The biologic effects of retinoids such as all-trans-retinoic acid (ATRA) and 9-cis-retinoic acid on proliferation and differentiation of hematopoietic cells are mediated by binding and activating two distinct families of transcription factors: the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs). The RARs require heterodimerization with RXRs; in addition, RXRs can form homodimers, which can bind to DNA response elements that are either distinct or the same as those bound by the RAR/RXR heterodimers. Therefore, the two retinoid pathways provide sequences that are specific for effective DNA binding and activation of target genes.

We have developed several series of novel synthetic retinoids that selectively interact with RXR/RXR homodimers and RAR/RXR heterodimers. We show here that SR11236 and SR11246, which are RXR-selective analogs, had little ability to inhibit clonal growth and induce differentiation of leukemic cells (HL-60 cells and fresh acute myeloid leukemia cells). However, SR11249, SR11256, and LGD1069, which activated both RXR/RXR homodimers and RAR/RXR heterodimers, could inhibit clonal growth and induce differentiation of HL-60 cells as well as leukemic cells from patients, including those with acute promyelocytic leukemia (APL). This is similar to results observed with RAR/RXR-specific ligands. Interestingly, the combination of ATRA and either SR11249, SR11256, or LGD1069 showed synergistic effects in inducing differentiation of HL-60 cells. A retinoid (SR11238) with strong anti-AP-1 activity that did not activate the RARs and RXRs for gene transcription from the response element TREpal was inactive in our assay systems, suggesting that the antiproliferative effects of retinoids on leukemic cells is not mediated by inhibiting the AP-1 pathway. We conclude that the RAR/RXR pathway is more important than RXR/RXR pathway for differentiation and proliferation of acute myeloid leukemia cells, and certain retinoids or combination of retinoids with both RAR and RXR specificities may synergistically enhance the differentiation activity of ATRA, which may be relevant in several clinical situations.

The retinoid all-trans-retinoic acid (ATRA) has a critical role in many aspects of proliferation and differentiation of a wide variety of cell types and is a clinically useful anticancer agent, having profound effects on hematopoietic cells. ATRA stimulates clonal growth of normal human myeloid and erythroid precursors in vitro and inhibits proliferation and induces differentiation of blast cells from some patients with acute myelogenous leukemia (AML). Investigations in vitro have shown that ATRA induces differentiation of the human myeloid leukemia cell line known as HL-60 and acute promyelocytic leukemia (APL) cells from patients. Also, a study has revealed that ATRA inhibited the proliferation of clonogenic acute myelogenous leukemic cells, even though these cells could not be induced to differentiate. Recent studies have shown that a high proportion of patients with APL achieve complete remission after treatment with ATRA.

Studies of the molecular mechanisms of action of retinoids have revealed that they exert their effects by binding to retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Both RARs (RARα, RARβ, and RARγ) and RXRs (RXRα, RXRβ, and RXRγ) are ligand-dependent transcription factors and belong to the steroid/thyroid receptor superfamily. Myeloid leukemia cells, including the HL-60 cell line, express RARα, RARβ, and RXRα, although RARα levels are higher than RARβ. The RXRs bind ATRA as well 9-cis-retinoic acid (9-cis-RA); the latter, but not the former, is the ligand for the RXRs. The RXRs act effectively by heterodimerizing to form RAR/RXR complexes; the RXRs act predominantly as coregulators enhancing the binding of the RAR and the vitamin D₃, thyroid hormone, and peroxisome proliferator-activated receptors to their response elements via heterodimers. In the presence of 9-cis-RA, the RXR can also form homodimers, a finding that suggests that several different pathways exist for retinoids to mediate their effects. Thus, 9-cis-RA may elicit a broad range of biologic responses.

Studies showed that 9-cis-RA and ATRA induced differentiation and inhibited proliferation of AML cell lines and fresh leukemic cells from patients, and 9-cis-RA often showed a modest increase in potency as compared with ATRA. Moreover, we found that 9-cis-RA in combination with ATRA was an effective inducer of differentiation of an RA-resistant HL-60 variant cell line. However, the biologic role of RXR/RXR homodimers in hematopoiesis is still unclear. Recently, a novel class of synthetic retinoids was demonstrated to have greater selectivity for activation of RXR/RXR homodimers rather than RAR/RXR heterodi...
In this study, we performed structure-function analysis to identify the characteristics of retinoids that allow them to induce differentiation and inhibit proliferation of acute myeloid leukemic cells.

