Expression of Selected Human HOX-2 Genes in B/T Acute Lymphoid Leukemia and Interleukin-2/Interleukin-1 β-Stimulated Natural Killer Lymphocytes

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Although the key role of human homeobox (HOX) genes in development is well established, their function in adult cells is still under scrutiny. We have analyzed, in normal adult blood cell subpopulations, acute lymphoid leukemia (ALL) cell lines, and primary blasts, the RNA expression of all HOX-2 cluster genes (5' - 2.5, 2.4, 2.3, 2.2, 2.1, 2.6, 2.7, 2.8, 2.9, 3') and nine genes in the HOX-1, -3, and -4 cluster by Northern blotting, RNase protection, and/or reverse transcriptase polymerase chain reaction (RT-PCR). The analyzed HOX-1, -3, and -4 genes were never expressed in all tested cell populations. Natural killer (NK) cells activated in interleukin-2 (IL-2)/IL-1ß-treated cultures exhibit a gradually increasing, expressed in quiescent lymphocytes (NK, B and T [T-cell receptor (TCR) α/β, γ/δ lymphocytes, thymocytes] cells), granulocytes, and monocytes. In B- and T-ALL cell lines, HOX-2 genes are expressed according to different patterns: (1) widespread transcription (seven of nine genes, including 2.3 and 2.6) in the Peer line bearing the TCR γ/δ; (2) expression of 2.5, 2.2, and 2.6 in the SEZ 627 line, which derives from an HTLV-1 T-helper leukemia; (3) transcription of 2.3 and 2.6 in both the T-ALL CEM line and four B-ALL lines (interestingly, CALLA- B-ALL lines are constantly 2.3/2.6 RNA+); (4) no HOX-2 gene expression was detected in one T- and two B-ALL lines. Primary blasts from five T- and five pre-B-ALL showed selective expression of one or more HOX-2 genes, namely 2.5, 2.2, 2.6, and 2.7. Our data are compatible with the hypothesis that selected HOX-2 genes play a role in the IL-2/IL-1ß-induced activation and/or proliferation of normal NK lymphocytes and possibly in the oncogenic process of some T- and B-ALL.

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MATERIALS AND METHODS

Natural Killer (NK) Cells Purification and Culture

Peripheral blood mononuclear cells (PBMC) were isolated by the standard Ficoll-Hypaque method. One hundred milliliters of heparinized blood was mixed with an equal volume of sterile Iscove’s medium (IMDM, Flow, Irvine, UK). Thirty milliliters of diluted blood layered on 20 mL of Ficoll-Hypaque (Nyegaards, Oslo, Norway) in a sterile plastic tube was centrifuged at 350g for 40 minutes at 20°C. The buffy coat was removed and the cells were washed three times with IMDM followed by centrifugation at 180g for 10 minutes.

PBMC were partially depleted of adherent cells (essentially monocytes) by two 1-hour incubation cycles in plastic culture flasks (Becton-Dickinson, Mountain View, CA). Cells were then sequentially depleted by a four-step procedure to sequentially remove CD14+ (residual monocytes), CD19+ (B lymphocytes), CD3+ (T lymphocytes), CD4+ (T-helper lymphocytes), and CD8+ (T-...
suppressor/cytotoxic lymphocytes) cells with magnetic microbeads (Dynal, Oslo, Norway) conjugated with CD14, CD19, CD3, CD4, and CD8 monoclonal antibodies (MoAbs) (Dynal), respectively. Thus, 200 × 10^6 nonadherent lymphocytes were mixed with 100 × 10^6 CD14+, 100 × 10^6 CD19+, 300 × 10^6 CD3+, 300 × 10^6 CD4+, and 200 × 10^6 CD8+ microbeads and incubated for 60 minutes at 4°C with each microbead-conjugated MoAb. Cells interacting with microbeads were removed by passage under a magnetic field. Residual cells were again incubated with magnetic beads as described above.

NK lymphocytes were grown in IMDM containing 5% heat-inactivated human AB serum, 10^6 U/mL recombinant human (rh) IL-2 (Roche, Nutley, NJ), and 25 ng/mL rhIL-1β (Selvaco, Siena, Italy).

