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HUMAN MEMORY T LYMPHOCYTES EXPRESS INCREASED LEVELS OF THREE CELL ADHESION MOLECULES (LFA-3, CD2, AND LFA-1) AND THREE OTHER MOLECULES (UCHL1, CDw29, AND Pgp-1) AND HAVE ENHANCED IFN-γ PRODUCTION

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Studies of cell-surface molecules involved in human T cell interaction reveal that differential expression of each of three adhesion molecules (LFA-3, CD2, and LFA-1) subdivides human peripheral blood T cells into major subpopulations. Systematic analysis of the relationship between expression of these and other markers of T cell subsets demonstrates a single major subset of human peripheral blood T lymphocytes distinguished by enhanced expression of LFA-3, CD2, LFA-1, and three other markers (CDw29 [4B4], UCHL1, and Pgp-1). Large differences in relative expression are observed for UCHL1 (29-fold) and LFA-3 (>8-fold), and smaller differences (2- to 4-fold) are seen for CDw29, CD2, LFA-1, and Pgp-1. Bimodal distribution of LFA-3 is found on both $CD4^+$ cells and on $CD8^+$ cells as well as on B lymphocytes ($CD19^+$). Neonatal T cells ($CD3^+$) are comprised almost exclusively of the subset expressing low LFA-3, CD2, LFA-1, CDw29, and UCHL1. Activation of cord peripheral blood mononuclear leukocytes with PHA leads to uniform enhanced expression of each of these molecules on $CD3^+$ cells. Functional analyses of these T cell subsets were performed after sorting of adult T cells based on differential LFA-3 expression. Only the $LFA\text{-}3^+$ subset proliferated in response to the Ag tetanus toxoid, even though the $LFA\text{-}3^-$ subset proliferated more strongly to PHA. Furthermore, the $LFA\text{-}3^+$ subset made greater than fivefold more IFN-γ than the $LFA\text{-}3^-$ subset in response to PHA, despite the fact that both subsets made equivalent amounts of IL-2. This phenotypic and functional analysis of resting and activated newborn and adult T cells indicates that human memory T cells express enhanced levels of LFA-3, CD2, LFA-1, UCHL1, CDw29, and Pgp-1; we speculate that the increase in expression of T cell adhesion molecules LFA-3, CD2, and LFA-1 on memory cells is functionally important in their enhanced responsiveness.

During cell differentiation, changes in expression of surface molecules modifies a cell's ability to communicate and interact with its environment. Because cell-cell adhesion is critical to T cell thymic education (1), homing (2), recognition, and response (3, 4) modulation of expression of adhesion molecules may be important during T cell differentiation. We have observed that levels of expression of three adhesion molecules (LFA-3, CD2, and LFA-1) are enhanced on a major subset of adult T cells. Our functional and phenotypic studies indicate that this subset of T cells is memory cells.

Recent studies indicate that Ag-independent T cell adhesion is an important early event in T cell recognition (5, 6). These studies have focused our interest on three T cell-surface molecules which mediate Ag-independent adhesion: LFA-3, CD2 (also known as T11, LFA-2, and the E-rosette-R), and LFA-1. LFA-3 is a widely expressed glycoprotein which recently has been shown to be a target cell-surface adhesion ligand for the T cell-restricted glycoprotein CD2 (6–8). LFA-1 is a cell-surface glycoprotein which has been implicated in adhesion of lymphocytes, macrophages, and granulocytes (3, 4).

LFA-3, CD2, and LFA-1 may function not only in adhesion but also in cell activation. T cells can be activated by mAb binding to CD2 (9); furthermore, LFA-3 can participate in CD2-mediated T cell activation (10, 11). Binding of mAb to surface LFA-3 can induce IL-1 secretion by human monocytes and thymic epithelium (12). Finally, binding of one mAb to LFA-1 has been reported to induce murine T cell proliferation and IFN-γ production (13).

There have been a number of previous studies of cell surface markers which distinguish memory T cells, i.e., cells which have undergone primary stimulation with Ag and are capable of mounting a rapid anamnestic response. CDw29 (also known as 4B4), CD45R (also known as 2H4, HB-11 or Leu-18), UCHL1, and Ta1 each have been reported to be altered in expression on a subset of human T lymphocytes which mount strong secondary in vitro proliferative responses to recall Ag (14–18). However, the interrelationship between the subsets defined by these mAb has not been defined, other than an almost reciprocal relationship between CDw29 and CD45R on $CD4^+$ T cells (14). Recently, Budd et al. (19–21) have shown that expression of another glycoprotein, Pgp-1, distinguishes a subset of mouse T lymphocytes which contains memory cytotoxic T cell precursors and shows enhanced IFN-γ production; these studies have not been extended to human T cells.

The present study: 1) correlates increased expression of

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three adhesion molecules (LFA-3, CD2, and LFA-1), with increased expression of three previously defined memory cell markers (CDw29, UCHL1, and Pgp-1) on a single major subset of adult T lymphocytes, 2) demonstrates the absence of this subset in neonatal cord blood, 3) demonstrates phenotypic conversion of neonatal T cells to the enhanced expression phenotype with activation, 4) demonstrates proliferative response to recall Ag in this subset when adult T cells are sorted based on LFA-3 expression, and 5) extends to the human species the observation of enhanced IFN-γ production by this subset. These data are consistent with the hypotheses that LFA-3$^+$, UCHL1$^+$, CDw29-high-, CD2-high-, LFA-1-high-, and Pgp-1 high-expressing T lymphocytes represent the in vivo-primed, i.e., memory, T cell population, and that human memory T lymphocytes are characterized by enhanced production of IFN-γ.

