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Differential expression of homeobox genes in functionally distinct CD34$^+$ subpopulations of human bone marrow cells

(cDNA/hematopoiesis/Hox genes/reverse transcription PCR/stem cell-specific genes)

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ABSTRACT **Class I homeobox (Hox) genes encode a major group of transcription factors controlling embryonic development and have been implicated in the continuing process of hematopoietic cell differentiation. They are clustered on four chromosomes and, in early development, exhibit spatially restricted expression with respect to their 3′ → 5′ chromosomal position. By using an improved PCR-based method for amplifying total cDNA derived from limited cell numbers, we now describe the expression of class I Hox genes in highly purified CD34$^+$ cell subpopulations isolated from normal human bone marrow that represent functionally distinct stem and progenitor cell compartments. Our data indicate that at least 16 different Hox genes, mainly from the A and the B clusters, are expressed in one or more of these subpopulations of human hematopoietic cells. Moreover, markedly elevated expression of some of the Hox genes found at the 3′ end of the A and B clusters (e.g., HoxB3) was a unique feature of the subpopulations that contained the most primitive functionally defined cells, whereas genes located in the 5′ region of each cluster (e.g., HoxA10) were found to be expressed at nearly equal levels in the CD34$^+$ subpopulations analyzed. In contrast to the findings for CD34$^+$ cells, expression of two selected Hox genes, HoxB3 and HoxA10, was virtually extinguished in the CD34$^-$ fraction of bone marrow cells. These results demonstrate the expression of a broad range of Hox genes in primitive hematopoietic cells and point to the existence of a regulated program of Hox gene expression during their normal development.**

Although the homeobox (Hox) genes were first recognized for their involvement in embryonic development, increasing evidence points to these genes playing important lineage-specific roles throughout life in various somatic tissues, including the hematopoietic system (reviewed in ref. 1). Studies of Hox gene expression during the differentiation of primitive normal hematopoietic cells have been hampered, however, by technical difficulties not only in obtaining the relevant subpopulations of appropriate phenotypic and functionally defined purity but also in the low numbers of such cells that would be available ($<10^4$) given their known frequencies, making standard mRNA analyses impossible. We have recently shown that the pattern of CD45RA and CD71 expression on CD34$^+$ cells in normal human bone marrow allows the reproducible isolation of relatively homogeneous progenitor populations of lineage-restricted erythroid, granulopoietic, and more primitive cells (2). Such populations might therefore be expected to provide suitable starting material for investigating possible changes in Hox gene expression during early stages of hematopoiesis. By using a modified reverse transcription polymerase chain reaction (RT-PCR) procedure that allows representative amplification of extended-length cDNAs from as few as 1000 cells, we have now documented expression of multiple Hox genes in primitive normal hematopoietic cells and identified striking differences in the patterns of expression of certain Hox genes within functionally distinct subpopulations of these cells.

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MATERIALS AND METHODS

Cell Purification. Low-density cells (<1.077 g/cm^3) of five different heparinized cadaveric human bone marrows (CAD3, CAD6, CAD7, CAD9, and CAD10) were isolated by centrifugation on Ficoll-Paque (Pharmacia LKB) and kept frozen in Iscove's medium containing 2.5% human serum albumin and 7.5% dimethyl sulfoxide. Cells from CAD3, CAD6, and CAD9 were thawed and stained with directly conjugated fluorescent antibodies to CD34 (8G12-Cy5), CD45RA (8d2-R-phycoerythrin), and CD71 (OKT9-fluorescein isothiocyanate), washed twice, and resuspended in 2 μg of propidium iodide per ml (P-5264; Sigma) prior to sorting on a FACStarPlus (Becton Dickinson Immunocytometry Systems) as described (2). After initial separation of CD34$^+$ cells on the basis of CD45RA and CD71 expression (see Fig. 1 *A* and *C*), CD34$^+$ CD45RA^{lo} CD71^{lo} cells were stained with the CD38 (Leu17-phycoerythrin; BD)-conjugated antibody and further separated into subpopulations expressing different levels of CD38 (see Fig. 1 *B* and *D*). Each subpopulation was subjected to two rounds of sorting and cell purity was assessed after the first sort. Cells from each of the five marrows were also stained with 8G12-Cy5 alone and separated into total CD34$^+$ and CD34$^-$ subpopulations.

