Retinoic Acid Receptors in Myeloid Leukemia: Characterization of Receptors in Retinoic Acid-Resistant K-562 Cells

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Although mRNA for the retinoic acid receptor α (RAR-α) is expressed in many different myeloid leukemias, most of these leukemia cells exhibit little if any phenotypic response when exposed to retinoic acid (RA). To determine whether such RA resistance is related to altered RA receptor structure or function, we performed a detailed analysis of nuclear RA receptors in RA-resistant K-562 cells. These cells exhibit RA receptors of the same approximate molecular weight and similar kD as those exhibited by the RA-sensitive HL-60 leukemia cell line, but the number of RA receptors in the RA-resistant K-562 cells (80 per cell) is significantly lower than that exhibited by RA-sensitive HL-60 cells (550 per cell). Retroviral-mediated transduction of RAR-α cDNA into K-562 significantly increased the number of RA receptors to 2,000 per cell. These RAR-α-transduced K-562 cells, when incubated with RA, exhibit diminished cell proliferation associated with decreased c-myc expression and an accumulation of cells in G1/G0. In addition, these RA-treated cells exhibit downregulation of the CD15 surface antigen and a slight increase in hemoglobin production but manifest no other evidence of significant erythroid, megakaryocytic, or myeloid differentiation. These results indicate that an elevated number of nuclear RA receptors can be involved in altering proliferation but not necessarily the differentiation of certain RA-treated myeloid leukemia cells.

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A CUTE MYELOGENOUS leukemia (AML) is a disease in which a block to normal myeloid cell differentiation occurs, resulting in progressive and lethal accumulation of immature myeloid precursors. Attempts to treat AML with certain therapeutic agents capable of relieving this block to normal myeloid differentiation have met with limited success. Retinoic acid (RA) induces terminal granulocytic differentiation of the HL-60 promyelocytic leukemia cell line and is a particularly attractive therapeutic agent for AML because it exhibits minimal marrow and systemic toxicity. Indeed, as a single agent, RA induces complete remissions in certain patients with AML, particularly those with M subtype. Nevertheless, this subset of patients constitutes less than 15% of all AML patients, and most AML patient samples appear to be resistant to RA-induced differentiation. Why some AML cells are sensitive to RA while most others are resistant is not known.

Recent molecular cloning of specific receptors for RA provided considerable insight into the molecular basis for the biologic effects of RA. These RA receptors are members of the steroid/thyroid hormone superfamily of transcription factors and possess discrete DNA-binding and ligand-binding domains. Their structure suggests that RA induces its biologic effects by binding to and activating specific nuclear RA receptors (RARs), leading to binding of the RAR to specific cis-acting DNA sequences that regulate transcription of certain target genes. To date, at least three different RARs have been molecularly cloned, including RAR-α, which is expressed in a wide variety of different hematopoietic cells; RAR-β, which is expressed in a variety of epithelial cell types; and RAR-γ, which is expressed primarily in skin.

We recently isolated an HL-60 variant subclone that, unlike parental HL-60 cells, is resistant to RA-induced differentiation. This RA-resistant variant subclone exhibits nuclear RA receptors with a decreased affinity for RA as well as a decreased molecular weight (mol wt) and fewer receptors per cell as compared with nuclear RA receptors from the RA-sensitive parental HL-60 cells. Retroviral vector-mediated transduction of a single copy of the RA receptor (RAR-α) into these RA-resistant HL-60 cells restores their sensitivity to RA. These observations indicate that RA-induced differentiation of HL-60 cells is mediated directly through the RAR-α. Presumably, RA-induced differentiation of other myeloid leukemia cells is also mediated through RAR-α. Moreover, the defect in RAR-α that we observed in the RA-resistant HL-60 variant suggests the possibility that other types of human myeloid leukemia cells resistant to RA might similarly harbor defects in RAR structure and function.

