# Hematopoietic Progenitor Cell Expression of the H-CAM (CD44) Homing-Associated Adhesion Molecule

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**We explored the expression of a lymphocyte homing-associated cell adhesion molecule (H-CAM, CD44) on hematopoietic progenitors. We demonstrate that immature myeloid and erythroid leukemic cell lines stain intensely with monoclonal antibodies Hermes-1 and Hermes-3, which define distinct epitopes on lymphocyte surface H-CAM, a glycoprotein involved in lymphocyte interactions with endothelial cells. Using fluorescence-activated cell sorting (FACS), human marrow cells were fractionated into Hermes$^{hi}$, Hermes$^{med}$, and Hermes$^{lo}$ populations according to the expression of both the Hermes-1 and Hermes-3 epitopes. Granulocyte-macrophage colony-forming unit and erythroid burst-forming unit precursors were found predominantly in the brightly positive fractions. Two-color FACS analysis confirmed that the My10 (CD34) positive populations of cells in bone marrow, which contain most of the progenitor cell activity, are brightly positive for Hermes-1. Finally, we demonstrate that among bone marrow cells, the highest levels of H-CAM are expressed on myeloid and erythroid progenitors as well as mature granulocytes and lymphocytes. Thus we provide evidence that molecules related or identical to the H-CAM homing receptor are expressed on marrow progenitor cells. H-CAM may contribute to progenitor cell interactions with marrow endothelial and stromal cell elements important to the maintenance and regulation of hematopoiesis.**



IN THE ADULT HUMAN, hematopoiesis normally occurs exclusively in the bone marrow (BM). However, the mechanism of this predilection is unclear, as stem cells circulate continuously[1]; can reconstitute irradiated BM in murine,[2] canine,[3] and human[4] systems; and can give rise to extramedullary sites of hematopoiesis under the appropriate circumstances. Current theory has stressed the importance of a hematopoietic inductive microenvironment,[5] which has been supported by the demonstration that a wide variety of colony stimulating factors (CSFs) are required for stem cell differentiation and proliferation in vitro.[6] In long-term marrow cultures, growth of hematopoietic cells occurs in association with an adherent layer of stromal cells, which are necessary for the maintenance of long-term in vitro hematopoiesis.[7,8] There is substantial evidence that the hematopoietic microenvironment, composed of stromal cells, soluble factors, and extracellular matrix (ECM), is crucial to the growth and differentiation of hematopoietic cells. Determinants expressed on the stem cell surface may be important in targeting these cells to hematopoietic sites, as well as in mediating specific interactions with stromal cells or ECM in the marrow. Indeed, studies have suggested that murine marrow granulocyte macrophage progenitor cells (CFU-GM) use specific membrane lectins which act as homing receptors for supportive marrow stroma,[9] and that leukemic cells from patients with chronic myeloid leukemia, which often establish extramedullary sites of hematopoiesis, have a markedly lowered affinity for and adhesivity to steroid-treated stromal cells in vitro in comparison with normal hematopoietic precursors.[10]

One experimental model that has helped to elucidate the role of cell-surface determinants in cell trafficking has been the study of lymphocyte homing. Lymphocytes circulate continuously between blood and lymphoid organs, leaving the blood through specialized high endothelial venules (HEVs). A lymphocyte surface glycoprotein of molecular weight 85- to 95-Kd, defined originally by monoclonal antibody (MoAb) Hermes-1,[11] is involved in lymphocyte recognition of peripheral node, mucosal, and synovial HEV.[12] Antibodies against certain epitopes of this 85- to 95-Kd glycoprotein can inhibit lymphocyte binding to HEV in an in vitro assay. Interestingly, the Hermes antibodies define related or identical molecules expressed by a variety of nonhematopoietic cells, including follicular dendritic cells, glial cells, keratinocytes, and fibroblasts, leading to the proposal that the Hermes-defined glycoprotein(s) may be a widely used cell adhesion molecule.[13,14] Indeed, the 85- to 90-Kd Hermes antigens isolated from lymphocytes, fibroblasts, and glial cells (but not an ~150-Kd keratinocyte form) can bind saturably and reversibly to the isolated mucosal vascular addressin, a 60-Kd endothelial cell adhesion molecule for lymphocytes.[13] Recent analyses of cDNAs encoding this homing-associated cell adhesion molecule (H-CAM) show that it is distinct from other known cell adhesion molecules, but displays features related to proteoglycan core proteins, including potential chondroitin sulfate linkage and O-glycosylation sites, and an N-terminal domain homologous to cartilage proteoglycan core and link protein domains involved in glycosaminoglycan interactions.[15-17] Thus, H-CAM, defined by the Hermes antibodies, is a widespread adhesion molecule likely to play a role in multiple cell–cell