MATERIALS AND METHODS

Cells and reagents. Human PBML[2] from normal donors were separated by Ficoll-Hypaque density sedimentation. T lymphocytes were obtained by either: 1) elutriation (22) (generously performed by Dr. L. Wahl, NIDR, Bethesda, MD), or 2) removal of plastic- and nylon wool-adherent cells. Cord blood was obtained at birth from the placenta of healthy neonates, anticoagulated with heparin, and mononuclear cells isolated by sedimentation of E with 0.08% dextran followed by density centrifugation of the supernatant over Ficoll-Hypaque. Antibody reagents were as follows: LFA-3 mAb TS2/9 (23); biotinylated CD2 mAb 95-5-49, specific for the $CD2_1$ epitope (Dr. R. Quinones, National Cancer Institute, Bethesda, MD); mAb MHM24, specific for the α-chain of LFA-1 (Dr. J. Hildreth, Johns Hopkins University, Baltimore, MD); human Pgp-1 mAb E1/2 (24) (Dr. C. Isacke, Salk Institute, San Diego, CA); mAb UCHL1 (16) (Third International Workshop on Leukocyte Differentiation Antigens); mAb TAC, specific for the low affinity IL-2R (Dr. D. Nelson, NCI, Bethesda, MD); FITC-conjugated CDw29-specific mAb 4B4 and PE-conjugated CD45R-specific mAb 2H4, 4B4, B1, CD19-specific mAb B4 and Ta1 (Coulter Immunology, Hialeah, FL); biotin-conjugated CD3 mAb anti-Leu-4, CD4 mAb anti-Leu-3a, CD8 mAb anti-Leu-2a, anti-Thy-1.2 mAb, and FITC-conjugated CD45R mAb anti-Leu-18 (Becton Dickinson Immunocytometry Systems, Mountain View, CA); mouse Qa-specific control mAb 1-9-9 (Dr. D. Sachs, NCI, Bethesda, MD); FITC-conjugated Fab'_2 goat anti-mouse IgG (Boehringer Manheim, Indianapolis, IN) and Texas Red-conjugated streptavidin (Bethesda Research Laboratories, Bethesda, MD). Human IgG was prepared by ammonium sulfate precipitation and was a gift from Dr. R. Quinones. All antibodies were used at saturating concentrations as purified Ig except 1-9-9, MHM24, and TAC, which were culture supernatants, and UCHL1, for which a dilution of ascites fluid was used.

FCM. T cells or PBML were washed twice with PBS and then incubated with purified human IgG for 10 min to inhibit subsequent mAb binding to FcR. Cells (1×10^6) were incubated for 30 min with a first step mAb. After two washes with HBSS containing 0.2% human serum albumin and 0.2% sodium azide, cells were stained for 30 min with Fab'_2 goat anti-mouse IgG FITC conjugate. After two washes, the cells were incubated 20 min with excess mouse IgG to block free goat anti-mouse IgG-binding sites. The goat anti-mouse IgG and blocking steps were omitted for samples stained with the directly FITC-conjugated mAb 4B4 or Leu-18. After the blocking step, biotinylated or directly PE-conjugated mAb was added for 30 min, followed by two washes. For staining with biotinylated mAb, TR-conjugated streptavidin was added for 10 min, and followed by two final washes. All incubations and washes were performed at 4°C. Cells were analyzed with a modified Becton Dickinson FACS II, equipped with 1) the manufacturer's filters and photomultiplier tubes, 2) argon ion laser for FITC excitation (488 nm), and 3) pumped dye (rhodamine 6G) laser for Texas Red excitation (590 nm), interfaced to a Digital Equipment Corporation PDP 11/84 (Digital Equipment Corp., Maynard, MA). Data were collected on 50,000 cells per sample and analyzed by using hardware and software designed by the Division of Computer Research and Technology at the National Institutes of Health. Logarithmic amplification was provided by a three-decade logarithmic amplifier constructed from a National Institutes of Health-modified design of R. Hiebert (Los Alamos, NM) and the data converted to the linear units of millivolts. Viability was determined by forward light scatter and, except for PE-stained cells, propidium iodide exclusion. Cell size was estimated from "electronic cell volume" measured on a Becton Dickinson FACS analyzer calibrated wth microbead standards.

Activation of PBML. PBML from normal adult donors or cord blood were stimulated with PHA at 1/100 final dilution of stock (GIBCO, Grand Island, NY) and cultured for 3 to 14 days as indicated. Cells were cultured at 1×10^6/ml in RPMI 1640 (GIBCO) supplemented with 10% pooled normal human heparinized plasma in a humidified incubator with 6% CO_2 at 37°C. Where indicated, control unstimulated cells from the same donor were cultured simultaneously and under identical conditions except that cell density was 2×10^6/ml. Culture media were periodically exchanged to maintain pH within the optimal range for cell growth.