MATERIALS AND METHODS

Cells. Myeloid leukemia lines used in this study were KG-1 and HL-60 cells. Leukemic samples from bone marrow or peripheral blood from eight consenting AML patients were collected in heparinized tubes before any therapy. Three patients had APL M3, three had AML M2. The percentage of circulating blast cells was more than 80% of the mononuclear population at the time of harvesting the cells. The diagnosis was established according to French-American-British (FAB) criteria. The leukemic cells were isolated by Ficoll-Hypaque (Lymphoprep; Nycomed Pharma AS, Oslo, Norway; density, 1.077 g/mL) gradient centrifugation, washed twice in phosphate-buffered saline (PBS), and enriched after monocyte adherence to plastic for 1 hour at 37°C. The blast cells were immediately placed into suspension culture containing RPMI 1640 medium with 10% fetal bovine serum (FBS; GIBCO BRL, Grand Island, NY), 100 U/mL penicillin, and 100 μg/mL streptomycin in humidified air/5% CO2.

Retinoids and transfection assays. ATRA was purchased from Sigma Chemical Co (St. Louis, MO), and 9-cis-RA was a gift from Dr M. Klaus (F. Hoffmann-La Roche, Basel, Switzerland). Synthetic ligands used in this study included 2-(4-carboxyphenyl)-4-(1E,3E-3-methylbut-2-enylbenzoic acid (SRI 1246); 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)cyclopropyl]benzoic acid (SRI 11246); 4-(4-carboxy-1E,3E-3-methylbutadienyl)-2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1,3-dioxane amnomium salt (SR11236); 2-(4-carboxyphenyl)-2-(3,4-dihydro-4,4-dimethyl-2H-1-benzopyran-6-yl)-1,3-dithiane (SR11238); 4-[1-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)cyclopropyl]benzoic acid (SR11249); 2-(4-carboxy-1E,3E-3-methylbutadienyl)-2-(5,6,7,8-tetrahydro-3,5,5,8,8-pentamethyl-2-naphthalenyl)-1,3-dioxolane (SR 11249); and (2E,4E)-6-(5,6,7,8-tetrahydro-5,8,8-tetramethyl-2-naphthalenyl)-3,7-dimethyl-2,4,6-octatetraenoic acid (SR11269) (Table 1), which were prepared as described by Dawson et al. Retinoid 4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethenyl]benzoic acid (LGD1069) was synthesized and characterized by Boehm et al (Fig 1). Retinoids were dissolved in 100% ethanol to a stock concentration of 10^{-4} mol/L, stored at -20°C, and protected from light. In each experiment, controls were run using the same concentration of ethanol as was present in the experimental plates. This concentration of diluant had no effect on proliferation of cells.

Table 1. Retinoid Receptor Transcriptional Activation

<table>
<thead>
<tr>
<th>Retinoid</th>
<th>RARα (mol/L)</th>
<th>RARβ (mol/L)</th>
<th>RARγ (mol/L)</th>
<th>RXRα (mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR11236</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>SR11238</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
</tr>
<tr>
<td>SR11246</td>
<td>-1</td>
<td>-11</td>
<td>-11</td>
<td>-11</td>
</tr>
<tr>
<td>SR11249</td>
<td>4</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
<tr>
<td>SR11256</td>
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<td>11</td>
<td>11</td>
<td>11</td>
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<tr>
<td>SR11269</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>LG1069</td>
<td>-5</td>
<td>-3</td>
<td>-3</td>
<td>-3</td>
</tr>
</tbody>
</table>

Relative response compared with 10^{-6} mol/L ATRA (100%) for RARs and 10^{-6} mol/L 9-cis-RA (100%) for RXRα.

Fig 1. Structures of synthetic retinoids.
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cell suspension ($2 \times 10^6$ cells per milliliter) was mixed with an equal volume of solution containing 1.25 mg/mL NBT (Sigma), 17 mg/mL bovine serum albumin (fraction V; Sigma), and 1 mg/mL 12-O-tetradecanoylphorbol-13-acetate (TPA; Sigma) for 30 minutes at 37°C. After incubation, the medium was discarded, and the formazan deposits were dissolved by adding 0.1 mL of dimethyl sulfoxide (DMSO; Sigma) and measured at 580 nm.

For analysis of cell-surface antigens, a two-color direct immunofluorescence staining technique was used. Cells were incubated for 60 minutes with human AB serum (Sigma) to block Fc receptors and then exposed to phycoerythrin (PE)-conjugated mouse IgG1 isotype antibody (Beckton Dickinson). Analysis of fluorescence was performed on a FACScan flow cytometer (Beckton Dickinson).

