Retinoids

Structure–Function Relationship in Normal and Leukemic Hematopoiesis In Vitro

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Abstract

Retinoids were studied both to identify what skeletal components are important in the modulation of normal and leukemic human myeloid clonal proliferation and differentiation in vitro and to elucidate the mechanism by which retinoids modulate proliferation of hematopoietic cells. Retinoids with a derivatized terminal carboxyl group were significantly less active than all-*trans*-retinoic acid, and those with the addition of two methyl groups to the cyclohexenyl ring of retinoic acid or substitution of its β-cyclogeranylidene group with a 1,1,3,3-5-indanyl ring system were markedly more active than all-*trans*-retinoic acid. Five of the retinoids strongly inhibited clonal growth of the HL-60 and KG-1 human leukemic cell lines (50% inhibition in the range of 3×10^{-10}–1×10^{-8} M) and markedly stimulated normal human myeloid colony formation (granulocyte-macrophage colony-forming cells [GM-CFC] 150% stimulation in the range of 3×10^{-9}–3×10^{-8} M). Further studies suggested that: (*a*) Common structural requirements of the retinoids were important in the modulation of both normal and leukemic hematopoiesis. (*b*) The retinoids were able to inhibit leukemic proliferation without induction of differentiation of the neoplastic cells. (*c*) Studies on normal human GM-CFC suggested that the retinoids did not act by themselves as a colony-stimulating factor (CSF), or by stimulating accessory cells to produce CSF, but either required earlier progenitor cells to become GM-CFC or enhanced the sensitivity of GM-CFC to the action of CSF.

Introduction

All-*trans*-retinoic acid affects normal and leukemic hematopoiesis in vitro and perhaps in vivo. The compound stimulates the clonal growth of normal human myeloid and eythroid precursors in vitro (1, 2). In contrast, all-*trans*-retinoic acid inhibits the clonal growth of the HL-60 and KG-1 human acute myeloid leukemic cell lines and the growth of the neoplastic blast cells harvested from patients with acute myelogenous leukemia (3). All-*trans*-retinoic acid also induces the differentiation of the human HL-60 promyelocytes to granulocyte-like cells (4). A small clinical trial suggests that 13-*cis*-retinoic acid may have a beneficial effect on hematopoiesis in patients with the myelodysplastic syndrome (5).

A new generation of retinoids has been synthesized that differs at their ring, side-chain, and polar functional units. We utilized all-*trans*-retinoic acid and these new retinoids in order: (*a*) to explore the importance of the influence of various structural modifications of the retinoid skeleton on normal and leukemic myeloid clonal proliferation and differentiation in vitro; and (*b*) to attempt to elucidate the mechanism of action of retinoids on the proliferation of normal and neoplastic hematopoietic cells.

Methods

Retinoids. The structure of the retinoids used in these experiments is shown in Table I. All-*trans*-retinoic acid (No. 1), RO 13-4306 (No. 2), RO 13-7410 (No. 5), RO 13-6298 (No. 7), and RO 22-4164 (No. 8) were generous gifts from Dr. M. I. Sherman, Hoffman-LaRoche, Inc., Nutley, NJ. SRI-5631-2 (No. 3), SRI-5397-41X (No. 4), and SRI-5442-28 (No. 6) were synthesized at Stanford Research Institute (SRI) International, Menlo Park, CA. The retinamides (Nos. 9 and 10) were generous gifts from Dr. R. C. Moon, Illinois Institute of Technology Research Institute, Chicago, IL. All compounds were stored at −20°C in sealed containers under a nitrogen atmosphere. Stock solutions of 0.25×10^{-2} M were prepared in 100% ethanol and preserved at −20°C under a nitrogen atmosphere for no longer than 14 d. Before use, the retinoids were diluted in phosphate-buffered saline (PBS) to final concentrations of 1×10^{-5}–1×10^{-12} M. The concentration of ethanol did not exceed 0.4%. This concentration had no influence on colony formation as compared with cultures containing no ethanol. To eliminate light, all experiments were performed in subdued light and the tubes containing the retinoids were covered with aluminum foil.