Electronic cell sorting. Elutriated T cells or purified T cells from donors with a history of prior tetanus immunization were stained for LFA-3 by incubation for 30 min with mAb TS2/9, followed by two washes with HBSS containing 0.2% human serum albumin. The cells were then incubated 30 min with goat anti-mouse IgG FITC conjugate, and washed twice. All incubations were performed at 4°C, and all reagents were sterile filtered and supplemented with gentamycin. LFA-3$^+$ and LFA-3$^-$ populations were sorted by using the Becton Dickinson FACS II cell sorter. Sort windows were set such that less than 15% of cells from the middle of the profile were intentionally discarded. Cells were collected in sterle polystyrene tubes containing RPMI 1640 supplemented with 25% FCS, and were resuspended in RPMI 1640 after centrifugation.

Proliferation assays. Sorted or stained unsorted cells were plated at 1×10^5/well in sterile 96-well culture plates in RPMI 1640 supplemented with 8% normal human plasma and gentamycin. Unstained autologous elutriated monocytes or plastc adherent cells, irradiated with 2000 R, were added at 1×10^4/well to serve as APC. Stimulation was performed for 3 days with PHA 1/100 final dilution of stock, and for 6 days with tetanus toxoid (Massachusetts Department of Public Health, Boston, MA) used at 10 FL U/ml. Cultures were maintained in a humidified incubator with 6% CO_2 at 37°C. Cultures were pulsed overnight with 1 μC/well [^{3}H]tymidine (sp. act. 2.0 Ci/mmol, New England Nuclear, Boston, MA) and harvested onto glass fiber filters. Incorporation was determined by liquid scintillation counting and data expressed as geometric mean cpm of triplicate cultures.

Lymphokine assays. Supernatants from the PHA-stimulated cultures were collected at 48 h. IFN-γ was assayed using a commercially available RIA (Centocor, Malvern, PA), according to the manufacturer's instructions. IL-2 assay was performed by using the murine IL-2-dependent cell line CTLL-2 (American Type Culture Collection, Rockville, MD). Aliquots of 100 μl of test supernatants were incubated with 5×10^4 CTLL in 100 μl RPMI 1640/10% FCS in a humidified CO_2 incubator at 37°C for 24 h. Tritiated thymidine, 1 μCi/well was added 8 h before harvest onto glass filters. Thymidine incorporation was determined by liquid scintillation counting, and units expressed in relation to a curve produced with serial dilutions of Biological Response Modifiers Program standard IL-2 (made from the Jurkat cell line).

RESULTS

Expression of LFA-3 on lymphoid cell subsets and monocytes in adult PBML. FCM analysis revealed a trimodal expression of LFA-3 on PBML (shown in Fig. 1, in two-color FCM by using a negative control mAb (Thy1.2) for red fluorescent staining). Two-color analysis with a variety of leukocyte markers was undertaken to identify the cells in each LFA-3 subpopulation. Analysis with mAb Leu-M3 demonstrated that the population with highest LFA-3 expression was monocytes. Analysis of LFA-3 expression by B lymphocytes by using either of two different B cell markers (CD19 (B4) in Fig. 2 and B1 in other studies) revealed an approximately equal distribution of LFA-3 expressors and nonexpressors.

LFA-3 expression was bimodal among CD3$^+$ cells (Fig. 1). LFA-3 expression was compared with other human cell surface molecules whose expression subdivides human peripheral blood T lymphocytes into subpopulations. Note that in each of these panels the population with highest expression of LFA-3 is monocytes and about 95% of the other cells are T cells. LFA-3 expression further subdivides both the CD4$^+$ and the CD8$^+$ T cell subsets; about half of the cells of each subset expressed LFA-3. CDw29 (also known as 4B4) and CD45R (also known as 2H4 or Leu-18) are

[2] Abbreviations used in this paper: PBML, peripheral blood mononuclear leukocyte; FCM, flow cytometry; PE, phycoerythrin.

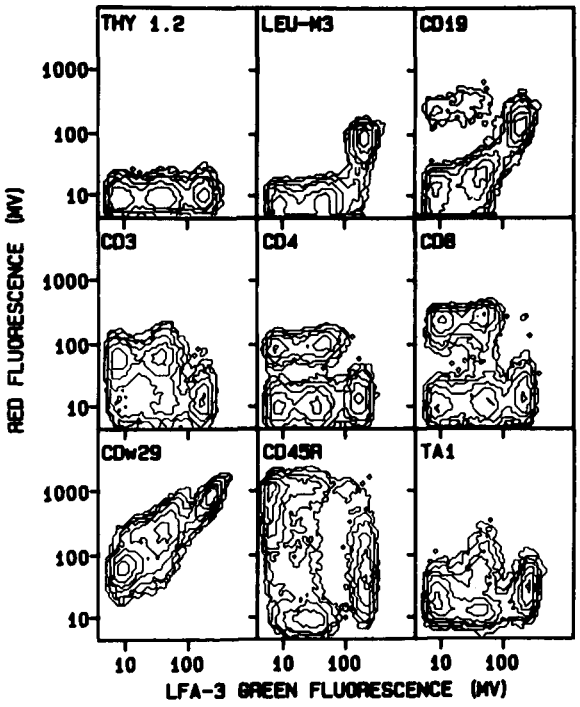

Figure 1. Two-color FCM analysis of adult PBML for LFA-3 (x-axis) vs other markers of hemopoietic cells (y-axis): control mAb anti-Thy-1.2; anti-Leu-M3; CD19 (B4); CD3 (anti-Leu-4); CD4 (anti-Leu-3a); CD8 (anti-Leu-2a); CDw29 (4B4); CD45R (2H4); Ta1. Constant values of the percentage of total cell number on the z-axis were selected to draw the contours around peaks of cells correlating green and red fluorescence.