RESULTS

Effect of retinoids on transactivation of a reporter gene having RAR and RXR response sequences. In Table 1 are presented the retinoid receptor transcriptional activities on the TREpal of nine retinoids in the presence of RARα, RARβ, RARγ, and RXRα. The TREpal response element, which was used in the transfection assay, is responsive to both RAR and RXR dimer complexes that have been activated by retinoids. The retinoids, the structures of which are presented in Table 2, show a range of activities for these retinoid receptors, particularly RXRα.

Table 2. Summary of Retinoid Activities

<table>
<thead>
<tr>
<th>Retinoid</th>
<th>Inhibition of Proliferation, of HL-60 Cells</th>
<th>Induction of CD11b on HL-60 Cells</th>
<th>Transcriptional Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10⁻⁷ mol/L</td>
<td>ED₅₀ (nmol/L)</td>
<td>% at 10⁻⁷ mol/L</td>
</tr>
<tr>
<td>SR11236</td>
<td>11 NR</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>SR11238</td>
<td>18 NR</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>SR11246</td>
<td>19 NR</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>SR11269</td>
<td>17 NR</td>
<td>0</td>
<td>N</td>
</tr>
<tr>
<td>LGD1069</td>
<td>37 NR</td>
<td>7</td>
<td>Y (B)</td>
</tr>
<tr>
<td>SR11249</td>
<td>89 20</td>
<td>3</td>
<td>Y (B)</td>
</tr>
<tr>
<td>SR11256</td>
<td>98 10 6</td>
<td>6</td>
<td>Y (13)</td>
</tr>
<tr>
<td>ATRA</td>
<td>97 10 10</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

Abbreviations: EC₅₀, concentration of retinoid giving 50% maximal response or response at 10⁻⁸ mol/L retinoid, whichever is greater, for retinoids whose maximal response is >30% of that of 10⁻⁸ mol/L ATRA for RARs and 10⁻⁸ mol/L 9-cis-RA for RXRα. NR, not reached; ND, not determined.

* ED₅₀, concentration of retinoid giving 50% inhibition of clonal growth.
† NR, no; Y, yes; ( ) fold-induction of CD11b on HL-60 cells in the presence of 10⁻¹ mol/L ATRA plus the novel retinoid (10⁻¹ mol/L) compared with ATRA (10⁻¹ mol/L)-treated control.
‡ Activation (%) at 10⁻⁶ mol/L retinoid compared with 10⁻⁶ mol/L ATRA for RARs and 10⁻⁶ mol/L 9-cis-RA for RXRα.

Effects of retinoids on proliferation of leukemic cells in soft gel culture. The retinoids were examined for their effect on clonogenic proliferation of HL-60 cells (Fig 2, Table 2). Retinoids SR11236, SR11238, SR11246, and SR11269 were poor inhibitors (less than 20%) of leukemic colony formation, and LGD1069 (10⁻⁶ mol/L) inhibited colony formation by 37%. These compounds showed greater selectivity for RXR than RAR. Analogs SR11249 and SR11256 also demonstrated panagonist activity by activating both retinoid receptor subclasses: RARα (38%), RARβ (52%), and RXRα (72%) at 10⁻⁶ mol/L. SR11256 also demonstrates some panagonist activity by activating RXRα (70%) at 10⁻⁵ mol/L, but not (15%) at 10⁻⁶ mol/L, and by activating the RARs, on which it is far more potent, giving activations greater than 60% for RXRα and RARβ at 10⁻⁴ mol/L.

For example, retinoid SR11236 selectively activates RXRα (59%) at 10⁻⁸ mol/L. It does not activate either RARα or RARβ at 10⁻⁸ mol/L, but does activate RXRα (34%) at 10⁻⁷ mol/L. SR11236 also lacks anti-AP-1 activity, as it does not inhibit the activation of the collagenase promoter in a reporter construct by the AP-1 Jun/Fos complex in the presence of any of the retinoid receptors (Table 2). In contrast, its analog, SR11238, does not activate any of these receptors to induce gene transcription but is capable of inhibiting AP-1 activity. SR11246 is the most RXR-selective of the seven retinoids because it does not activate any of the RARs at 10⁻⁷ mol/L, but does activate RXRα 65% at 10⁻⁷ mol/L. SR11249 demonstrates selective activity for RARβ and RXRα at concentrations as low as 10⁻⁸ mol/L (34% and 49%, respectively), whereas its analog SR11269 activates RARβ (45%) and RXRα (104%) at 10⁻⁸ mol/L but only activates RXRα at 10⁻⁷ mol/L (45%). LG1069 also demonstrates panagonist activity by activating both retinoid receptor subclasses: RARα (38%), RARβ (52%), and RXRα (72%) at 10⁻⁶ mol/L. SR11256 also demonstrates some panagonist activity by activating RXRα (70%) at 10⁻⁵ mol/L, but not (15%) at 10⁻⁶ mol/L, and by activating the RARs, on which it is far more potent, giving activations greater than 60% for RXRα and RARβ at 10⁻⁴ mol/L.