Clonogenic Progenitor Assays. Aliquots from purified CD34$^+$ subpopulations were assayed for colonies derived from colony-forming unit, erythroid (CFU-E), mature burst-forming unit, erythroid (BFU-E), primitive BFU-E, CFU, granulocyte/macrophage (CFU-GM), or CFU, granulocyte/erythroid/macrophage/megakaryocyte precursors (CFU-GEMM) in methylcellulose cultures containing fetal calf serum, 3 units of highly purified human erythropoietin per ml (100,000 units/mg, StemCell Technologies, Vancouver, BC Canada), 50 ng of human Steel factor per ml (Amgen), and 20 ng/ml each of human interleukin 6 (IL-6) (Immunex), human

Abbreviations: MDR1, multidrug resistance 1 or P-glycoprotein; Hox, Hox homeobox gene; LTC-IC, long-term culture-initiating cell(s); RT-PCR, reverse transcription polymerase chain reaction; CFU-E, colony-forming unit, erythroid; BFU-E, burst-forming unit, erythroid; CFU-GM, CFU, granulocyte/macrophage; CFU-GEMM, CFU, granulocyte/erythroid/macrophage/megakaryocyte; CSF, colony-stimulating factor; IL, interleukin; FACS, fluoroescence-activated cell sorting.

GM colony-stimulating factor (GM-CSF) (Sandoz), human G-CSF (Amgen), and human IL-3 (Sandoz) (3).

Long-Term Culture. The long-term culture-initiating cell (LTC-IC) content of each CD34$^+$ subpopulation was evaluated essentially as described by placing 2400 cells on irradiated (8000 cGy) murine fibroblast feeder layers consisting of 1.5×10^5 cells per 35-mm dish of both M2-10B4 cells (4) and S1/S1 cells (5) engineered by retroviral gene transfer to produce 10 ng of human IL-3 per ml, 130 ng of human G-CSF per ml, and 10 ng of human Steel factor per ml. After 6 weeks, the clonogenic progenitor content of the LTC was determined and the number of LTC-IC was calculated by dividing this number by 4 based on previous studies (6) and also confirmed here by limiting dilution analysis (data not shown).

cDNA Generation and Amplification. A previously described method (7) for generating representative amplified total cDNA from small numbers of hematopoietic cells using an oligo(dT)-based primer and poly(A) tailing strategy was used after modifications designed to improve cDNA yield of even rare transcripts and to provide amplified sequence extending up to 2 kb 5′ of the poly(A) site. Briefly, cells were lysed in guanidinium isothiocyanate and the nucleic acids were precipitated as described (7). For reverse transcription, the RNA was resuspended in a solution containing 6 μl of diethyl pyrocarbonate-treated water, 2 μl of 5× RT buffer (GIBCO/BRL), 1 μl of 0.1 M dithiothreitol, 0.2 μl of 25 mM dNTPs (Pharmacia), 0.2 μl of a special 60-mer oligo(dT) primer (1 μg/μl) (7), 0.1 μl of placental RNase inhibitor (10 units/μl, GIBCO/BRL), and 0.5 μl of Moloney murine leukemia virus SuperScript (200 units/μl; GIBCO/BRL). The sample was left at 40°C for 1 hr, heated to 75°C for 10 min, and ethanol precipitated with ammonium acetate and a linear polyacrylamide carrier. The washed pellet was resuspended in 5.5 μl of tailing solution [1 μl of 5× tailing buffer (GIBCO/BRL), 0.5 μl of 100 mM dATP (Pharmacia), 3.5 μl of water, and 0.5 μl of terminal deoxynucleotidyl transferase (15 units/μl, GIBCO/BRL)] for 15 min at 37°C. The solution was directly added to a PCR amplification mixture consisting of 25 μl of a 2× buffer (20 mM Tris, pH 8.8/100 mM KCl/10 mM $MgCl_2$), 4 μl of the 60-mer primer (7), 0.5 μl of nuclease-free bovine serum albumin (10 mg/ml, Sigma), 0.25 μl of Triton X-100, 5 μl of water, and 2 μl of d(GCT) deoxynucleotide adjusted at 25 mM each. Four micrograms of gene32 protein (Pharmacia) and 5 units of *Taq* polymerase (GIBCO/BRL) were added to each tube and the cDNA was amplified using an Ericomp thermal cycler (Ericomp, San Diego) using the following parameters: 94°C for 1 min; 55°C for 2 min except for the first cycle, which was performed at 37°C; and 72°C for 10 min for 40 cycles.

