## *Brief Definitive Report*

# GENETICALLY DETERMINED LACK OF CD45R$^-$ T CELLS IN HEALTHY INDIVIDUALS

## Evidence for a Regulatory Polymorphism of CD45R Antigen Expression

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The family of human leukocyte common antigens (LCA; CD45) is composed of at least four glycoprotein members with molecular masses of 180, 190, 205, and 220 kD resulting from alternative splicing (1–4). On the protein level, the heterogeneity of different CD45 isoforms can be resolved by mAbs that react with restricted epitopes specific for distinct isoforms. In humans, mAb UCHL1 detects the CD45RO epitope specific for the low molecular mass isoform (180 kD) of CD45; several mAbs detect the CD45R epitope present only on the high molecular mass isoform (205 and 220 kD) (5–7).

The CD45R and CD45RO isoforms have been reported to define complementary subsets among CD4$^+$ and CD8$^+$ T cells (8) that differ in functional properties. Whereas CD45RO$^+$ T cells respond well to recall antigens such as tetanus toxoid, weak or no reactivity has been reported for CD45R$^+$ T cells (5, 9). After in vitro activation, CD45R$^+$ T cells have been shown to lose CD45R antigens and to express CD45RO molecules (10–12). These results led to the assumption that expression of the CD45RO isoform and loss of CD45R molecules are typical features of primed or memory T cells (13, 14). We now report on a genetically determined variant pattern of CD45R expression.

## Materials and Methods

*Blood Donors and Cell Separation.* Heparinized peripheral venous blood was taken from 52 male and female healthy individuals; furthermore, three families were studied. PBMC were isolated by Ficoll gradient centrifugation.

*mAbs and Flow Cytometry.* The low molecular mass isoform of LCA (180 kD) was detected by mAb UCHL1 (CD45RO) (5), donated by Dr. P. C. L. Beverley, London, UK. The high molecular mass isoform (205 and 220 kD) was identified by mAb Leu-18 (Becton Dickinson & Co., Heidelberg, FRG) and by several antibodies included in the CD45R panel of the Fourth Workshop of Leucocyte Typing (6). Common epitopes expressed on all isoforms of LCA were identified by mAb GRT2. Binding of the various mAbs to the respective CD45 epitopes was detected by indirect immunofluorescence. Coexpression of high and low molecular mass isoforms of CD45 was analyzed by two-color flow cytometry using directly labeled FITC–Leu-18 in combination with indirect staining for UCHL1. PBMC were incubated with mAb

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UCHL1 followed by phycoerythrin(PE)-conjugated rat anti-mouse κ (Becton Dickinson & Co.). After blocking the remaining active sites on the PE reagent with 20 μl normal mouse serum, the cells were washed and stained with FITC-Leu-18. Analysis was performed on a FACStar flow cytometer (Becton Dickinson & Co., Mountain View, CA) gated on lymphocytes.

*Cell Culture.* The kinetics of CD45R and CD45RO expression were studied in $CD45R^+$ cells obtained after incubation of PBMC with mAb UCHL1 and negative immunoselection using magnetic beads coated with anti-mouse Ig (Dynabeads; Dianova, Hamburg, FRG). $10^6$ negatively selected $CD45R^+$ cells/ml were stimulated with 1 μg/ml PHA (Wellcome Diagnostics, Burgwedel, FRG) in RPMI 1640 medium (Gibco Laboratories, Paisley, Scotland) supplemented with 4 mM glutamine, 15 mM Hepes buffer, 100 IU/ml penicillin-streptomycin, and 10% FCS (Biochrom, Berlin, FRG). Cell proliferation was assayed by stimulating $0.5 \times 10^6$ PBMC/ml ($10^5$/well) with PHA (1 μg/ml), OKT3 (15 ng/ml; Muromonab-CD3; Ortho Diagnostic Systems Inc., Westwood, MA), or tetanus toxoid (1:300 dilution; Behringwerke, Marburg, FRG) in round-bottomed microtiter plates (Nunc, Roskilde, DK) for 4 d. Cultures were pulsed with [$^3$H]thymidine 16 h before harvesting.