Cells. The human myeloid leukemia cell lines HL-60 and KG-1, and the murine myeloid leukemia cell line M1 were maintained in suspension culture in T flasks (Miles Laboratories, Inc., Naperville, IL) containing alpha modified minimum essential medium (Flow Laboratories, Inc., McLean, VA) and 10% fetal calf serum (FCS;[1] Irvine Scientific, Santa Ana, CA). The cells were incubated in a humidified atmosphere containing 7% CO_2. The medium was changed twice weekly. The HL-60 cell line is predominantly blocked at the promyelocytic stage (6) and the KG-1 line is composed of myeloblasts (7). The M1 myeloblast line was derived originally from an SL murine myeloid leukemia (8). All of our experiments were performed in the logarithmic growth phase of the cells.

Normal human bone marrow was obtained from healthy volunteers. The bone marrow cells were layered over Ficol-Hypaque (density, 1.077 g/ml) and centrifuged at 450 *g* for 40 min at room temperature. The light density, mononuclear cell fraction was washed twice in PBS and resuspended in alpha medium plus 10% FCS.

Colony formation assay by two-layer agar technique. The underlayer

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Dr. Koeffler is a member of the Jonsson Comprehensive Cancer Center and is the recipient of a Career Development Award from the National Institutes of Health.

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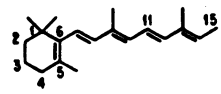
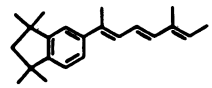
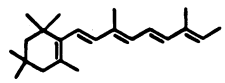
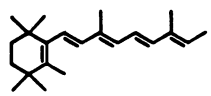
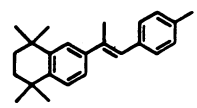
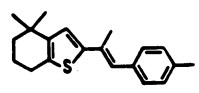
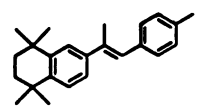
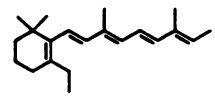
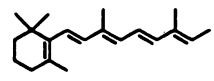
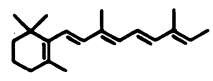
Received for publication 16 October 1985.

J. Clin. Invest.

0021-9738/86/07/0303/07 $1.00
Volume 78, July 1986, 303–309

1. *Abbreviations used in this paper:* CM, conditioned medium; CSF, colony-stimulating factor; cRABP, cytoplasmic retinoic acid–binding protein; ED_{50}, dose effective in achieving a half-maximal response; FCS, fetal calf serum; GCT, giant cell tumor line; GM-CFC, granulocyte-macrophage colony-forming cells; GM-CSF, granulocyte-macrophage colony-stimulating factor; NBT, nitroblue tetrazolium; TTNBP, (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid.

Table I. Structure of Retinoids

Compound No.	Structure		Code No./trivial name
1		COOH	all-*trans*-retinoic acid
2		COOH	RO 13-4306
3		COOH	SRI-5631-2 (3, 3-dimethyl analogue of retinoic acid)
4		COOH	SRI-5397-41X (4, 4-dimethyl analogue of retinoic acid)
5		COOH	RO 13-7410 (TTNPB)
6		COOH	SRI-5447-28 (tetrahydrobenzo[b]thiophene analogue of TTNPB)
7		COOEt	RO 13-6298 (TTNPB ethyl ester)
8		COOEt	RO 22-4164
9		CONHEt	*N*-ethyl retinamide (ER)
10		CONH-4-C_6H_4OH	*N*-(4-hydroxyphenyl) retinamide (4HPR)

was plated in 1-ml portions in 35-mm petri dishes (Miles Laboratories, Inc.) containing 0.5% agar, alpha medium, 16% FCS, and the various retinoids, and colony-stimulating factors (CSF). Conditioned medium (CM) obtained from a human T-lymphocyte line was used as a source of CSF for most experiments (9). In selected experiments, recombinant human granulocyte-macrophage colony-stimulating factor (GM-CSF) (10) or CSF derived from a giant cell tumor line (GCT) (11) was used. The top layer (1 ml) contained cells, alpha medium, 16% FCS, penicillin, streptomycin, and 0.3% agar. The HL-60 and M1 cells were plated in the absence of CSF; the KG-1 cells and normal human bone marrow require CSF for clonal growth and these plates received 1.5% CSF-rich CM. The cloning efficiency of KG-1 cells (in the presence of submaximally stimulating concentrations of CSF) was 4%; that of HL-60 and M1 cells was 34 and 25%, respectively. The cloning efficiency of normal bone marrow cells varied between human volunteers in a range of 0.03–0.06%. The culture dishes were incubated at 37°C in a humidified atmosphere containing 7% CO_2 for 10–13 d. Colonies (>40 cells) were counted using an inverted microscope. Each experiment was performed at least twice, and most experiments were repeated three times. All experiments contained three dishes per experimental point.