The effects of ATRA and retinoids SR11236, SR11238, and SR11249 on proliferation of fresh leukemic cells from patients paralleled those observed with HL-60 cells (Fig 3). Analogs SR11236 and SR11238 (10⁻⁷ mol/L) had little inhibitory effect on the clonal growth of leukemic blast cells and, in combination with 10⁻⁷ mol/L ATRA, did not enhance the potency of ATRA alone. SR11249 (10⁻⁷ mol/L) inhibited the proliferation of leukemic cells by 60%, whereas the com-

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Combination of SR11249 and ATRA had a subadditive inhibitory effect.

Effects of retinoids on differentiation of leukemic cells. Induction of differentiation of myeloid cell lines and fresh leukemic cells from patients with AML into more mature granulocyte-like cells by these novel retinoids either alone or in combination with either ATRA or 9-cis-RA was assayed by NBT reduction activity and by expression of CD11b antigens using fluorescence-activated cell sorter (FACS) analysis. None of the analogs induced differentiation of KG-1 cells and fresh leukemic cells from M1 and M2 samples (data not shown). Retinoids SR11236 and SR11238 (10^{-7} mol/L) did not induce HL-60 cells to reduce NBT (Fig 4, top panel). The combination of either of these analogs with either ATRA or 9-cis-RA (10^{-7} mol/L) had little additional effect on the ability of HL-60 cells to reduce NBT as compared with the effect of ATRA or 9-cis-RA alone. SR11249 (10^{-7} mol/L) moderately induced the differentiation of HL-60 cells, having about 35% to 40% of the ability to reduce NBT as compared with that of either ATRA or 9-cis-RA (Fig 4, upper left panel). In combination with either ATRA (10^{-7} mol/L) or 9-cis-RA (10^{-7} mol/L), SR11249 showed a slightly additive effect (Fig 4, upper right panel). The induction of NBT activity in leukemic cells from patients with APL by these same synthetic retinoids either alone or in combination with either ATRA or 9-cis-RA showed results comparable with those observed with the HL-60 cells (Fig 4, lower panels).

The expression of CD11b increases as myeloid cells differentiate towards granulocytes. Exposure of HL-60 cells to increasing concentrations of ATRA (10^{-11} to 10^{-7} mol/L) resulted in an increase in expression of CD11b in a dose-response manner, with 10^{-7} mol/L ATRA producing an approximately 10-fold greater expression of CD11b compared with that of untreated HL-60 cells (Fig 5). The RXR-selective retinoids SR11236, SR11249, and SR11269 (10^{-11} to
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Fig 4. Comparison of the differentiation-inducing activity (NBT reduction) of synthetic retinoids. HL-60 cells (right panel) and fresh APL cells (left panel) were cultured with either SR11236, SR11238, or SR11249 (10⁻⁷ mol/L), or were combined with either ATRA (10⁻⁷ mol/L) or 9-cis-RA (10⁻⁷ mol/L) for 6 days. Differentiation was determined by NBT reduction. Results are expressed as the percentage of control dishes that contained no retinoids (mean ± SD from three experiments). The SD were within 10% of the mean.

10⁻⁷ mol/L) alone had negligible effects on expression of CD11b and did not enhance the ability of ATRA (10⁻¹¹ to 10⁻⁷ mol/L) to increase expression of CD11b. The anti-AP1 retinoid SR11238 behaved similarly. In contrast, SR11249, SR11256, and LGD1069 alone increased expression of CD11b (threefold to sevenfold at 10⁻⁷ mol/L), as compared with cells not exposed to a retinoid; but when combined with an equal molar concentration of ATRA, a dramatic and synergistically increased expression of CD11b occurred. For example, at 10⁻¹¹ mol/L, none of the retinoids induced CD11b over background levels, but SR11249, SR11256, or LGD1069 (10⁻¹¹ mol/L) combined together with ATRA (10⁻¹¹ mol/L) increased CD11b expression by 5-fold, 8-fold, and 13-fold, respectively. Comparable results were noted when analyzing another myeloid differentiation antigen, CD14 (data not shown).

