activation of unprimed CD4+CD45RA+/RO- T cells results in a gradual loss of CD45RA expression concomitant with the acquisition of CD45RO. It has been suggested that this conversion occurs in vivo through a CD45RAbright/RObright stage. Next to this small CD45RAbright/RObright subset (Dbright), a larger subpopulation that expresses both RA and RO isoforms at low levels (Ddull) can be found in the circulating CD4+ T-cell population of all donors. The properties of the latter population are largely undefined. Here, we show that Ddull cells have an intermediate phenotype for antigens such as CD31, CD62L, CD58, and CD95 that are differentially expressed on unprimed versus primed T cells. In addition, they are able to provide help for B-cell differentiation and contain substantial numbers of tetanus toxoid (TT)-specific precursor cells. Remarkably, both intracellular cytokine staining and analysis of T-cell clones showed that Ddull cells and CD45RO+ T cells produce comparable high amounts of both interferon (IFN)-γ and interleukin (IL)-4, which clearly distinguishes them from CD45RA+ and Dbright T cells. Finally, prolonged culture of sorted Ddull cells in a mixture of IL-2, IL-6, and tumor necrosis factor (TNF)-α showed that about half of the population retained the Ddull phenotype. Part of the cells upregulated the CD45RA isoform, whereas only a minority switched to single CD45RO expression. Our findings indicate that the Ddull population contains primed T cells, some of which may reacquire an “unprimed” phenotype in the absence of antigenic stimulation.

**MATERIALS AND METHODS**

Reagents. The monoclonal antibodies (MoAbs) CLB-T11.1/1, CLB-T11.2/1, CLB-HIK27 (all CD2), CLB-CD4/1, CLB-CD8/1, CLB-CD14/1, CLB-FcR gran1 (CD16), CLB-CD19/1, CLB-HeC/75 (CD31), CLB-CD25/1, and CLB-CD27/1 were all produced at the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB). CD95 MoAb was kindly provided by Dr Yonehara (Tokyo, Japan) and CD58 MoAb (TS2/9) was a gift of Dr T.A. Springer (Boston, MA). CD62L MoAb (Leu-8) was from Becton Dickinson (San Jose, CA). CD45RA MoAbs 2H4 and 2H4-RD1 were obtained from Coulter Immunology (Hialeah, FL). CD45RO MoAb UCHL-1 FITC was purchased from DAKO (Glostrup, Denmark) and UCHL-1-Quantum Red from Sigma (St Louis, MO). Biotinylated CLB-IL-4/1 MoAb (antihuman IL-4, mouse immu-
noglobin G1 [IgG1], kindly provided by Dr T. van der Pouw-Kraan, CLB) and biotinylated MD-1 MoAb\(^{32}\) (anti-human IFN-\(\gamma\), mouse IgG1, obtained from Dr P. van der Meide, TNO, Rijswijk, The Netherlands) were used for intracellular staining of cytokines. Mouse IgG1-B (biotinylated mouse IgG1, CLB) was used as a control reagent. Streptavidin Red 670 was obtained from Life Technologies (Gaithersburg, MD). Antibodies for IL-5 and IL-10 enzyme-linked immunosorbent assay (ELISA) were generously provided by DNAX Institute (Palo Alto, CA).

Tetanus toxoid (TT) was purchased from RIVM (Zeist, The Netherlands).

**Cell preparation and flow cytometry.** Human peripheral blood mononuclear cells (PBMC) were isolated from buffy coats of healthy donors by Ficoll-Isopaque density centrifugation (Pharmacia, Uppsala, Sweden). CD4\(^+\) T cells were purified by incubating the PBMC with CD8, CD14, CD16, and CD19 MoAbs followed by negative depletion with Dynabeads-M450 (Dynal A.S., Oslo, Norway). CD4\(^+\) cells (purity > 95%) were subsequently stained with CD45RA-PE and CD45RO-FITC and sorted into CD45RA\(^+\), CD45RA\(^-\)RO\(^+\) (Ddull), and CD45RO\(^+\) populations (purity > 98%) using a FACScan (Becton Dickinson, Mountain View, CA).