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and cell–substrate adhesion events throughout the body. H-CAM has been shown to be CD44.[18]

Because of the involvement of H-CAM in lymphocyte adhesion to endothelial cells, its possible role in diverse cell adhesion events, and its expression on mature granulocytes and monocytes (see below), it was of interest to study the expression of this glycoprotein on hematopoietic progenitor cells. These progenitors are characterized by clonogenicity in vitro under the stimulus of CSFs and erythropoietin[19,20] and express the surface antigen My10[21] (CD34). In this report, we demonstrate that the Hermes-1 and Hermes-3 epitopes are expressed at high levels on human BM myeloid and erythroid progenitors; that H-CAM is expressed on My10 (CD34) positive cells; and that among BM cells, Hermes expression is bimodal, with highest levels being expressed on myeloid and erythroid progenitors, immature and mature granulocytes and lymphocytes, and lower levels on some lymphocytes, normoblasts, and some intermediate granulocytes. The findings define H-CAM expression as an additional phenotypic marker of human hematopoietic progenitor cells, and raise the possibility that this cell-adhesion molecule may play an important role in the interaction of hematopoietic progenitor cells with elements of the BM microenvironment.

## MATERIALS AND METHODS

*Marrow samples.* BM samples were obtained from normal subjects according to the guidelines established by the Stanford University Human Experimentation Committee. Three to five milliliters of marrow aspirate were drawn into a heparinized syringe. The BM were diluted with phosphate buffered saline, layered over Ficoll-Hypaque (density 1.077 g/mL), centrifuged at 400 × *g* for 20 minutes, and the mononuclear cells (MNCs) at the interface were collected, washed in Iscove's Modified Dulbecco's Media (IMDM), and counted.

*Cell lines.* The human myeloid/erythroid leukemic cell lines KG1, HL60, and U937 were obtained from the ATCC, and grown in RPMI containing 10% fetal calf serum (FCS), at 37°C with 5% $CO_2$.

*Isolation of human neutrophils and peripheral blood mononuclear leukocytes.* Human venous blood was obtained, diluted in cell suspension media containing 5mmol/L EDTA, and mononuclear cells purified on a Histopaque (Sigma, St Louis, MO) density 1.077 gradient. The pellet, containing red blood cells and neutrophils, was resuspended, and neutrophils purified on a Histopaque density 1.119 gradient. All cells were washed two times in RPMI 1640 containing 5mmol/L EDTA, and the cells were labeled as described below.

*Immunofluorescence staining.* To detect the presence of H-CAM on the surface of BM MNCs, cells were stained with biotinylated Hermes-1 (0.5 $\mu g/10^6$ cells; antibody biotinylated as described[22]) followed by FITC-avidin (Sigma, 1:50). Hermes-1 is a rat immunoglobulin G2a (IgG2a).[11] For Hermes-3 staining, cells were stained with Hermes-3 (0.5 $\mu g/10^6$ cells) followed by FITC anti-mouse Ig (Fab fragments, Sigma, 1:50). Hermes-3 is a mouse IgG2a.[12] Class-matched antibodies were used as negative controls: either biotinylated 4G10 (a rat IgG2a anti-mouse Ig idiotype antibody, a gift of R.Levy, Stanford, CA) as a control for biotinylated Hermes-1; or unconjugated A20 (a mouse IgG2a against an irrelevant antigen, a gift of M. Jalkanen, Stanford, CA). All incubations were performed at a volume of 10 $\mu L/10^6$ cells for 30 minutes on ice.

In studies analyzing expression of H-CAM on peripheral blood lymphocytes and neutrophils (see Fig 2), leukocytes were separated from erythrocytes on a histopaque gradient (see above) and stained with either biotinylated Hermes-1 or control biotinylated 30G12 (0.5 $\mu g/10^6$ cells in 10 $\mu L$) followed by FITC-avidin as described above. 30G12 is a rat IgG2a against mouse T200.[23] In studies comparing H-CAM on cell lines and peripheral blood leukocytes (see Table 1), Hermes-1 and Hermes-3 were used unconjugated (1 $\mu g/10^6$ cells) with second-stage FITC anti-rat Ig and anti-mouse FAb reagents (Sigma, 1:50), respectively. 30G12 and MAR 18.5, an anti-mouse $\kappa$ MoAb (from the ATCC), were used as rat IgG2a and mouse IgG2a controls, respectively.

