

Selective Expression of CD45 Isoforms on Functional Subpopulations of CD34⁺ Hemopoietic Cells from Human Bone Marrow

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Summary

We have found that the small population of cells in human marrow that are characterized by their expression of CD34 can be readily subdivided into two apparently nonoverlapping subpopulations of approximate equal size, one expressing CD45RO and one CD45R. Functional studies of these subpopulations revealed that all of the primitive erythroid colony-forming cells (BFU-E) are CD34⁺ CD45RO⁺. Similarly, more primitive cells that give rise to both erythroid and granulopoietic colony-forming cells after being maintained for 5 wk on confluent irradiated long-term marrow culture feeder layers, also show this phenotype. In contrast, most granulopoietic colony-forming cells are CD34⁺ CD45RO⁻ cells. The differential expression of CD45 isoforms on distinct functional subpopulations of hemopoietic cells is consistent with the concept that these molecules play an important role in the differentiation or activation of primitive, normally quiescent, hemopoietic cells. The presence of CD45RO and the lack of CD45R on human cells capable of initiating hemopoiesis in the long-term marrow culture system correspond to the reported lack of CD45R on transplantable hemopoietic stem cells in rodents and may be a useful addition to strategies for human stem cell purification, or for purging CD45R⁺ leukemic cells.

The leukocyte common antigen (LCA or CD45) is now known to identify a family of glycoproteins that are expressed at different concentrations on the surface of different types of hemopoietic cells (1). The CD45 complex includes proteins of molecular mass 180 kD, 190 kD, 205 kD, and 220 kD. All are encoded by the same gene but are translated from different mRNAs which result from alternative exon splicing (2). The biologic functions of CD45 molecules in hemopoietic cells are not known: however, the demonstration of a 30–40% amino acid identity of two regions of the cytoplasmic domain of CD45 with a soluble placental protein phosphotyrosine phosphatase (3) suggests that these molecules may be involved in the regulation of protein phosphorylation and signal transduction. This notion is supported by the observed increase in phosphorylation of the *src*-related leukocyte-specific tyrosine kinase p56^{lck} in T cells that have lost CD45 (4).

Some mAbs to human CD45 recognize epitopes shared by all CD45 isoforms (2), whereas others appear to bind selectively either to the 180 kD or to the 220 kD isoform. Alterations in the expression of CD45 molecules are known to occur during normal hemopoietic cell development in humans with low levels being characteristic of more primitive cells defined by their co-expression of CD34 (5). Less is known about the

expression of the various CD45 isoforms on hemopoietic progenitor cells. CD45R has been variously reported to be expressed on a minority (6) or most (7) granulocyte/monocyte progenitor cells. In view of these conflicting reports and because antibodies against murine 220 kD (8) and rat B220 (9) can be used to obtain enriched populations of pluripotent hemopoietic stem cells from rodents, we studied CD45 isoform expression on human hemopoietic progenitor cells, including the most primitive populations currently detectable in normal marrow (10, 11).

Materials and Methods

mAbs. UCHL-1 (12) specific for CD45 180 kD (anti-CD45RO) was a gift from Dr. P.C.L. Beverley (ICRF, London, UK). RFB5, a mAb against CD45R (220 kD) was obtained from the IV International Workshop on Leucocyte Differentiation Antigens. 8G12, a mAb specific for CD34, was described previously (13). Purified 8G12 antibody was labeled with FITC using standard procedures.

Cells and Immunofluorescence Staining Procedures. Normal human bone marrow obtained from consenting donors was separated using Ficoll Hypaque. The low density leukocytes were washed in HBSS with 2% FCS and 0.1% sodium azide (HFN) and incubated at 10⁷ cells/ml with an equal volume of UCHL-1 (undiluted tissue culture medium), RFB5 (ascitic fluid diluted 1 in 200 to HFN),

or control mouse IgG1 antibody for 30 min on ice. After two washes in HFN the cell pellets were resuspended in biotinylated goat anti-mouse Ig (Jackson ImmunoResearch Laboratories, West Grove, PA), and incubated for 1 h followed by incubation with streptavidin-R-PE (Molecular Probes, Eugene, OR) together with 8G12-FITC. After 30 min the cells were washed and resuspended at a concentration of 2×10^6 /ml in HFN for sorting.