PCR Amplification of Hox Homeodomains. The amplified total cDNA derived from each different bone marrow subpopulation or cell line was subjected to a second round of PCR amplification using degenerate primer sets designed to match all of the different sequences represented at the conserved α-1 helix [5′-(TC)(TCGA)GA(AG)(TC)T(CG)-GA(AG)AA(AG)GA-(AG)TT-3′] and the α-3 helix (5′-C (GT)(ACGT)C(GT)(AG)TTCTG(AG)AACCA(ACGT)A-3′] regions of the human Hox gene homeodomains (8). One percent of the total cDNA (0.5 μl) of each subpopulation was amplified and the resulting 118-bp products were subcloned and sequenced. Control preparations from original RNA not reverse transcribed failed to yield a band indicating that contamination by genomic DNA was not encountered.

Southern Blot Analysis of Total Amplified cDNA. One-fifth of the total amplified cDNA prepared from each purified subpopulation or from cell lines was analyzed by standard Southern blot procedures. Homeodomain-free probes for all Hox genes but HoxB3 and HoxB4 were prepared from subcloned sequences of cDNA or genomic clones initially kindly provided by E. Boncinelli. Probes for HoxB3, HoxB4, CD71, and MDR1 (multidrug resistance 1 or P-glycoprotein) were derived from clones obtained from the American Type Culture Collection. Full-length HB24 cDNA and CD34 cDNA sequences were kindly provided by J. Kehrl and B. Seed, respectively. Densitometric analysis was performed by using two different autoradiogram exposure times for each blot to ensure linearity of the results.

RESULTS

Characterization of Purified CD34$^+$ Bone Marrow Cell Subpopulations. Antibodies directed against CD34, CD45RA, and CD71 were used to isolate by fluorescence-activated cell sorting (FACS) three phenotypically and functionally distinct subpopulations (I, IIM, and IIIE) from the low-density fraction of three different normal human bone marrow samples (CAD3, CAD6, and CAD9). Fig. 1 *A* and *C* show the FACS profiles of two of these three marrows: CAD3 and CAD6 (CAD9 profiles were virtually superimposable to CAD6; not shown). In each case, subpopulation I was further subdivided into two (CAD3, Fig. 1*B*) or three (CAD6, Fig. 1*D*; CAD9, data not shown) subpopulations by sorting the cells based on their surface expression of the CD38 antigen. After the first round of sorting, each of these subpopulations was analyzed and found to be >98% pure. For CAD3 and CAD6, functional assays were performed on aliquots from each of the purified subpopulations (Table 1). For both marrows, LTC-IC were detected exclusively in subpopulation I, and subdivision of these cells according to their expression of CD38 revealed further segregation of the LTC-IC to the CD38$^{low\text{-}med}$ cells. In contrast, cells of subpopulation I capable of proliferating in semisolid assays in response to a potent cocktail of soluble growth factors (Steel factor plus G-CSF plus GM-CSF plus IL-3 plus IL-6) were more concentrated in the CD38$^{med\text{-}high}$ fraction. As a result, for both marrows it was possible to demonstrate that in the CD38^{low} fractions, LTC-IC constituted the major class of detectable hematopoietic cells, and at least 50% (CAD3; population I.1) and up to 98% (CAD6; population IA) of these could not be detected as clonogenic