Sorted cell populations were either used in functional assays or cultured for 14 days in Iscove’s modified Dulbecco’s medium (IMDM) supplemented with 10% fetal calf serum (FCS) and antibiotics in the presence of a combination of recombinant (r)IL-2 (250 U/mL; kind gift of Sandoz, Vienna, Austria), recombinant tumor necrosis factor (rTNF-\(\alpha\)) (20 ng/mL; Genzyme, Cambridge, MA), and rIL-6 (500 U/mL; generously provided by Professor L. Aarden, CLB) as described by Ununatz et al.\(^{2,22}\)

Immunofluorescence analysis was performed according to standard protocols\(^{13}\) with either GAM-FITC (CLB-GM17-FITC, CLB) or directly labeled MoAb for staining and a FACScan (Becton Dickinson, Mountain View, CA). Figure 1 shows three-color staining of PBMC with CD4, CD45RA, and CD45RO. Values are expressed in percentages or as the number of cells per million.

Flow cytometric cytokine production measurement. Flow cytometric measurement of cytokine production was based on the stimulation of cells in the presence of an inhibitor of protein secretion resulting in the cytoplasmic accumulation of the synthesized cytokines. After cell fixation and permeabilization, intracytoplasmic staining was performed according to a protocol, originally described by Jung et al.\(^{24}\).

Briefly, 1 x 10\(^6\) cells/mL were stimulated for 5 hours with phorbol myristate acetate (PMA) 5 ng/mL and ionomycin 1 \(\mu\)g/mL in the presence of the protein-secretion-inhibitor monensin 1 \(\mu\)g/mL (all from Sigma). Cells were washed twice with cold phosphate-buffered saline (PBS) and fixed with PBS/4% paraformaldehyde (at 4°C, 10 minutes). Fixation was followed by permeabilization with PBS containing 0.1% saponin (Sigma) supplemented with 10% human pooled serum (at 4°C, 10 minutes). For all subsequent incubation and washing steps, PBS/0.1% saponin/0.5% bovine serum albumin (BSA) was used. Staining of the cytoplasm with a biotinylated cytokine MoAb (5 \(\mu\)g/mL) was followed by incubation with Streptavidin Red 670 (both at 4°C, 20 minutes). Biotinylated mouse IgG1 was used to verify the staining specificity of the cytokine MoAb. Analysis was performed on a FACScan.

Induction and measurement of Ig synthesis. Increasing amounts of T cells were cocultured with E-rosette-negative cells of an allogeneic donor (5 x 10\(^5\)/well) in cD3-coated (1:1,000 final dilution of ascites) flat-bottom microtiter plates (96-well; Greiner, Nürnberg, Germany). Supernatants of triplicate cultures were obtained after 14 days and tested for their Ig content. IgM and IgG production was measured by ELISA, as described previously.\(^{25}\)

Limiting dilution assay. To determine the precursor frequency of TT-reactive T cells, a limiting dilution analysis was performed as previously described.\(^{26}\) CD4\(^+\) T cells were sorted into CD45RA\(^+\), Ddull, and CD45RO\(^+\) subsets and graded numbers of T cells (40 x 10\(^3\) to 0.3 x 10\(^3\)) were cocultured with 5 x 10\(^3\) autologous irradiated (3,000 rad) plastic-adherent cells in the presence of TT (15 limit dilution [LF]/mL). No antigen was added to control wells. Cells were cultured in IMDM supplemented with 10% human pooled serum (HPS) for 9 days, and during the last 18 hours, 0.2 \(\mu\)Ci/well of \(^{3}H\)thymidine (2 Ci/mmol; Amersham, Buckinghamshire, UK) was added. Twenty-four replicate cultures were set up at each cell concentration. Only those cultures were scored as positive in which the counts per minute incorporated exceeded the mean ± 3 SD in the set of corresponding nonstimulated cultures. Frequency estimates were made using the “single-hit Poisson model” described by Strijbosch et al.\(^{27}\)

