Brief Definitive Report

DIFFERENTIAL EXPRESSION OF CD45RO (UCHL1) AND ITS FUNCTIONAL RELEVANCE IN TWO SUBPOPULATIONS OF CIRCULATING TCR-γ/δ^+ LYMPHOCYTES

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Human T cells express various isoforms of CD45 (leukocyte common antigen), which arise from alternate mRNA splicing (1). The mAb UCHL1 recognizes a 180 kD molecular mass isoform, CD45RO (2). Recent studies on major two ($CD4^+$ and $CD8^+$) subpopulations of circulating T cells have suggested that UCHL1 identifies a primed population of T cells that includes memory cells (3). Within $CD4^+$ cells, the CD45RO population mediates proliferative responses to recall antigens and is able to support B cell differentiation. A reciprocal population of $CD4^+$ cells, identified by mAbs to the 205- and 220-kD molecular mass isoforms termed CD45RA, do not show these capabilities. It has also been shown that the CD45RO population of $CD8^+$ cells contains precursors exerting memory-dependent cytotoxicity (4).

While the majority of mature T cells express the TCR-α/β–CD3 complex, a minor population of blood T cells lacking both CD4 and CD8 bear a distinct TCR composed of γ and δ subunits. Although the function and the repertoire of TCR-γ/δ remain to be fully defined, it has been shown that blood TCR-γ/δ^+ T cells can be subdivided largely into two nonoverlapping subsets defined by BB3 and δTCS1 mAb (5, 6). In this paper we examined the expression of CD45RO by TCR-γ/δ^+ in healthy children and adults to elucidate some of their biological roles. We will show here that CD45RO is preferentially expressed on $BB3^+$ subsets among circulating TCR-γ/δ^+ cells. $BB3^+$ cells appear to represent a population of Tγ/δ^+ cells capable of responding to some antigens.

Materials and Methods

Blood Samples. Cord blood was collected from the umbilical vein immediately after the uneventful delivery of full-term newborn babies. Venous blood from healthy infants and children of different ages was obtained after the informed parental consent was given. Adult subjects were healthy volunteers between the ages of 25 and 35 yr.

Monoclonal Antibodies. The mAb UCHL1 (IgG2a) against CD45RO (2) was purchased from Dakopatts a/s (Copenhagen, Denmark). The BB3 mAb (IgG1) specific for human TCR-γ

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chain (5) was the kind gift of Dr. L. Moretta (University of Geneva, Italy). Anti-TiγA mAb (IgG2a), generously provided by Dr. T. Hercend (Institut Gustave-Roussy, Villejuif, France), recognizes the human TCR-γ molecule (6). FITC-conjugated TCRδ1 mAb (IgG1) (7) and FITC-conjugated δTCS1 mAb (IgG1) (8), both of which have been found to be directed to human TCR-δ chains, were obtained from T Cell Sciences (Cambridge, MA). FITC-conjugated anti-Leu-3a mAb (IgG1) specific for CD4 was from Becton Dickinson & Co. (Mountain View, CA).

Immunofluorescence Analysis. Viable mononuclear cells from heparinized blood were isolated on Ficoll/Hypaque gradients (Lymphoprep, Nycomed AS, Oslo, Norway) and stained with mAb. Two-color staining was performed to evaluate expression of CD45RO by TCR-γ/δ^+ cells. The cells were first incubated with UCHL1 or isotype-matched control mouse IgG, and counterstained with biotinylated rabbit anti-mouse IgG2a antibody (Zymed Laboratories, San Francisco, CA), and further with phycoerythrin (PE)-conjugated streptavidin (Becton Dickinson & Co.). Next, the cells were stained for determination of TCR-γ/δ^+ cells with FITC-conjugated TCRδ1, FITC-conjugated δTCS1, or unconjugated BB3 mAb. For BB3 staining, the cells were finally treated with FITC-conjugated horse anti-mouse IgG1 antibody (Zymed Laboratories). The labeled cells were analyzed by an Epics C flow cytometer (Coulter Electronics, Inc., Hialeah, FL).