**Purification of Other Blood Cell Populations**

Briefly, T cells, as well as their α/β and γ/δ subpopulations, were purified by incubation with standard, specific MoAbs and magnetic microbeads. Thymocytes (the thymus specimen was obtained from a 3-year-old boy admitted to cardiac surgery [fully informed consent had been obtained from the parents]) were essentially isolated by a standard Ficoll-Hypaque gradient. Granulocytes were purified by sedimentation of peripheral blood cells on Plasmagel (Pharmacia Fine Chemicals, Uppsala, Sweden). Monocytes were obtained by standard plastic adherence procedures.

**Primary Blasts From ALL**

Samples of peripheral blood of ALL patients were obtained after informed consent. Leukemic blasts were isolated by centrifugation on a gradient of Ficoll-Hypaque under the above conditions. The cushion of white blood cells, largely composed by leukemic blasts, was recovered and washed three times with RPMI-1640 medium. The cell pellet was stored in liquid nitrogen until analyzed. Primary blasts were classified according to French-American-British (FAB) criteria and standard membrane immunologic markers. According to these criteria, ALL pertains to the T-cell lineage and five are pre-B CALLA+ type.34

**Cell lines.** The expression of HOX genes was investigated in several human leukemic lines pertaining to different hematopoietic lineages: T-cell leukemia (PEER, SEZ 627, CEM-CCRF, and TALL-1); B-cell leukemia (8392, SB-CCRF, GM1500, ALL202, ALL1, ALL2B, and Daudi); erythroleukemia (K562); myeloblastic (KG1); promyelocytic (HL-60, AML-193, and MV4-11); monocytic (U-937 and AML-1).

Cell lines were grown under standard conditions in RPMI 1640 medium containing 10% fetal calf serum (FCS), with the exception of AML-193 cells that were grown in IMDM containing 10% FCS and 10 ng/mL rh granulomonocytic colony-stimulating factor (rhGM-CSF).

All cell lines were negative for mycoplasma. No lymphoid cell line was infected by lymphocyte-toxic viruses, with the exception of the SEZ 627 cell line that contains the HTLV-1 genome integrated into cellular DNA.35

**Analysis of Differentiation Markers in ALL/Lymphoma Cell Lines**

Immunofluorescence experiments were performed using the following MoAbs: anti-HLA ABC (Dakopatt, Copenhagen, Denmark), anti-CALLA, -IgM, -IgD, -α, and -λ chains (Technogenetics, Turin, Italy), anti-CD2, -CD3, -CD4, -CD7, -CD8, -CD19, -CD20, -CD22, -CR1, -CR2. HLA-DP, -β-2m, -α-chain (Becton-Dickinson), anti-IL-2R β-chain (TU-207) (courtesy of Dr Kazuo Sugamura, Department of Microbiology, Tohoku University, Sendai, Japan). Briefly, cells were washed three times at 4°C with Hanks’ balanced saline solution and incubated (5 × 10^6) for 60 minutes at 4°C in the presence of an appropriate dilution of MoAb. After three washes with Hanks’ solution, the cells were incubated 60 minutes at 4°C in the presence of fluorescein isothiocyanate (FITC)-conjugated F(ab')2 sheep antimouse affinity-purified Ig's (Technogenetics, Turin, Italy). After three additional washes in cold Hanks’ solution, cells were resuspended in phosphate-buffered saline (PBS) and analyzed using a fluorescence-activated cell sorter (FACScan, Becton-Dickinson).

**Probes.** The following probes were used for Northern blot analysis:

1. **HOX-2:** 2.5, HaelIII-HaeIII, 300 bp; 2.4, HaeIII-HaeIII, 600 bp; 2.3, HaelIII-HaeIII, 500 bp; 2.2, ApaI-EcoRI, 300 bp; 2.1, PvuII-PvuII, 500 bp; 2.6, HindIII-HindIII, 300 bp; 2.7, EcoRI-Bgl II, 900 bp; 2.8, Smal-Smal, 100 bp (17); 2.9, PvuII-PvuII, 600 bp from the genomic clone BC206; all of these probes are localized 3’ to the HOX region.

