Expression of Retinoic Acid Receptor Alpha mRNA in Human Leukemia Cells

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The expression of the newly described human retinoic acid receptor alpha (RARα) in six nonlymphoid and six lymphoid leukemia cell lines and nine freshly obtained samples of leukemia cells from patients with acute nonlymphoid leukemia was assessed by Northern blot analysis, using a full length cDNA clone of RARα as probe. RARα was expressed in all 12 cell lines and in all fresh leukemia samples as two major transcripts of 2.6 and 3.5 kb in size. Levels of RARα expression and transcript sizes in retinoid-sensitive cells (such as HL60 or fresh promyelocytic leukemia cells) were not different from those in other samples. Moreover, expression of RARα was not significantly modulated by exposure to cis-retinoic acid (cisRA) in either cisRA-responsive or unresponsive cells. By using a 3' fragment of the RARα gene as a probe, we confirmed that the transcripts visualized did not represent the homologous RARβ gene. RARα appears to be expressed in most human leukemia cells regardless of the type of biologic response to retinoic acid.

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Materials and Methods

Cell samples. The legends of Figs 1, 3, and 4 outline the leukemic cell lines studied and the morphologic subtypes of the fresh leukemia samples studied according to the French-American-British (FAB) classification.11 All patients involved in this study were advised of the methods and possible risks of marrow aspiration, in accordance with institutional guidelines, and gave informed consent. Heparinized samples of patients' bone marrow or peripheral blood were obtained and light-density mononuclear cells isolated by a standard centrifugation step with Ficoll-Hypaque (Pharmacia, Piscataway, NJ).12

Materials. cisRA was provided by Dr Peter Sorter, Hoffman-LaRoche (Nutley, NJ). The probe for the retinoic acid receptor alpha was a full length cDNA provided by Dr Vincent Giguere, Salk Institute (San Diego). A second probe was prepared by restricting the plasmid pH1K containing the RARα cDNA with PvuII. An 891 bp fragment (bases 1880 to 2771) derived from the 3' untranslated region of the RAR cDNA was recovered following electrophoresis through agarose. This fragment lacks significant homology with the published sequence for RARβ.4

RESULTS

As shown in Fig 1, Northern blot analysis of poly-A enriched RNA from five nonlymphoid leukemia cell lines...
more primitive T cell lymphoblastoid cell lines (lanes 1-3) appear lower than in the three more mature lymphoid lines (lanes 4-6).

Figure 4 shows the Northern blot analysis of total cellular RNA from eight samples of fresh leukemic cells representing a spectrum of morphologic subtypes of ANLL and myelodysplasia including two cases of acute promyelocytic leukemia (APL; FAB-M3). The figure demonstrates some expression, albeit variable, of RARα in each case. In all samples, two major transcripts of 2.6 and 3.5 kb were visualized as well as faint signals for 1.8 and 4.1 kb transcripts, a pattern identical to that seen in the myeloid cell lines. Levels of expression of RARα when normalized to actin were variable between samples (data not shown), and no novel transcript sizes were seen. Six of the fresh leukemic samples were tested for differentiation with cisRA 10⁻⁶ mol/L in vitro. Only one sample of APL cells (patient no. 4—the other sample of APL cells from no. 8 were not tested) demonstrated morphologic differentiation to granulocytes (data not shown). However no modulation of RARα mRNA levels was seen in any of the six samples treated with cisRA. We have studied an additional patient with APL (data not shown) whose cells showed differentiation with cisRA and found no modulation of RARα expression by treatment with the vitamin.

Figure 1. Northern blot analysis of RARα expression in five nonlymphocytic leukemia cell lines. Poly-A enriched RNA was electrophoresed and probed with RARα (upper panel) and actin (lower panel). Lane 1, KG-1; lane 2, HEL; lane 3, K562; lane 4, HL60; lane 5, U937.