Nitroblue tetrazolium (NBT) reduction. The HL-60 cells (2×10^5/ml) were exposed to the various retinoids for 6 d in liquid culture containing alpha medium and 10% FCS in a humidified atmosphere of 7% CO_2. On day 6 the ability of HL-60 cells to generate superoxide (O_2^-) was measured by the reduction of NBT (12, 13). The reduction of NBT was measured qualitatively; 1×10^6 cells/ml were incubated with an equal volume of 1.25 mg NBT/ml (Sigma Chemical Co., St. Louis, MO), 17 mg bovine albumin/ml (Sigma Chemical Co.), and 10^{-5} M 12-*O*-tetra-decanoylphorbol-13-acetate (Consolidated Midland Corp., Brewster, NY) in alpha medium plus 10% FCS for 30 min at 37°C. After the incubation, the cells were washed once in PBS, cytocentrifuged, fixed in methanol for 5 min, and stained for 5 min with safranin (Difco Laboratories, Inc., Detroit, MI). The percentage of cells containing reduced blue-black formazan deposits was determined by differentially counting 200 cells with the light microscope.

Results

Effect of retinoids on clonal growth and differentiation of cells from myeloid leukemic cell lines. We examined the ability of various retinoids to inhibit clonal growth of the HL-60 cell line (Fig. 1). The HL-60 cells were strongly inhibited by retinoids with a terminal carboxyl group (Nos. 1–5). A slight inhibition was observed at concentrations as low as 1×10^{-10} M. Concentrations required for achieving a half maximal clonal growth inhibition are shown in Table II. The 2,2-dimethyl indanyl analogue (No. 2) and the (E)-4-[2-(5,6,7,8-tetrahydro-5,5,8,8-tetramethyl-2-naphthalenyl)-1-propenyl]benzoic acid (TTNBP) analogue (No. 5) were nearly 10-fold more active than all-*trans*-retinoic acid (dose effective in achieving a half-maximal response [Ed_{50}], $\sim 2 \times 10^{-9}$ M). The addition of two methyl groups at either the 3- (No. 3) or 4-position (No. 4) of the retinoic acid skeleton produced a 10-fold increase of activity as compared with all-*trans*-retinoic acid. Replacement of the tetrahydrotetramethylnaphthalenyl group of No. 5 by a tetrahydrodimethylbenzo[b]thienyl group (No. 6) decreased activity to values approaching those of all-*trans*-retinoic acid. Derivatization of the carboxyl group significantly decreased activity. Conversion of the terminal carboxyl function to a carbethoxy group (No. 7) decreased activity ~100-fold as compared with the carboxyl-containing analogue (No. 5). The amide derivatives of retinoic acid (Nos. 9 and 10) were almost inactive, even at 1×10^{-6} M. Four other retinamides, *N*-(hydroxyethyl) retinamide, *N*-(2-hydroxypropyl) retinamide, *N*-(3-hydroxypropyl)