Generation of T-cell clones. Purified CD4\(^+\) T cells (>95%) were sorted into CD45RA\(^+\), CD45RA\(^-\)RO\(^+\) (Ddull), and CD45RO\(^+\) subsets and cultured under limiting dilution conditions (0.3 cells per well) in the presence of 1 \(\mu\)g/mL phytohemagglutinin (PHA; Wellcome, Beckenham, UK), 20 U/ml rIL-2 (Sandoz) and irradiated (3,000 rad) allogeneic PBMC (10\(^3\)/mL) in IMDM supplemented with 10% human pooled serum.\(^{23}\) T-cell clones from all subsets were generated with similar cloning efficiencies (>30%) and passed weekly by culturing 0.3 x 10\(^6\) cells/mL with PHA, rIL-2, and feeder cells as described earlier. To assess the cytokine profile of each clone, triplicate cultures of T-cell blasts (5 x 10\(^4\)/0.2 mL), harvested 7 days after restimulation, were stimulated with a mitogenic combination of CD2 MoAb (CLB-T11.1/1, CLB-T11.2/1, and CLB-HIK27, all 1:1,000 final dilution of ascites) and CD28 MoAb (CLB-CD28/1, 1:1,000 final dilution of ascites). Supernatants were collected at 24 hours (IL-2) and at day 4 (IFN-\(\gamma\), IL-4, IL-5, and IL-10) after initiation of the culture and stored at -20°C. IL-2 production was determined by means of the IL-2-dependent CTLL-2 line as previously described.\(^{25}\) All other cytokines were measured with specific ELISAs.\(^{20,21,30,31}\)

_Statistics._ The Mann-Whitney U test was used to compare functional and phenotypic data between the different subsets of T-cell clones.
Table 1. Phenotypic Analysis of CD4' T-Cell Subsets

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>CD45RA+</th>
<th>Ddull</th>
<th>CD45RO-</th>
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<tbody>
<tr>
<td>CD31</td>
<td>47.3±14.6</td>
<td>15.3±8.4</td>
<td>4.1±2.0</td>
</tr>
<tr>
<td>CD62L</td>
<td>84.9±20.7</td>
<td>72.6±31.0</td>
<td>54.2±26.8</td>
</tr>
<tr>
<td>CD58</td>
<td>14.7±8.1</td>
<td>42.7±19.3</td>
<td>73.8±15.8</td>
</tr>
<tr>
<td>CD95</td>
<td>67.3±3.8</td>
<td>36.0±19.0</td>
<td>73.7±16.4</td>
</tr>
</tbody>
</table>

*Freshly isolated CD4' cells (n = 5) were stained by 3-color immuno-fluorescence using Quantum Red-conjugated CD45RO and PE-conjugated CD45RA in combination with unlabeled MoAbs that were detected with GAM-FITC.

† Percentage positive cells.

RESULTS

Two intermediate phenotypes exist between CD45RA' /RO- and CD45RA'/RO' T cells. Next to the well-described CD45RA' /RO- (unprimed) and CD45RA'/RO' (primed) cells, two distinct cell populations can be detected within the peripheral blood CD4' T-cell subset that coexpress both CD45 isoforms either at high (CD45RAbright/RObright) or low density (CD45RA dull/RO dull) (Fig 1). In all healthy donors tested (n = 0), both phenotypes were present, although the CD45RA bright/RO bright subset that constitutes less than 1% (0.37% ± 0.1%) of the circulating CD4' T-cell population is much smaller than the CD45RA dull/RO dull population (17.4% ± 2.5%). The four subpopulations that can be characterized by differential expression of CD45 isoforms were designated CD45RA', D bright (CD45RA bright/RO bright), D dull (CD45RA dull/RO dull), and CD45RO-.