2. **HOX-1:** 1.2, TaqI-TaqI, 700 bp containing part of the homeobox and its 3’ region and 1.3, EcoRI-EcoRI, 3.5 kb containing the HOX (kindly provided by E. Boncinelli, International Institute of Genetics and Biophysics, CNR, Naples, Italy), 1.4, XbaI-HindIII, 420 bp 3’ to the HOX (L. Cianetti, unpublished results, March 1988).

3. **HOX-3:** 3.3, 1.8 kb full-length cDNA; 3.4, Smal-Smal, 350 bp (kindly provided by E. Boncinelli); 3.5, RsaI-RsaI, 150 bp.

4. **HOX-4:** 4.5, BamHI-EcoRI, 500 bp 3’ to the HOX, 4.4, EcoRI-EcoRI, 800 bp containing the HOX and its 3’ sequence; 4.2, ApaI-EcoRI, 500 bp containing the first exon.

In the RNAase protection analysis riboprobes synthesized from the following fragments were used: 2.2, 128 Smal-XhoI, 40 bp, 198 Smal-PstI, 26, 121 bp ApaI-ApaI (our unpublished results), 2.3, 140 bp Smal-TaqI, 42, 190 bp AanalHinII all from the region 3’ to the HOX; human β-actin, 93 bp RsaI from the 3’ region of the cDNA (our unpublished results).

**RNA Analysis by Northern Blotting, RNAse Protection, and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)**

Total RNA was extracted from cells by the standard guanidinium thiocyanate technique.

**Northern blotting.** Poly(A)+ was selected by one passage on oligo (dT)-cellulose columns.41 Four micrograms of poly(A)+ eluted from 200 μg of total RNA was run on 1.0% agarose-formaldehyde gels, transferred to nylon membranes (Hybond N; Amersham, Buckinghamshire, England) by capillary blotting, and hybridized to 10^6 cpm DNA probe labeled by multiprimed random priming to a specific activity of 3 × 10^9 dpm/μg.

Prehybridization and hybridization were performed as previously described.19 After stringent washing (15 mmol/L NaCl/1.5 mmol/L sodium citrate/0.1% NaDodSO4 at 65°C) the blots were exposed for 3 to 7 days at ~70°C to Kodak X-Omat AR film (Eastman Kodak, Rochester, NY) in an X-omatic intensifying...
screen box. Probes were then removed by a solution consisting of 0.005 mol/L Tris-HCl pH 8.0; 0.002 mol/L Na₂ EDTA; Ficoll 0.002%, polyvinyl pyrrolidone 0.002%, bovine serum albumin 0.002%, at 65°C for 1 hour and the filters rehybridized. β-Actin probe was used for normalization.

**RNAse protection.** RNA probes were synthesized to high specificity from DNA fragments cloned in the pGEM4Z vector (Promega, Madison, WI) to generate 32P-labeled antisense RNAs according to standard protocols (Promega). In each experiment, 20 μg of total RNA was hybridized to 2×10⁶ cpm of each probe. Hybridization and RNAse digestion were performed according to standard procedures. Samples were run on an 8 mol/L urea/6% acrylamide gel. Gels were dried and exposed for 1 to 10 days to Kodak X-OMAT AR film in an X-omatic intensifying screen box. Probes were then removed by a solution consisting of 5×SSC/0.1% SDS.

Hybridization and RNAse digestion were performed according to standard procedures. “In each experiment, 20 pg of total RNA was hybridized to 2×10⁶ cpm of each probe.”

