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Phenotypic changes associated with activation of CD45RA$^+$ and CD45RO$^+$ T cells

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SUMMARY

Resting CD45RO$^+$, mature/memory, T cells are phenotypically distinct from intermediate CD45RO$^+$/CD45RA$^+$ and CD45RA$^+$, immature/virgin, T cells, and are characterized by high levels of expression of a number of adhesion molecules, such as CD2, CD18, CD58 and CD29. The kinetics of up-regulation of molecules, like CD25 and CD54 associated with activation, were similar in both subsets and suggested that their high level expression was associated with later events rather than initial recognition and signal transduction. CD45RA$^+$ T cells, unlike CD45RO$^+$ T cells, were unable to proliferate in response to mitogenic combinations of CD2 monoclonal antibodies (mAb), although in combination with submitogenic doses of PMA both up-regulation of cell-surface molecules and proliferation occurred. In addition, recruitment of CD45RA$^+$ T cells by CD2 mAb-activated CD45RO$^+$ T cells can occur.

INTRODUCTION

CD45RA and CD45RO monoclonal antibodies (mAb) identify reciprocal subsets of resting human T lymphocytes. Both subsets proliferate and generate cytotoxicity in response to alloantigens. CD45RO$^+$ CD4$^+$ T cells respond by proliferation to soluble recall antigens and provide help for pokeweed mitogen (PWM) or antigen-induced immunoglobulin synthesis (Morimoto *et al.*, 1985a; Smith *et al.*, 1986), whilst CD8$^+$ CD45 RO$^+$ T cells contain increased numbers of cytotoxic memory precursors (Merkenschagler & Beverley, 1989). In contrast, the CD45RA$^+$ subset does not respond to recall antigens but contains T cells that have been shown to act as inducers for CD8$^+$ suppressor cells (Morimoto *et al.*, 1985b). Recent studies have shown that, following *in vitro* stimulation, CD45RA$^+$ T cells acquire CD45RO and lose CD45RA (Akbar *et al.*, 1988). This, together with the ability of CD45RO$^+$ T cells to respond to recall antigens, supports the view that the CD45RA$^+$ subset represents immature/virgin T cells whilst the CD45RO$^+$ subset contains the memory population, and that these represent different stages of a maturational pathway rather than distinct lineages (Beverley, 1987).

Abbreviations: FITC, fluorescein isothiocyanate conjugate; [^{3}H]TdR, tritiated thymidine; LCA, leucocyte-common antigen; LFA, lymphocyte function-associated antigen; mAb, monoclonal antibody; PHA, phytohaemagglutinin; PMA, phorbol myristate acetate; PWM, pokeweed mitogen.

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Little is known regarding the mechanisms underlying the vigorous secondary responses of memory cells and whether different functional molecules mediate interactions between different T-cell subsets and between T cells and their accessory/target cells. A number of cell-surface molecules are now recognized as playing roles in transmembrane signalling and/or cell–cell adhesion interactions. Some of these molecules, in particular CD58 (LFA-3), CD29 (4B4), CD2 and CD44 (the human equivalent of murine Pgp-1; Sanders *et al*, 1988), are expressed to a greater extent on CD45RO$^+$ memory cells. However, the relationship between the expression of these molecules and their role in early signal transduction and intermediate events, such as stimulation of IL-2 production and subsequent cell cycle progression, are not well understood, The up- or down-regulation of a number of these cell surface molecules on subsets of CD45RA$^+$ and CD45RO$^+$ T cells was therefore examined to determine whether there was any correlation between the phenotypic changes observed and the stage of activation and whether expression of different surface molecules influenced the effectiveness of utilization of different activation pathways.

MATERIALS AND METHODS

Monoclonal antibodies

The following mAb were used. OKT11 (CD2; ATCC, Rockville, MD), D66/1 (CD2R; Bernard *et al.*, 1982), SN130 (CD45RA; Akbar *et al.*, 1988), UCHL1 (CD45RO; Smith *et al.*, 1986), MHM23 (CD18; Hildreth *et al.*, 1983), TS2/9 (CD58; Sanchez-Madrid *et al.*, 1982), UCHT1 (CD3; Beverley & Callard, 1981), UCHT2 (CD5; Beverley & Callard, 1982),

UCHM1 (CD14; Hogg *et al.*, 1984), TAC (CD25; Leonard *et al.*, 1982), B-1 (CD20); Stashenko *et al.*, 1980); 4B4 (CD29; Morimoto *et al.*, 1985a), Leu 11b (CD16; Becton-Dickinson, Mountain View, CA); and L243 (MHC class 11; ATCC). BRIC5(CD58) and 7F7 (CD54) were obtained from the Fourth International Workshop on Leucocyte Differentiation Antigens. Fluorescein isothiocyanate-conjugated goat anti-mouse IgG1 and IgG2a antibodies were from Southern Biotechnology Associates Inc., Birmingham, AL.