In two-color immunofluorescence studies of H-CAM versus My10 expression on BM mononuclear cells, cells were stained with either biotinylated Hermes-1 or biotinylated 30G12, at the concentrations above, in combination with phycoerythrin-conjugated My10 (1 $\mu g/10^6$ cells). PE-My10 was the kind of gift of Ann Jackson, PhD, Becton-Dickinson, Mountain View, CA. After incubation with the combined first-stage antibodies, FITC-avidin (Sigma, 1:50) was added as a second-stage for the biotinylated antibodies. Dead cells were stained by adding 1 $\mu L$ of a 2 $\mu g/mL$ propridium iodide solution after the final wash. Incubations and washes were performed as described,[24] except that cells were handled in the presence of 5 mmol/L EDTA without serum.

Fluorescence-activated cell sorter (FACS) analyses were performed on a modified Becton-Dickinson FACS II equipped with a 4-decade logarithmic amplifier. Individual cells were scored for fluorescence-staining intensity, forward light scatter (a cell-size related parameter[25]), and, where possible, obtuse light scatter. FACS and computer software were made available through the shared FACS users group, Stanford University. To assure data collection on only viable cells, dead cells were excluded both by light scatter and propridium iodide staining.[25]

*FACS.* The FACS was used to sort Hermes-1 and Hermes-3 stained human BM mononuclear cells into high, medium, and low fractions. Sorting gates, shown in Fig 2, were set such that Hermes-1 or -$3^{hi}$ cells stained at a level equivalent to or greater than that of brightly staining peripheral blood lymphocytes. Hermes-$1^{lo}$ gates were set such that cells falling within this gate stained at a level equivalent to staining with the negative control antibody. Because Hermes-1(3) staining on BM was characteristically heterogeneous, a Hermes$^{med}$ gate was set to isolate the population of positive but less intensely stained cells. Control sorts were performed using cells stained with the irrelevant control antibodies 4G10 (rat IgG2a control for Hermes-1) or A20 (mouse IgG2a control for Hermes-3), as described above.

*Morphologic analysis.* Cytocentrifuge preparations of the sorted BM cells were stained with Wright's-Giemsa for morphologic analysis. Morphology was assessed by evaluating 200 consecutive cells per slide.

*Hematopoietic colony-formation assays.* Colony-formation assays were performed on antibody-stained FACS-sorted fractions (described above), or on antibody-stained cells passed through the cell sorter without sorting into fractions (whole unsorted BM). The BM mononuclear cells ($1.5 \times 10^5$ cells/mL) were cultured in IMDM with 15% FCS, 0.9% bovine serum albumin (Armour Pharmaceutical, Tarrytown, NY), 50 $\mu$mol/L 2 mercaptoethanol, 1% penicillin/streptomycin, 1% L-glutamine, and methylcellulose (final concentration 1.1%) as previously described.[26] Cultures were generally seeded as $0.375 \times 10^5$ cells in duplicate in 0.25-mL volumes ($1.5 \times 10^5$ cells/mL) in Costar Mark II tissue culture plates (Costar, Cambridge, MA). Cultures for colony-forming units-granulocyte-monocyte (CFU-GM) were plated with 15% placental conditioned media (PCM). Cultures for burst-forming units erythroid (BFU-E) were plated with 1% Mo cell-conditioned media (a

T-cell line media kindly provided by Dr David Golde, UCLA Medical Center, Los Angeles, CA) as a burst-promoting activity source, and 0.5 $\mu$/mL purified human urinary erythropoietin (1,140 $\mu$/mg protein, obtained from The British Columbia Cancer Research Centre, Vancouver, BC, Canada). Cultures were incubated in humidified 5% $CO_2$ at 37°C. CFU-GM colonies were scored on day 10, and BFU-E on day 14. To compare the data with the prior literature, colony formation was extrapolated to enumerate colonies per $10^5$ cells plated.

CFU-GM recovery in each sorted fraction was calculated as:

$$\text{CFU-GM recovery} = \frac{\text{CFU-GM}/10^5 \text{ Sorted Fraction} \times \text{No. Cells Sorted Fraction}}{\text{CFU-GM}/10^5 \text{ Unfractionated Cells} \times \text{No. Cells Collected Without Sorting}} = \frac{\text{Total Colonies per Sorted Fraction}}{\text{Total Colonies in "Unsorted" Bone Marrow}}$$

Similar calculations were made for BFU-E recovery.