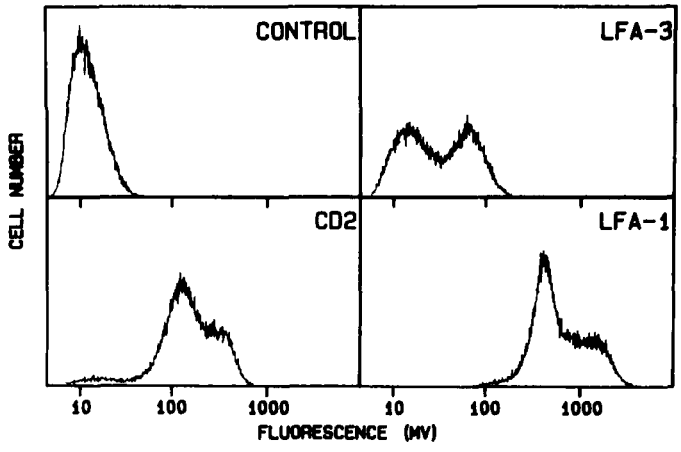

Figure 2. Bimodal expression of adhesion molecules on peripheral blood T cells. Data shown are for elutriated T cells which were more than 95% $CD2^+$. Panels show staining profiles with mAb to mouse Qa (control), LFA-3, CD2, and LFA-1. Fluorescence intensity (in millivolts) is plotted on a logarithmic scale on the x-axis vs cell number on the y-axis.

markers of two other major essentially reciprocal T cell subsets (14, 15). Evaluation of LFA-3 expression on PBML indicated a high degree of concordance between the subsets identified by LFA-3 and by these two markers. The correlation was most striking with CDw29 which identified the same subsets as LFA-3 and its level of expression correlated with expression of LFA-3 on these subsets. In contrast, CD45R was largely concordant with LFA-3 with respect to the subsets identified, but its expression was generally increased on the lymphocytes which lacked LFA-3 and vice versa. The concordance of LFA-3 subsets with CDw29 subsets was one of multiple lines of evidence which suggested that the LFA-3^+ T cells are memory cells (see below). Expression of Ta1, which also has been reported as a marker of human memory cells (17), did not correlate in a simple way with expression of LFA-3.

Bimodal expression of LFA-3, CD2, and LFA-1 on adult peripheral blood T lymphocytes. Since LFA-3 expression on adult T cells was bimodal, we extended the analysis to other molecules involved in T cell adhesion (Fig. 2). FCM analysis revealed bimodal expression of all three adhesion molecules, LFA-3, CD2, and LFA-1, indicating the existence of major T cell subsets differing in expression of LFA-3, CD2, and LFA-1. The existence of such bimodal expression of LFA-3, CD2, and LFA-1 has been observed in multiple donors. Two characteristics distinguish expression of LFA-3 from expression of CD2 or LFA-1: 1) the difference in LFA-3 expression is qualitative (either present or essentially absent), whereas the difference in CD2 and LFA-1 is quantitative (two to four times differences in level of expression); 2) LFA-3 expression always resolves T cells into two distinct subsets, whereas CD2 and LFA-1 expression does not always discriminate subsets so clearly in one-color cytometric analysis (see below). For each of seven normal donors studied, LFA-3 expression readily distinguished two subsets, each representing from 40 to 60% of peripheral blood T lymphocytes. In contrast, the one-color profile shown for CD2 in Figure 2 was selected as a particularly good example of CD2 bimodality. Wth other donors, the existence of subsets differing in CD2 expression was seen as a shoulder, or as a broad distribution which could be resolved into distinct subpopulations only with two-color analysis (see discussion below). A broad or bimodal distribution for CD2 is seen not only with the mAb used in this study (95-5-49) but with other CD2 mAb by ourselves (e.g., CD2 mAb 35.1, data not shown) and by others (T. A. Fleisher with CD2 mAb Coulter Clone T11, personal communication).

Coordinate expression of six cell-surface molecules on a subset of T lymphocytes. Two-color FCM analysis was performed to determine the relationship between the subsets of T cells distinguished by differential expression of LFA-3, CD2, and LFA-1 (Fig. 3). Two-color analysis of LFA-3 expression (x-axis) vs CD2 expression (y-axis) reveals only

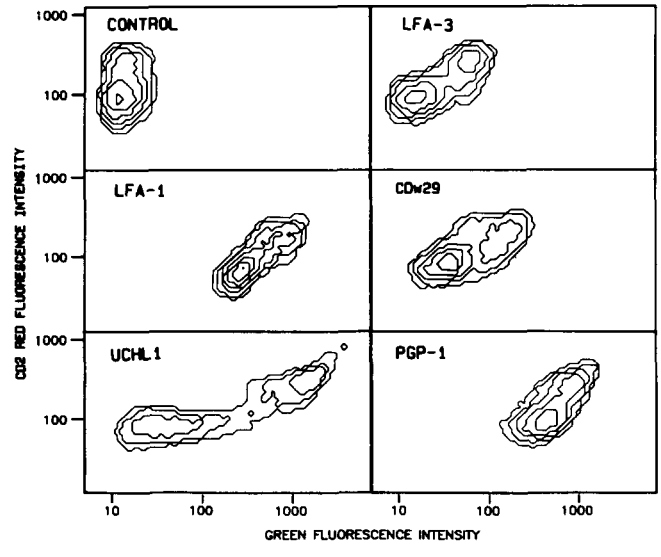

Figure 3. Concordance of T cell subsets identified by staining with CD2 and five other markers. Two color FCM contour plot of elutriated peripheral blood T lymphocytes comparing CD2 staining (y-axis) with staining (shown on the x-axis) using: negative control mAb (mouse Qa), LFA-3, LFA-1, CDw29 (4B4), UCHL1, and Pgp-1.