Reagents

Purified phytohaemagglutinin (PHA; Wellcome Pharmaceuticals, Beckenham, Kent) was added to cultures at an optimal concentration of 1μg/ml. Phorbol myristate acetate (PMA; Sigma Chemical Company, Poole, Dorset) was dissolved in ethanol diluted in medium and used at a final concentration of 0·25 ng/ml. Sheep anti-mouse immunoglobulin-coated Dynabeads and Dynal magnetic particle concentrator came from Dynal A.S. (Oslo, Norway). Rabbit complement was obtained from Cedarlane Laboratories Ltd (Hornby, Ontario, Canada). Tetanus toxoid was a gift from Wellcome Research Laboratories, Beckenham, Kent.

Lymphocyte preparation

Peripheral blood mononuclear cells (PBM) were isolated from normal healthy adults by Ficoll–Hypaque fractionation (Nycomed, Oslo, Norway). PBM were depleted of adherent cells by incubating on plastic tissue culture plates for 90 min at 37°. CD45RA$^+$ and CD45RO$^+$ subpopulations were prepared for proliferation assays and phenotyping by negative selection procedures from non-adherent cells. 10^7 cells/ml in RPMI-1640 containing 5% fetal calf serum (FCS) were incubated with saturating doses of either SN130 (CD45RA) or UCHL1 (CD45RO) mAb (as determined by previous titration) together with anti-monocyte (UCHM1) and anti-B cell (B-1) mAb for 40 min at 4°. Cells were then washed twice and resuspended in 100 μl of medium. Ten microlitres of sheep anti-mouse immunoglobulin-coated Dynabeads were added to 10^7 cells, mixed and incubated for 50 min at 4°. Cells were resuspended in 5 ml of medium and incubated with a Dynal magnetic particle concentrator for 3 min, after which the supernatant containing non-magnetized cells was removed. These cells were centrifuged and the procedure then repeated. Natural killer (NK) cells were removed by incubating the cells with a saturating dose of Leu 11b for 30 min at 4°. Cells were washed twice in RPMI-1640 containing 1% FCS and resuspended at 3×10^6/ml in rabbit complement diluted according manufacturer's instructions and incubated at 37° for 60 min. Cells were then washed twice in RPMI-1640 containing 10 FCS. Cells were routinely >95% CD45RA$^+$ or CD45RO$^+$ and contained less than 5% contaminating cells from the reciprocal subset.

Proliferation assays

2×10^5 purified CD45RA$^+$ or CD45RO$^+$ T cells together with $1{\cdot}5 \times 10^4$ autologous irradiated adherent cells were incubated in triplicate in flat-bottomed microtitre plates (Titertek, Flow Laboratories Ltd, Rickmansworth, Herts) together with a mitogenic combination of CD2 mAb (OKT11,1/200 dilution of ascites, +D66/1, 1/800 dilution of ascites) or PHA 1 μg/ml in RPMI-1640 containing 5% FCS. Cultures were incubated for 3/4 days and pulsed for the last 6 hr with 1 μCi/well of [^{3}H]TdR; (thymidine Amersham International, Amersham, Bucks). The contents of each well were harvested onto glass fibre filters (Whatman, Maidstone, Kent) using an automash harvester (Dynatech, Billinghurst, Sussex). Radioactivity was measured in a LS 1800 liquid scintillation counter (Beckman, Palo Alto, CA). T cells stimulated with tetanus toxoid [1 limiting flocculation value (LF)/ml] were incubated in medium containing 5% autologous serum and pulsed and harvested on Day 6.

Single-colour immunofluorescence

2×10^5 purified T cells/well were incubated in round-bottomed flexible microtitre plates (Dynatech) together with 50 μl of antibody at a saturating dose of ascites or hybridoma supernatant in phosphate-buffered saline (PBS) containing 0·1% bovine serum albumin (BSA) for 30 min at 4°. Cells were washed four times with cold PBS/0·1% BSA, resuspended in 50 μl of a pretitrated amount of fluorescein isothiocyanate-conjugated goat anti-mouse IgG1 or IgG2a, and incubated for 30 min at 4°. Cells were washed four times in PBS/0·1% BSA; 5000 cells/sample were analysed on a FACScan (Becton-Dickinson).

Dual-colour immunofluorescence

Purified T lymphocytes (4×10^5/sample) in PBS containing 0·1% BSA were incubated in flexible microtitre plates for 40 min at 4° with 50 μl of unlabelled antibodies at a saturating dose of ascites or hybridoma supernatant. Cells were washed four times with cold PBS/0·1% BSA; supernatants were discarded and the cells were incubated with 50 μl of an optimal amount of fluorescein-isothiocyanate-conjugated goat anti-mouse IgG1 or IgG2a for 40 min at 4°. Cells were washed four times, supernatant discarded and cells mixed with 50 μl of an optimal concentration of biotinylated UCHL1 mAb. After 40 min incubation at 4°, cells were washed four times then incubated with an optimal amount of PE-conjugated avidin (Biogenesis, Bournemouth, Hants) for 40 min at 4°, then washed four times. 5000 cells/sample were analysed on a FACScan.