Flow Cytometry and Functional Assays. Flow cytometric analyses and cell sorting were performed with a FACStar Plus (Becton Dickinson, San Jose, CA). A gate was set using forward light scatter and 90° scatter signals that would include all lymphocytes, blast cells, and all hemopoietic progenitor populations to be assayed but that would exclude most erythrocytes and granulocytes and other cells with a high 90° scatter signal as described previously (10). Cells in the light scatter window were sorted on the basis of green and red fluorescence and diluted in tissue culture medium to give a desired concentration for functional analyses. Primitive erythropoietic progenitors (BFU-E), multilineage progenitors (CFU-GEMM), and all classes of granulopoietic progenitors (CFU-GM) were assayed in methylcellulose cultures as described previously (10). The relative frequency of long-term culture initiating cells (LTC-IC) was estimated by measuring the total number of clonogenic cells (BFU-E, CFU-GM, and CFU-GEMM) present in LTC initiated 5 wk after seeding the original cells on feeder layers from pre-established, irradiated, normal marrow adherent layers (10, 11).

Results

Low density cells in normal human marrow samples were double stained with antibodies against either CD45R or CD45RO, and against CD34. Without exception (more than 10 normal individuals tested), both anti-CD45 isoform antibodies subdivided the CD34⁺ cells into two distinct subpopulations. These appear to represent mutually exclusive phenotypes in that the percentage of CD34⁺ CD45R⁺ cells corresponded roughly to the percentage of cells that were CD34⁺ CD45RO⁻, and in that almost all CD34⁺ cells

were stained if CD45R and CD45RO antibodies were used together (data not shown). An example of the staining profiles obtained is shown in Fig. 1. Only a few percent of the cells present in the light scatter window are stained by anti-CD34 (Fig. 1 A). Antibodies against CD45R and CD45RO react with variable numbers of CD34⁻ cells (Figure 1, B and C), but each divides the CD34⁺ cells into two populations of about equal size (Fig. 1, E and F). These CD34⁺ cells do not stain with the control antibody (Fig. 1 D). Note that the staining intensity of CD45R and CD45RO on CD34⁺ cells is significantly less than the staining intensity observed with subsets of CD34⁻ cells.

The distribution of functionally distinct hemopoietic cells was investigated by sorting of CD34⁺ cells on the basis of CD45RO fluorescence (as shown in Fig. 1) and assaying for directly clonogenic cells and their precursors (LTC-ICs). As shown in Table 1, BFU-E are found consistently and exclusively in the fraction of CD34⁺ CD45RO⁺ cells, although a significant number of these cells may be lost during the sorting procedure (recovery values of 15–27%). CFU-GM are found in both the CD34⁺ CD45RO⁻ and the CD34⁺ CD45RO⁺ cell fractions, with the majority being found in the CD34⁺ CD45RO⁻ cell fraction. LTC-ICs are found predominantly in the CD34⁺ CD45RO⁺ cell fraction. However, occasionally (as in Exp. 3), a significant number of clonogenic cells can be generated in the LTC system from cells with a CD34⁺, CD45RO⁻ phenotype. Nevertheless, even in this particular experiment, the cells in the LTC giving rise to BFU-E were enriched in the CD34⁺ CD45RO⁺ cell fraction sixfold more than in the CD34⁺ CD45RO⁻ fraction (data not shown), whereas cells giving rise to only CFU-GM were approximately equally distributed. Because most CFU-GM are CD34⁺ CD45RO⁻, it is possible that some granulopoietic progenitors may persist or generate more mature forms of CFU-GM even after 5 wk in LTC and thus