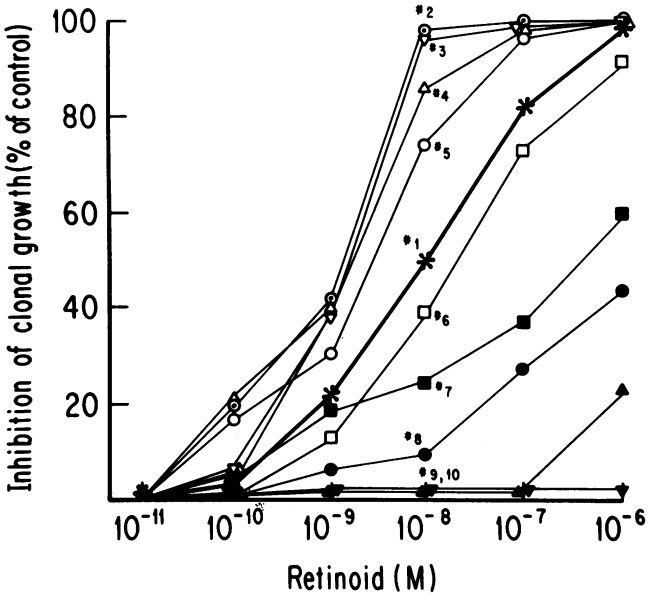


Figure 1. Dose-response curves of clonal growth inhibition of HL-60 cells by 10 retinoids. Compound No. 1 (•), No. 2 (⊙), No. 3 (▽), No. 4 (△), No. 5 (○), No. 6 (□), No. 7 (■), No. 8 (●), No. 9 (▲), No. 10 (▼). Cells were plated in soft agar in the presence of different concentrations of the various retinoids. Results are expressed as the percentage of clonal growth inhibition in culture dishes containing the retinoid as compared with control dishes without the retinoid. Each point represents the mean percentage of three independent experiments performed with triplicate dishes per point.

retinamide, and *N*-(2,3-dihydroxypropyl) retinamide were also inactive at concentrations of 10^{-11}–10^{-6} M (data not shown).

We determined whether the retinoids had a similar structure-activity relationship on the clonal growth of a less mature human myeloid leukemia cell line, known as KG-1. These cells are at the myeloblast stage of differentiation, and early passage of the cells are unique because their clonal growth is dependent on CSF (7). The various retinoids had a structure-activity pattern with the KG-1 cells that was similar to that of the clonal growth of HL-60 cells (Fig. 2). The KG-1 cells were even more sensitive than HL-60 cells to growth inhibition by the more potent analogues. For example, a 50% inhibition of growth was achieved by retinoid Nos. 3 and 4 at 3×10^{-10} and 4×10^{-10} M, respectively (Table II). The only exception was the TTNPB analogue (No. 5), which was more inhibitory of HL-60 than KG-1 clonal growth. In a pattern similar to our findings with HL-60, the compounds with a derivatized terminal carboxyl moiety were weakly active (Nos. 7 and 8) or inactive (Nos. 9 and 10) in clonal growth inhibition of KG-1 cells.

We examined the ability of the retinoids (Nos. 1–10), (10^{-10}–10^{-6} M) to affect the clonal growth of the M1 murine myeloblast cell line. This cell line is induced to differentiate by a wide variety of compounds (14). We found that only high concentrations (1×10^{-6} M) of the indanyl analogue of retinoic acid (No. 2) inhibited clonal growth (26%) as compared with control plates containing no retinoid. All other retinoids ($\leq 10^{-6}$ M) had no effect on clonal growth of M1 cells.

We studied the potency of different retinoids (Nos. 1–10) over a wide range of concentrations (10^{-10}–10^{-6} M) to induce differentiation of HL-60 cells as assessed by the ability of the cells to reduce NBT. Table II shows the concentration of each of the compounds required to achieve differentiation of 50% of HL-60 cells. The ability of the retinoids to induce differentiation of HL-60 cells paralleled their inhibition of clonal growth of HL-60 and KG-1 cells. Compounds with a terminal carboxyl function were potent inducers. Derivatization of the carboxyl group (Nos. 7 and 8) significantly reduced the activity to induce

Table II. Concentration of Retinoids Inhibiting 50% Clonal Growth or Inducing 50% Differentiation of Leukemic Cells

	Inhibition of clonal growth ED_{50}		Induction of differentiation* ED_{50}
Retinoid	KG-1	HL-60	HL-60
	M	*M*	*M*
Analogues containing a terminal carboxyl group			
No. 1‡	4×10^{-9}	1×10^{-8}	1×10^{-7}
No. 2	1×10^{-9}	2×10^{-9}	1×10^{-8}
No. 3	3×10^{-10}	2×10^{-9}	2×10^{-8}
No. 4	4×10^{-10}	2×10^{-9}	1×10^{-8}
No. 5	7×10^{-9}	3×10^{-9}	2×10^{-8}
No. 6	5×10^{-9}	2×10^{-8}	5×10^{-7}
Analogues with a derivatized terminal carboxyl group			
No. 7	$>10^{-6}$	4×10^{-7}	$>10^{-6}$
No. 8	$>10^{-6}$	$>10^{-6}$	$>10^{-6}$
No. 9	$>10^{-6}$	$>10^{-6}$	No induction at 10^{-6}
No. 10	$>10^{-6}$	$>10^{-6}$	No induction at 10^{-6}

* Induction of differentiation determined by ability of cells to reduce nitroblue tetrazolium.
‡ All-*trans*-retinoic acid.