RESULTS

Phenotypic analysis of resting peripheral blood T cells

Figure 1 shows the immunofluorescence profiles of resting peripheral blood T cells double stained with CD45RO mAb UCHL1 and a variety of adhesion/signal transduction/activation molecules.

Previous studies (Smith *et al.*, 1986) showed that the CD45RO mAb (UCHL1) and CD45RA mAb (2H4) define reciprocal populations of T cells. However, the use of more sensitive flow cytometers (FACScan) reveals that not only are CD45RA$^+$/CD45RO$^-$ and CD45RA$^-$/CD45RO$^+$ subpopulations detected, but also that a subset of cells expressing intermediate levels of both molecules is present (Fig. 1a). The proportion of CD45RA$^+$ and intermediate cells varies considerably between individuals, although the number of CD45RO$^+$ cells is relatively constant (about 30%). The properties of these three subpopulations are under investigation (L. D. Wallace *et al.*, manuscript in preparation).

CD29 (4B4) and CD58 (LFA-3) have both been reported to define cells with memory function (Morimoto *et al.*, 1985a; Sanders *et al.*, 1988). CD58 is present only on CD45 RO$^+$ cells, whereas CD29 is present on all cells but at a higher level on

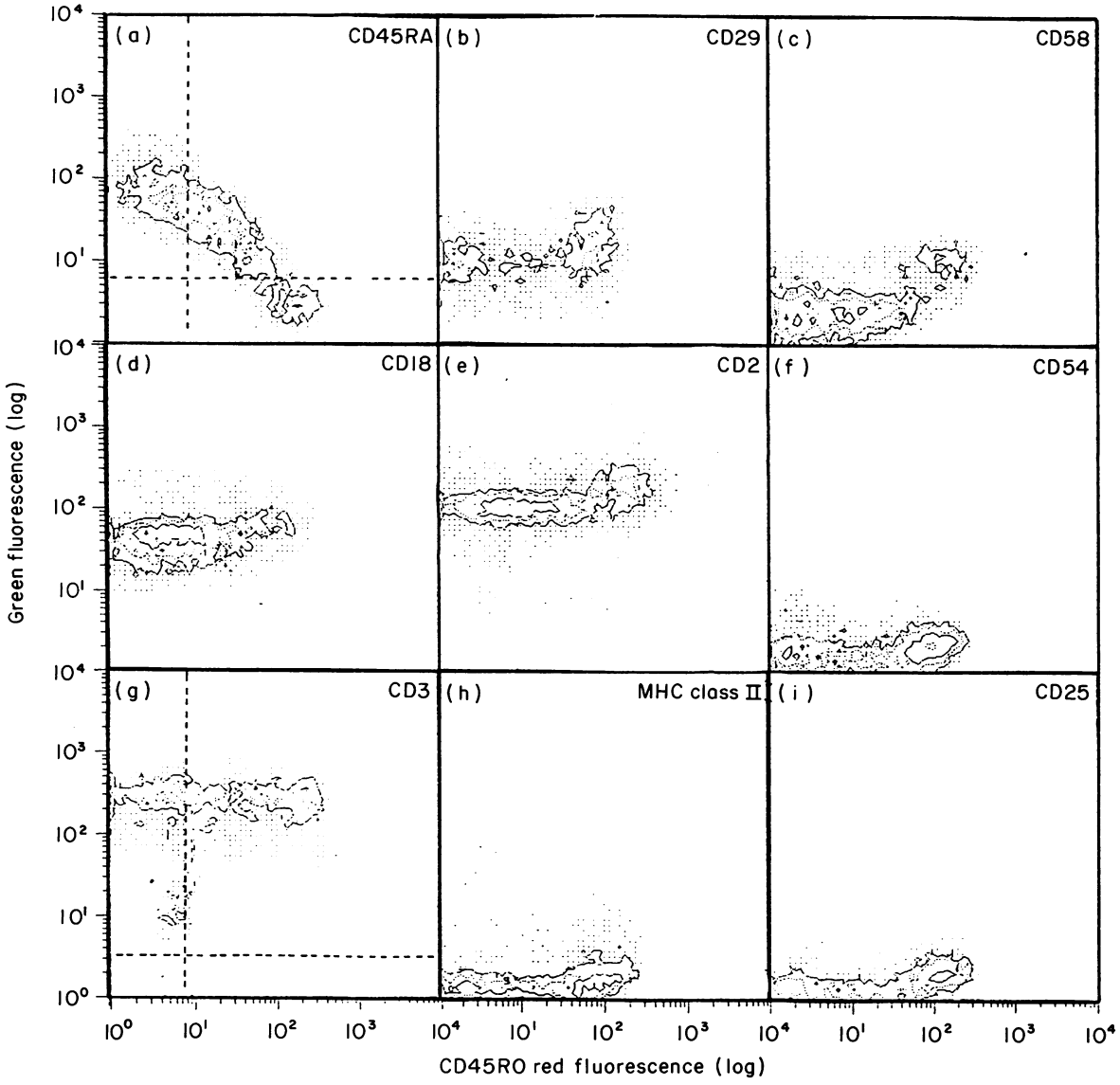

Figure 1. Two-colour immunofluorescence analysis of the expression of a variety of lymphocyte antigens in relation to CD45RO expression on resting human peripheral blood T lymphocytes taken from three individuals. Goat anti-mouse IgG1 and IgG2a FITC conjugates were used to detect mAb against surface antigens (*y*-axis), while biotinylated UCHL1 mAb followed by phycoerythrin-conjugated avidin was used to detect CD45RO (*x*-axis). The cut-off point for positive/negative cells as determined by reference to an irrelevant negative control mAb is shown by the dotted lines in (a) for (a–e) and (g) for (f–i). Samples were analysed on a FACScan using the Consort 30 program.