Proliferative Responses. TCR-γ/δ^+ cell (TiγA^+ and $\delta TCS1^+$) subsets were isolated using an Epics C flow cytometer from mononuclear cells stained with the corresponding mAbs as above. These cells were seeded on flat-bottomed microtiter plates (Corning Glass Works, Corning, NY) at 5×10^4 cells/well in 0.2 ml of RPMI 1640 supplemented with 10% AB serum in the presence of 5×10^3 irradiated adherent monocytes from the same donor. Cultures were stimulated with 1 μg/ml of purified protein derivative (PPD; Japan BCG Co., Ltd., Tokyo, Japan) or 10 ng/ml of anti-CD3 mAb (OKT3; Ortho Pharmaceutical Co., Raritan, NJ) for 120 h and were pulsed with 0.5 μCi of [^{3}H]TdR 12 h before harvesting. As controls, the similar cultures were carried out for CD45RO$^+$ and CD45RO$^-$ CD4$^+$ cells separated by the cell sorting.

Results and Discussion

We used TCRδ1 mAb to examine the frequency of TCR-γ/δ^+ cells and their expression of CD45RO, since TCRδ1 has been shown to detect the whole population of TCR-γ/δ^+ cells (6). The appearance of blood TCR-γ/δ^+ cells was scarce (<0.5%) at birth, gradually increased with age, and reached the adult values in older children (Table I). On the other hand, the proportions of TCR-α/β^+ cells with CD4 or CD8 in the neonatal blood are similar to those observed in the adult blood (9). Consistent

TABLE I

Frequency of TCRδ1$^+$ Cells and Their Expression of CD45RO in Peripheral Blood of Different Ages

Age	*n*	Percentage of TCRδ1$^+$ cells	Percent CD45RO expression by TCRδ1$^+$ cells
Newborn	6	<0.5	ND
0-1 mo	7	1.5 ± 0.2*	72.5 ± 4.6
2-3 mo	8	1.9 ± 0.3	63.8 ± 3.3
4-11 mo	6	2.3 ± 0.4	61.0 ± 5.1
1-2 yr	12	2.7 ± 0.5	69.6 ± 4.0
3-6 yr	14	3.9 ± 0.4	72.4 ± 3.5
7-15 yr	16	4.4 ± 0.6	69.3 ± 3.1
Adult	12	5.8 ± 1.1	72.4 ± 3.7

* Means ± SEM.

with the current concept of naive and memory T cells, we observed that the circulating pool of $CD4^+$ and $CD8^+$ cells expressing CD45RO increased as a function of age (unpublished observations). In adult subjects, the mean percentages of $CD45RO^+$ cells within $CD4^+$ and $CD8^+$ cells are 35 and 25%, respectively. In marked contrast to $CD4^+$ and $CD8^+$ cells, expression of CD45RO was observed on a sizable portion (~70%) of blood TCR-γ/δ^+ cells in all donors examined (Table I). The remarkable finding was that most TCR-γ/δ^+ cells possessed CD45RO on their surfaces even around 1 mo of life shortly after birth.

We often observed that some of donors exhibited a clear bimodal distribution of CD45RO expression on TCR-γ/δ^+ cells (Fig. 1 *A*). This suggests the presence of a small $CD45RO^-$ population of TCR-γ/δ^+ cells in blood. The recent availability of other mAb specific for human TCR-γ/δ molecules have allowed to identify two mutually exclusive subsets defined by BB3 and δTCS1 mAbs (5, 6). In blood TCR-γ/δ^+ cells, most cells reacts with BB3, whereas a few cells are positive for δTCS1. Based on our observations, we next asked whether expression of CD45RO by these two TCR-γ/δ^+ cells might be different from each other. Fig. 1 shows a representative of two-color immunofluorescence analysis for CD45RO expression by Tγ/δ^+ subsets. Interestingly, a large percentage of $BB3^+$ cells were considered $CD45RO^+$, whereas CD45RO expression was absent from $\delta TCS1^+$ cells. As presented in Table II, this was the rule in all of seven subjects studied.

