, both positively (Fig. 1A) and negatively (Fig. 1C) isolated, were also stimulated with PHA. These cells remained UCHL1+ and essentially SN130-. The small increase in SN130 staining represents non-specific uptake by non-viable cells present in cultures. No double staining for SN130 and UCHL1 was observed upon PHA stimulation of UCHL1+ cells. This shows that the change from CD45R+ to UCHL1+ upon PHA activation is unidirectional. We also analyzed

<table>
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<td>Cell Type</td>
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<td>Mononuclear cells</td>
<td>52 (50-54)</td>
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<tr>
<td>Polymorphonuclear leucocytes</td>
<td>95 (94-96)</td>
</tr>
<tr>
<td>E+</td>
<td>40 (27-49)</td>
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<tr>
<td>E-</td>
<td>72 (35-85)</td>
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<td>Molecular size (kDa)</td>
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* Percentage of positive cells was determined by indirect immunofluorescence. Each result is the mean and range of at least three experiments.
* E-rosette: mononuclear cells.
* E-rosette: mononuclear cells.

Figure 1. The change in phenotype of UCHL1 or SN130 sorted T cells after PHA activation. After UCHL1 sorting (see Materials and Methods), UCHL1+ (A) or UCHL1+ (B) cells were cultured with PHA for 7 days and stained for UCHL1 (V) or SN130 (O) by double label immunofluorescence and analyzed on the EPICS V. Similarly, after SN130 sorting, SN130+ (C) or SN130+ (D) cells were activated and double stained for UCHL1 (V) or SN130 (O). The percentage of sorted T cells that double stain for both SN130 and UCHL1 after activation is also indicated. The results shown are a representative experiment of three performed to date. The highest numbers of double labeled cells can be seen at day 3 (see Fig. 1).
There was also an increase in UCHL1 staining on autolymphocytes with 18% of cells double stained for SN130 and UCHL1. Blast cells, of which the majority were UCHL1+. On day 5 and 7, the percentage of double staining cells decreased to 15 and 9%, respectively. The intensity of the SN130 stain was gradually reduced as compared with the UCHL1 stain, further showing that a SN130 (CD45R-like) to UCHL1 switch in phenotype occurs upon activation.

**The effect of autologous or allogeneic MLR activation on CD45R phenotype.** SN130+ T cells were cultured with either autologous (Fig. 3B) or autologous (Fig. 3C) non-T cells in order to determine whether with these forms of stimulation CD45R determinants are lost with a concomitant gain of the 180-kDa determinant. Controls consisted of SN130+ T cells cultured alone (Fig. 3A). After 6 days the cultured cells were double stained for SN130 and UCHL1 and analyzed on the EPICS in two ways: first, as the intensity of staining for SN130 (Fig. 3, column 1) or UCHL1 (Fig. 3, column 2) as a function of cell number; second, as a two-color histogram of SN130 and UCHL1 staining (Fig. 3, column 3). Non-stimulated cultures remained SN130+, any apparent double staining or gain in UCHL1 labeling being due to non-specific cytoplasmic uptake by non-viable cells. Alloantigen-stimulated SN130+ (CD45R+) cells showed two different populations: small unactivated lymphocytes and large activated blast cells. The former resting population remained SN130+, UCHL1+. About 40 to 45% of the cultures were blast cells, of which the majority were UCHL1+. On day 6 after alloantigen stimulation, 39% of cells were UCHL1+ with 18% of cells double stained for SN130 and UCHL1. There was also an increase in UCHL1 staining on autologous MHC activation, but to a lesser extent compared with the allo-MLR. When UCHL1+ cells were stimulated in a primary allogeneic MLR, similar proliferation to SN130+ T cells was obtained with similar kinetics of proliferation (Fig. 4). These cells, however, remained 95% UCHL1+ with no appearance of SN130+ or double stained cells (data not shown). The switch in phenotype from SN130+ (CD45R+) to UCHL1+ in allo-MLR is therefore also unidirectional.

**Response of SN130+ or UCHL1+ T cells to a secondary alloantigenic challenge.** The previous results suggested that the acquisition of UCHL1 reactivity by CD45R+ T cells upon PHA or MLR stimulation identified a primed population of cells. To investigate this further we rechallenged separated subsets in a secondary MLR which allowed the quantitation of T cell priming. Following the activation for 6 days by alloantigen, T cells were positively or negatively sorted into SN130+ or UCHL1+ populations. These cells were then rechallenged by the primary alloantigen (Fig. 5). When the thymidine incorporation was measured in unfractionated cultures, T cells showed typical secondary MLR kinetics characterized by accelerated thymidine incorporation as compared with the primary MLR (Fig. 5). Furthermore, when UCHL1+ cultures of primed T cells were used the accelerated proliferative activity resided in the UCHL1+ population which showed 7- to 20-fold more proliferation than the SN130+ population. The increased incorporated [3H]thymidine by UCHL1+ T cells during the secondary challenge with alloantigen was paralleled by an increase in the overall number of cells in the active phases of the cell cycle.