## RESULTS

*Immature myeloid and erythroid cell lines express the Hermes-1 antigen.* The immature myeloid/erythroid cell lines U937, HL-60, and KG-1 have proven useful in the study of hematopoietic differentiation, as all are capable under the appropriate circumstances, of undergoing further differentiation along myeloid and erythroid lines.[27] All three lines were stained with the Hermes-1 and Hermes-3 antibodies, and all expressed both epitopes at levels comparable with peripheral blood lymphocytes (Table 1).

*Myeloid and erythroid progenitor cells in BM express high levels of the Hermes-1 and Hermes-3 epitopes.* Ficoll-Hypaque isolated BM mononuclear cells were stained with either Hermes-1 or Hermes-3, analyzed by flow cytometry, and separated into Hermes$^{hi}$, Hermes$^{med}$, and Hermes$^{lo}$ fractions (see Fig 1 for representative gates; 95% of peripheral blood lymphocytes, neutrophils, and monocytes would fall within the Hermes$^{hi}$ gate). Sorted cells were assessed for hematopoietic progenitor cells by plating them in the methylcellulose clonogenic system.[26] Additionally, cytocentrifuge preparations of the sorted fractions were prepared for morphologic evaluation of each fraction.

As shown in Tables 2 and 3, virtually all of the myeloid and erythroid progenitor cells (both CFU-GM and BFU-E) segregated with the Hermes-1$^{hi}$ and Hermes-3$^{hi}$ fractions. In contrast, when BM MNCs stained with negative control antibodies and sorted using identical gates were plated, all recoverable progenitor cells were present in the negative fraction (Tables 2 and 3). Thus, the presence of progenitor cells in the brightly Hermes-1$^{hi}$ and Hermes-3$^{hi}$ fractions cannot be explained on the basis of autofluorescence or nonspecific staining. In control experiments not using the cell sorter, neither Hermes or control antibodies altered colony formation of stained BM cells in vitro (data not shown).

**Table 1. Expression of H-CAM by Immature Hematopoietic Cell Lines**

| | Mean Fluorescence | | | |
|---|---|---|---|---|
| | 30G12 | Hermes-1 | Mar 18.5 | Hermes-3 |
| KG1 | 5.0 | 1,523 | 8.0 | 1,138 |
| HL60 | 3.0 | 165 | 3.0 | 123 |
| U937 | 4.0 | 567 | 4.0 | 512 |
| Normal lymphocytes | 2.0 | 339 | — | — |
| Blood neutrophils | 4.0 | 313 | — | — |

Cell lines or peripheral blood mononuclear cells were stained with the indicated MoAbs followed by FITC-conjugated second-stage antibodies. The mean fluorescence determined by FACS analysis is given. Lymphocytes and neutrophils were discriminated by forward and obtuse light scatter parameters.

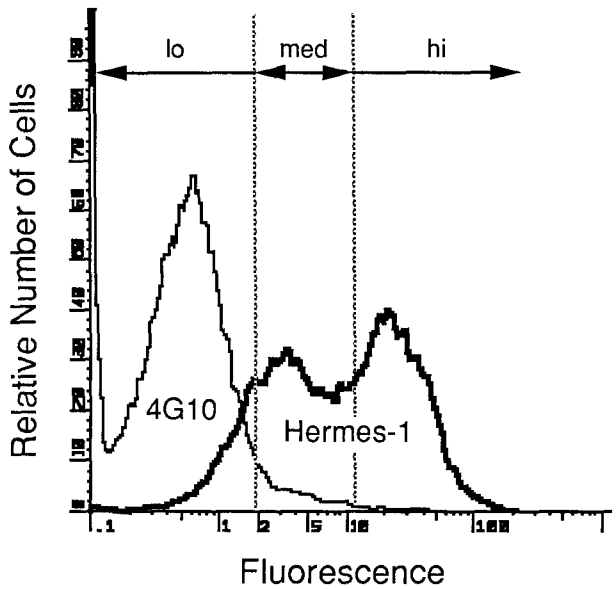


**Fig 1. The FACS was used to sort Hermes-1–stained human BM cells into high, medium, and low fractions. The sorting gates shown were set such that Hermes-1$^{hi}$ cells stained at a level equivalent to or greater than that of brightly staining peripheral blood lymphocytes. Hermes$^{lo}$ gates were set such that cells falling within this gate stained at a level equivalent to staining with the negative control antibody. Because Hermes-1 staining on BM was characteristically heterogeneous, a Hermes-1$^{med}$ gate was set to isolate the population of positive but less-intensely stained cells. The same gates were used to sort cells stained with isotype-matched (rat IgG2a) antibody 4G10.**