After antigenic activation, unprimed CD4' T cells not only switch from CD45RA to CD45RO expression, but also undergo coordinate changes in the expression of adhesion molecules and homing receptors. Surface antigens that are differentially expressed on unprimed compared with primed T cells were analyzed on the D dull population (Table 1). CD45RA' cells contained a higher percentage of cells that expressed the homing receptors CD31 and CD62L compared with CD45RO- cells. CD58, an adhesion antigen, and CD95 were upregulated in the course of transition from unprimed to primed cells. The transitional phenotype of the D dull cells resulted either from an intermediate expression level (CD58), the presence of both high and low expressing cells (CD31, CD62L), or both (CD95) (Fig 2).

D dull cells have the capacity to provide help for B cells and contain antigen-specific precursor cells. Functionally, CD45RA' and CD45RO' differ in their capacity to provide help for B-cell Ig synthesis. To compare helper activity of D dull cells with that of the other subsets, sorted T cells were cocultured with E-rosette-negative cells of allogeneic donors in CD3 MoAb-coated wells and Ig synthesis was measured after 14 days. In parallel cultures [3H]-thymidine incorporation was determined on day 3 to test the proliferative capacity of the different subsets to the given stimulus. Despite comparable proliferation of all subsets after optimal stimulation with immobilized CD3 MoAb, CD45RA' cells failed to provide B-cell help. D dull cells had considerable helper activity, although the amount of secreted IgG reached only about half the level of that in the presence of CD45RO- cells (Fig 3A). To compare D dull and CD45RO' cells in more detail, increasing numbers of sorted cells were cocultured with E-rosette-negative cells. Again, no Ig secretion could be detected in the presence of CD45RA' cells. To generate equal amounts of IgG, approximately five times more D dull than CD45RO- cells were required (Fig 3B).

Responses to recall antigens such as TT are compartmentalized almost exclusively in the primed CD45RO- subset. Subsets of CD4' cells from two healthy blood donors were sorted and a limiting dilution analysis was performed. The
sorted into CD45RA⁺, Dnull, and CD45RO⁺ subsets. Subsequently, the sorted populations were stimulated for 5 hours with PMA (1 ng/mL) and ionomycin (1 μM) in the presence of the protein secretion-inhibitor monensin. After fixation and permeabilization of the cell membranes, accumulated IFN-γ and IL-4 were detected with specific MoAbs (Fig 4). In contrast to the CD45RA⁺ population where, as expected, the number of IFN-γ- or IL-4-producing cells was low, the Dnull subset contained substantial numbers of IFN-γ-producing cells compared with the CD45RO⁺ subset. Notable, its frequency of IL-4 producers was comparable to the CD45RO⁺ population.

Thus far, Dbright cells have been only phenotypically characterized. Due to the very low frequency of Dbright cells in the circulation, T-cell cloning is the only possibility to compare functional properties of Dbright and Dnull cells. Purified CD4⁺ T cells were sorted into CD45RA⁺, Dbright, Dnull, and CD45RO⁺ subsets and random cloning was performed with PHA and IL-2 (for the sort windows, see Fig 1). T-cell clones from all subsets were obtained with similar cloning efficiency (30% to 40%) and 24 T-cell clones per subset were analyzed. Although all T-cell clones expressed CD45RO after 3 weeks of culture, the clones were named after the cell population they were generated from. Cytokine secretion of the T-cell clones was analyzed 4 to 6 weeks after their generation. No differences in proliferation of the T-cell clones obtained from the four populations to stimulation either with CD3 MoAb or a combination of three CD2 MoAbs and CD28 MoAb were observed (data not shown). The CD45RA⁺ group of T-cell clones appeared to be mainly composed of IL-2 producers, whereas the CD45RO⁺ group contains high IFN-γ, IL-4, and IL-5, as well as IL-10 producers (Fig 5). The differences between the two subsets were found to be significant for all cytokines measured. T-cell clones derived from Dnull cells had a cytokine secretion that was largely comparable to the CD45RA⁺ group. The main difference was the higher frequency of clones secreting IL-4, IL-5, and IL-10 secretion. Concerning cytokine secretion, both freshly isolated Dnull

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**Table 2. Precursor Frequency of TT-Reactive T Cells**