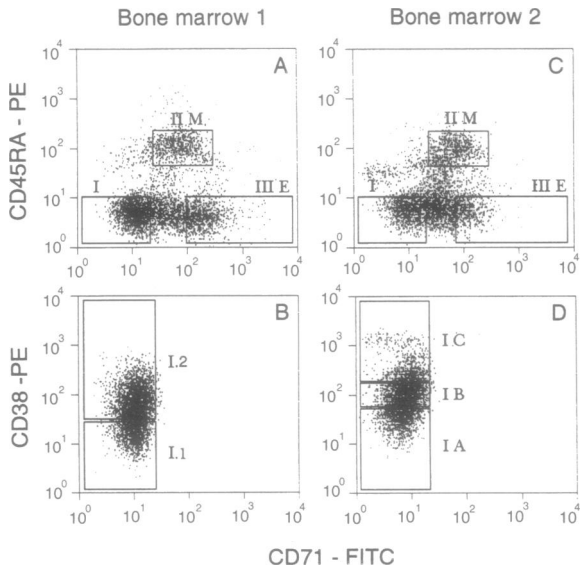

FIG. 1. FACS profiles of the CD34$^+$ subpopulations isolated from bone marrows no. 1 (CAD3) and no. 2 (CAD6). For both marrows, the cells in subpopulation I (CD34$^+$, CD45RA$^-$, and CD71$^-$) shown in *A* and *C* were further fractionated according to their expression of CD38 into two fractions for marrow no. 1 (I.1 and I.2) (*B*) and into three fractions for marrow no. 2 (IA, IB, and IC) (*D*). Each of these subpopulations represents between 0.4% and 3% of the low-density bone marrow cells and are >99% pure. FITC, fluorescein isothiocyanate; PE, phycoerythrin.

Table 1. Clonogenic progenitor* and LTC-IC† content of the different purified CD34+ bone marrow subpopulations

	CFU-E		BFU-E		CFU-GM		CFU-GEMM		LTC-IC	
Fraction sorted	% purity	Enrichment	% purity	Enrichment	% purity	Enrichment	% purity	Enrichment	% purity	Enrichment
CAD3										
I.1	0	0	0	0	2.5	2	0	0	4.3	>2100
I.2	0	0	8.4	9	26	24	5.1	113	5.3	>2600
IIM	0	0	0	0	26	24	0	0	0	<20
IIIE	4.3	13	13	14	6.5	6	5.6	124	0	<20
Total marrow‡	0.3	1	0.95	1	1.1	1	0.05	1	<0.002§	1
CAD6										
IA	0	0	0	0	0.5	1	0	0	29	1450
IB	0	0	1.5	3	5.8	8	0.5	167	29	1450
IC	0.8	1	4	7	17	24	0.5	167	0	0
IIM	0	0	0	0	24	34	0	0	0	0
IIIE	14	18	22	37	2.8	4	0	0	0	0
Total marrow	0.8	1	0.6	1	0.7	1	0.003	1	0.02	1

*Based on analysis of 400 cells.
†Based on analysis of 2400 and 1000 cells for marrows no. 1 and no. 2, respectively.
‡This part of the experiment was performed on a different aliquot of the same marrow thawed and assayed at a later time.
§Based on the analysis of 6.6×10^4 cells.

cells in a 4-week direct assay. Those few clonogenic cells that were identified as copurifying with the LTC-IC in the $CD38^{low}$ fraction, although classified as CFU-GM, all showed delayed initiation and generated very large colonies of cells of a morphology *in situ* (i.e., without staining) of granulocytes and macrophages. Subpopulations IIM and IIIE were highly and differentially enriched in granulopoietic and erythroid clonogenic cells, respectively, in each case to a purity of 20% with ≤5% of clonogenic cells of the opposite lineage still present.