One prototype human myeloid leukemia cell type that is resistant to RA-induced differentiation is the K-562 CML blast crisis cell line. Certain chemical inducers, including hemin and butyrate, induce erythroid differentiation of K-562, whereas phorbol esters (tetradecanoyl phorbol acetate [TPA]) induce megakaryoblastic differentiation of this cell line. Nevertheless, despite this demonstrable capacity to differentiate, K-562 cells are resistant to RA, and RA has no effect on proliferation or differentiation of the K-562 cell line. To determine whether the resistance to RA involves a defect(s) in the structure and function of the RAR in K-562, we performed a detailed analysis of nuclear RARs in K-562. We also transduced a copy of the RAR-α gene into K-562 to determine whether this would alter the sensitivity of the cells to RA.

MATERIALS AND METHODS

Cell culture. The K-562 CML blast crisis cell line and the HL-60 promyelocytic leukemia cell line were maintained in liquid...
suspension culture in RPMI medium supplemented with 5% fetal bovine serum. All-trans RA (Sigma Chemical, St Louis, MO) was added to cultures from a stock solution of 2 mmol/L in ethanol made fresh daily and kept in the dark at -20°C until use. Morphological evaluation of cells was performed by sedimenting cells onto glass slides with a Shandon Cytospin 2 cytocentrifuge (Pittsburgh, PA), followed by Wright's staining.

**Northern and Southern blot hybridization.** DNA extractions were performed by digesting nuclei with protease K, followed by phenol-chloroform extraction and ethanol precipitation. Southern blots of restriction endonuclease-digested genomic DNA were performed as previously described. RNA was extracted with guanidine hydrochloride and subjected to Northern blotting in formaldehyde denaturing gels as previously described.

**Retroviral infection of K-562.** The retroviral vector LRARSN was constructed from the parent vector LXSN as previously described. The LRARSN construct harbors the complete coding sequence of the RAR-α gene driven by the Moloney murine leukemia virus (Mo-MuLV) long terminal repeat (LTR). The vector also harbors the neo′ gene as previously described. 4 pg/mL. Supernatant from the PA317-LRARSN retroviral vector producer cell line was added at a multiplicity of infection of 0.1. After 24 hour incubation at 37°C, the cells were suspended in 96-well microtiter dishes in standard growth media supplemented with 1 mg/mL G418. Growth of G418-resistant K-562 cells was noted 8 to 10 days after plating, and individual clones of infected K-562 cells were obtained by cloning the G418-resistant cells in soft agar as previously detailed. 29 Genomic DNA from subclones was subjected to Southern blot analysis after cutting with HindIII, which has a single restriction site within the LRARSN vector. Blots were probed with a neo′ probe, and each subclone demonstrated only a single copy of the LRARSN proviral DNA incorporated into the infected cell genome. We noted little if any variation in experimental results among these infected K-562 subclones.

**Molecular probes.** The molecular probes used in the Northern and Southern blots included a 1.309-kb pair (bp) EcoRI-Smal fragment from the hTTR RAR-α cDNA clone; α-actin, the α1 β chicken actin 2-kilobase (kb) Pst fragment; c-myc, a 1.8-kb human myc cDNA clone obtained from David Bentley; neo′, a 0.9-kb PstI fragment from the bacterial neomycin phosphotransferase gene isolated from N. proviral DNA; and bcr-abl, a 600-bp EcoRI-PstI 5′ bcr cDNA fragment that hybridizes to the bcr-abl fusion gene. All probes were labeled by nick-translation before hybridization.

**Hemoglobin assay.** Cellular hemoglobin was determined by a colorimetric assay with 2,7-diaminofluorene (DAF). Cells were sedimented at 700 g for 5 minutes, lysed with 0.01% NP-40, stained with DAF, and the absorbance was determined on a Beckman Du-20 spectrophotometer 7 to 8 minutes after the DAF reagent was added.