Probed with the RARα probe revealed two major transcripts of 2.6 and 3.5 kb size in all five cell lines, representing a wide range of hematopoietic phenotypes (erythroid: K562 and HEL; monocytic: U937; promyelocytic: HL60; and primitive myeloblast: KG-1). Levels of RARα expression, when normalized to β-actin, appeared to be highest in KG-1 cells but were readily evident in all five cell lines, and no variant transcript sizes were observed. Faint bands were also seen with electrophoretic mobilities of 4.1 and 1.8 kb, which correspond to the migration positions of the ribosomal subunits, and probably represent nonspecific hybridization to these major RNA species. We also compared the expression of RARα in KG-1 with that in the subclone KG-1a, which differs from the parent cell line in being resistant to inducers such as TPA ¹⁷ and found that the KG-1a line expressed both major transcripts in somewhat lower levels than KG-1 (data not shown).

Figure 2 demonstrates that induction of differentiation of U937 and HL60 cells by treatment with cisRA 10⁻⁶ mol/L did not result in modulation of RARα mRNA levels. Similarly induction of monocytic differentiation of HL60 cells with phorbol ester and 1,25-dihydroxy-Vitamin D₃ was not associated with changes in RARα expression.

Figure 3 demonstrates that RARα was expressed in all four T cell and both B cell lines tested with transcript sizes identical to that seen in the myeloid cell lines. Levels in the...
Fig 3. Northern blot analysis of RARα expression in lymphoid cell lines. Poly-A enriched RNA was electrophoresed and probed as described in Figs 1 and 2. Lanes 1 through 3: T cell lymphoblastic lines (lane 1; CCRF-CEM; lane 2, MOLT 3; lane 3, MOLT 4); lane 4: T cell hairy cell leukemia line (Mo); lanes 5 and 6: B-cell plasma cell lines (lane 5, Su; lane 6, UCD-PC1); lane 7: HEL; lanes 8 and 9: U937. The two major bands visualized in each lane of the upper panel are 3.5 kb and 2.6 kb in size, an identical pattern to that seen in Figs 1 and 2. The actin bands in the lower panel are 2.2 kb in size.

To exclude the possibility that some of the bands seen on our Northern blots might represent cross hybridization with transcripts from the RARβ gene, the filters used in Fig 1 and 4 were rehybridized with a 3' PvuII fragment of the RARα gene, a region with no significant homology (22%) with RARβ. The pattern of bands visualized with this fragment were identical to that seen with the full length RARα cDNA (data not shown).

DISCUSSION

These data indicate that the RARα gene is uniformly expressed in human leukemia cell lines and fresh leukemic cells from patients regardless of morphologic subtype or biologic response to treatment with cisRA. The major transcript sizes are roughly equivalent to those previously reported for normal tissues. The bands do not represent transcripts of RARβ, which have been previously reported in HL60 and K562 with sizes of 2.5 and 3.0 kb and which share substantial homology (>90%) in the DNA-binding and retinoic acid-binding domains with RARα.

We had previously hypothesized that the heterogenous effects of retinoids might be related to variable presence of the cytoplasmic retinoic acid binding protein (CRABP), described by Chytil and Ong. This protein had been postulated as an intracellular mediator of retinoic acid's effects. In earlier studies, we and others were unable to detect measurable levels of CRABP in any leukemic samples tested, and we concluded that this protein probably did not mediate the actions of cisRA on human leukemic cells.

Since the RARα gene has been reported to be localized on chromosome 17q21.1, it was important to evaluate the expression of RARα in promyelocytic leukemia cells, which uniformly possess a 15:17 translocation with a breakpoint at 17q21-23 or q22-24 and which consistently differentiate to granulocytes when exposed to retinoic acid. However the heterogenous biologic effects of cisRA on human myeloid leukemia cells, and particularly the differentiating effects of retinoids on APL cells, are not apparently related to altered levels of RARα or the presence of abnormal transcripts. These data do not exclude the possibility of a point mutation in the RAR gene, leading to functional alterations in the protein.

The role of RARα in normal hematopoiesis and as a possible oncogene remains to be explored. The expression of RARα may be necessary, although not sufficient, for a maturation response to retinoic acid. The proof of this hypothesis requires further study of variant HL60 cell lines.
which lack RARα and no longer respond to cisRA, or of the effect of anti-sense RARα on retinoid-responsive cell lines. The expression of RARα in inducible leukemic cell lines makes these cells an interesting model for the study of RARα and differentiation.

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REFERENCES

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