**RT-PCR.** Twenty micrograms of total RNA was reverse-transcribed into cDNA (MMLV-RT; BRL, Gaithersburg, MD) using 250 ng of oligo-dT as primer. An aliquot of each RT reaction was incubated in the presence of α³²P-dCTP tracer and the reverse transcribed RNA/DNA was normalized accordingly. One microliter of reverse-transcribed mRNA was amplified for each sample. The amplification procedure involved denaturation at 95°C for 1 minute, annealing for 1.30 minutes at 58°C for 2.3, 2.2, 2.8, 4.2, and 1.4, at 54°C for 2.5, 2.4, 2.1, 2.7, 3.3, and extension at 72°C for 1.30 minutes during 30 PCR cycles, ie, within the range of linear amplification. The following 5’, 3’ primers and probes were used: (1) HOX-2 5’ primer 5’GAGACGGGCACAGTAA3’, 3’ primer 5’CTTCTCTGACAGACTTG3’, probe 5’ATCTCAGCTATGATGACGG3’; (2) 2.4, 5’ primer 5’GCCTCCITGTGCT3’, 3’ primer 5’GTAACATTTGGCCACGG3’, probe 5’GAACGTGCTTTTCTGTAATGACCAAGGTACCGATTTC-3’ (our unpublished results); (3) 2.3, 5’ primer from 809 bp to 828 bp, 3’ primer from 1,235 bp to 1,254 bp, probe from 1,108 bp to 1,145 bp; (4) 2.2, 5’ primer from 1,816 bp to 1,835 bp, 3’ primer from 2,030 bp to 2,049 bp, probe from 1,983 bp to 2,026 bp; (5) 2.1, 5’ primer from 174 bp to 191 bp, 3’ primer from 326 bp to 343 bp, probe from 196 bp to 232 bp; (6) 2.7, 5’ primer from 1,184 bp to 1,201 bp, 3’ primer from 1,474 bp to 1,491 bp, probe from 1,258 bp to 1,296 bp; (7) 2.8, 5’ primer from 731 bp to 750 bp, 3’ primer from 1,121 bp to 1,139 bp, probe from 993 bp to 1,037 bp; (8) 1.4, 5’ primer 5’AGATGGCATCTCTAAATCCGG3’, 3’ primer 5’CATGGTTAAAGATGTGGTCG3’, probe 5’ACCCGAGCACCTCCACGG3’; (9) 3.3, 3’ primer from 841 bp to 858 bp, 3’ primer from 1,002 bp to 1,019 bp, probe from 872 bp to 909 bp; (10) 4.2, 5’ primer from 2,873 bp to 2,890 bp, 3’ primer from 3,157 bp to 3,174 bp, cDNA probe 187 bp fragment Apal-HindIII; (11) β₂-microglobulin, 5’ primer 5’AAACACGTCATTCTTTCACACGG3’, 3’ primer 5’CTGCTCATCACATACACATTG3’, probe from 311 bp to 360 bp.

**PCR was performed in a total volume of 100 μL; 10 μL of each sample was separated in a 2% agarose gel and transferred to a nylon filter. Filters were hybridized with a probe end-labeled with γ³²P-ATP and polynucleotide kinase. The same negative samples were semi-quantified by densitometer scanning with a laser densitometer (LKB) after normalization with β₂-microglobulin. As mentioned above, an additional control was performed by normalization of the amount of the reverse-transcribed RNA/DNA.**

**DNA Analysis**

High molecular weight DNA was extracted from 10⁷ cells, digested with restriction endonucleases, electrophoresed in 1% agarose gels, transferred onto nylon filters, and hybridized to 2×10⁶ cpm of radiolabeled probe as described.⁴⁴

**RESULTS**

We analyzed 10 ALL (4 pertaining to the T- and 6 to the B-cell lineage), 1 B lymphoma, and 7 AML cell lines (1 erythroid and 6 myelo-monocytic), as well as the primary blasts from 5 T- and 5 pre-B-ALL.³⁴

**HOX** gene expression was also investigated in purified populations of normal peripheral blood cells (granulocytes, monocytes, T, B, and NK lymphocytes) and thymocytes, as well as in IL-2/IL-1β-stimulated NK cells.

**Expression of HOX Genes in ALL Cell Lines**

Genes pertaining to the **HOX**-2 cluster are widely expressed in human ALL cell lines as evaluated by Northern blotting of poly(A)⁺ RNA (Table 1). Four patterns were observed: (1) transcription of seven **HOX**-2 genes in the Peer T-ALL line; (2) transcription of 2.5, 2.2, 2.6 in the SEZ 627 T-ALL line; (3) selective activation of both 2.3 and 2.6 in four B-ALL and the CEM T-ALL line; (4) no expression of **HOX**-2 genes in two B- and one T-ALL line. Negative results were confirmed by RT-PCR analysis.