two peaks: one with high expression of LFA-3 and CD2 and the other with essentially no LFA-3 and lower expression of CD2. It is important to note that no other populations were appreciated with discordant expression of these two molecules. Similar results were obtained for the comparison of LFA-1 with CD2. Thus, the combined results of the analyses with LFA-3 or LFA-1 mAb indicate the existence of a single T cell subset with coordinate higher expression of CD2, LFA-3, and LFA-1 and a reciprocal subset with lower expression of these molecules. Such subsets were evident in every donor examined using two-color FCM.

Comparison of CD2 with CDw29 on purified T cells (Fig. 3) confirmed the concordance of these markers predicted by LFA-3 correlation with CDw29 (Fig. 1) and by CD2 correlation with LFA-3. Two additional molecules were identified whose expression marks this same pair of T cell subsets: UCHL1 and Pgp-1. UCHL1 is a mAb which, like CDw29, has been reported to identify those human T cells capable of in vitro proliferative response to recall Ag (16). Pgp-1 is a molecule whose expression was analyzed because of reports that it was a marker of murine memory T cells (19–21). Thus, these two T cell subsets are characterized by differental expression of six distinct molecules, including three adhesion molecules and three markers of memory cells.

Some of the six coordinately expressed molecules discriminate these two populations better than others. One important parameter for each molecule is its relative expression on the high-expressing vs low-expressing subsets, i.e., "H/L ratio"; this parameter was calculated for each marker from the data shown in Figure 3 (Table I). The division into high and low subsets was established based on expression of CD2; median fluorescence of other markers was then determined for each subset. Two molecules showed particularly large H/L ratios: UCHL1 (29-fold increase) and LFA-3 (at least an eightfold increase); other markers had H/L ratios from 2.1 to 3.7.

The ability to detect bimodal expression of a marker on subsets depends not only on the H/L ratio for that marker, but also on the heterogeneity in expression of that marker within each subset. The Pgp-1 marker does not show obvious bimodal distribution on T lymphocytes by using single-color FCM (24) (data not shown) because it has the lowest H/L ratio of the six informative markers and its distribution of expression within subsets is not narrow. However, LFA-1, a molecule with only slightly larger H/L ratio, can be seen to be bimodal on one-color analysis (Fig. 2) because of narrow distribution of its expression, particularly in the lower expressing subset. One-color profiles for CD2 expression vary between donors from obviously bimodal (Fig. 2) to a wide unimodal distribution. The possibility that the observed differences in fluorescence were due to a difference in volume between the cells in these two T cell subsets was explored by measurement of mean electronic cell volume. The estimated difference in surface area between the subsets (7%) was much too small to explain the observed differences in Ag expression. Thus, these data demonstrate two major subpopulations which differ quantitatively in their expression of each of these six markers.

TABLE I

Quantitation of differential expression of markers on CD2-high vs CD2-low subsets of T cells

Molecule	Expression on		High/low ratio
	High subset	Low subset	
LFA-3	40[a]	<5	>8.0
CD2	283	106	2.8
LFA-1	787	333	2.4
CDw29	106	29	3.7
UCHL1	1004	34	29.4
Pgp-1	1004	483	2.1

[a] Fluorescence is expressed as median fluorescence (converted to the physical unit millivolts) for the high- or low-expressing peak and corrected by subtraction of fluorescence of cells stained with negative control antibody. LFA-3 expression is shown as <5 mV because values less than 5 may not be reliably distinguished from zero. The high/low ratio can be directly compared between all mAb. The millivolt data are not directly comparable for CD2 staining performed with Texas Red and for the CDw29 staining performed with directly FITC-conjugated mAb. Staining for the other four mAb was with unmodified antibody and developed with a FITC-conjugated goat anti-mouse IgG.

Lack of expression of LFA-3 and low expression of coordinate markers by neonatal lymphocytes. To further investigate the hypothesis that the LFA-3$^+$ T cells are memory cells, we examined neonatal cord blood T cells for expression of LFA-3 and the other markers coordinately expressed on adult T cells. Cord blood should have few memory T cells because of limited antigenic exposure of the neonate. Histograms for fluorescence of simultaneously stained adult and neonatal T cells (CD3$^+$) have been superimposed to facilitate comparison (Fig. 4). Unlike adult T cells, neonatal T cells are all LFA-3$^-$. Similar results are seen for CDw29$^+$ and UCHL1$^+$ T cells; neonatal cells have only a single major peak with mean fluorescence which is close to that for the low-expressing population of adult T cells. Biomodal expression of LFA-1 by CD3$^+$ cells is present only in the adult (seen as a sizable shoulder) and not in cord blood. CD45R (Leu-18), a marker whose expression is decreased on the LFA-3$^+$ subset, is largely positive on cord blood CD3$^+$ cells unlike adult CD3$^+$ cells, which show bimodal expression of this marker with a clearly negative subset. These findings demonstrate that neonatal T cells are comprised predominantly of one of the two subsets found in adults, i.e., the LFA-3$^-$, CDw29-low, UCHL1$^-$, CD45R$^+$, LFA-1-low subset. These findings are those predicted by the hypothesis that this phenotype is characteristic of naive T cells.