DISCUSSION

Recent studies have shown that RARs require heterodimerization with RXRs for effective DNA binding and function and that RXRs bind several other ligand-regulated receptors, including thyroid hormone receptors (TR) and vitamin D3 receptors. The RXRs can form either RXR/RXR homodimers or RAR/RXR heterodimers in the presence of 9-cis-RA. We have previously shown that 9-cis-RA was slightly more potent than ATRA in inhibiting proliferation and inducing differentiation of both myeloid leukemia cell lines and fresh APL cells. How retinoids...
regulate various aspects of differentiation and proliferation of leukemic cells still remains unclear. The role of RXR/RXR homodimers and RXR/RAR heterodimers in hematopoiesis is unknown. To examine this issue, we investigated the effects of several new retinoids with defined RAR and RXR specificities, either alone or in combination with either ATRA or 9-cis-RA, on the in vitro growth and differentiation of the myeloid leukemic cell line, HL-60, as well as of fresh leukemic blasts from patients with AML, including those with APL. A summary of the salient data is shown in Table 2.

Retinoids SR11236, SR11246, and SR11269 were extremely weak inhibitors of proliferation and inducers of differentiation of myeloid leukemic cells. SR11236, SR11246, and SR11269 predominantly activate RXR/RXR homodimers in the cotransfection assay, but do not activate RARα and RARβ at concentrations up to $10^{-3}$ mol/L (SR11236 and SR11246) or weakly activate these receptors (SR11269), resulting in the modulation of RXR-selective response pathways. We conclude, therefore, that RXR/RXR-selective retinoids do not have a prominent effect on growth and differentiation of leukemic cells. SR11217, another ligand selective for RXR/RXR homodimers, also had little effect on either inducing differentiation or inhibiting clonal growth of myeloid leukemic cells. The retinoids SR11249 and SR11256 in this series were nearly as active as ATRA in our leukemic models and had the ability to activate both RAR/RXR and RXR/RXR (Table 2). The retinoid 4-(5,6,7,8-tetrahydro-5,8,8-trimethyl-2-naphthalenyl)benzoic acid (TTAB) is also a potent inducer of differentiation and inhibitor of clonal growth of leukemic cells, and it too is RAR/RXR-specific. These results suggest that RAR/RXR probably is the specific target that modulates proliferation and differentiation of hematopoietic cells.

The situation may be more complicated when the effects of two retinoids are examined simultaneously. When ATRA and the RXR/RXR-selective ligands SR11236, SR11238, SR11246, or SR11269 are combined, the expression of CD11b by HL-60 cells was no greater than when ATRA was added alone (Fig 5). In contrast, when either SR11249, SR11256, or LGD1069 was added to ATRA, a marked synergy occurred in the expression of CD11b (Fig 5). If these retinoids mediated their activities exactly like ATRA (eg, through the same RAR/RXR retinoic acid response elements), synergy should not have occurred. We do not know how to explain this phenomenon. Because the RXR/RXR-selective analogs did not synergize with ATRA, perhaps the synergy requires the somewhat hybrid nature (RAR/RXR- and RXR/RXR-activating capacities) of retinoids SR11249 and SR11256. This may also explain why 9-cis-RA is slightly more active than ATRA and why it may act synergistically with ATRA. Another receptor or response element may also be involved. Perhaps these compounds bind to either RARβ or RXRα to activate a heterodimer on this response element. Clarification of the mechanism of enhancement will require the identification and study of the genes critical for retinoid modulation of hematopoieses.