In Peer cells (Fig 1A), the size of **HOX**-2 transcripts is comparable to that observed in normal human embryos,¹⁷ with the exception of 2.2, which shows a 1.3-kb band in addition to the 1.6-kb mRNA, probably representing the alternative transcript previously reported⁴⁰ and 2.8 expressing two distinct transcripts of 1.7 and 1.5 kb, respectively. Because Northern blot was performed in a strictly standardized fashion, the level of expression of **HOX**-2 genes may be evaluated on a roughly quantitative basis. In this regard, we noted that the 2.5 and 2.3 mRNA is particularly abundant.

Figure 1B shows the Northern blot of **HOX**-2 RNAs expressed in SEZ 627 cells. The size of the 2.6 mRNA is similar to that described in human embryos.¹⁷ The faint 2.5 transcripts comprise both the 3.4-kb mRNA and an undescribed smaller band; similarly, 2.2 is transcribed in two RNA species, one of which is smaller than the 1.6-kb mRNA, as also observed in Peer cells.

<table>
<thead>
<tr>
<th>HOX-2 Locus Gene Expression in ALL Cell Lines</th>
<th>T-ALL</th>
<th>PEER</th>
<th>SEZ627</th>
<th>CEM-CCR</th>
<th>TALL-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2.2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2.3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

Results were confirmed in at least two separate experiments. Northern blot analysis has been performed under the same standard conditions (see Materials and Methods). Semi-quantitative results are indicated, as compared with the level of expression of 2.6 in the Peer line (considered as +) by densitometer scanning.

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3.4-

A Peer

HOX-2.5
HOX-2.4
HOX-2.3
HOX-2.2
HOX-2.1
HOX-2.6
HOX-2.7
HOX-2.8
HOX-2.9

1.6-

2.1-

1.5-

1.8-

2.3-

1.7-

3.6-

actin-

2.4-

1.6-

1.3-

3.4-

2.3-

1.3-

3.4-

2.3-

1.3-

3.4-

2.3-

1.3-

actin-

Fig 1. Northern blot analysis of 4 μg poly (A)^+ RNA from two T-ALL cell lines: expression of a number of HOX-2 genes in the Peer line (A), and 2.5, 2.2, and 2.6 in the SEZ 627 line (B). Sizes are in kb. β-Actin was used as control.

B SEZ 627

HOX-2.5
HOX-2.2
HOX-2.6

3.4-

2.3-

1.6-

1.3-

actin-

Cytogenetic analysis of both cell lines showed no significant abnormality of chromosome 17 (deletion, translocation or hyperploidy) (data not shown). Furthermore, Southern blotting of the genomic DNA from Peer and SEZ 627 showed a normal restriction map of HOX-2 genes for a variety of restriction enzymes (BamHI, PvuII, BglII, EcoRI, and HindIII) (results not presented).

Analysis of the membrane phenotype showed that the Peer line pertains to the T-lymphocyte population expressing the γ/δ TCR, and the SEZ 627 line corresponds to a T-helper HTLV-1^+ leukemic line.

Figure 2 shows the constant expression of 2.3 and 2.6 transcripts in the third group of lymphoid cell lines, including one T- and four B-ALL lines: 2.3 and 2.6 transcripts are variably abundant and of the same size as described in human embryos.

A detailed analysis of the membrane phenotype of B-ALL cell lines (Table 2) was performed to explore a possible correlation between their differentiation stage and the expression of 2.3/2.6. Interestingly, three CALLA^- lines exhibit both 2.3 and 2.6 transcripts. On the other hand, CALLA^+ lines are either 2.3/2.6 RNA^- (ALL1, ALL2B) or RNA^+ (ALL202). In this regard, the Daudi B-cell lymphoma line is both CALLA^+ and HOX-2 RNA^- (data not shown).

Experiments were performed to evaluate the expression of HOX-1, -3, and -4 cluster genes in ALL cell lines. No expression of 1.4, 1.3, 1.2 (HOX-1), 3.3, 3.4, 3.5 (HOX-3) or 4.2, 4.4, 4.5 (HOX-4) was detected by poly (A)^+ RNA Northern blot analysis.