Hox Gene Expression in CD34+ Subpopulations. To assess Hox gene expression in purified bone marrow cell subpopulations, we generated amplified total cDNA from 1000–5000 cell aliquots using a modified RT-PCR procedure. Amplified total cDNAs from the purified CD34+ subpopulations were analyzed for Hox gene expression by using two different approaches. The first of these was aimed at obtaining an initial indication of the range and potential pattern of Hox gene expression in the CD34+ purified subpopulations. It was performed on one marrow (CAD3) and consisted of reamplifying the cDNA derived from each subpopulation with a set of Hox gene-specific degenerate primers that spanned a 118-bp region of the homeodomain. Resulting products were subcloned and a total of 92 clones were sequenced. As summarized in Table 2, this analysis revealed the expression of 12 different Hox genes in the various CD34+ subpopulations: 7 from cluster A, 3 from cluster B, and 2 from cluster C. No expression of any cluster D genes was detected in these cells. In all, 82 of the 92 clones sequenced were found to belong to cluster A. This difference probably reflects a higher expression of cluster A genes in these cells since, as reinforced by the analysis of the cell line data described below, it is unlikely that the degenerate primers used preferentially amplified this particular group of Hox genes. Interestingly, most of the cluster A sequences detected (34 of 46; 76%) in the most primitive subpopulations (I.1 and I.2) were found to be within the 3′ region (A1–A6) of this cluster, whereas 27 of the 38 (71%) cluster A sequences found in the more differentiated subpopulations (IIM and IIIE) were concentrated at the 5′ region of this cluster (A7–A13). Also, of the 8 sequences belonging to cluster B, 6 were limited to subpopulation I.1 and I.2, suggesting a more restricted expression of this Hox cluster to the most primitive hematopoietic cells.

For comparison, and to assess both the sensitivity and specificity of this approach, a similar analysis was carried out using equivalent numbers of cells from the K562 (erythroleukemic) and HL-60 (promyelocytic) cell lines. Homeodomain sequences were obtained from 27 clones derived from K562 cells and 22 clones derived from HL-60 cells. As also shown in Table 2, five of the seven expressed Hox genes detected in HL-60 were Hox A genes, while six of the eight genes detected in K562 were Hox B genes. These results are in agreement with previous reports of Hox gene expression in these two cell lines (9). Moreover, they are consistent with

Table 2. Expression of Hox genes in purified bone marrow subpopulations and in hemopoietic cell lines determined by sequence analyses of amplified homeobox containing cDNA

	Purified subpopulation† (CAD3)				Cell line	
Hox gene*	I.1	I.2	IIM	IIIE	HL-60	K562
A1					1	2
A2		1				
A3						
A4	3	6	1			
A5	5	11	7	2	5	
A6	4	3		1	1	
A7			6	5	8	
A9	5	6	8	4		
A10			1	3	2	
A11						
A13						
B1						
B2						2
B3	1					3
B4						
B5						2
B6						
B7		4	1	1		2
B8						3
B9		1				3
C4–6						
C8				1	1	
C9	1				4	12
C10–13						
D1–13						
Total	19	32	24	17	22	27

The total number of independent clones identified for a specific gene is shown. One or more cDNA clones containing Hox gene-specific homeobox sequence are indicated.
*For each cluster, the genes are listed as found 3′ → 5′ on their respective chromosome.
†Functional characteristics of each of these subpopulations are presented in Table 1.

previous observations of preferential expression of Hox A genes in myeloid cell leukemias and Hox B genes in leukemic cell lines with erythroid phenotypes (1).

cDNA Southern Blot Analysis of Hox Gene Expression in CD34$^+$ Subpopulations. To characterize further the extent and possible differential expression of Hox genes in early hematopoietic cells, the initial amplified total cDNA prepared from the various CD34$^+$ subpopulations was also analyzed by Southern blot using homeodomain-free Hox gene-specific probes. For marrow CAD6, the five CD34$^+$ subpopulations were analyzed using 11 different Hox gene probes (Fig. 2*A*): 7 of the 9 known cluster B genes (B2, B3, B4, B5, B6, B8, and B9), 4 of the 11 cluster A genes (A4, A5, A9, and A10), and 1 noncluster homeodomain-containing gene (HB24: Fig. 2*B*) whose expression was recently described as restricted to CD34$^+$ cells (10). Expression of 10 of these 11 Hox genes was detected in all subpopulations with the exception of B6, which was not detected in any of the primitive subpopulations or in HL-60 or K562 cells (Fig. 2*A*). On the other hand, HB24 appeared to be equally expressed in all subpopulations paralleling that of actin (Fig. 2*B*). Densitometric analysis of these blots showed that HoxB9, which is located toward the 5′ end of the B cluster, was expressed at similar levels in all CD34$^+$ subpopulations, whereas B3 and B4, which are more 3′ cluster B genes, were expressed at much higher levels (17- and 6-fold, respectively) in subpopulation IA, which is highly enriched in LTC-IC, than in IIM, which is enriched in myeloid progenitors and devoid of LTC-IC. This differential expression of 3′ cluster B genes was similarly observed when subpopulation IA was compared with the erythroid cell-enriched subpopulation IIIE. These results suggest that the 3′ genes are preferentially expressed in the most primitive hemopoietic cells in contrast to more 5′ genes, which appear to be more uniformly expressed at least in the early stages of differentiation. This finding was reinforced by the findings for Hox genes located in the middle of the B cluster (B5 and B7) whose expression was only slightly more restricted to the more primitive subpopulations IA and IB.