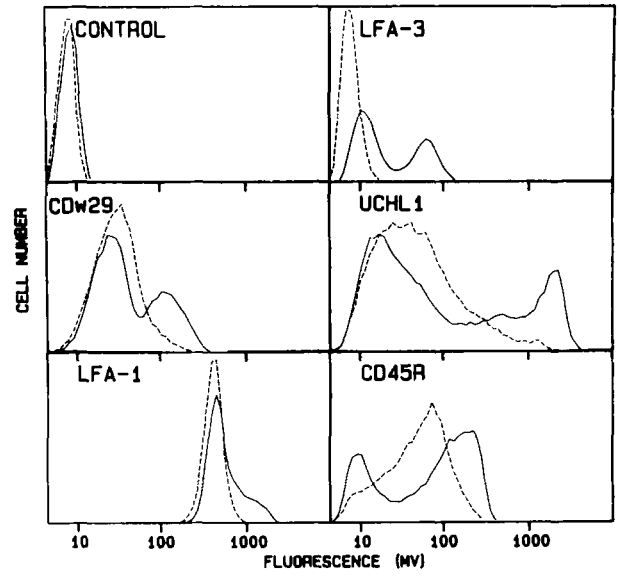

Figure 4. FCM analysis of simultaneously stained cord and adult PBML gated to show expression of various markers on CD3$^+$ lymphocytes: Profiles for cord blood are shown with *dashed lines* and adult blood with *solid lines*. Control mAb is anti-mouse Qa; other markers are LFA-3, CDw29 (4B4), UCHL1, LFA-1, and CD45R (anti-Leu-18).

Expression of LFA-3, and coordinate markers after activation of cord PBML: Markers of memory T cells should appear on neonatal cells after activation. Activation of cord blood PBML with PHA for 3 days leads to uniform expression of LFA-3 and UCHL1 on $CD3^+$ cells, as well as augmentation of expression of CD2, LFA-1, and CDw29 (Fig. 5). Thus, activation of naive neonatal T cells results in their phenotypic conversion from a population resembling LFA-3^- adult T cells to one resembling LFA-3^+ adult T cells. Expression of CD45R (Leu-18) by cord T cells decreased marginally (15%) following activation; this probably reflects the beginning of loss of expression of a molecule whose membrane turnover is slow (see *Discussion*). Activation of adult PBML with PHA leads to uniform conversion within 3 days of LFA-3^- T cells to LFA-3^+, at the same level of expression of LFA-3 as seen on the LFA-3^+ subset in adult PBML (data not shown); this expression of LFA-3 persists for at least 14 days in culture, after loss of most (>85% of peak) of the expression of a transient marker of T cell activation, the IL-2R.

Enhanced proliferation of the high-expressing T lymphocyte subset to soluble Ag. To analyze functional properties of these two subsets, human T lymphocytes were separated by electronic cell sorting. LFA-3 was chosen as the marker on which to sort because: 1) it showed good discrimination of subpopulations, and 2) pretreatment of T cells with LFA-3 mAb did not interfere with the functional response of T cells cultured in the presence of untreated autologous monocytes (data not shown). FCM analysis of sorted cells showed the purity to be greater than 95% for the LFA-3^+ population and greater than 97% for the LFA-3^- population. The two subpopulations showed reciprocal differences in their responses to lectin or recall Ag stimulation. Stimulation with tetanus toxoid resulted in proliferation only in the LFA-3^+ subset (Fig. 6, first panel). In contrast, the LFA-3^- subset proliferated better to PHA than the LFA-3^+ subset (Fig. 6, second panel).

Enhanced production of IFN-γ by the high expressing T lymphocyte subset. Based on the finding of enhanced IFN-γ production by murine memory cells (20), we analyzed lymphokine production by the LFA-3 sorted subsets. Greater than five times more IFN-γ was produced by the PHA-stimulated LFA-3^+ cells than by the LFA-3^- cells (Fig. 6, fourth panel). For IL-2, the amount detected varied between donors by more than 10-fold, as is commonly seen, but for both donors the amounts were within readily quantifiable ranges (10 to 100 U). Despite the large differences in IFN-γ production between the two subsets, IL-2 accumulation

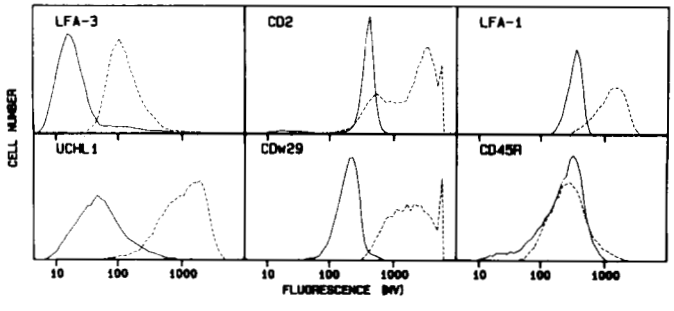

Figure 5. FCM analysis of PHA-activated cord PBML gated to show expression of various markers on $CD3^+$ lymphocytes. Cord PBML were cultured for three days without PHA (*solid lines*) or with PHA (*dashed lines*). Markers shown are LFA-3, CD2, LFA-1, UCHL1, CDw29 (4B4), and CD45R (anti-Leu-18).