$CD45RO^+$ cells. No third population with intermediate levels of CD29/CD58 staining is seen (Fig. 1b, c). Similarly, molecules associated with adhesion/signal transduction. CD18 (LFA-1 β-chain) and CD2 (Fig. 1d, e) show a pattern of staining where high CD18/CD2 expression correlates with high expression of CD45RO. In contrast, CD54 (ICAM-1), another molecule associated with memory and adhesion (Buckle & Hogg, 1989) is expressed at a low level and only on a proportion of $CD45RO^+$ cells (30% of $CD45RO^+$). Low levels of activation markers like CD25 (TAC/IL-2R) or MHC class 11 (Fig. 1h, i) can be found on a few cells. $CD45RO^+$ bright cells therefore form a phenotypically distinct population characterized by high levels of expression of CD45RO, CD2, CD58 and CD29 and with variable levels of activation molecules such as CD54 and CD25.

In contrast to those molecules involved in the adhesion pathways, those molecules involved in the CD3/Ti activation pathway, such as CD3 (Fig. 1) and MHC class 1/CD4/CD8 (data not shown), express equal levels on all subpopulation of T cells. The same is true for other molecules of unknown function, for example, CD5 (data not shown).

Phenotypic analysis of activated T cells

Many cell-surface molecules involved in cell interactions are up- or down-regulated during the course of activation. It has previously been shown that PHA induces $CD45RA^+$ T cells to become $CD45RO^+$ (Akbar *et al.*, 1988). Figure 2 shows the up- or down-regulation on $CD45RA^+$ cells of a variety of adhesion