Two independent factors may have influenced our results. First, the rapid proliferation in the secondary MLR might have been due to the continuation of proliferation that had been initiated in the primary MLR rather than representing a second response to the alloantigens. On culturing the sorted UCHL1+ cells alone in the absence of restimulation, however, the thymidine incorporation was <2,500 cpm in three experiments studied, as compared with 16,000 to 32,000 cpm seen in the restimulated cultures on day 2. We have therefore concluded that the increased secondary thymidine incorporation is alloantigen driven. Second, the SN130+, UCHL1+ and UCHL1+, SN130+ populations can be obtained by positive or negative selection (see Materials and Methods). The SN130+ cells showed minimal proliferation irrespective of positive or negative selection. Nevertheless, the prolif-
CHANGE IN HUMAN T200 Ag EXPRESSION AND T CELL PRIMING

Figure 3. The effect of autologous or allogeneic MLR activation on CD45R phenotype. SN130+ (CD45R) T cells were unstimulated (A) or were stimulated with allogeneic (B) or autologous (C) mitomycin C-treated non-T cells for 6 days. After culture the cells were double labeled with SN130 (column 1) and UCHL1 (column 2) and the results are shown as 256 channel fluorescent profiles of 10,000 SN130-FITC+ and UCHL1-PE+ FALS gated live cells. In column 3 double staining for both SN130 and UCHL1 is shown and the results are expressed as 64 x 64 channel two-color histograms, as defined in Figure 2.

Figure 4. The response of unfractionated (A), UCHL1 (B), or SN130+ T cells (C) to alloantigen in a primary MLR. The results from two separate experiments are shown. In experiment A, cells were sorted for SN130 (UCHL1 negatively selected); in experiment B cells were sorted for UCHL1 (SN130 negatively selected). Each point represents the mean of triplicate determinations.

DISCUSSION

The 220-, 200-, and 180-kDa molecules all belong to the LCA or T200 complex (CD45) which includes a further 190-kDa component recognized by antibody MT2 (28, 29). Some epitopes defined by mAb are restricted to only certain members of this family of molecules. One group of reagents including 2H4 (24), WR16 (25), MD4.3 (8), and SN130 (20) defines an epitope present on 205,200-kDa surface structures (CD45R), whereas UCHL1 recognizes a molecule of 180-kDa on a reciprocal subset of T cells. Our earlier studies appeared to show no relationship of the UCHL1 (180 kDa) defined polypeptide to CD45 (10) but more recent data (L. Terry, A. Pickford, and P. C. L. Beverley, manuscript in preparation) as well as data from the Third International Workshop on leukocyte Ag (27) show that UCHL1 is indeed part of the CD45 complex.

Upon activation by either PHA (7), Con A (8, 30), or PWM (9, 22), the expression of the 220-kDa determinant is diminished. These findings have now been extended by showing that when CD45R-like determinants are lost on activation by either PHA, or allogeneic and autologous
lymphocytes, there is a concomitant increase of UCHL1 (180-kDa) surface Ag expression after PHA stimulation (31). This change of expression of LCA components has been further documented by double staining of T cells for both 180-kDa and CD45R determinants during the course of activation.

The reciprocal loss of CD45R and gain of novel cell surface determinants upon activation has also been shown by other workers. In one study, it was found that Con A activation of T cells resulted in a loss of CD45R (2H4) reactivity with a concomitant gain of a determinant recognized by the antibody 4B4 which recognizes a reciprocal resting T cell subset to 2H4 (30). Unlike UCHL1, however, which recognizes the 180-kDa glycoprotein of the LCA, 4B4 recognizes a 135-kDa structure (11). In another study, it was found that PWM stimulation resulted in a loss of CD45R reactivity (WR16) with a concomitant increase of reactivity with the antibody WR19 (9). The m.w. of the WR19 reactive component is undefined at present but unlike UCHL1 (10) and 4B4 (11), WR19 does not bind to CD8+ lymphocytes (9). It has been shown recently that an antibody known as S6F1 binds to 180- and 95-kDa surface structures which are expressed on a S6F1+ population of CD8+ cells after alloantigen activation (32). Immunoprecipitation studies have shown that this antibody recognizes an epitope of the LFA-1 Ag (32). The relationship between UCHL1, 4B4, WR19 and S6F1 expression on T cells upon activation is unclear at present. It is of note, however, that whereas UCHL1 reactivity in tissue sections is leukocyte specific, WR19 binds to epithelium and vascular endothelium (9) whereas 4B4 binds to vascular endothelium (M. Bofill, unpublished observations).

The newly generated UCHL1 reactivity appears to be different from the temporary appearance of activation markers such as receptors for IL-2 and transferrin (6), and reactivity with 4F2 (33). Unlike UCHL1, the expression of these latter Ag diminishes when the cells return to the resting state in culture. From our results it appears that the switch in the phenotype of the stimulated T cells from CD45R– to UCHL1+ is unidirectional because during the culture period we studied, UCHL1+ cells have not reverted to a CD45R+ phenotype. Interestingly, cells can also switch their phenotype in an autologous MLR. Although the significance of the autologous MLR is unclear, it is possible that a change from CD45R– to UCHL1+ may also result from activation by self Ag. The possibility that UCHL1+ cells arise in culture from a residual small subset of UCHL1+ precursors is made unlikely by the demonstration of double labeled (CD45R+,UCHL1+) cells after the activation of CD45R+ T cells in culture.