<table>
<thead>
<tr>
<th>Donor No.</th>
<th>CD45RA⁺</th>
<th>Dnull</th>
<th>CD45RO⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1:110,000*</td>
<td>1:900</td>
<td>1:350</td>
</tr>
<tr>
<td>95% CI</td>
<td>333,000-62,500</td>
<td>1,500-650</td>
<td>560-260</td>
</tr>
<tr>
<td>2</td>
<td>1:333,000</td>
<td>1:5500</td>
<td>1:260</td>
</tr>
<tr>
<td>95% CI</td>
<td>1,000,000-145,000</td>
<td>9,100-4,200</td>
<td>770-150</td>
</tr>
</tbody>
</table>

Abbreviation: 95% CI, 95% confidence interval.

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very low number of precursors found in the CD45RA⁺ subset corresponded to the expected low frequency of TT-specific T cells in the primary T-cell repertoire. Both donors tested showed a high precursor frequency within their CD45RO⁺ populations. Notably, also the Dnull subsets contained high numbers of TT-specific precursors (Table 2).

_Dnull cells can secrete both IL-4 and IFN-γ._ The data on the phenotypic and functional characteristics suggested that the Dnull population has mixed properties when compared with CD45RA⁺ and CD45RO⁺ populations. To explore this further, the ability of this subset to secrete cytokines was assessed. Freshly isolated CD4⁺ T cells were

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**Fig 3.** Helper capacity of CD4⁺ T-cell subsets. (A) Sorted T cells (5 × 10⁴/well) were cocultured with allogeneic E-rosette-negative cells (5 × 10⁴/well) in plates coated with increasing amounts of CD3 MoAb. Proliferation was determined on day 3 by adding [³H]thymidine to the cultures and IgG content in the supernatant was measured on day 14 with ELISA. (B) Increasing numbers of sorted T cells were cocultured with allogeneic E-rosette-negative cells (5 × 10⁴/well) in CD3 MoAb-coated (5 μg/mL) plates. IgG production was measured on day 14 with ELISA. (■) CD45RA⁺ T cells; (□) Dnull T cells; (■) CD45RO⁺ T cells.

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cells and T-cell clones generated from this subset clearly have features of primed cells. In contrast, the other intermediate subset, the Ddull cells, most closely resembles the unprimed CD45RA+ subset.

**Heterogeneity of the Ddull after in vitro culture.** To follow the default differentiation pathway of Ddull cells in vitro, sorted cells have to be kept in culture for prolonged periods without stimulation of the T-cell receptor, since this will eventually lead to a switch from CD45RA to CD45RO expression. Unutmaz et al. described that both unprimed and primed CD4+ T cells can survive for weeks when cultured in the presence of a combination of IL-2, IL-6, and TNF-α. Remarkably, under these conditions, unprimed CD45RA+ cells do not switch from CD45RA to CD45RO expression, although part of the cells become CD25+.

CD4+ T cells were sorted into CD45RA+, Ddull, and CD45RO+ subsets. The different cell populations were kept in medium that contained 100 U/mL IL-2, 20 ng/mL TNF-α, and 500 U/mL IL-6 for 14 days. Thereafter, three-color fluorescence was performed to simultaneously analyze expression of CD25, CD45RA, and CD45RO antigens (Fig 6). Approximately 20% of the cells in all three subsets were CD25+ (data not shown). In accordance with this, no significant differences between the studied populations were found when proliferation was measured on day 8. Furthermore, the percentage of living cells after the 14-day culture period was approximately 90% in all subsets (data not shown).

In agreement with published findings, unprimed CD45RA+ cells, although proliferating, did not switch to CD45RO expression, which supports the notion that stimulation of the T-cell receptor is necessary to induce the switch in CD45 isoform expression. The majority of primed cells did not alter their CD45 expression pattern. However, a small subset reexpressed the CD45RA isoform, which indicates that part of the CD45RO+ cells may gain a Ddull phenotype in the absence of T-cell receptor crosslinking. Analysis of the Ddull subset after culture showed a marked heterogeneity in CD45 isoform expression. The original homogeneous population could be subdivided into, first, cells that switched to a CD45RA+/RO− phenotype, second, cells that upregulated the CD45RA isoform, and, third, cells that maintained the Ddull phenotype.