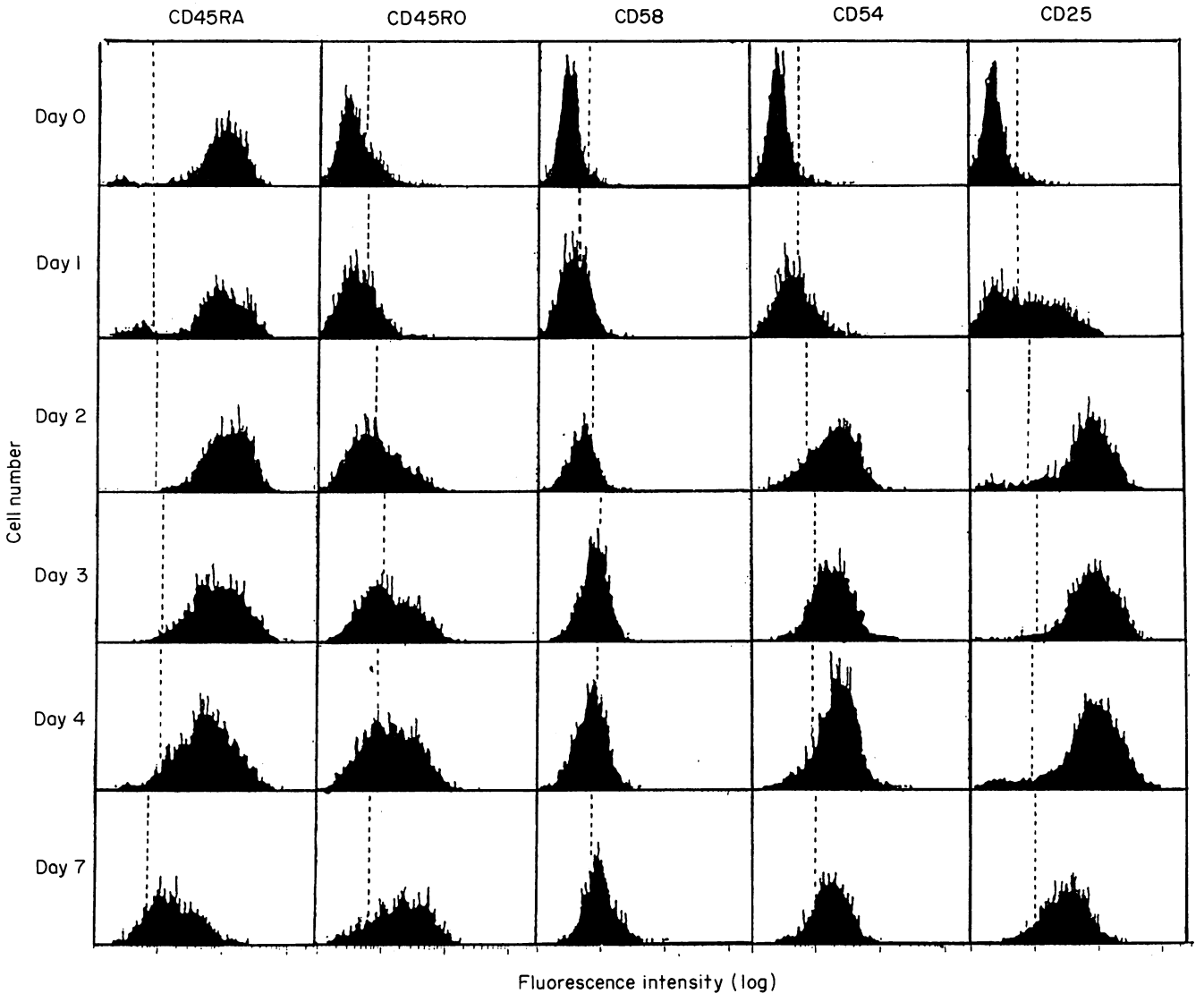

Figure 2. Alterations in expression of CD45RO (UCHL1), CD45RA (SN130), CD58 (TS2/9) CD54 (7F7) and CD25 (TAC) over a period of 7 days, on purified CD45RA$^+$ T cells stimulated with PHA (1 $\mu g/10^6$ cells). Surface antigens were detected by indirect immunofluorescence using FITC-conjugated anti-subclass-specific antisera. The negative control for each subclass (an irrelevant mAb of the same subclass) is represented by the dotted line.

molecules and activation antigens at various times after PHA-induced stimulation of CD45RA$^+$ T cells.

CD54 and CD25 expression reaches a maximum level at Day 3/4. The level of CD25 then starts to decrease after Day 4; however, the level of CD54 at Day 7 is still greater than that found on CD54$^+$, unstimulated, circulating CD45RO$^+$ T cells. CD58 is also up-regulated, but a lesser extent. Median fluorescence on Day 0 is similar, 115 for CD54 and 116 for CD58, but by Day 3 the median fluorescence of CD54 is 375 compared with 238 for CD58.

Maximum expression of both CD54 and CD25 correlates with the time of peak proliferation (Day 3/4). In contrast, CD45RA down-regulation and CD45RO up-regulation are more gradual and do not reach completion till after proliferation has ceased; by this time cells are decreasing in size and returning to G0. Initial stages of activation therefore do not appear to be dependent on upregulation of CD45RO.

When CD45RO$^+$ T cells are stimulated with PHA, the kinetics of CD25 and CD54 expression are similar to those obtained with PHA-stimulated CD45RA$^+$ T cells (Fig. 3). Levels of CD58 and CD45RA expression do not alter after stimulation (data not shown). The level of CD25 on CD45RO$^+$ T cells begins to decrease after Day 3, while that of CD54 even on Day 11 is still high. In contrast, MHC class 11 expression is up-regulated more slowly, reaching a maximum at Day 7/8 and thereafter declining.

Since on both CD45RA$^+$ and CD45RO$^+$ T cells up-regulation of CD58 adhesion molecules occurs only after 24 hr, this suggests that this molecule is less likely to be involved in initial signal transduction than in amplification signals and