Comparison of the clonal proliferation and differentiation results of HL-60 cells with the retinoid receptor transcriptional activation results for these retinoids is hampered because the retinoid response elements responsible for retinoid action in HL-60 cells have yet to be identified. Retinoid activation effects on particular receptors will depend on the response element involved (M.P. and M.I.D., unpublished data, August 1995). Therefore, the authentic HL-60 retinoid response elements may provide somewhat different results than those obtained using the synthetic TREpal. Nevertheless, several conclusions can be made on comparison of these data. Only three of the synthetic retinoids, SR11249, SR11256, and LG1069, were able to inhibit effectively the proliferation of HL-60 cells at $10^{-6}$ mol/L and induce the expression of the differentiation marker CD11b at $10^{-7}$ mol/L. The first two retinoids were the most potent and showed $ED_{50}$ values comparable with those of ATRA and 9-cis-RA. Only SR11256 was capable of activating RARα at $10^{-6}$ mol/L, but only activated RXRα by 15% at this concentration. SR11249 and LG1069 were potent activators of RXRα at $10^{-6}$ mol/L (57% and 95%, respectively), but their activation...
of RARα was ≤10%. All three retinoids were able to activate RARβ at 10⁻⁶ mol/L. Therefore, activation of RARβ may be involved in inhibition of proliferation and induction of differentiation mediated by retinoids. Of the other retinoids evaluated, only SR11269 showed a similar transcriptional activation profile on the TREpal; however, it was a far less potent activator of RARβ. At 10⁻⁶ mol/L, SR11269 did not activate RARα but did activate RARα. Although its activation of RARβ (46%) at 10⁻⁶ mol/L was comparable with that (52%) of LG1069, its activation of RARβ at 10⁻⁷ mol/L dropped to 3% compared to 23% for LG1069.

Takahashi and Breitman39 have recently reported that the RARα-selective retinoid Am80 is able to induce the differentiation of HL-60 cells in medium containing serum, but is unable to do so in the absence of serum. Addition of ATRA to Am80 in the absence of serum fully induced HL-60 cell differentiation. These results indicate that activation of RARα alone is not sufficient to induce HL-60 cell differentiation and that another receptor(s) is involved. Our results using SR11249, SR11256, and LG1069 suggest that the other retinoid receptor involved in inhibition of proliferation and induction of differentiation by retinoids may be either RARβ or an RXR. Because the RXR-selective retinoid SR11246, which efficiently activates the RXR homodimer, is inactive in the HL-60 cell assay and because SR11256, which is a potent activator of RARs but not RARα, is active in this assay, it appears likely that RARβ has an important role in proliferation inhibition and the induction of CD11b.

Our experiments, which are shown in Fig 4, demonstrate that the ability of the various analogs to induce differentiation of fresh APL cells from patients paralleled their abilities to induce differentiation of HL-60. These results suggest two points. First, HL-60 cells may be a good model for APL cells, even though they have different genetic changes. Second, perhaps the activity of retinoids on APL cells is independent of its genetic alterations. APL cells have a balanced translocation of part of the PML gene and the RARα gene and express PML/RARα and RARα/PML fusion proteins as well as a normal RARα and PML. We have found that NB4 cells (APL cell line; data not shown), APL cells, and HL-60 cells respond similarly to the various retinoids. Nevertheless, HL-60 cells do not express PML/RARα. Likewise, some AML samples at the late M-2 stage (FAB classification) of development, but not at an earlier stage of differentiation, are responsive to ATRA and 9-cis-RA.29 Perhaps the sensitivity of APL blast cells to induction of differentiation by retinoids is not related directly to their PML/RARα fusion gene product, but is more related to their relatively mature stage of differentiation. Both APL and HL-60 have this in common. Clearly, more studies are required to determine how retinoids induce differentiation of AML cells.

SR11238 is a member of a new class of retinoids that selectively inhibits AP-1 activity but does not activate transcription.27 This retinoid did not induce the differentiation of F9 neuroblastoma cells but was able to inhibit the proliferation of the lung and breast cancer cell lines Calu-6 and T-47D, as well as HeLa cervical cancer cells.40 We have found that SR11238 had very little effect on either the differentiation or clonal proliferation of either the HL-60 and KG-1 (data not shown) leukemic cells or fresh AML cells. Therefore, this class of transcriptional antagonists probably is not a candidate for further clinical consideration as an antileukemic agent.

Several lines of indirect evidence point to the critical role of RAR in the modulation of myeloid proliferation and differentiation. (1) As shown in this study, RXR-selective retinoids have little effect on hematopoietic cells, but those with RAR selectivity are very active. (2) An analog that functioned as a selective RARα antagonist counteracted the ability of ATRA to induce differentiation of HL-60 cells.41 (3) Two independent HL-60 sublines that cannot undergo differentiation in the presence of ATRA have mutations in the ligand-binding region of RARα,22,43 and replacement of the RARα partially restored responsiveness of the cells to ATRA.44

Our results suggest that certain combinations of retinoids may have greater therapeutic activity than either alone. Several of these compounds are undergoing independent clinical testing; however, eventual trials using combinations of these retinoids are possible.

ACKNOWLEDGMENT

We thank Gail Jao for her assistance in the preparation of the manuscript.

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Effects of novel retinoid X receptor-selective ligands on myeloid leukemia differentiation and proliferation in vitro

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