Our percentages of progenitor cell recovery after fractionation by the cell sorter were variable (38% to 134% of CFU-GM, and 21% to 48% BFU-E, after sorting with Hermes-1 or Hermes-3; see Tables 2 and 3), but overall were similar to those reported by others (eg, see Civin, et al[28]). Cell loss and/or damage during FACS sorting is a known technical problem. Selective damage to sorted populations may explain the finding that, even though the Hermes$^{hi}$ fractions contained almost all of the progenitor activity among fractionated populations, the frequency of progenitors capable of forming colonies was not enriched over that of the unseparated whole BM populations. (Given that the percent of cells recovered in the Hermes$^{hi}$ fractions ranged from 31% to 45%, one would have predicted two- to threefold enrichment in progenitor cell incidence in these fractions after sorting.) However, the frequency of progenitor cells in the Hermes$^{hi}$ fractions is substantially enriched over that observed in the control antibody stained and sorted populations in all experiments (ie, the 4G10$^{lo}$ or A20$^{lo}$ fractions; Tables 2 and 3); these fractionated control populations may

**Table 2. Distribution of BM Hematopoietic Progenitor Cells Relative to Their Expression of the Hermes-1 Epitope**

| Hermes-1 Expression | Total Cell No. ($\times 10^6$) | BM Cells: Cell Recovery (%) | CFU-GM*: Colonies/$10^5$ Cells | CFU-GM*: Total No. of Colonies | CFU-GM*: CFU-GM Recovery (%) | BFU-E†: Colonies/$10^5$ | BFU-E†: Total No. of Colonies | BFU-E†: BFU-E Recovery (%) |
|---|---|---|---|---|---|---|---|---|
| Donor 1 | | | | | | | | |
| High | 2.0 | 45 | 121 ± 4 | 2,412 ± 30 | 62 | 46 ± 4 | 911 ± 30 | 21 |
| Medium | 1.75 | 39 | 0 | 0 | 0 | 0 | 0 | 0 |
| Low | 0.7 | 16 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unsorted‡ | 4.45 | 100 | 88 ± 15 | 3,916 ± 656 | 100 | 99 ± 4 | 4,405 ± 67 | 100 |
| Donor 2 | | | | | | | | |
| High | 0.35 | 31 | 160 ± 4 | 560 ± 14 | 134 | 168 ± 3 | 588 ± 11 | 48 |
| Medium | 0.38 | 34 | 0 | 0 | 0 | 5 ± 1 | 19 ± 4 | 1 |
| Low | 0.4 | 35 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unsorted‡ | 1.13 | 100 | 37 ± 7 | 418 ± 79 | 100 | 109 ± 22 | 1,231 ± 249 | 100 |
| Control Antibody (4G10) Sort | | | | | | | | |
| High | 0.02 | 1 | § | | | | | |
| Medium | 0.06 | 3 | § | | | | | |
| Low | 2.15 | 96 | 43 ± 1 | 924 ± 21 | 35 | 16 ± 8 | 320 ± 63 | 9 |
| Unsorted‡ | 2.23 | 100 | 118 ± 25 | 2,631 ± 209 | 100 | 153 ± 19 | 3,412 ± 147 | 100 |

*CFU-GM were counted after 10 days of incubation, stimulated with 15% PCM. Colony numbers are calculated as mean ± SE (plating was performed in duplicate).

†BFU-E were counted after 14 days of incubation, stimulated with 1% MoCM and 0.5 U/mL human erythropoietin. Colony numbers are calculated as mean ± SE (plating was performed in duplicate).

‡Unsorted cells were stained with antibody and passed through the FACS without sorting into fractions.

§Insufficient number of cells in medium and high control fractions for plating.

offer a more appropriate comparison for the fractionated Hermes$^{hi}$, Hermes$^{med}$, and Hermes$^{lo}$ populations.