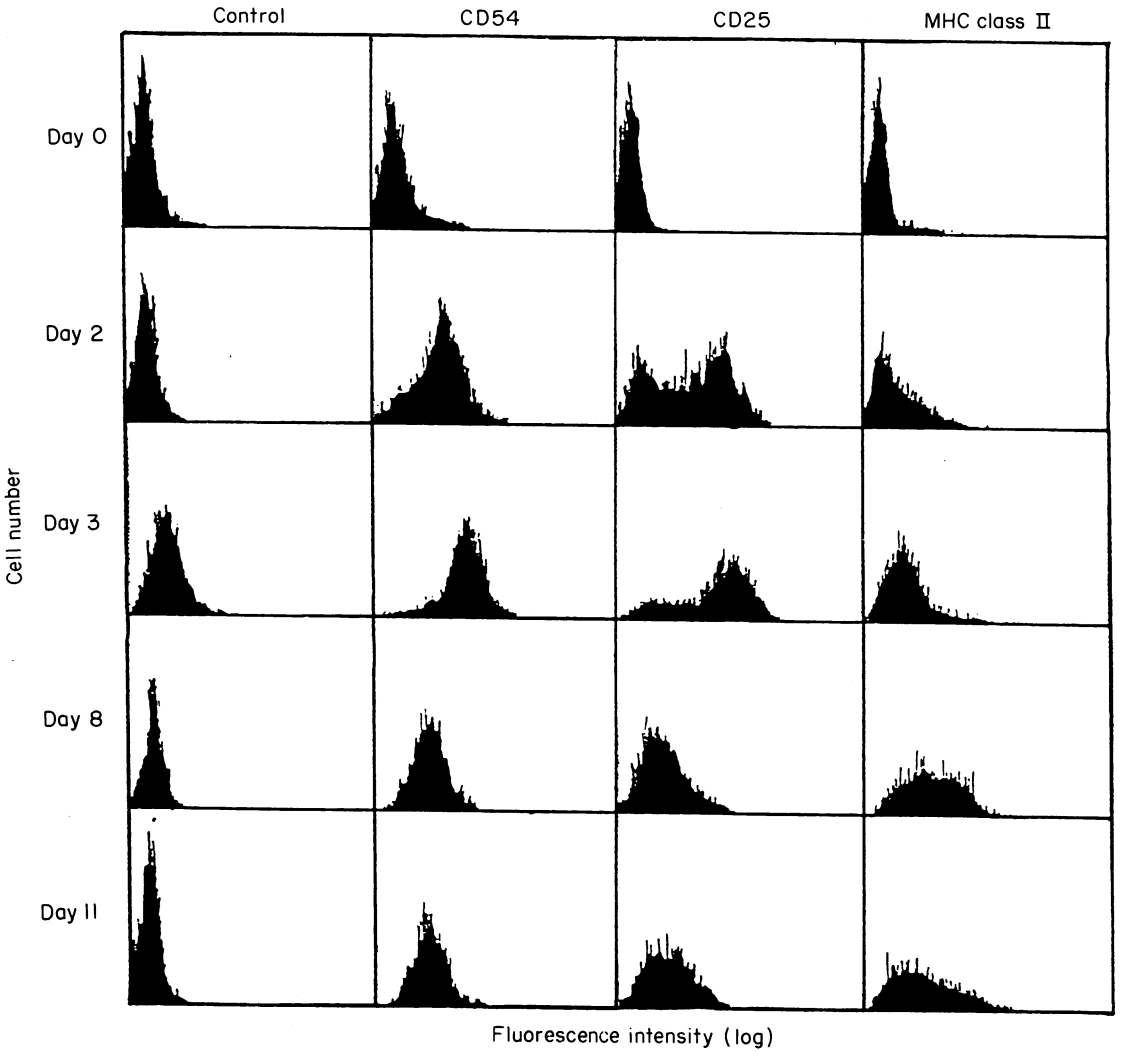

Figure 3. Alterations in expression of CD54 (7F7), CD25 (TAC) and MHC class 11 (L243) on negatively selected CD45R$^+$ T cells stimulated with PHA (1 μg/10^6 cells). Indirect immunofluorescence using FITC-conjugated anti-subclass antisera was carried out prior to stimulation (Day 0) and on Days 2, 3, 8 and 11 after stimulation. The negative control consisted of an irrelevant antibody the same immunoglobulin subclass.

Table 1.

Stimulus	[^{3}H]TdR incorporation (c.p.m. ± SD) of responding T cells	
	CD45RO$^+$	CD45RA$^+$
OKT11 + D66/1	43,337 ± 6563	2265 ± 138
PMA	6500 ± 604	7200 ± 597
OKT11 + D66/1 + PMA	197,200 ± 6700	201,000 ± 3480
PHA	71,500 ± 2532	106,800 ± 1906
Tetanus toxoid	17,753 ± 1412	4350 ± 2430

2×10^5 negatively selected CD45RO$^+$ and CD45RA$^+$ T cells (supplemented with 10% irradiated autologous adherent cells) were stimulated with CD2 mAb (OKT11 1/200 ascites + D66/1 1/800 ascites) with or without addition of 0·25 ng/ml of PMA, or with PHA alone (1 μg/ml), or tetanus toxoid at 1 LF/ml. Cells were pulsed with [^{3}H]TdR for 6 hr on Day 3 (Day 6 for tetanus toxoid).

effector function. In contrast, other molecules that are already well expressed, such as CD2 and CD18, may play a role in signal transduction.

Response of CD45RA$^+$ and CD45RO$^+$ subpopulations of T cells to CD2 mAb

Although both subpopulations seem to respond identically to polyclonal mitogens such as PHA-P (Table 1), CD45RA$^+$ virgin cells and CD45RO$^+$ memory cells have been shown to have different functional properties (Smith *et al.*, 1986), the ability to respond to recall antigens being restricted to CD45RO$^+$ T cells (Table 1). In addition, Table 1 shows that CD2 mAb, although able to bind to CD45RA$^+$ T cells (Fig. 1e), are unable to induce proliferation of these cells, even though CD45RO$^+$ T cells respond well. The data suggest that the CD2 pathway is more effectively utilized in CD45RO$^+$ T cells than in CD45RA$^+$ T cells. This difference is not dependent on accessory cells, as even