This pattern also extended to the cluster A genes. As shown in Fig. 2*A Right,* expression of the 3′ gene HoxA4 was 7-fold higher in subpopulation IA than in IIM, and a relatively uniform expression for the 5′ genes A9 and A10 was found. Again, for a gene from the middle of the cluster, HoxA5, expression was moderately elevated in subpopulation IA (3-fold higher than in subpopulation IIM). Moreover, the relative expression of the six paralogs analyzed (i.e., A4 and B4, A5 and B5, and A9 and B9) was strikingly similar in each of the five subpopulations, suggesting that, as in embryonic development, a simultaneous expression of the Hox paralogs may occur during hematopoietic cell maturation.

To examine the reproducibility of this apparent trend, Southern blot analyses were similarly carried out on the four subpopulations of CAD3 and the five subpopulations of a third marrow (CAD9) fractionated exactly as for CAD6 (Fig. 1). Two selected Hox probes (one 3′, HoxB3; and one 5′, HoxA10) were used for this analysis (data not shown). Densitometric analysis of Hox gene expression normalized to actin revealed again that HoxB3 expression was mainly limited to the most primitive subpopulations (15- and 28-fold higher in the CD34$^+$ CD38$^-$ cells of subpopulation IA than in subpopulation IIM, respectively, for CAD9 and CAD3) and that HoxA10 expression was invariant among all of the subpopulations.

The significance of these patterns of Hox gene expression in the various CD34$^+$ populations analyzed was reinforced by several controls. First, the level of CD34 expression in these subpopulations as detected by cDNA Southern analysis was found to correlate with the surface expression of this antigen as studied by FACS analysis with a progressive decline: IA