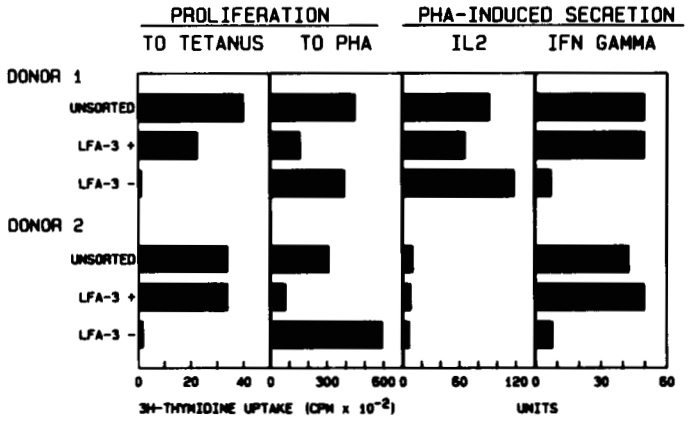

Figure 6. Proliferation and lymphokine production by T lymphocytes sorted for expression of LFA-3. Values for proliferation represent geometric mean cpm for triplicate cultures after subtraction of mean cpm for media control (which was less than 454 cpm for each condition).

in the cultures of LFA-3^- cells was almost equivalent to (donor 2) or greater than (donor 1) the accumulation in cultures of LFA-3^+ cells (Fig. 6, third panel).

DISCUSSION

The foregoing studies systematically analyze a pair of reciprocal T cell subsets characterized by differences in expression of six distinct surface molecules and striking differences in their functional capacity. Three adhesion molecules and three previously identified markers of memory T lymphocytes are increased on one of these subsets of human peripheral blood T cells. This high-expressing subset is not found in neonatal T cells, although neonatal T cells can be induced to express these markers with activation. The high-expressing subset contains the cells which proliferate in response to the recall Ag tetanus toxoid and which are the most potent producers of IFN-γ in response to polyconal activation. The reciprocal low-expressing subset is fully competent to proliferate and produce IL-2 in response to lectin stimulation, although it does not proliferate to recall Ag. These data indicate that the high-expressing subset is memory T cells.

These results integrate and extend a variety of previously reported findings regarding human memory T cells. At least five prior reports with five different markers have indicated the existence of a phenotypically identifiable subset of T cells which responds to recall Ag. However, the relationship among these subsets was unclear, apart from the reciprocal relationship between 4B4 and 2H4 on $CD4^+$ T cells. Our direct correlation of markers establishes that the subsets previously identified by 4B4 (and 2H4), HB-11, and UCHL1 (but not Ta1) are identical to each other and to the subset we have identified (by increased expression of adhesion molecules LFA-3, CD2, LFA-1). The data regarding proliferative response to recall Ag can therefore be compared for the same T cell subsets discriminated by different markers. T cells positively sorted for expression of LFA-3 (present data), 4B4 (14) or UCHL1 (16) or negatively sorted for 2H4 (15) or HB-11 (18) proliferate in response to recall Ag, whereas their reciprocal subsets do not. An additional internal consistency of results is evident from the finding that the PHA-response of our LFA-3^- subset is greater than that of the LFA-3^+ subset, paralleling previous findings with cells sorted based on expression of 4B4 (14), 2H4 (15), or HB-11 (18).

Thus, the comparisons shows a complete consistency between our data and data from four previously reported studies by using markers other than LFA-3.

The subsets defind in this report are clearly distinct from those discriminated with the Ta1 marker, which has also been reported (17) to delineate a subset of human peripheral T cells which includes memory T cells. The smaller size of the Ta1 subset (10 to 15%) (17), clearly distinguishes it from the UCHL1$^+$ subset (about 40%) (16), the CDw29 high subset (41%)(14) and from the high-expressing subset (40 to 60%) in our studies. Direct comparison of Ta1 expression to LFA-3 expression reveals Ta1$^+$ and Ta1$^-$ cells in both the LFA-3$^+$ and LFA-3$^-$ subsets. A possible explanation to reconcile the findings of Hafler et al. (17) with Ta1 with our data is that memory cells may be only in the subset of cells positive for Ta1 as well as the other six markers we report. Further experiments using dual sorting techniques are required to resolve this discrepancy.

Budd et al. (19–21) have reported the first marker of murine memory T cells, a molecule Pgp-1, whose function is not yet known. A previous one-color analysis of Pgp-1 expression on human lymphocytes provided no evidence that it could distinguish subsets of T cells (24). We find by two-color analysis that Pgp-1 shows differences in level of expression which correlate with other markers of human memory cells, but differences in level of Pgp-1 expression on the two subsets are too small to make it useful in discriminating them by one color analysis.