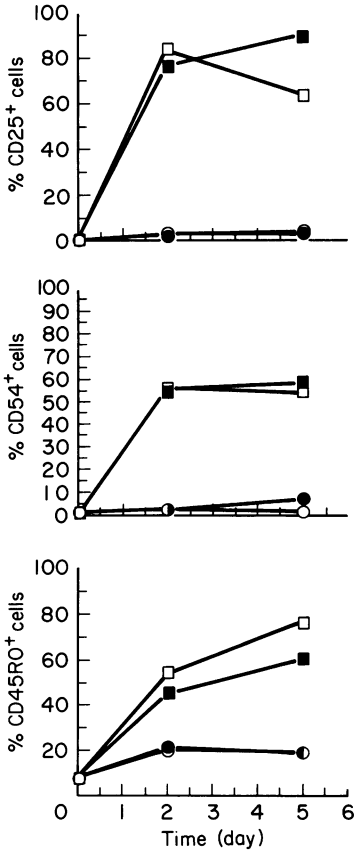

Figure 4. Negatively selected CD45RA⁺ T cells at 2×10^6/ml were stimulated with a combination of CD2 mAb that were mitogenic for unseparated T cells (●) (D66/1,1/800 dilution of ascites + OKT11 1/200 dilution of ascites), with a submitogenic dose of PMA (○) (0·25 ng/ml), a combination of both (■) or with PHA at 1 μ/ml (□). The percentage of cells expressing CD25 (TAC), CD54 (7F7) and CD45RO (UCHL1) was examined by indirect immunofluorescence using FITC-conjugated anti-subclass antisera.

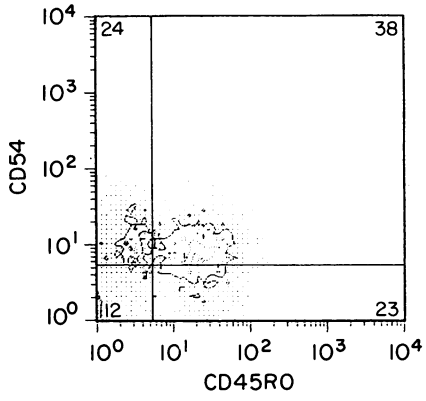

Figure 5. Purified T cells were stimulated with a mitogenic pair of CD2 mAb (OKT11 + D66/1). On Day 3 cells were stained indirectly with CD54 mAb and FITC-conjugated anti-subclass antisera (*y*-axis) and then double-stained with biotinylated CD45RO mAb (UCHL1) and PE-conjugated avidin (*x*-axis). Samples were analysed on a FACScan using the Consort 30 program. Samples were gated (solid lines) with reference to an irrelevant control mAb of the same subclass.

the addition of 30% autologous adherent cells does not reconstitute the response (data not shown). Although these results are consistent with those obtained by some groups (Sanders *et al.*, 1989; Byrne *et al.*, 1989), conflicting results have been obtained by others (Matsuyama *et al.*, 1988). One explanation may be the method of cell preparation used. In the experiments described here negative selection was used. The resulting subpopulations consist of CD45RA⁺ and CD45RO⁺ cells, while intermediate cells are discarded. This method was preferred as recent experiments (Schraven *et al.*, 1988) have shown that CD45RA mAb in combination with CD2 mAb can provide a co-mitogenic signal for CD45RA⁺ cells.

Previous data has shown that PMA in combination with CD2/CD3 mAb can provide a co-mitogenic signal for T cells (Holter *et al.*, 1986, 1985). Table 1 shows that PMA at submitogenic concentration is able to provide a co-mitogenic signal for the CD45RA⁺ T cells in combination with CD2 mAb. In addition the level of proliferation of both CD45RA⁺ and CD45RO⁺ cells is four times that of CD45RO⁺ T cells stimulated with CD2 mAb alone.

Phenotyping of CD2-stimulated CD45RA⁺ T cells was carried out to determine whether expression of activation molecules such as CD25 and CD54 could occur. Figure 4 indicates that CD25 and CD54 are not detected even when autologus presenting cells are provided, even though PHA-P does induce up-regulation of these molecules. Low concentrations of PMA (0·25 ng/ml) had no effects on up-regulation. However, when used in combination with CD2 mAb, it is able to induce expression of CD25/CD54.

Recruitment of CD45RA⁺ T cells by CD45RO⁺ T cells

The possibility that activated CD45RO⁺ cells can in turn stimulate CD45RA⁺ T cells was investigated using CD2 mAb-induced stimulation of unseparated T cells, as these mAb have been shown to stimulate only CD45RO⁺ T cells. Because CD54 is expressed on activated CD45RA⁺ T cells before they have lost CD45RA and acquired CD45RO, cells were double-stained on Day 3 with CD45RA mAb and CD54 mAb. Figure 5 shows that 24% of the cells are CD45RO⁻ (CD45RA⁺)/CD54⁺. Similar results were found with CD25 (data not shown). This may indicate that *in vivo* non-specific 'recruitment' of CD45RA⁺ T cells can occur.

DISCUSSION

Molecular analysis has indicated that several different leucocyte-common antigen messenger RNAs, coding for at least four different polypeptides (220,000, 205,000, 195,000 and 180,000 MW), can be obtained from a single gene by alternative splicing (Ralph *et al.*,1987). CD45RA mAb that recognize high molecular weight polypeptides (220,000, 205,000 MW) distinguish a functionally distinct subset of T cells with suppressor/inducer activity, while a non-overlapping subset of CD45RO⁺ cells with helper/inducer activity is identified by the CD45RO mAb UCHL1, which recognizes the 180,000 MW polypeptide (Terry *et al.*, 1988). The switch from one pattern of splicing (CD45RA⁺) to another (CD45RO⁺) is now thought to be linked to maturation or activation of T cells, the CD45 RA⁺ subset being immature/virgin cells and the CD45RO⁺ subset

being mature/memory cells (Akbar *et al.*, 1988; Merkenschlager *et al.*,1988; Merkenschlager & Beverley, 1989).