## Results

The characteristic pattern of CD45R antigen expression (15) was found in 48 of 52 tested individuals. They possessed $CD45R^+$ as well as $CD45R^-$ lymphocytes (77.5 ± 7.3% and 22.2 ± 7.5%, respectively) (Fig. 1, *a–c*). In four individuals, however, the $CD45R^-$ subset was absent (Fig. 1, *f–h*). This was confirmed with an additional 13 mAbs included in the CD45R panel of the Fourth Workshop of Leucocyte Typing (data not shown). Repeated analysis over a period of several months demonstrated that the aberrant CD45R expression was a stable individual trait. In addition to the modified expression on lymphocytes, an increased density of CD45R antigens was observed on macrophages (data not shown). The reactivity pattern of mAbs detecting common epitopes of LCA (CD45 mAb) was not different (Fig. 1, *d* and *i*). Moreover, no significant difference was observed for the density of CD45RO detected by mAb UCHL1 (Fig. 1, *e* and *j*).

For three of the four individuals displaying the variant pattern, the analysis of other family members was possible. In all instances, the variant pattern could also be identified in other members of the family. The parents in the three families uniformly consisted of one parent with normal expression of CD45R and one parent

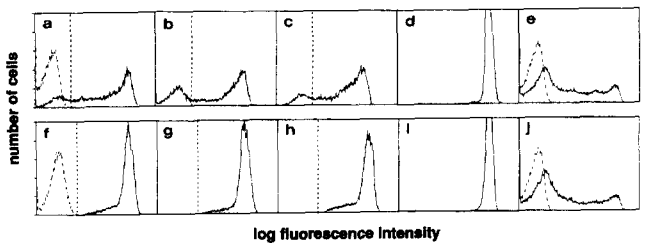


FIGURE 1. Two patterns of CD45R expression. Cells from an individual displaying the normal pattern (*top*) and from an individual displaying the variant pattern (*bottom*) were stained by indirect immunofluorescence with the CD45R mAb MB1 (*a* and *f*), 4KB5 (*b* and *g*), and Leu-18 (*c* and *h*). Histograms *d* and *i* were obtained after staining with the CD45 mAb GRT2; *e* and *j* represent binding of mAb UCHL1 (CD45RO). Binding of MB1, 4KB5, Leu-18, and GRT2 was detected by FITC-conjugated goat anti-mouse IgG; UCHL1 binding was visualized by PE-conjugated anti-mouse κ. Histograms of broken lines represent fluorescence obtained with the secondary reagents alone. The vertical broken line separates negative and positive cells. Whereas in panels *a–c*, $CD45R^-$ cells can be detected, this subset is absent in panels *f–h*.

displaying the variant pattern. A total of 12 children could be tested. In six children the variant pattern was found, and in six the normal pattern of CD45R expression was observed. Females and males were present in both groups. This distribution of the variant pattern of CD45R expression establishes it as a genetically determined trait and strongly suggests an autosomal dominant mode of inheritance.

Correlated analysis of CD45R and CD45RO expression by two-color flow cytometry revealed three subsets in individuals with normal CD45R phenotype. (Fig. 2 *a*). 55% of cells were $CD45R^+CD45RO^-$, 23% expressed the $CD45R^-CD45RO^+$ phenotype, and 22% were double labeled ($CD45R^+CD45RO^+$ cells). The inverse relation between CD45R and CD45RO antigen density is explained by the loss of CD45R antigens and the gain of CD45RO determinants occurring during priming of T cells by antigen (11). In contrast, the two-color analysis of lymphocytes from individuals with aberrant CD45R expression again demonstrated coexpression of CD45R on all $CD45RO^+$ cells (Fig. 2 *b*). Although CD45R antigen density was reduced in bright $CD45RO^+$ cells, complete absence of CD45R was never observed.