**Analysis of nuclear RA receptors.** Nuclear extracts for evaluation of RA receptors were prepared by a modification of the technique of Shapiro et al as exactly as previously described. The approximate mol wt, affinity, and number per cell of RARs were determined by incubating [3H] RA (50 Ci/mmol; Dupont, NEN Research Products) with nuclear extracts followed by size-exclusion high-pressure liquid chromatography (HPLC) (Vario Instruments, Sunnyvale, CA) over a Bio-Sil TSK 250 size-exclusion column (Bio-Rad Laboratories, Richmond, CA) exactly as described previously.

**FACS analysis.** For fluorescent antibody binding assays, 2 x 10⁶ cells were pelleted at 700 g for 5 minutes and washed with phosphate-buffered saline containing 2% human AB serum (PBS-AB). The cells were resuspended in 100 µL primary antibody in PBS-AB, incubated at 4°C for 15 to 20 minutes, and washed with PBS-AB. The pellet was then mixed with FITC-conjugated goat anti-mouse IgG-M (TAG) diluted 1:40 with PBS-AB for a total volume of 100 µL. After incubation for 15 to 20 minutes at 4°C, the cells were washed and resuspended in 300 µL PBS-AB. Cells were examined by fluorescence microscopy with a Leitz Ortholux microscope with a Poelm illuminator or by flow microfluorimetry using a FACS II system (Becton Dickinson), and cell labeling was expressed as relative fluorescent intensity.

**Analysis of DNA content.** Cells were stained with propidium iodide and analyzed on the FACS II system. The percentage of cells in G0/G1 was determined using the Multicycle software program from Phoenix Flow Systems (San Diego, CA).

**RESULTS**

**Analysis of nuclear RA receptors in K-562 cells.** RA induces granulocytic differentiation of HL-60 cells as well as certain other myeloid leukemic cells. We previously identified a subclone of HL-60 cells that, in contrast to parental HL-60 cells, is resistant to RA-induced differentiation. These variant HL-60 cells harbor aberrant nuclear RA receptors with a reduced affinity for RA and fewer receptors per cell. Because K-562 is similarly resistant to RA, we performed a detailed analysis of nuclear RA receptors in K-562 to determine whether these cells harbor similarly aberrant nuclear RA receptors. Analysis of RAR-α mRNA in K-562 by Northern blot analysis using an RAR-α-specific probe shows normal-sized endogenous RAR-α transcripts of 3.3 and 2.5 kb (Fig 1). As compared with RA-sensitive HL-60 cells, about half as many RAR-α transcripts consistently appeared to be present (Fig 1; lane 1 as compared with lane 2). Further characterization of nuclear K-562 RARs by size-exclusion HPLC of K-562 nuclear extracts after incubation with [3H] RA identified RA binding at a mol wt of about 100,000, which is specifically competed out with a 100-fold excess of cold RA (Fig 2A).

**Equilibrium binding analysis of the K-562 nuclear extracts at various concentrations of [3H] RA demonstrated a dissociation constant (kd) of 1.5 nmol/L (Fig 3A), similar to wild-type RA-sensitive HL-60 cells.** A Scatchard plot of the binding data indicated that K-562 cells exhibit about 80 nuclear RAR receptors per cell (Fig 3A, inset), as compared with RA-sensitive HL-60 cells that harbor approximately 550 nuclear receptors per cell.

**Retroviral-mediated gene transfer of RAR-α cDNA into K-562.** We wished to determine whether an increased number of RAR-α receptors would render K-562 cells sensitive to RA. As detailed in the Materials and Methods section, we infected K-562 cells with the LRARSN retroviral vector.
ral vector that harbors a cDNA fragment containing the complete coding sequences of RAR-α and isolated cells exhibiting a single integrated copy of the retroviral-transduced RAR-α gene. The infected cells (now designated K-562 + LRARSN) exhibit the endogenous RAR-α transcripts as well as the more abundant retroviral RAR-α 4.9-kb and 3.1-kb RAR-α transcripts (Fig 1, lane 3). The 3.1-kb transcript does not hybridize to a neo' probe and probably represents a Mo-MuLV LTR-initiated transcript terminating with a cryptic poly(A) site within the 500 bp 3'untranslated region of the RAR-α cDNA.11

To determine the number of nuclear RA receptors in the retroviral vector infected K-562 cells, we examined specific binding of [3H]-RA to nuclear extracts of the infected K-562 + LRARSN cells using size-exclusion HPLC. As expected, the infected K-562 + LRARSN nuclear extract exhibits specific [3H] RA binding that eluted at a mol wt of about 100,000 (Fig 2B). Scatchard plot analysis indicated that the RARs in K-562 + LRARSN cells have a kd of about 1.8 nmol/L, and these RAR-α-transduced cells exhibit approximately 2,000 nuclear RA receptors per cell (Fig 3B).