Our data, integrated with that of others, indicates that the CD45 molecule (also previously known as T200 or LCA) undergoes important changes concurrent with activation. The findings are as follows: There are two reports of markers characterized by lack of expression on human memory cells: 2H4 (15) and HB-11 (18). Results of L. Terry et al. and F. Sanchez-Madrid et al. (Third International Workshop on Human Leukocyte Differentiation Antigens) unify these observations by showing that HB-11 and 2H4 both recognize epitopes on some forms of the CD45 molecule (the 200-kDa and 220-kDa forms). Different forms of the CD45 molecule are generated by differences between cell types in their splicing of a single mRNA (25). It is therefore of particular interest that UCHL1 may be an epitope on a different M_r form (180 kDa) of the CD45 molecule (F. Sanchez-Madrid et al., Third International Workshop on Human Leukocyte Differentiation Antigens). Thus, it appears that one form of CD45 (CD45R, 2H4, HB-11) is downregulated in memory cells and an alternate form (UCHL1) upregulated.

Activation of cord blood results in augmentation of expression of each of the six coordinately expressed markers. Serra et al.[3] have observed similar enhanced CDw29 expression on adult CDw29$^-$ T cells after PHA activation. We would predict that CD45R (2H4) would be lost with activation, since it is absent from the subset we propose to be memory cells. We observed a slight decrease in expression of CD45R after 3 days of PHA-actvation. Ledbetter et al. (26) and Serra et al.[3] have reported substantal loss of CD45R expression by day 6 after PHA activation. The rate of loss of expression of a cell surface molecule after cessation of transcription will depend on stability of mRNA and rate of turnover of the membrane protein. CD45 may have very slow membrane turnover, inasmuch as there is very slow loss of CD45 antibody from the surface of stained T cells (J. Hansen, personal communication). These findings, combined with the findings of enhanced expression of each of the six molecules on a single subset of adult T cells generally lacking CD45R, suggest that initial activation of postthymic T lymphocytes is associated with acquisition within 3 days of stably enhanced expression of LFA-3, CD2, LFA-1, CDw29, UCHL1, and Pgp-1, and somewhat slower loss of expression of CD45R.

LFA-3$^+$ T lymphocytes produce much more IFN-γ than do LFA-3$^-$ T lymphocytes. This extends the recent finding of Budd et al. (20) that both murine L3T4$^+$ and Lyt2$^+$ subsets can be further subdivided by Pgp-1 expression, and that both Pgp-1$^+$ subsets have markedly enhanced IFN-γ production. In both their studies and ours, IL-2 production by the various subsets were approximately equivalent. Production of IL-2 and IFN-γ have been posulated to involve shared regulatory mechanisms (27–29); however, discordant production of these two lymphokines by these two subsets suggests the existence of some distinct regulatory mechanisms. The coordinate expression shown in our studies raises the possibility that there may be shared regulatory elements involved in expression of each of six cell surface molecules and of production of IFN-γ. Furthermore, our finding of the absence of LFA-3$^+$, UCHL1$^+$, CDw29-high, CD45R-low T lymphocytes in neonatal cord blood is consistent with previous reports by others of the lack of proliferation to soluble memory Ag and lack of production of IFN-γ by cord blood lymphocytes from most normal neonates (30, 31).

Enhanced expression of the three adhesion molecules LFA-3, LFA-1, and CD2 on previously activated T lymphocytes could be critical to their ability to respond rapidly upon restimulation. Inasmuch as cell-cell interactions are important early events in many immunologic reactions, enhanced expression of these molecules could promote Ag-independent T cell adhesion to other types of cells, possibly facilitating promptness of secondary immune responses through enhancement of Ag presentation, helper function, or T cell migration. Although two- to fourfold increases in expression of LFA-1 and CD2 may not seem dramatic, studies in other systems demonstrate profound effects of small differences in adhesion molecule density (32); for example, a twofold increase in density of neural cell adhesion molecule in liposomes results in a greater than 30-fold increase in adhesion (32, 33). Data recently reported by Cavendar et al. (34) indicate that a population of normal human peripheral blood T lymphocytes with enhanced expression of CD18 (β-chain for the LFA-1 family of adhesion molecules) and slightly enhanced cell volume show increased adhesiveness for endothelial cells. It is likely that the T cell population described in their studies is coincident with the population we are describing. In addition, enhanced LFA-3 expression could make a T lymphocyte more susceptible as a target to immunoregulatory cytolytic suppressor T lymphocytes, facilitating its removal during an immune response.

Inasmuch as LFA-3, LFA-1, and CD2 have each been reported in some systems to have signaling function of relevance to T cell activation (9, 12, 13), their enhanced expression on previously activated T cells could promote subsequent reactivation of those cells. For instance, enhanced expression of CD2 on the same cell with enhanced

[3] **Serra, H. M., J. A. Ledbetter, J. F. Krowka, and L. M. Pilarski.** 1987. Loss of CD45R (Lp220) represents a post-thymic T cell differentiation event. *J. Immun. 140:1435.*

expression of its ligand LFA-3 possibly could lead to an interaction of CD2 with LFA-3 on the same cell, resulting in an incomplete activation signal which augments responses to exogenous signals.

Activation of T cells induces increased expression of a wide variety of cell surface molecules. One parameter which distinguishes them is transient vs sustained increased expression. Some, such as IL-2R or class II HLA molecules are induced only transiently and are useful markers of recently activated human T cells. On the other extreme are molecules whose expression is stably increased after activation, and which therefore serve as markers of previously activated, i.e., memory T cells. Because of the magnitude of their increase in expression, LFA-3, UCHL1, and CDw29 are particularly useful markers of prior activation. Further studies are needed to understand the physiologic roles and mechanisms of regulation of this family of molecules whose expression is stably enhanced on memory T lymphocytes.

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