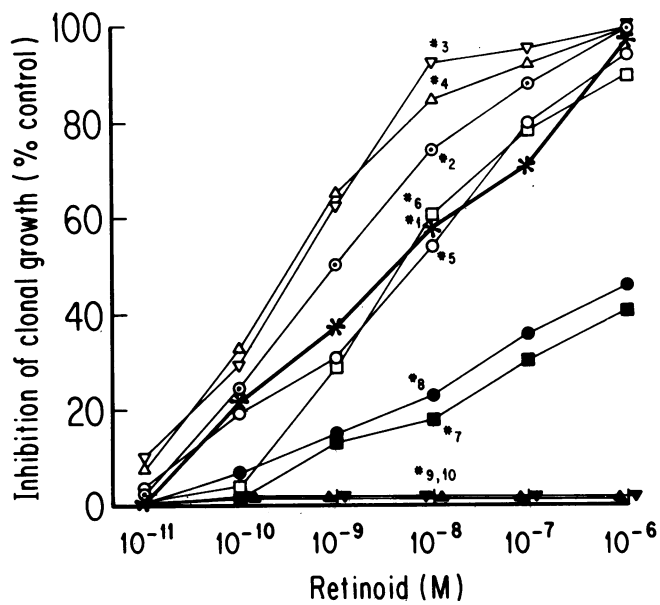


Figure 2. Dose-response curves of clonal growth inhibition of KG-1 myeloblasts by 10 retinoids. Compound No. 1 (•), No. 2 (⊙), No. 3 (▽), No. 4 (△), No. 5 (○), No. 6 (□), No. 7 (■), No. 8 (●), No. 9 (▲), No. 10 (▼). Cells were plated in soft agar in the presence of different concentrations of the various retinoids. Results are expressed as described in the legend of Fig. 1.

differentiation in HL-60 cells. Retinoids with an amide terminus (Nos. 9 and 10) had no impact on the differentiation process. We also examined the ability of the retinoids (Nos. 1–10) to induce differentiation of KG-1 cells as measured by morphology and capability to reduce NBT. None of the retinoids induced differentiation of KG-1 cells.

Effect of a combination of retinoids on clonal growth of HL-60 cells. We studied the effect of a combination of two retinoids with different ring and side-chain structures on leukemic cell growth to help elucidate the mechanism of action of the different retinoids on the clonal growth of leukemic cells. We tested a combination of retinoid No. 2 (which is a 2,2-dimethyl indanyl analogue) and No. 4 (which differs from all-*trans*-retinoic acid by the addition of two methyl groups) (Fig. 3). This combination additively inhibited clonal growth of HL-60 cells at each concentration of the compounds tested (10^{-11}–10^{-9} M).

Effect of retinoids on clonal proliferation of normal human myeloid stem cells. We studied the effect of retinoids (Nos. 1–10) on the clonal growth of normal human myeloid progenitor cells (GM-CFC). We found that retinoids Nos. 1–8 had a 1.5–2.1-fold stimulatory effect on GM-CFC formation in the presence of a submaximal stimulating concentration of CSF (Fig. 4). The addition of analogues with a free carboxyl group (Nos. 1–6) to the culture plates markedly increased the formation of myeloid colonies. These compounds achieved a maximal enhancement at 1×10^{-7} M, increasing the number of GM-CFC in the range of 155–208% as compared with plates not containing a retinoid.

The concentration of the ethyl ester retinoids (Nos. 7 and 8) required to maximally enhance the formation of normal human myeloid colonies was ~10–100-fold higher (1×10^{-6} M) than that for the compounds with a terminal carboxyl group. At this concentration, the most potent analogues (Nos. 1–5) became less active, probably because of toxic effects. At 1×10^{-5} M, all retinoids inhibited GM-CFC formation. The amide derivatives (Nos. 9 and 10) showed no stimulation of normal human progenitor cells. Table III shows the concentrations of the retinoids that achieved 150% stimulation of GM-CFC as compared with control plates containing no retinoid. In general, the rank-order of the retinoids in their potency to stimulate normal