To assess the morphology of the sorted fractions, Wright's-Giemsa–stained cytocentrifuge preparations were made of each fraction. As shown in Table 4, the Hermes-1$^{hi}$ fractions consisted predominately of lymphocytes (44%), immature myeloid cells (25%), mature granulocytes (15%), and monocytes (6%). The Hermes-1$^{lo}$ fraction was composed predominately of mature granulocytes (55%) and normoblasts (25%), with relatively few lymphocytes, monocytes, or immature myeloid cells. In contrast, the control 4G10$^{lo}$ sorted fraction, which comprised more than 90% of the total cells, had a composition similar to unsorted cells: 46% mature granulocytes, 17% intermediate granulocytes, 15% lymphocytes, 12% normoblasts, 7% immature granulocytes, and 3% monocytes. Similar results were obtained from morphologic assessment of Hermes-3 sorted fractions (data not shown).

In separate studies, neutrophils (Fig 2) and monocytes (not shown) from peripheral blood were found to stain intensely with Hermes-1.

*My10 (CD34) positive cells express the Hermes-1 antigen.* My10 is a cell-surface marker expressed on 1% to 4% of normal BM cells. The My10 positive population is highly enriched for hematopoietic progenitors.[28] We used two-color immunofluorescence to study the coexpression of the Hermes-1 antigen on these BM My10 (CD34)$^+$ progenitor cells. As shown in Fig 3, the My10 positive cells express high levels of the Hermes-1 antigen.

## DISCUSSION

We found that human immature myeloid/erythroid leukemic cell lines, immature and mature myeloid cells, BM hematopoietic progenitor cells, BM progenitor cells capable

**Table 3. Distribution of BM Hematopoietic Progenitor Cells Relative to Their Expression of the Hermes-3 Epitope**

| | Total Cell No. ($\times 10^6$) | BM Cells: Cell Recovery (%) | CFU-GM*: Colonies/$10^5$ Cells | CFU-GM*: Total No. of Colonies | CFU-GM*: CFU-GM Recovery (%) | BFU-E†: Colonies/$10^5$ | BFU-E†: Total No. of Colonies | BFU-E†: CFU-GM Recovery (%) |
|---|---|---|---|---|---|---|---|---|
| Hermes-3 Expression | | | | | | | | |
| High | 1.0 | 37.0 | 161 ± 7 | 1,610 ± 70 | 38 | 227 ± 27 | 2,270 ± 270 | 45 |
| Medium | 0.75 | 27.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Low | 1.0 | 37.0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Unsorted | 2.75 | 100.0 | 155 ± 0 | 4,562 ± 0 | 100 | 184 ± 12 | 5,060 ± 330 | 100 |
| Control Antibody Expression A20 | | | | | | | | |
| High | 0.06 | 5.0 | 0 | 0 | 0 | 0 | 0 | 48 |
| Medium | 0.25 | 19.0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Low | 1.0 | 76.0 | 86 ± 4 | 860 ± 53 | 56 | 19 ± 0 | 188 ± 0 | 11 |
| Unsorted | 1.31 | 100.0 | 118 ± 4 | 1,545 ± 53 | 100 | 137 ± 2 | 1,794 ± 26 | 100 |

*CFU-GM were counted after 10 days of incubation, stimulated with 15% PCM. Colony number was calculated as ± SE (plating was performed in duplicate).

†BFU-E were counted after 14 days of incubation and stimulated with 1% MoCM and 0.5 U/mL human erythropoietin. Colony number was calculated as ± SE (plating was performed in duplicate).

**Table 4. Differential Counts of BM Cell Fractions Sorted Based on Hermes-1 or Control Antibody Staining**

| Fraction | % of Unsorted | Lymphocytes | Granulocytes* | | | Monocytes | Normoblasts |
|---|---|---|---|---|---|---|---|
| | | | Immature | Intermediate | Mature | | |
| Hermes-1 | | | | | | | |
| High | 45 | 44 ± 8 | 25 ± 11 | 5 ± 1 | 15 ± 6 | 6 ± 5 | 5 ± 2 |
| Medium | 37 | 1 ± 1 | 8 ± 1 | 11 ± 9 | 59 ± 12 | 1 ± 1 | 20 ± 9 |
| Low | 15 | 9 ± 4 | 2 ± 1 | 9 ± 1 | 55 ± 17 | 0 | 25 ± 12 |
| Unsorted | 100 | 19 ± 1 | 3 ± 1 | 11 ± 4 | 42 ± 11 | 5 ± 2 | 20 ± 2 |
| 4G10† | | | | | | | |
| Low‡ | 94 | 15 ± 3 | 7 ± 1 | 17 ± 2 | 46 ± 1 | 3 ± 1 | 12 ± 1 |
| Unsorted | 100 | 18 ± 2 | 4 ± 2 | 13 ± 3 | 45 ± 6 | 5 ± 2 | 15 ± 9 |

Results expressed as mean ± SE, n = 3. Cytocentrifuge preparations were made for Wright's-Giemsa staining and morphology was assessed by evaluating 200 consecutive cells.