These results taken together show a unidirectional switch from CD45R to 180-kDa determinant phenotype upon activation. We have further investigated this phenomenon using the secondary MLR as a model which enables the priming of T cells to be quantitated in vitro (13, 14). Priming in a MLR is manifested as an accelerated response to rechallenge by the original alloantigen (13, 14). We have observed that after a primary MLR the accelerated proliferative activity upon rechallenge resides almost exclusively in the UCHL1+ T cell population which supports the concept that the 180-kDa, UCHL1 reactive determinant identifies a primed population of T cells.

In primary MLR both CD45R+ and UCHL1+ T cells show similar kinetics of proliferation and magnitude of response to an alloantigenic stimulus. The SN130+ cells recruited by the priming alloantigen are stimulated and switch their phenotype to UCHL1 positivity. The residual SN130+ T cells are depleted of cells which react to the priming alloantigen and thus remain unresponsive to a second challenge exerted by the same alloantigen. A proportion of resting UCHL1+ T cells can also respond to the primary (cross-reactive?) challenge but these remain UCHL1+. Hence the exclusive presence of primed cells among UCHL1+ T cells.

Whereas both CD45R+ and UCHL1+ T cells respond to alloantigens, only the latter are stimulated by soluble Ag in vitro (10, 12, 24). The frequency of Ag-specific proliferating cells and Ag-specific cytotoxic cells has also been shown to be greatly enhanced in the UCHL1+ T cell subset (M. Merkenschlager, L. Terry, and P. C. L. Beverly, manuscript in preparation). These observations are probably attributable to the fact that during primary responses conventional soluble Ag recruit fewer responsive T cells than complex alloantigens do, and these few cells apparently remain undetectable in the CD45R+ population when studied in vitro. As the responding cells stimulated by Ag in vivo expand, they are likely to switch from a CD45R to a UCHL1+ phenotype. On subsequent secondary challenge in vitro, the Ag-specific proliferative and cytotoxic responses thus reside in the previously primed UCHL1+ population.

In the mouse it has already been demonstrated that virgin and memory T lymphocytes can be distinguished by the stable acquisition of a glycoprotein known as Pgp-1 upon antigenic stimulation of Pgp-1+ cells (34). This Ag, however, shows an 80- to 90-kDa molecular size and is not part of the murine T200 complex (35). Thus, the relationship, if any, between Pgp-1 and the T200 complex is presently unknown. Recently, the human Ta1 determinant was shown to identify a minor subset of
CD45R antibodies have been used previously to identify subsets of CD4+ inducer T cells (9–11, 24, 25). The determinants recognized by these antibodies, however, are not expressed constantly, and it is possible that the inducer capabilities of T cells may depend upon their state of differentiation. It has been shown, for example, that T cells produce B cell growth factor at an early stage of differentiation, and that after Ag activation acquire the capability of producing B cell differentiation factor. At the same time these cells show memory proliferative responses to soluble Ag (38).

On peripheral T cells, the presence of UCHL1 seems to be related to previous priming and perhaps memory function. The molecule itself, however, is not synonymous with “memory” T cells. Monocyte/macrophages and polymorphonuclear leukocytes also have 180-kDa (UCHL1) determinants (10). The presence of the UCHL1 reactive determinant on monocyte/macrophages, polymorphonuclear leukocytes, and primed T cells may perhaps be linked to responsiveness to chemotactic stimuli and/or to a capability of enhanced migration out of vascular endothelium in response to injury or antigenic stimulus.Immature cortical thymocytes are also >95% positive for UCHL1 but negative for CD45R, while <50% of mature medullary thymocytes are CD45R+ (P. C. L. Beverley, manuscript in preparation). This suggests that the expression of UCHL1 on T cells during their life-span may be biphasic. Immature UCHL1+ thymocytes may lose this marker with concomitant gain of CD45R determinants on maturation to virgin mature T cells. These T cells then leave the thymus and do not reexpress UCHL1 until antigenic challenge has occurred. At this time CD45R determinants are lost and UCHL1 determinants reappear.

These observations about the phenotypic and functional changes during T cell activation are in line with the possibility that the UCHL1+, CD45R− population contains recently activated and memory T cells. Accordingly, it has been demonstrated that the majority of T cells in the synovium of rheumatoid arthritis patients are almost exclusively UCHL1+, CD45R− (M. Boßli, D. Rowe, G. Janossy, and P. C. L. Beverley, manuscript in preparation). The infiltration of CD8+ T cells observed in the lymph nodes of HIV-infected patients who develop persistent generalized lymphadenopathy has also been reported to be the consequence of an increased number of CD45R− T cells (39). These cells are almost exclusively UCHL1+ (M. Boßli, et al., manuscript in preparation). These findings are consistent with the possibility that the chronic immune stimulation of various T cell populations leads to an accumulation of primed T cells. These cells then readily seed to the tissues and express distinctive positivity for UCHL1, a feature which may indicate that these T lymphocytes are memory cells.

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