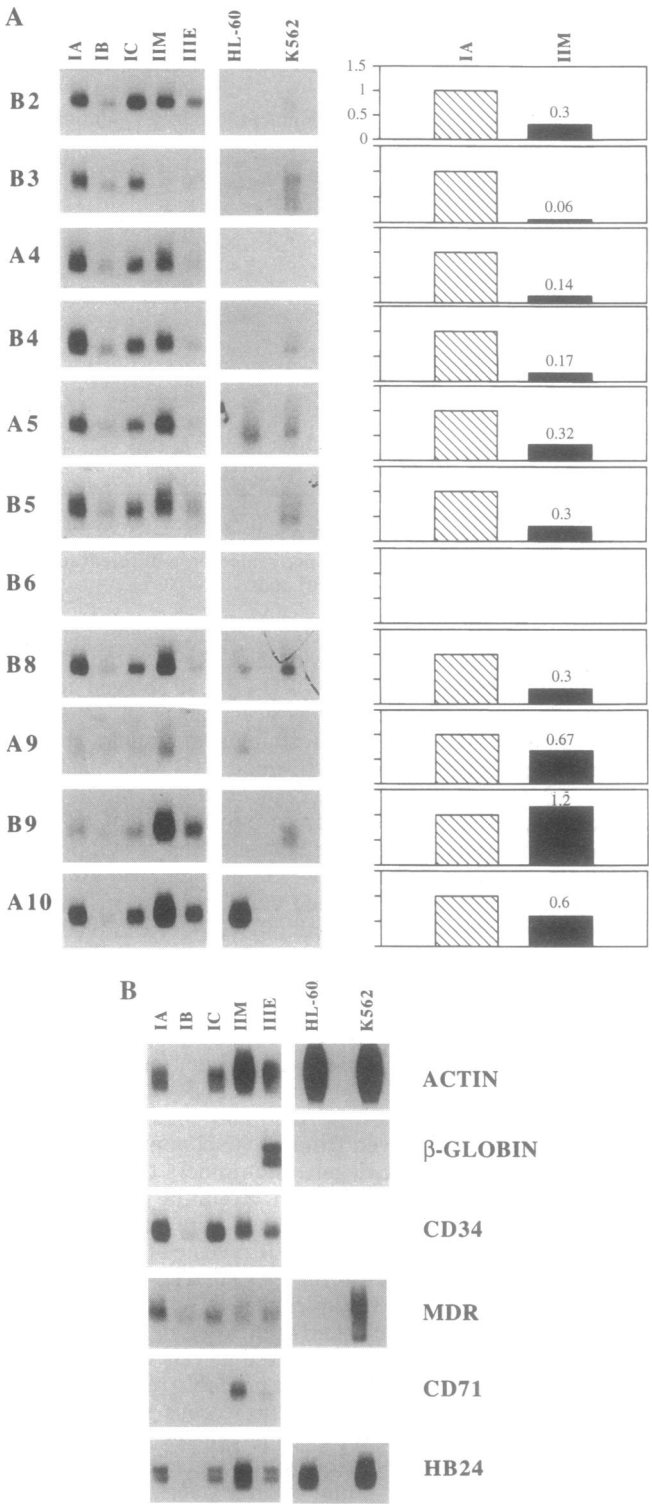

FIG. 2. (*A*) Southern blot analysis of the total amplified cDNA derived from each CD34$^+$ subpopulation from marrow no. 2 (CAD6) and from HL-60 and K-562 cells. Exposure times were adjusted to facilitate comparison of the expression of individual Hox genes between the five subpopulations studied. For HoxA4, hybridization to HL-60 or K-562 was not done. Vertical alignment of the blots follows the relative positions 3′ → 5′ of each Hox gene in its cluster. Each blot is accompanied by a display (*Right*) that shows the normalized expression (to actin and to subpopulation IA) of the different Hox genes found in subpopulations IA and IIM as determined by densitometric analysis. (*B*) The same membrane was also hybridized to probes for actin, CD34, β-globin, P-glycoprotein (MDR1), CD71, and HB24.

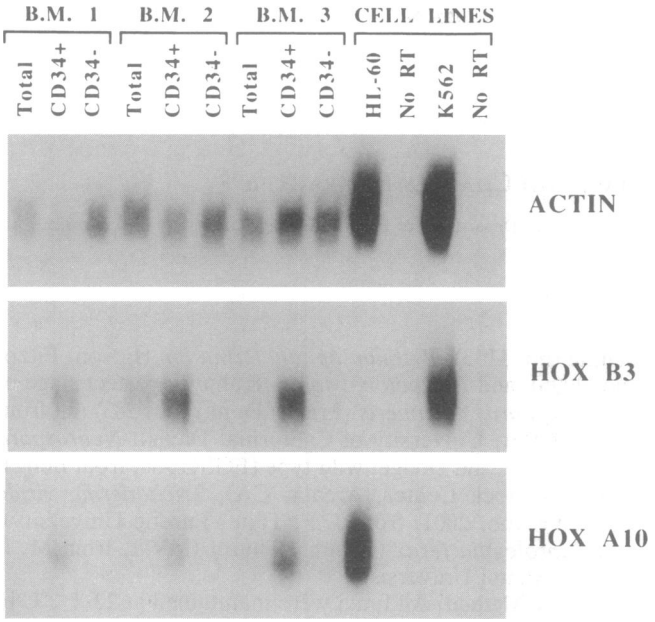

FIG. 3. Southern blot analysis of total amplified cDNA derived from the low-density cells fraction of three different marrows. Cells were sorted in unfractionated, CD34-enriched, and CD34-depleted subpopulations. $CD34^+$ content of the unfractionated, $CD34^+$ and $CD34^-$ fractions were 5.5%, 95.8%, and 0.4% for marrow no. 1 (CAD7), 16.1%, 95.2%, and 1.8% for marrow no. 2 (CAD9), and 7.3%, 95.5%, and 0.7% for marrow no. 3 (CAD10). Viability was >95% in each fraction. No RT, no reverse transcriptase.