*Immature granulocytes are myeloblasts and promyelocytes. Intermediate granulocytes are myelocytes and metamyelocytes. Mature granulocytes are band forms and segmented neutrophils.

†Insufficient number of cells in medium and high control fractions for morphologic analysis.

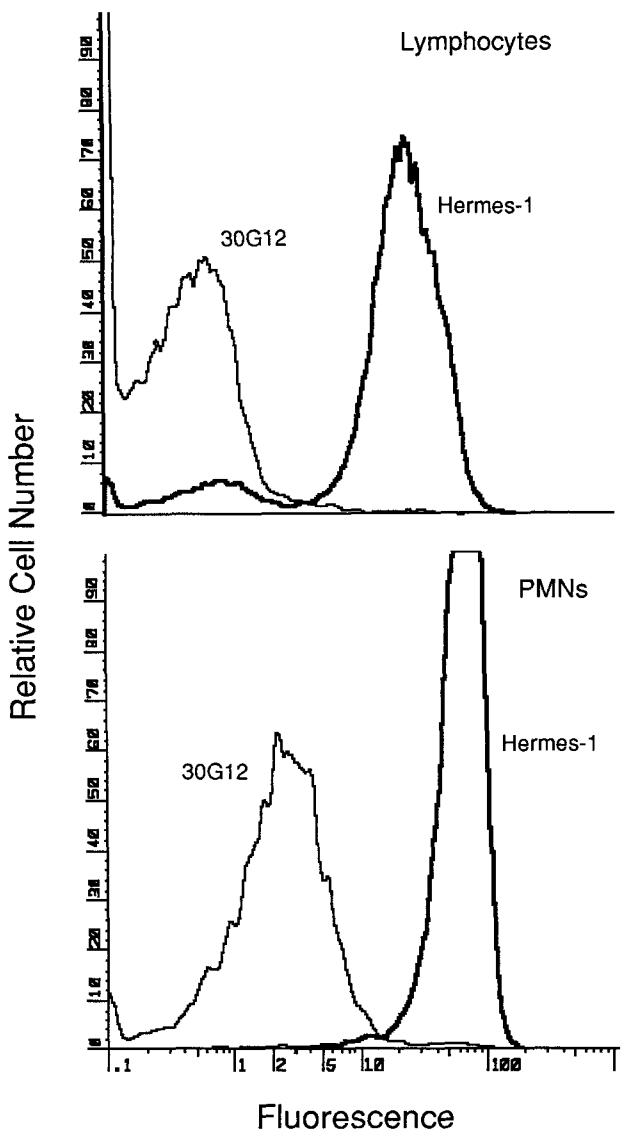


Fig 2. Staining of peripheral blood leukocytes with Hermes-1. Normal human leukocytes were stained with biotinylated Hermes-1 or control antibody (30G12) followed by FITC-avidin. Shown here are the histograms of lymphocytes and neutrophils, identified by their characteristic scatter profiles.

of generating CFU-GM and BFU-E colonies in vitro, and My10 positive BM cells stain intensely with MoAbs Hermes-1 and Hermes-3, which recognize distinct epitopes on H-CAM, a lymphocyte surface-adhesion molecule.[12,13] Whether H-CAM on progenitor cells is identical to, or only closely related to, the lymphocyte glycoprotein is unknown. While its role in hematopoietic progenitor cell physiology is speculative, by analogy with the importance of H-CAM in lymphocyte adhesion to HEV, it seems reasonable to hypothesize that this molecule may also serve a cell adhesion/recognition function on progenitor cells. For example, progenitor cell H-CAM could be involved in homing of progenitor cells to BM through selective interaction with sinusoidal endothelial cells. Alternatively, these molecules could be involved in the interaction of progenitor cells with marrow stromal cells, or with extracellular matrix components.