Expression of HOX-2 Genes in AML Lines

Seven myeloid leukemic lines (K562, HL60, U937, AML-193, MV4-11, AML-1, and KG1) were screened for the expression of HOX-2 genes by Northern blot poly(A)^+ or RT-PCR analysis. The erythroleukemic K562 line expresses 2.5, 2.3, 2.6, and 2.8, the myeloblastic KG1 line expresses 2.5, 2.3, 2.2, and 2.7, whereas the other five AML lines (promyelocytic, myelomonocytic, or monocytic) do not exhibit HOX-2 transcripts (data not shown).
Expression of HOX-2 Genes in Primary ALL Blasts

We screened five T- and five pre-B-ALL by RT-PCR for HOX-2 genes (Table 3 and Fig 3). Because RT-PCR analysis of 2.6 mRNA was unsatisfactory, possibly due to the presence of inverted repeats in its sequence, the expression of this gene was analyzed by RNase protection (data not shown). Of the five T-ALL, all were positive for 2.5, three for 2.2, and three for 2.7. In regard to pre-B ALL, 3/5 were positive for 2.5, 3/5 for 2.2, 3/4 for 2.6, and 3/5 for 2.7. One, two, or four HOX genes were expressed in each sample. Cluster genes 2.4, 2.3, 2.1, and 2.8 were never expressed in the analyzed leukemias. The expression levels of HOX-2 genes in primary ALL blasts were variable, ranging from 5% to 73% as compared with the levels observed in the Peer line, as evaluated by densitometer scanning of the autoradiographs, after normalization with β2-microglobulin.

Table 3. HOX-2 Locus Gene Expression in T and pre-B Primary Blasts

<table>
<thead>
<tr>
<th>Case No.</th>
<th>HOX-2 Genes</th>
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<tbody>
<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>T-ALL</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>+</td>
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<tr>
<td>4</td>
<td>+</td>
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<tr>
<td>5</td>
<td>+</td>
</tr>
<tr>
<td>Pre B-ALL</td>
<td></td>
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<tr>
<td>1</td>
<td>+</td>
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<td>2</td>
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<td>4</td>
<td></td>
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<td>5</td>
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</tr>
</tbody>
</table>

Samples were analyzed by RT-PCR (with the exception of 2.6 expression, which was analyzed by RNase protection).

Abbreviation: ND, not done.
expression in 24-day activated NK cells was semi-quantified by densitometer scanning of the autoradiographs after normalization with β-actin: 2.6 and 2.8 expression levels are comparable with the Peer line, while 2.2 expression is more abundant. To evaluate if the induction of these genes occurs at earlier times, we have analyzed by RT-PCR the 2.2 and 2.8 expression in 4-day cultures: the RNA level of both genes is very low, as compared with the 9- to 24-day activated NK cells (data not shown).

Finally, RT-PCR analysis indicated a low level of expression of 2.3, 2.1, and 2.7 in 24-day activated NK cells, while no expression was detected in 9-day cultures (Fig 4B).

**DISCUSSION**

Previous studies have analyzed the expression of HOX genes in human AML cell lines pertaining to the erythroid and granulo-monocytic lineage. Indeed, (1) a high proportion (four of six) of B-ALL lines express both 2.3 and 2.6, whereas no HOX-2 gene is transcribed in normal circulating B lymphocytes; (2) three of four T-ALL cell lines express several genes of the HOX-2 cluster (ie, the Peer line expresses all HOX-2 genes except 2.4 and 2.9, the SEZ line only 2.2, 2.5, 2.6, the CEM line both 2.3 and 2.6), whereas quiescent T lymphocytes from adult peripheral blood or thymus did not show transcription of HOX-2 genes. Moreover, 1.4, 1.3, 1.2, (HOX-1), 3.3, 3.4, 3.5, (HOX-3) and 4.2, 4.4, 4.5, (HOX-4) genes were not expressed in any of the above-mentioned lymphoid cell populations, thus suggesting the specificity of HOX-2 gene expression.