Activation of negatively selected $CD45R^+$ ($UCHL1^-$) cells by PHA usually resulted in a decrease of CD45R antigen expression (Fig. 3). In contrast, CD45R expression on cells from individuals displaying the variant CD45R pattern remained stable over a period of several weeks. No difference was observed between both groups with respect to CD45RO antigens that were induced from day 3 onwards. Thus, the in vitro activation of PBMC from controls resulted in blast cells expressing CD45RO but not CD45R antigens, whereas activated cells obtained from individuals with aberrant CD45R expression coexpressed CD45R and CD45RO molecules (Fig. 3, *e* and *j*).

It has been suggested that the memory T cell pool is included within the $CD45RO^+CD45R^-$ subset (5, 11). To test whether the absence of $CD45RO^+CD45R^-$ cells in some individuals may also result in a deficient memory cell function, we compared the in vitro response of their PBMC to tetanus toxoid with that of control cells. PBMC from individuals lacking $CD45R^-$ cells responded as well to tetanus toxoid as control cells. Moreover, neither PHA reactivity nor the proliferative response to CD3-triggered stimulation differed significantly between both groups (Table I).

## Discussion

A variant pattern of CD45R expression was found with a frequency of ~8% among normal individuals. This pattern is characterized by persistent expression of the CD45R epitope in T cells that have undergone activation. Family studies indicate

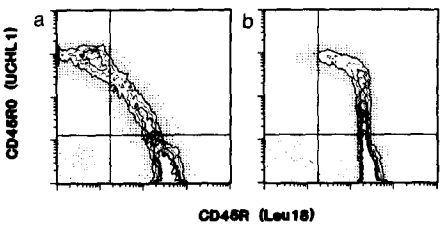


FIGURE 2. Correlated analysis of CD45R and CD45RO expression by two-color flow cytometry. PBMC were stained by mAb FITC-Leu-18 and UCHL1 as outlined in Materials and Methods. The normal pattern is shown in *a*; a representative example of the variant pattern is depicted in *b*.

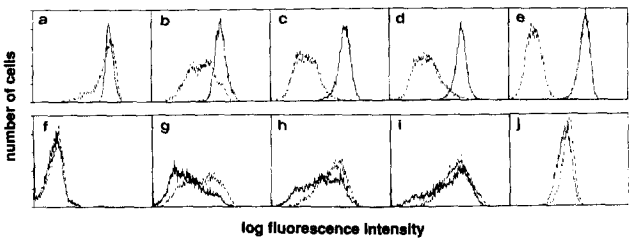


FIGURE 3. Kinetics of CD45R and CD45RO expression during in vitro activation. (*Top*) Staining with mAb FITC-Leu-18 (CD45R). (*Bottom*) Staining with mAb UCHL1 (CD45RO). Broken lines represent fluorescence of cells from a control person; solid lines from an individual displaying the variant CD45R pattern. Negatively selected $CD45R^+$ cells were analyzed at day 0 (*a* and *f*), stimulated with PHA, and subsequently analyzed at day 3 (*b* and *g*), 5 (*c* and *h*), and 7 (*d* and *i*). Histograms *e* and *j* show the phenotypes of cells cultured in IL-2 containing medium for 3 wk. In contrast to the control cells, all cells from the individual displaying the variant CD45R pattern remain $CD45R^+$.

an autosomal dominant mode of inheritance with full penetrance. Therefore, all individuals described in this study to carry the variant phenotype are heterozygous for this trait. To define the homozygous variant pattern, one would have to find a family with two parents displaying the variant pattern and informative offspring. The analysis of such an individual would certainly be of major interest for the functional evaluation of the described polymorphism.