A number of adhesion molecules and/or adhesive activities have been found to be involved in the interaction of

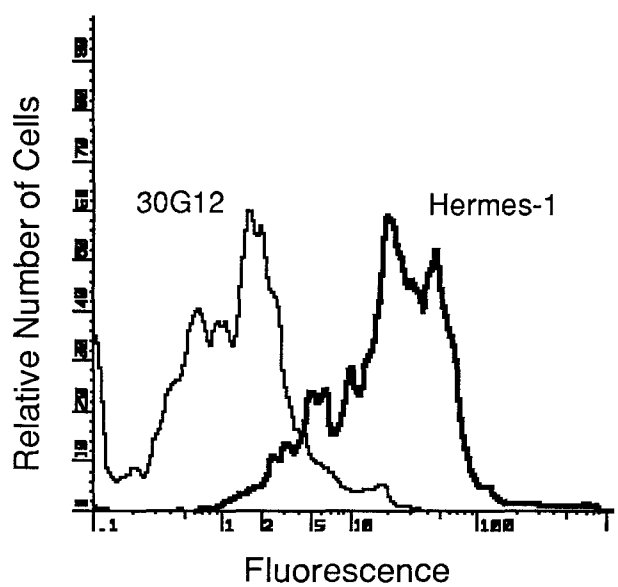


Fig 3. Marrow mononuclear cells were stained with phycoerythrin-conjugated anti-My10 and biotinylated Hermes-1 followed by FITC-avidin. Shown here are the Hermes-1 and 30G12 (control antibody) histograms of the brightly My10 (CD34) positive population (4% of total cells).

marrow progenitor cells and marrow stromal components. Recently, Campbell et al[29] described a protein, haemonectin, in extracellular matrix that is a lineage and organ-specific attachment molecule for cells of granulocyte lineage. Tavassoli et al found specific galactosyl and mannosyl membrane lectins on murine CFU-GM capable of binding to supportive stroma, which may be involved in the homing of hematopoietic stem cells to hematopoietic tissues.[9,30,31] They further defined receptors with specificity for galactosyl residues of glycoconjugates on the lumenal surface of murine marrow sinus endothelium cells.[32] The relationship between these lectin activities on murine progenitor cells within the marrow microenvironment and the expression of H-CAM remains to be determined. However, in this context it is interesting that a mouse lymph node homing receptor defined by MoAb MEL-14[33] appears to be a mammalian lectin, as cDNA sequence analysis of the MEL-14 antigen shows homology to other lectins,[34,35] and functional studies suggest that $gp90^{MEL-14}$ can bind mannose-6-phosphate and related carbohydrates.[36] It will be of interest to ask whether the MEL-14 antigen and/or other putative lymphocyte adhesion systems are expressed on progenitor cells like H-CAM, and if so, what role they might play in interactions with stromal cells.

Within the Ficoll-purified marrow population, lymphocytes and immature and mature myeloid cells expressed the highest levels of the Hermes-1 and -3 epitopes. It is intriguing that the distribution of Hermes antigen among cells of the myeloid lineage appears to be bimodal, with greatest expression occurring on very immature and on mature cells. Because mature peripheral blood neutrophils and lymphocytes express high levels of H-CAM, we interpret the data in the following manner: Lymphocytes are brightly positive for the H-CAM, as are monocytes. Granulocytes express high levels of the antigen while immature, losing expression during development, but regain high levels on or before entering the peripheral circulation. Normoblasts express lower amounts of the antigen. One possible explanation for this developmental pattern of expression is that H-CAM is involved in distinct interactions or targeting events on immature and on mature hematopoietic cells. The antigen on progenitor cells could be involved in cell adhesive interactions in the hematopoietic microenvironment whereas the antigen on mature myeloid cells may be able to participate in interactions with peripheral endothelial cells during leukocyte egress into inflammatory sites. In this context, it is interesting that Campbell et al[29] recently found that peripheral blood granulocytes are only one tenth as efficient as marrow granulocyte precursors in binding to haemonectin. A similar finding was obtained with mature erythrocytes and BM fibronectin.[37]

In summary, we found that human immature myeloid and erythroid leukemic cell lines, normal immature and mature myeloid cells, BM myeloid and erythroid progenitor cells, and BM My10 (CD34) positive cells all express high levels of H-CAM (CD44), an adhesion molecule involved in the interaction of peripheral blood lymphocytes with HEV during lymphocyte homing. It will be important to evaluate the specific role of H-CAM in cell targeting and/or in progenitor cell interactions with stromal cell and endothelial cell components of the hematopoietic microenvironment.

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