Conversely, HOX-2 genes are occasionally expressed in the seven analyzed AML cell lines, ie, they are not transcribed in five AML lines, while the K562 erythroleukemic and KG1 myeloblastic line express 2.5, 2.3, 2.6, 2.8, 2.9 and 2.5, 2.3, 2.2, 2.7, respectively. These results are essentially in agreement with the observation that HOX-2 genes are expressed in erythroleukemic but not in myelomonocytic cell lines.

Membrane phenotype analysis of the lymphoid lines showed no clear relationship between the maturation status of the leukemic cells along the T-cell pathway and the pattern of expression of HOX-2 genes. However, analysis of B-ALL lines indicates that all CALLA− lines (8392, GM 1500, and SB) express 2.3 and 2.6, whereas CALLA+ ones may or not express these two genes. The functional relationship between CALLA and 2.3 and 2.6 expression is obscure because the role of the CALLA/CD10 antigen on human lymphoid cells is still uncertain. In this regard, the recently cloned CALLA gene is identical to the membrane-associated enzyme neutral endopeptidase in the bone marrow microenvironment molecules surrounding CALLA/CD10+ lymphoid precursors may represent substrates for the CD10 antigen. At the clinical level, the CALLA/CD10− phenotype is associated with high white blood cell count, splenomegaly, and shorter disease-free intervals.

The Peer T-cell line, presenting an almost complete activation of the HOX-2 cluster, pertains to the T-cell subset expressing the γ/δ TCR. Recent studies have shown that γ/δ lymphocytes represent a minor subset (1% to 7%) of circulating T lymphocytes and exhibit molecular and functional properties usually displayed by T cells and NK lymphocytes. The pattern of HOX-2 gene expression in the Peer cell line is reminiscent of that displayed by proliferating NK lymphocytes, as discussed below.
Our studies on primary ALL blasts indicate that 2.5, 2.2, 2.6, and 2.7, but not 2.4, 2.3, 2.1, and 2.8 are expressed in T- and pre-B-ALL. The pattern of HOX-2 expression in primary ALL blasts is variable, in that these ALLs exhibit transcription of one, two, three, or four of these genes. Normal cells from adult peripheral blood (ie, TCR α/β or γ/δ T cells, thymocytes, B lymphocytes, CD3− NK cells, granulocytes, and monocytes) do not express any of the HOX-2 genes.

Interestingly, CD3− activated NK cells grown in IL-2/IL-1β-supplemented culture transcribe 2.2, 2.6, and 2.8 at a gradually increasing, abundant level, while 2.3, 2.1, and 2.7 are expressed at low level and late culture times. The transcription of HOX-2 genes in activated NK cells may be linked to their proliferative status and/or acquisition of new functional properties (ie, induction of LAK antitumor cytotoxic activity). The kinetics of HOX-2 gene expression is compatible with a role in NK proliferation control. On the other hand, the LAK activity is monitored before the onset of significant HOX-2 gene expression, thus rendering unlikely a role of HOX-2 genes in this phenomenon.

Our preliminary data further indicate that activated TCR α/β or γ/δ T lymphocytes express a variety of HOX-2 genes (results not shown here). In this regard, 2.3 expression has been linked to the activation of T and B lymphocytes.51,52 Therefore, we suggest that selected HOX-2 genes may play a role in the proliferation of normal NK and T cells.
In conclusion, these studies indicate the specific expression of a variety of HOX-2 genes in a majority of T- and B-ALL cell lines and primary blasts, as well as in activated NK cells. These findings may reflect an important functional role of selected HOX-2 genes in these cell populations, via interaction of the corresponding homeoproteins with target genes. Because HOX genes are considered to play a key role in the early development of a variety of mammalian tissues and body parts, they may be involved in the control of the proliferation/differentiation of restricted populations of adult cells (eg, NK and T lymphocytes). Moreover, growing evidence supports a role for mammalian HOX genes as cofactors in myeloid/lymphoid leukemogenesis; therefore, it is also suggested that the expression of selected HOX-2 genes in B- and T-ALL may represent a significant oncogenic cofactor.

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