**DISCUSSION**

Expression of T-cell differentiation and activation antigens can be used to distinguish discrete stages in postthymic T-cell development. Within the CD4+ T-cell compartment, CD45RA and CD45RO antigens are expressed on unprimed and primed T cells, respectively, whereas loss of CD27 expression is a feature of differentiated effector-type T cells. To explore further the human T-cell subset origin in vivo, we analyzed two putative transitional subsets between unprimed and primed T cells in the circulation that can be distinguished on the basis of coexpression of CD45RA and CD45RO either at high (Dbright) or at low (Ddull) levels.

In the secondary lymphoid organs, the development of an unprimed CD45RA+ into a primed CD45RO+ phenotype has
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Fig 5. Cytokine production of T-cell clones generated from different subsets of peripheral blood CD4 T cells. T-cell clones were stimulated with CD2 and CD28 MoAbs and supernatants were harvested at 24 hours (IL-2) and 4 days (IFN-γ, IL-4, IL-5, and IL-10) after initiation of the culture. No differences in proliferation of the T-cell clones from the different subsets to the given stimulus were observed (data not shown). Each dot represents the cytokine production of one individual T-cell clone and the median of all clones is shown. The mean production of the T-cell clone subsets is given in brackets below the subset names. The detection limit for all cytokine ELISAs was 50 pg/ml. Data for the different groups of T-cell clones were compared by using the Mann-Whitney U test. Only significant differences are shown (*P < .05, **P < .01, ***P < .001, ****P < .0001).

been suggested to occur via a Dbright stage. In the periphery, these cells are only found in low frequency (<1%), and the majority of intermediate cells express the CD45 isoforms at low density. Phenotypically, Ddull cells have intermediate expression of all surface markers that are differentially expressed on unprimed versus primed cells, eg, adhesion antigens such as CD58 and homing receptors such PECAM (CD31) and L-selectin (CD62L). Moreover, also with respect to helper activity for B-cell differentiation and frequency of recall antigen-specific precursors, Ddull cells displayed an intermediate phenotype. However, in marked contrast to these phenotypic and functional findings, Ddull cells were found to have a cytokine secretion pattern that is quite comparable to that of primed CD45RO cells. Ddull CD4 T cells were able to secrete IL-2, IFN-γ, IL-4, IL-5, and IL-10, whereas Dbright cells produced mainly IL-2 and IFN-γ and thus resembled unprimed CD45RA cells. The capacity of the Dbright cells to secrete IFN-γ next to IL-2 further indicates that this small population in the peripheral blood could indeed represent recently stimulated cells, since IFN-γ production capacity is rapidly acquired after antigenic stimulation. However, since lymphokine secretion was determined after a cloning procedure that included several rounds of stimulation, it cannot be excluded that the Dbright cells may represent TH1-like cells.

How may Ddull cells be generated in vivo? First, it might be postulated that next to the transitional Dbright stage, a relatively high number of recently activated CD45RA T cells are compartmentalized within this subset. A prerequisite for this notion would be that the capacity to secrete a large array of cytokines is acquired before complete change of membrane phenotype and the acquisition of B-cell-helper activity. However, in contrast to the Dbright T cells, the majority of which express activation markers and are in the S or G2/M phase of the cell cycle, only a few cells of the Ddull subset have these characteristics of recently activated cells. This makes it questionable whether a large proportion of Ddull T cells are actually converting from a CD45RA/RO phenotype to a CD45RA/RO phenotype due to recent antigenic stimulation.

Another possibility is that the Ddull subset encompasses primed CD45RA/RO cells that, upon waning of their specific antigen, reexpress the CD45RA isoform. Indeed, during in vitro culture, in the absence of stimulation of the T-cell...
In vitro culture of CD4+ T-cell subsets. Sorted T cells were cultured with a combination of IL-2 (250 U/mL), rTNF-α (20 ng/mL), and rIL-6 (500 U/mL). Expression of CD45RA and CD45RO isoforms on the different subsets was analyzed immediately after sorting (A). Fourteen days after initiation of the culture, cells were restained with CD45RA and CD45RO MoAb (B).