Growth inhibition of infected K-562 + LRARSN cells by RA. As shown, the RA-resistant parental K-562 cells exhibit approximately 80 nuclear RA receptors per cell and the RAR-α-transduced K-562 + LRARSN cells harbor about 2,000 nuclear RA receptors per cell. To determine whether the increased number of RAR-α receptors expressed by the retroviral infected K-562 cells altered the sensitivity of these cells to RA, we first compared the

Fig 1. Expression of RAR-α transcripts in leukemic cell lines. Total RNA (5 μg per lane) was subjected to Northern blot analysis with a human RAR-α probe. The 3.3- and 2.5-kb endogenous RAR-α transcripts as well as the 4.9- and 3.1-kb LRARSN retroviral vector RAR-α transcripts are indicated. As a control, the same samples were also hybridized with an actin probe. Lane 1, uninfected K-562 cells; lane 2, HL-60 cells; lane 3, K-562 cells infected with the LRARSN retroviral vector.

Fig 2. Specific nuclear RA-binding activity in K-562 (A) and K-562 + LRARSN (B) cells. Nuclear extracts from each cell line were incubated with [3H] RA with or without a 100-fold excess of unlabeled RA and subjected to size-exclusion HPLC analysis as detailed in the Materials and Methods section. The radioactivity in each fraction (0.5 mL) was then determined. [3H] RA only (Φ); [3H] RA and a 100-fold excess of unlabeled RA (Ο). Elution times of specific mol-wt markers (in thousands) as determined by A260 (arrows). These protein markers include thyroglobulin 670,000, γ-globulin 158,000, human albumin 68,000, ovalbumin 44,000, myoglobin 17,000, and vitamin B12 1,300.
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Fig 3. Equilibrium binding of [3H] RA to parental and LRARSN infected K-562 cells. Increasing concentrations of [3H] RA were incubated with nuclear extracts and analyzed by size-exclusion HPLC (described in legend to Fig 2). Specific binding is shown on the ordinate and represents total bound [3H] RA within the peak HPLC fraction minus [3H] RA bound in the same relative fractions when the extracts were incubated with a 100-fold excess of unlabeled RA. Scatchard plots of the equilibrium binding data are shown (inset).

Fig 4. Growth of uninfected and LRARSN infected K-562 cells in RA. The cell lines were incubated in liquid suspension in the concentrations of RA indicated, and the total viable cell number was determined at daily intervals. The experimental points represent the mean of four independent experiments.

growth in liquid suspension of the cells with RA added (Fig 4). The wild-type K-562 cells continued to exhibit logarithmic growth when cultured with RA, although at a slightly reduced rate (Fig 4A). In contrast, the infected K-562 + LRARSN cells showed marked growth inhibition in the presence of RA; growth was completely inhibited in 10^{-5} mol/L RA (Fig 4B). Propidium staining and FACS analysis indicated that these RA-induced cells accumulate in G_0/G_1, with K-562 + LRARSN cells incubated in RA for 2 days, exhibiting an average of 59% of cells in G_0/G_1, as compared with 37% of cells in G_0/G_1, exhibited by uninfected cultures (n = 2).