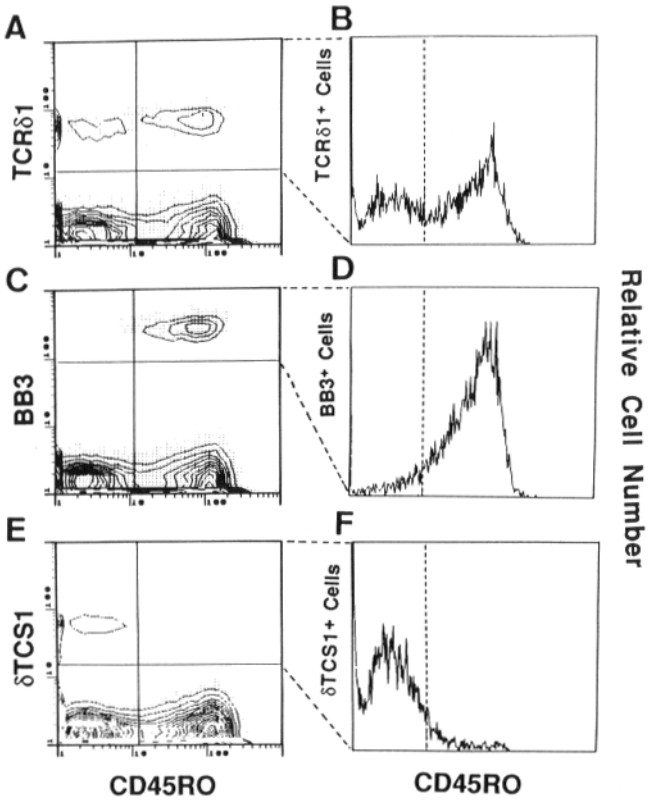

FIGURE 1. Two-color immunofluorescence analysis of CD45RO expression by TCRδ1+, BB3+ and δTCS1+ cells. Contour maps (A, C, and E) show two-color staining patterns of lymphocytes gated by forward and 90° light scatters. A second gate was set to include the whole populations of TCRδ1+ (B), BB3+ (D) and δTCS1+ cells (F) and 5,000 cells accumulating within the gate were analyzed for CD45RO expression, which are expressed as the histogram patterns. A vertical dashed line in the histograms indicates the negative control.

TABLE II

Expression of CD45RO by TCR-γ/δ⁺ Subsets

Subjects	Percent CD45RO Expression by		
	TCRδ1⁺ Cells	BB3⁺ Cells	δTCS1⁺ Cells
NT	50.1 (4.6)*	92.1 (3.5)	1.4 (1.6)
NY	84.5 (6.6)	94.0 (5.8)	ND (<0.5)
TT	82.6 (6.6)	93.4 (5.1)	0.9 (0.9)
NK	62.3 (6.2)	97.2 (4.3)	0.8 (0.9)
AY	86.0 (12.0)	91.6 (11.2)	ND (<0.5)
YH	92.0 (4.5)	92.0 (1.2)	2.6 (1.4)
YK	38.2 (11.0)	92.0 (7.8)	1.7 (3.8)
	61.8 ± 8.9‡	93.2 ± 0.7‡	1.5 ± 0.3‡
	(7.4 ± 2.8)	(5.6 ± 1.2)	(1.7 ± 0.5)

CD45RO expression by each TCR-γ/δ⁺ subset was examined for the blood from adult subjects.

* Parentheses indicate the proportions of each subset in lymphocytes.

‡ Mean ± SEM.