CD45RA

CD45RO

63% CD4+ 54% CD4+
32% 19%

100% CD45RA+ 98% CD45RA+
4% 2%

36% Ddull 37% Ddull
17% 13%

100% CD45RO+ 87% CD45RO+
4% 13%

Fig 6. In vitro culture of CD4+ T-cell subsets. Sorted T cells were cultured with a combination of IL-2 (250 U/mL), rTNF-α (20 ng/mL), and rIL-6 (500 U/mL). Expression of CD45RA and CD45RO isoforms on the different subsets was analyzed immediately after sorting (A). Fourteen days after initiation of the culture, cells were restained with CD45RA and CD45RO MoAb (B).

ACKNOWLEDGMENT

We thank M.H.G. Rep for help with the statistical analyses and S.M.A. Lens and L. Meyaard for critical reading of the manuscript. We are indebted to Dr J. de Vries (DNAX, Palo Alto, CA) for providing ELISAs for IL-5 and IL-10.

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receptor, part of the Ddull cells upregulated CD45RA. Moreover, although the majority of single CD45RO+ cells did not alter CD45 isoform expression, approximately 10% of the cells started to upregulate CD45RA expression. In support of the notion that the Ddull population contains reconvertinig primed T cells, in vivo data in rats, mice, and humans have provided evidence that the switch from expression of high- to low-molecular weight isoforms of the CD45 antigen may be reversible, but the conditions that would lead to the putative reexpression of CD45RA are not understood. Recently, it was found that primed T cells can survive for longer periods by entering a quiescent stage when cocultured with fibroblasts. Since it was shown that those cells stayed CD45RO+, it is possible that the Ddull population contains cells that have entered a resting stage and reexpressed the CD45RA isof orm. It seems unlikely that reexpression of CD45RA will eventually lead to a complete downregulation of CD45RO expression, since the CD45RA+ population appears both phenotypically and functionally homogeneous. Moreover, both studies on hypoxanthine phosphoribosyl transferase (HPRT) mutant frequency and telomeric repeat length have pointed to considerable differences in replicative history between unprimed and primed T cells, which argues against substantial reconversion from CD45RO to CD45RA single positive phenotype. Instead, resting memory cells could rather have a Ddull phenotype, and upon restimulation develop into functionally active effector cells. The capacity of the Ddull cells to produce a large array of cytokines characteristic for primed cells would agree with this assumption.

Finally, it could be possible that the Ddull population also contains helper T cells that have reached a stable CD45RA-low/CD45RO-high phenotype. Support for this notion is provided by the observation that the majority of the sorted Ddull cells maintained stable expression of both CD45RA and CD45RO isoforms even after 14 days of culture. Expression of different isoforms of the CD45 antigen is regulated in a complex manner and can be influenced by cytokines. In addition, it has recently been shown that distinct peptide/major histocompatibility complexes (MHCs) may deliver quantitative distinct activation signals to T cells. Therefore, it cannot be excluded that the activation of unprimed CD45RA T cells in vivo does not always result in a single CD45RO+ phenotype, but may, depending on both microenvironment and quality of the T-cell receptor signal, result in the generation of Ddull cells. The observation that in children with early-onset insulin-dependent diabetes mellitus, a relative increase of this population is found further argues for a specific place of Ddull cells in T-cell differentiation.

In summary, we have shown that in all donors a relatively large subset of CD45RA-high/CD45RO-low CD4+ T cells can be found with features that separate it from both transitional CD45RA-high/CD45RO-high CD4+ T cells and from primed CD45RO+ T cells.
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Heterogeneity of the human CD4+ T-cell population: two distinct CD4+ T-cell subsets characterized by coexpression of CD45RA and CD45RO isoforms

D Hamann, PA Baars, B Hooibrink and RW van Lier