Evaluation of K-562 and K-562 + LRARSN cells for RA-induced differentiation. K-562 cells exhibit erythroid differentiation when exposed to certain chemical inducers, including hemin and butyrate, and megakaryoblastic differentiation when treated with TPA. We wished to determine whether RA, which inhibits the growth of the infected K-562 + LRARSN cells (Fig 4B), also induces any differentiation in these cells. Wright's stains of cytopsin preparations of both RA-treated K-562 and RA-treated K-562 + LRARSN cells indicated that both retain their blast morphology with no evidence of myeloid, erythroid, or megakaryocytic morphologic differentiation. Moreover, we could detect no hemoglobin production in the RA-treated cells.
using benzidine staining (data not shown). Using the more sensitive diaminofluorene assay for hemoglobin content, we noted a slight increase in hemoglobin levels in the RA-treated K-562 + LRARSN culture, which was less than that exhibited by hemin-treated K-562 + LRARSN cells and by normal peripheral blood RBC (Table 1).

We also assessed differentiation of the RA-treated K-562 and K-562 + LRARSN cells by FACS analysis using various antibodies specific for surface markers of megakaryocytic, lymphoid, erythroid, and myeloid differentiation. There was no evidence of megakaryocytic glycoprotein Ib (GpIb) or lymphoid (CD2, CD19, CD20) surface antigen expression in either the parental K-562 or the K-562 + LRARSN cells before or after exposure of up to 10^{-5} mol/L RA for 7 days (data not shown). The erythroid marker glycophorin A is expressed at a moderate basal level in both LRARSN-infected and wild-type K-562 cells, but we found no change in this level of expression in either cell type on exposure to 10^{-4} and 10^{-5} mol/L RA for 2, 4, or 7 days. Similarly, we noted no change in the level of the erythroid/myeloid antigen recognized by 7B9 antibody in untreated or RA-treated cells. Both the K-562 and the K-562 + LRARSN cells express low levels of the early myeloid antigen CD33 but not CD34, and neither changes with exposure to RA. Four other granulocyte/monocyte-specific surface antigens were examined for expression: three of them, CD17, CD18, and CD36, were undetectable in both uninduced and RA-induced K-562 and K-562 + LRARSN cells. The fourth marker, CD15, is expressed at high levels in both K-562 cells and infected K-562 + LRARSN cells. K-562 cells incubated in 10^{-5} or 10^{-4} mol/L RA for 7 days show only a slight decrease in the level of expression of CD15 (Fig 5A). In contrast, surface expression of CD15 essentially disappears from the infected K-562 + LRARSN cells after incubation in 10^{-4} or 10^{-3} mol/L RA for 7 days (Fig 5B).

Cellular oncogene expression in RA-induced cells. We wished to determine by Northern blot analysis whether any change in expression of certain cellular oncogenes occurred during RA-induced growth inhibition of the K-562 + LRARSN cells (Fig 4B). For these studies, we chose to assess the expression of both the c-myc gene, which is transcriptionally downregulated during RA-induced HL-60 differentiation, and the bcr-abl fusion gene, whose expression appears to be critical to the pathogenesis of CML and that is genomically amplified approximately fourfold to eightfold in K-562 cells. We noted no significant changes in c-myc expression when the parental K-562 cells were treated with RA (Fig 6A). There was a significant decrease in c-myc expression in the growth-inhibited, RA-treated K-562 + LRARSN cells, however (Fig 6B). In contrast, there was no decrease in the bcr-abl fusion gene expression in either the RA-treated K-562 or the growth-inhibited, RA-treated K-562 + LRARSN cells (Fig 6). Indeed there was an increase in the amount of this transcript in RA-treated K-562 + LRARSN cells, the significance of which is unclear.

### DISCUSSION

The recent molecular cloning and characterization of specific nuclear receptors for RA that are members of the steroid/thyroid family of transcription factors provides con-

<p>| Table 1. Hemoglobin Production in K-562 and K-562 + LRARSN Cells |
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<table>
<thead>
<tr>
<th>Cells</th>
<th>Abs/10^8 Cells</th>
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<tr>
<td>K-562</td>
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<tr>
<td>K-562 + 10^{-4} mol/L RA</td>
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<td>K-562 + 10^{-5} mol/L RA</td>
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<tr>
<td>K-562 + LRARSN</td>
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<td>K-562 + LRARSN + 10^{-5} RA</td>
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<td>K-562 + LRARSN + 30 μmol/L hemin</td>
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<tr>
<td>HL-60</td>
<td>0.119</td>
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<tr>
<td>Human RBC</td>
<td>0.751</td>
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*Mean of three determinations.