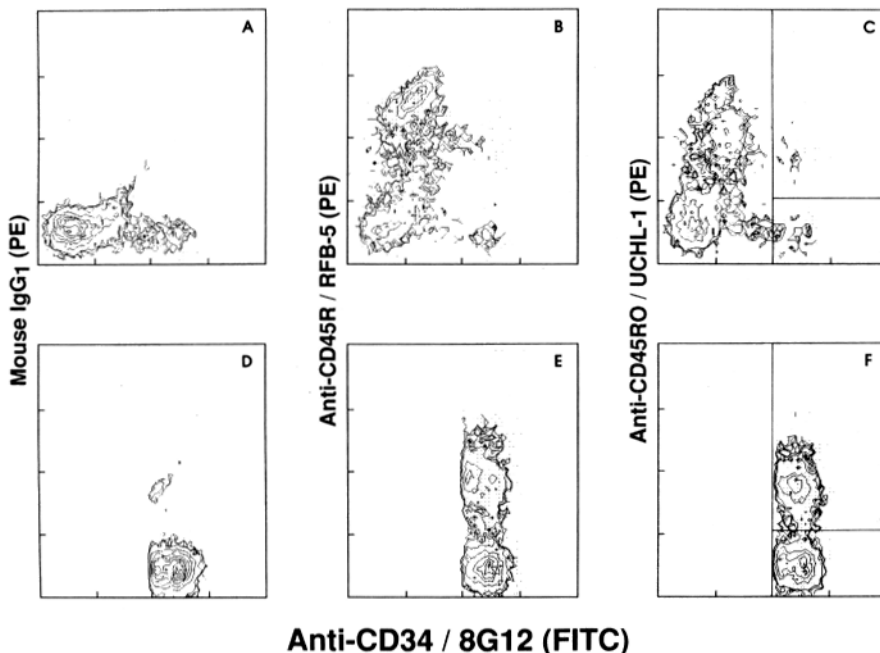


Figure 1. Expression of CD45 isoforms on cells that express CD34. Low density bone marrow cells were double stained for CD34 and either a control antibody (A and D), anti-CD45R (B and E), or anti-CD45RO (C and F). To facilitate analysis of the distribution of CD45 isoforms on (infrequent) cells that express CD34, an additional gate on green (CD34) fluorescence was set to obtain the contour plots shown in D–F. Fluorescence is plotted on a log scale. The lines shown in C and F mark the sort windows of CD34⁺ cells used for functional studies (Table 1, Exp. 3).

Table 1. Expression of CD45RO on Directly Clonogenic Cells and LTC-IC

Exp.	Fraction sorted	Percent sorted	BFU-E			CFU-GM			LTC-IC		
			Frequency (per 10 ⁵)	Enrichment	Recovery	Frequency (per 10 ⁵)	Enrichment	Recovery	Frequency (per 2 × 10 ⁶)	Enrichment	Recovery
1	Unsorted, stained	—	416	1	100	358	1		371	1	100
	CD34 ⁺ CD45RO ⁻	0.9	0	0	0	12,000	34	30	3,220	9	8
	CD34 ⁺ CD45RO ⁺	0.9	11,000	27	24	5,680	16	14	24,100	65	58
2	Unsorted	—	93	1	100	86	1		630	1	100
	CD34 ⁺ CD45RO ⁻	0.7	0	0	0	4,220	49	34	100	<1	<1
	CD34 ⁺ CD45RO ⁺	1.0	2,550	27	27	849	10	10	21,300	34	33
3	Unsorted	—	132	1	100	140	1		204	1	100
	CD34 ⁺ CD45RO ⁻	0.5	0	0	0	15,700	112	50	12,705	62	28
	CD34 ⁺ CD45RO ⁺	0.4	4,930	37	15	2,670	19	7	19,400	95	37

operationally overlap with LTC-IC. Additional experiments were undertaken with cells doubly stained with anti-CD34 and anti-CD45R. These confirmed the expression of CD45R on ~90% of CFU-GM and the lack of expression of CD45R on BFU-E (data not shown).