Although studies for established clones suggest that all TCR-γ/δ⁺ cell clones mediate non-MHC-restricted cytotoxicity (10), the antigen-specificity and immunological functions of these cells remain speculative. On the basis of the correlation between CD45RO expression and memory T cells, it seemed feasible to suppose that BB3⁺ cells, but not δTCS1⁺ cells lacking CD45RO, might contain a population responding to certain antigens in a similar manner to memory CD4⁺ cells. Preliminary experiments suggested that staining with BB3 mAb for the cell sorting resulted in somewhat enhanced background responses of isolated cells, indicating the activation of corresponding subsets by this antibody (11). It has been demonstrated that most BB3⁺ cells are identified by anti-TiγA mAbs as well (12). In addition, resting TiγA cells do not proliferate in response to anti-TiγA mAb in the absence of exogenous IL-2 (13). Thus, we substituted anti-TiγA for BB3 to isolate CD45RO⁺ TCR-γ/δ⁺ subsets. As shown in Table III, both TiγA⁺ and δTCS1⁺ cells proliferated well in response to anti-CD3 mAb. In PPD-reactive individuals, appreciable proliferative responses to PPD, though less than those seen in memory (CD45RO⁺) CD4⁺ cells, were obtained only from TiγA⁺ cells, but not from δTCS1⁺ cells. These results strongly suggest that a fraction of TiγA⁺ cells may contribute the immune response to a nominal antigen. Since 95% of BB3 cells, though not completely identical, coexpress TiγA antigen (13), we speculate that functional profiles of TiγA⁺ cells may represent those of BB3⁺ cells. Regarding the antigen-specific response of TCR-γ/δ⁺ cells, it has recently been reported that TCR-γ/δ⁺ clones established from blood lymphocytes respond to some antigens such as tetanus toxoid or PPD in a MHC-restricted fashion (14, 15).

Moretta et al. (16) have indicated that BB3⁺ cells express Cγ1-encoded TCR-γ/δ heterodimers, whereas δTCS1⁺ cells express Cγ2-encoded molecules. BB3⁺ cells predominate in the peripheral blood, but are very scantily found in the thymic tissues. In contrast, δTCS1⁺ cells are enriched in the thymus. Immunohistochemical analysis have shown that δTCS1⁺ cells are predominant in intraepithelial lymphocytes of the jejunum as compared with the blood (17). However, the reason for such different tissue distribution between BB3⁺ and δTCS1⁺ cells is unknown.

TABLE III
Proliferative Responses of TCR-γ/δ⁺ and CD4⁺ Subpopulations by PPD or Anti-CD3 mAb

Donors	Stimuli	TCR-γ/δ⁺ Cells		CD4⁺ Cells	
		TiγA⁺	δ-TCS1⁺	CD45RO⁺	CD45RO⁻
YH	None	266	501	562	722
	PPD	2,305	488	24,968	902
	Anti-CD3	31,600	28,098	53,338	48,221
YK	None	320	341	982	1,102
	PPD	14,614	256	34,682	1,490
	Anti-CD3	29,646	12,660	63,052	38,962
NT	None	664	125	902	562
	PPD	8,420	102	23,315	875
	Anti-CD3	23,528	32,628	100,192	48,802

Each subpopulation was isolated from PPD-reactive adult donors. The cells (5×10^4/well) were cultured with PPD (1 μg/ml) or anti-CD3 mAb (10 ng/ml) in the presence of autologous irradiated monocytes for 5 d. The results indicate the mean [^{3}H]TdR incorporation of triplicate cultures.

In conclusion, the present study showed that the BB3⁺ subset in the peripheral blood selectively expressed CD45RO, corresponding with their responding capability to recall antigens. These observations implicate a special role of this subset in the immune response to many pathogens and infectious agents.

Summary

We examined the developmental profile of TCR-γ/δ⁺ cells with respect to CD45RO expression. Although total TCR-γ/δ⁺ cells were negligible in the neonatal blood and increased with advancing age, most blood TCR-γ/δ⁺ cells markedly expressed CD45RO without a distinction of age, probably reflecting a different CD45RO expression of two subsets defined by BB3 and δTCS1 mAbs. The vast majority of BB3⁺ cells expressed CD45RO, whereas expression of CD45RO was virtually absent in the δTCS1⁺ population. Functional studies revealed that, while both TCR-γ/δ⁺ cell subsets showed CD3-mediated activation, only BB3⁺ (or TiγA⁺) cells, but not δTCS1⁺ cells, appeared to proliferate in response to PPD in PPD-reactive individuals. The results suggested that the CD45RO⁺ (BB3⁺ or TiγA⁺) subset among blood TCR-γ/δ⁺ cells may be mainly involved in the memory or primed component of the immune system responding to some foreign antigens.

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