Although CD45RA and CD455RO mAb are said to identify two reciprocal subsets, the double immunofluorescence staining described here (using one of the more sensitive current flow cytometers (FACScan) shows that resting peripheral blood T cells also contain a population with an intermediate pattern of staining. These could represent cells in the process of switching from CD45RA$^+$ to CD45RO$^+$ phenotype, although they do not express activation markers such as CD25 or MHC class 11 or increased levels of CD54 and CD58. An alternative view is that there is heterogeneity among immature/virgin T cells. Differences in proliferative responses to monoclonal antibodies among CD45RA cells support this idea (B. Dupont, personal communication).

Immunofluorescence analysis of resting cells showed that the CD45RO$^+$ subset is a phenotypically distinct population characterized by high levels of molecules involved in adhesion/signal transduction, including CD58, CD29 and CD2. In addition, a proportion of CD45RO$^+$ cells expresses low levels of CD54. This is in agreement with previous reports (Sanders *et al.*, 1988; Buckle & Hogg, 1989). Whether the presence of CD54 and the activation makes CD25 and MHC class II indicate that memory T cells are constantly antigen driven, as has been suggested for memory B cells, remains to be determined (Gray & Skarvall, 1988).

This study also investigated the phenotypic changes occurring as a result of activation. CD45RA$^+$ T cells rapidly up-regulate those molecules associated with memory cells, such as CD54 and CD58, as well as CD25. In contrast, CD45RO up-regulation is slow and does not reach its maximum until after cells have ceased to proliferate; this implies that CD45RO expression is not required for activation. However, as early biochemical events leading to signal transduction across the cell membrane occur within minutes, it is unlikely that CD54 and CD58 are involved in these initial events but rather that they are involved in secondary amplification events that allow cells to proceed to proliferation. In contrast, LFA-1 may play a role in initial recognition and T cell–target cell adhesion, as CD11a and CD18 mAb (LFA-1 α- and LFA-1 β-chains) can block proliferative responses to mitogens and antigen (Dougherty & Hogg, 1987). The kinetics of the up-regulation and maintenance of expression of CD54 and CD58 on both CD45RA$^+$ and CD45RO$^+$ subsets is very similar, implying that differences in responsiveness are related to transmission of initial signals rather than interactions occurring as a result of up-regulation of these molecules. The persistence of these molecules after proliferation has ceased suggests they may also play a role in the effector and regulatory functions of T cells. For example, they may facilitate the effects of paracrine growth factors like IL-2 by allowing clustering of activated cells. Recent evidence has demonstrated that CD11a/CD18 in association with specific homing molecules facilitate endothelial adherence (Mentzer *et al.*, 1986). Up-regulation of adhesion molecules may therefore influence the localization of activated T cells in secondary lymphoid organs.

Like others (Sanders *et al.*, 1989; Byrne *et al.*, 1989), in this study it was observed that CD45RA$^+$ T cells do not proliferate in response to stimulation with mitogenic pairs of CD2 mAb, although CD45RO$^+$ T cells respond well. Therefore it was investigated whether, since they can bind to CD2 molecules on all T cells, they could induce expression of surface markers associated with activation. Phenotypic analysis of CD2 mAb-stimulated CD45RA$^+$ T cells showed that expression of early activation molecules like CD25 and CD54 was not induced even when autologous presenting cells were provided. Interestingly, PMA at submitogenic doses could, in combination with CD2 mAb though not on its own, induce proliferation of CD45RA$^+$ T cells. PMA is said to short circuit the accessory cell requirements by directly activating protein kinase C (Niedal *et al.*, 1983), and also to up-regulate CD2 expression (Cantrell *et al.*, 1988). It may be that signals resulting from CD2 binding are not on their own sufficient to induce activation, and that a second signal leading to up-regulation of CD25 and CD58 is required before cells can differentiate into effector cells. Physical T cell–accessory cell interaction is likely to be necessary for activation; in CD45RA$^+$ T cells this interaction may be inefficient because of the low level of expression of accessory molecules. Increased expression, on CD45RO$^+$ mature/memory T cells, of those cell surface molecules involved in antigen-independent adhesion and signal transduction would be likely to enhance the vigorous secondary responses resulting from activation of memory cells via CD3/Ti while, in contrast, reduced expression of these molecules on virgin CD45RA$^+$ T cells would lessen the risk of non-specific activation of self-reactive cells.

These studies also showed that CD45RA$^+$ T cells can be activated in the presence of CD2-stimulated CD45RO$^+$ T cells, indicating that *in vivo* recruitment of CD45RA$^+$ T cells might occur. The nature of signals delivered to CD45RA$^+$ T cells by CD45RO$^+$ cells remains to be determined.

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