Cells were incubated with or without varying concentrations of RA for 4 days. Cells were sedimented, lysed, and stained with 2,7-diaminofluorene (described in the Materials and Methods section). Hemoglobin was quantified by absorbance at 610 nm after 7 to 8 minutes.

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siderable insight into how certain retinoids, including RA, exert their biologic effects. Indeed, we previously demonstrated that RAR-α plays a critical and central role in mediating RA-induced terminal granulocytic differentiation of HL-60 leukemia cells. Paradoxically, however, previous studies comparing different myeloid leukemia cell samples indicate no clear correlation between expression of the RAR (at the mRNA level) and the sensitivity of leukemia cells to RA. To explore this problem in more detail, we chose to analyze nuclear RA receptors in K-562 cells, which, like most human myeloid leukemia cells, are resistant to RA-induced differentiation. K-562 cells are of particular interest because they display phenotypic characteristics of the erythroid and megakaryocytic lineage when incubated with the appropriate inducers.

We noted that the approximate mol wt of RA receptors in the RA-resistant K-562 cells (100,000) is similar to the mol wt of the RA receptors previously noted in RA-sensitive HL-60 cells. Because cDNA sequence analysis predicts the mol wt of nuclear RA receptors to be approximately 50,000, the HPLC mol wt estimation of 100,000 is consistent with dimerization of the RA receptors in both K-562 and HL-60. Moreover, the receptors in both cell lines exhibit approximately the same kd and therefore have the same relative affinity for RA. Thus, by these criteria, the nuclear RA receptors in the RA-resistant K-562 cells are indistinguishable, in contrast to the nuclear RA receptors previously noted in an RA-resistant HL-60 variant subclone which exhibit an apparent mol wt of approximately 60,000 as well as a reduced affinity for RA (kd = 12.5 nM/L). Nevertheless, we did note a significant difference between K-562 and HL-60 in the calculated number of nuclear RA receptors per cell, with K-562 cells exhibiting approximately 80 receptors per cell as compared with the approximately 550 receptors per cell previously noted in RA-sensitive HL-60 cells. The relative RA resistance exhibited by K-562 cells may be related directly to this relatively reduced number of RA receptors expressed by these cells.

What might account for the reduced number of nuclear RA receptors in K-562 cells as compared with HL-60 cells? We consistently noted that K-562 cells express approximately half the amount of RAR-α mRNA as HL-60 cells (Fig 1). Nevertheless, the >80% reduction in the number of nuclear RA receptors in K-562 v HL-60 cells suggests that translational control or posttranslational modifications may also contribute to the reduced number of RA receptors in K-562. Alternatively, the observed numbers of nuclear RA receptors in K-562 may be reduced because of heterodimer formation of the RA receptor with other nuclear factors; e.g., the thyroid hormone receptor appears capable of forming heterodimers with the RA receptor, and such heterodimers may be incapable of binding RA, resulting in an apparent lower number of nuclear RA receptors. In this regard, we have observed mRNA transcripts for thyroid hormone receptors in K-562 but not in HL-60 cells (unpublished observations).