The persistent expression of CD45R in individuals displaying the variant pattern could be due either to reduced membrane turnover or to maintained synthesis in activated cells. A possible cause for a reduced membrane turnover would be a modified molecular structure of the CD45R antigen. This is unlikely for three reasons. (*a*) A reduced CD45R turnover should also lead to an increased CD45R expression on $CD45RO^-$ cells, which has not been observed (Fig. 2). (*b*) The analysis of individuals possessing the variant pattern with the CD45R antibody panel of the Fourth Workshop of Leucocyte Typing (6) did not provide evidence for a deviant antigenic structure of CD45R in these individuals. (*c*) A reduced turnover could hardly explain the persistent expression even after prolonged periods of in vitro culture (Fig.

TABLE I

*Proliferative Responses to Different Stimuli: Comparison of PBMC from Individuals Displaying the Normal and the Variant CD45R Pattern*

| CD45R expression | Donor | Percent $CD45R^-$ cells | PHA | OKT3 | Tetanus toxoid |
|---|---|---|---|---|---|
| | | | | *cpm* | |
| Normal | GE | 36 | 89,087* | 33,946 | 9,081 |
| | PW | 32 | 62,796 | 9,987 | 21,209 |
| | AJ | 19 | 88,375 | 16,846 | 8,144 |
| | RS | 23 | 103,082 | 23,058 | 21,701 |
| Variant | KB | <1 | 120,409 | 27,866 | 16,369 |
| | CL | <1 | 65,897 | 9,803 | 13,941 |
| | OS | <1 | 94,985 | 26,870 | 8,976 |

PBMC were stimulated as described in Materials and Methods.

* Results are expressed as mean cpm of triplicate cultures corrected by subtraction of media controls. Data were obtained in two different experiments.

3). Thus, the presented results point to a regulatory polymorphism of CD45R expression permitting maintenance of CD45R synthesis in activated T cells and memory cells.

The different isoforms of CD45 are products of the same gene and are derived by alternative splicing (1). How alternative splicing is coordinated with distinct cellular activation and differentiation steps is not yet known. A possible mechanism for the persistent synthesis found in certain individuals could be the mutation of a splice site required for alternative splicing. If one assumes heterozygosity of the affected individuals, the chromosome carrying the deviant CD45 gene should be responsible for the persistent CD45R expression. On the other hand, the normal CD45 gene on the homologous chromosome could regulate the otherwise normal CD45 expression and, in particular, the upregulation of CD45RO during activation. The reduced expression of CD45R in the cells with the highest CD45RO expression (Fig. 2 *b*) indicates that synthesis of CD45R mRNA has not fully escaped control. Definite clarification of the underlying mechanism has to come from a detailed analysis of the deviant CD45 gene by molecular biology techniques.

It has been suggested that the differential expression of CD45R and CD45RO antigens on unprimed and memory T cells might be related to the functional differences between both cell types (16). The clear-cut response of PBMC from individuals lacking $CD45R^-$ cells upon tetanus toxoid stimulation (Table I) suggests that they have a functional memory cell pool, although these cells exhibit an atypical phenotype ($CD45R^+CD45RO^+$). Thus, expression of CD45RO, but not loss of CD45R antigens, is correlated with a cell's memory function. The knowledge of the described polymorphism of CD45R antigen expression is important to avoid misinterpretation of memory cell phenotype and function in individuals lacking $CD45R^-$ cells.

## Summary

A genetic polymorphism of CD45R expression was identified resulting in a lack of $CD45R^-$ lymphocytes in ~8% of healthy individuals. Family studies revealed an autosomal dominant mode of inheritance of the variant CD45R expression pattern. PBMC from donors possessing the variant type did not lose the CD45R antigen after in vitro activation, whereas a decrease of CD45R molecules was readily detected in individuals with the normal pattern. The expression of CD45RO antigens, as well as memory cell function, did not differ between both groups. These data show that activation and in vivo priming of T cells is not necessarily associated with a loss of CD45R antigen expression.

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