Discussion

In this report we show that antibodies against CD45R and CD45RO can readily separate the small CD34⁺ cell population present in normal human marrow into two distinct subpopulations. These studies were greatly facilitated by the availability of a new anti-CD34 mAb that was recently developed in our laboratory (13) and that, unlike all other anti-CD34 to date, can be directly labelled with FITC without loss of binding properties. The two CD34 subpopulations detected with CD45 isoform antibodies are of about equal size but appear to contain hemopoietic cells at different stages of development.

Low expression of CD45 common determinants by normal human clonogenic cells was first reported by Beverley et al. (14), who used this finding to isolate a highly enriched progenitor cell population from low density bone marrow cell suspensions after depletion of CD15⁺ cells. Subsequently, Shah et al. (5) demonstrated that CD34⁺ cells express low levels of CD45. Because it had been shown that all cells capable of forming hemopoietic colonies express CD34 (15), these findings were not unexpected. Our results confirm these earlier observations and demonstrate, in addition, that most, if not all CD34⁺ cells express either CD45R or CD45RO.

The relatively low level of expression of CD45 on all CD34⁺ cells could explain the apparent discrepancy between our findings regarding the expression of CD45 isoforms and those of Katz et al. (6). In the latter study only 11% of CFU-GM were found to express CD45R, but in these experiments cells with low or intermediate CD45R expression were excluded from the analysis. Strauss et al. (7) reported reactivity of mAb My11 with most CFU-GM and lack of My11 reactivity with BFU-E. It now seems likely that My11 reacts with

CD45R. This pattern of reactivity of anti-CD45R antibodies with myeloid and erythroid colony-forming cells was confirmed here and is exactly complementary to the reactivity pattern seen with anti-CD45RO. Taken together the data on the expression of CD45 common determinants on colony-forming cells and CD34⁺ cells (5, 14) and CD45 isoforms on these cells (6, 7; this paper) strongly suggest that hemopoietic cells can be subdivided into mutually exclusive subpopulations based on their expression of CD45 isoforms. This situation is reminiscent of the mutually exclusive expression of CD45 isoforms on CD4⁺ CD8⁻ and CD4⁻ CD8⁺ peripheral T cells (2).

The intracellular domain of CD45 molecules appears to include a region with phosphatase activity (3), and CD45 has been implicated in signal transduction pathways of both T and B cells (16). The segregation of functionally distinct hemopoietic subpopulations using antibodies against CD45 isoforms suggests that these molecules may play analogous roles in developing erythroid and granulopoietic cells. We have shown that LTC-IC, the most primitive hemopoietic cells in human marrow currently detectable, express predominantly the CD45RO isoform. It will therefore be of interest to study in greater detail the regulation of CD45 isoform expression in these cells as they begin to proliferate and differentiate into clonogenic cells. Lymphokines such as IL-1 and IL-6 that have been implicated in the activation and proliferation of primitive hemopoietic cells (17) have also been reported to upregulate CD45R expression (18).

The lack of CD45R expression on the most primitive cells tested in our study is in agreement with studies on CD45R expression on transplantable hemopoietic stem cells in mice (8) and rats (9). Conservation during evolution of developmentally regulated expression of CD45 isoforms on hemopoietic cells further suggests that the strict regulation of CD45 isoforms may be related to their functional significance. The findings reported here also indicate that antibodies against CD45R may be useful for the depletion of committed (clonogenic) hemopoietic cells and thus for the purification of more

primitive human hemopoietic cell populations. Because a significant proportion of blast cells from acute myeloid leukemia patients express CD45R⁺ (6, 7), depletion of CD45R⁺

cells could also be considered in these cases as a strategy for removing leukemic cells from bone marrow for autologous bone marrow transplantation.

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