We increased the number of nuclear RAR-α receptors in K-562 cells by infecting the cells with the LRARSN retroviral vector harboring RAR-α coding sequences. Although the retroviral vector-infected K-562 cells harbored only a single copy of the retroviral-transduced RAR-α gene, the enhanced mRNA expression from this gene as compared with endogenous K-562 RAR-α transcripts (Fig 1) probably resulted in the relatively high number of nuclear RA receptors (2,000 per cell) displayed by the infected K-562 cells. In contrast, with uninfected K-562, proliferation of the infected K-562 cells was inhibited when the cells were incubated with RA and the growth inhibition was associated with accumulation of cells into G0/G1. RA induced decreased mRNA expression of c-myc but not bcr-abl in these cells. Although the physiologic function of the c-myc nuclear oncogene is not clear, its enhanced expression appears to be associated with progression through


the cell cycle, whereas diminished c-myc expression is associated with decreased proliferation. For example, mitogen-stimulated fibroblasts and lymphocytes display enhanced c-myc expression before initiation of S phase, whereas HL-60 cells induced with RA to terminally differentiate demonstrate decreased c-myc expression. Moreover, murine erythroleukemia cells in which c-myc expression is inhibited with anti-sense c-myc constructs accumulate in G1/G0. The activated RA receptors in the retroviral-transduced K-562 cells may interact directly with the c-myc locus, resulting in decreased c-myc expression and decreased cell proliferation. Nevertheless, specific RAR response elements have not yet been identified in the c-myc locus, and the reduced c-myc expression in the RA-treated K-562 + LRARSN cells may somehow be an indirect result of nuclear RA receptor activation.

Using a battery of different monoclonal antibodies directed against erythroid, myeloid, megakaryocytic, and lymphoid surface antigens, we detected virtually no change in expression of these antigens in RA-treated RAR-α-transduced K-562 cells. The only exception was the CD15 antigen, whose expression essentially disappears from the RA-treated K-562 + LRARSN cells (Fig 5). The significance of this observation is unclear. CD15 is widely expressed on a variety of different types of myeloid leukemias, but its normal cell function as well as its relationship to leukemia cell differentiation is uncertain.

Although RA clearly induced decreased proliferation of K-562 cells harboring the retroviral-transduced RAR-α cDNA, this decreased proliferation was not accompanied by any significant erythroid or megakaryocytic differentiation. The increased globin accumulation noted in the RA-treated K-562 + LRARSN cells (Table 1) was not accompanied by any other clear evidence of erythroid differentiation and might be related to the decreased cell division displayed by these induced cells. Apparently even the presumably increased number of activated RA receptors in the RA-treated K-562 cells does not serve as a master "switch" to trigger the erythroid, megakaryocytic, or myeloid differentiation program in these cells. This could result from the absence of specific RAR response elements in the target genes of interest, inaccessibility of the activated RA receptors to the target genes because of local chromatin structure, or lack of other specific transcription factors that might interact with activated RA receptors to optimally influence transcription of specific target genes. Alternatively, the retroviral-transduced RAR-α gene may provide an excess number of activated RA receptors that may interact with and inhibit (squelch) other critical transcription factors that are necessary for optimal differentiation. Such "self-squelching" by an excess of specific transcription factors was recently reported in cotransfection assays. A relatively narrow range of concentrations of activated nuclear RA receptors may be critical in exerting a specific biologic effect.

We demonstrated that enhancing the expression of RA receptors in K-562 cells alters the phenotypic response of the cells to RA. This, together with our previous observations in HL-60 cells, strengthens if not directly confirms the hypothesis, previously based on predicted RA receptor structure as well as cotransfection assays, that the biologic effects of RA are mediated directly through the nuclear RA receptors. Our observations indicate that proliferation or differentiation of K-562 and HL-60 leukemic cells is influenced by the interaction of RA with these nuclear receptors. An important but unanswered question is what role these nuclear RA receptors play in normal hematopoiesis. RAR-α is expressed in a wide variety of different hematopoietic cell lines, including myeloid, erythroid, and T- and B-lymphoid cells. In preliminary studies on normal bone marrow, we readily detected RAR-α mRNA as well as specific nuclear RA binding activity indistinguishable from that observed in RA-sensitive HL-60 cells. Thus, these nuclear RA receptors, which appear to be important in altering proliferation or differentiation of myeloid leukemic cells, may also play an important although currently unknown role in control of normal hematopoiesis.

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Retinoic acid receptors in myeloid leukemia: characterization of receptors in retinoic acid-resistant K-562 cells

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