> IB > IC > IIM = IIIE (see Fig. 2*B* for cDNA Southern results). MDR1 expression was also higher in the more primitive cell subpopulations, was not found in HL-60, and was present at high level in K562 consistent with the expected expression pattern for this gene (11). Similarly, CD71 expression determined by cDNA analysis correlated with its surface expression (i.e., more expressed in the IIM and IIIE subfractions, Fig. 2*B*), and only the subpopulation enriched for late erythroid progenitors (CFU-E, subpopulation IIIE) was found to express significant levels of β-globin mRNA.

cDNA Southern Analysis of Hox Gene Expression in $CD34^-$ Cells. To explore the possibility of additional changes in Hox gene expression with further hematopoietic differentiation, expression of two selected Hox genes, HoxB3 and HoxA10, was analyzed in the $CD34^-$ fraction of mononuclear cells from five different marrows compared to the total or $CD34^+$ fractions. Results of Southern blot analysis of total amplified cDNA generated from the various fractions of three representative marrows are shown in Fig. 3 (CAD7, CAD9, and CAD10). As expected from the analysis of $CD34^+$ subpopulations, expression of both HoxB3 and HoxA10 was readily detected in the total $CD34^+$ mononuclear cell fraction of the three bone marrows. In sharp contrast, however, expression of both genes was virtually extinguished in the $CD34^-$ fraction. Consistent with these observations, the signal intensity from total mononuclear cells was lower in proportion to the percentage of $CD34^+$ cells.

DISCUSSION

These studies document expression of at least 16 different Hox genes in highly purified subpopulations of primitive $CD34^+$ human marrow cells. Moreover, these data suggest that the lineage negative subfractions of these primitive cells are characterized by markedly higher levels of expression of certain Hox genes in comparison to their levels in lineage positive subfractions. Detailed functional characterization of these cell subpopulations indicates that the preferential expression is associated with a very primitive $CD34^+$ subpopulation containing all LTC-IC and relatively few lineage-restricted progenitors detectable in direct colony assays. This enhanced expression appears to involve genes located in the 3′ regions of the A and B clusters. On the other hand, 5′ located genes were expressed at relatively equal levels in each of the primitive $CD34^+$ subpopulations analyzed. Our results suggest that early differentiation events involve or are accompanied by a down-regulation (potentially 3′ → 5′) of class I Hox genes. For some, such as HoxB3, this down-regulation appears to coincide with the earliest stages of hematopoietic differentiation, whereas for others, such as HoxA10, expression may persist to later stages of development. Interestingly, however, for at least two Hox genes examined here, expression was virtually extinguished with further progression to the $CD34^-$ stage.

By using various cell lines as prototypes of cells at different stages of hemopoietic maturation, it has been suggested that expression of some Hox genes may be lineage-restricted (1, 9, 12). For example, HoxB3 has been described as erythroid-specific because it is expressed in both OCIM2 and K562 cells, and both of these cell lines exhibit some erythroid features (9, 12). Our results failed to demonstrate preferential expression of HoxB3 in normal human erythroid progenitors (subpopulation IIIE). In fact, this gene was expressed at about 20-fold higher levels in more primitive subpopulations in which no CFU-E or BFU-E was detectable by comparison to the only subpopulation in which these erythroid progenitors were found. Another Hox gene, HoxA10, has been similarly described as myeloid-restricted (9, 13). However, in the present study, we found this gene to be equally expressed in all of the $CD34^+$ subpopulations.

Taken together, our results point to the existence of a highly regulated program of Hox gene expression at the earliest stages of hemopoietic cell differentiation. Such findings set the stage for future identification of mechanisms regulating hematopoietic cell proliferation and lineage determination.

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