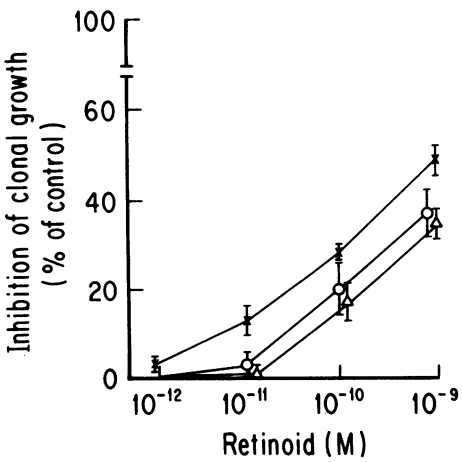


Figure 3. Effect of the combination of two retinoids on clonal growth inhibition of HL-60 cells. Compound No. 2 (○), No. 4 (△), Nos. 2 plus 4 (×). Cells were plated in soft agar in the presence of different concentrations of the two retinoids either alone or in combination. Results are expressed as mean percentage (±SD) of clonal growth inhibition in culture dishes containing the retinoid(s) as compared with control plates not containing the retinoid(s). The experiment was performed three times in triplicate dishes.

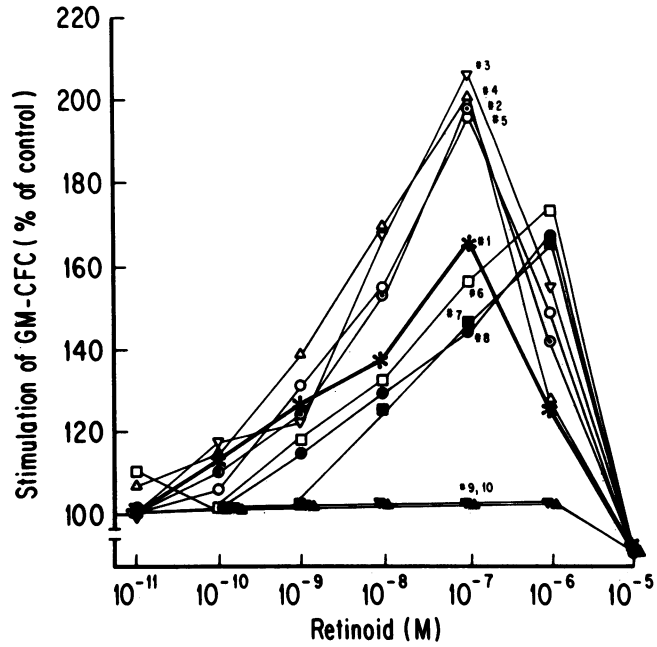


Figure 4. Effect of various concentrations of 10 retinoids on human marrow GM-CFC formation in the presence of 1.5% CSF-rich conditioned medium. Compound No. 1 (•), No. 2 (⊙), No. 3 (▽), No. 4 (△), No. 5 (○), No. 6 (□), No. 7 (■), No. 8 (●), No. 9 (▲), No. 10 (▼). Colony results are expressed as a mean percentage of control dishes that contained no retinoid. Each experiment was performed three times in triplicate plates.

Table III. Concentration of Various Retinoids Inducing a 150% Stimulation of GM-CFC

Retinoid	SD_{150}*
	M
Analogues containing a terminal carboxyl group	
No. 1‡	3×10^{-8}
No. 2	8×10^{-9}
No. 3	3×10^{-9}
No. 4	4×10^{-9}
No. 5	6×10^{-9}
No. 6	6×10^{-8}
Analogues with a derivatized terminal carboxyl group	
No. 7	1×10^{-7}
No. 8	1×10^{-7}
No. 9	No stimulation
No. 10	No stimulation

* SD_{150}, concentration effective in achieving a 150% stimulation of GM-CFC as compared with control dishes containing no retinoid.
‡ All-*trans*-retinoic acid.

human myeloid colony-forming cells paralleled their potency to inhibit leukemic colony-forming cells.

We examined the effect of the combination of two retinoids with different side-chain and ring structures (Nos. 2 and 4 at 1 $\times 10^{-7}$ M for each compound) on the proliferation of normal GM-CFC. Mean stimulation for each compound alone was 194%±5 (±SD) for retinoid No. 2, and a mean 194%±12 (±SD) for retinoid No. 4 as compared with control dishes which contained CSF alone. A mean stimulation of 226%±15 (±SD) occurred in experimental dishes that contained 10^{-7} M of both retinoid No. 2 and No. 4. (Control dishes formed 120±8 (±SD) colonies.) We subjected the data to an analysis of variants which showed that the combination of the two agents had neither an additive effect ($P < 0.001$) nor the same effect ($P < 0.001$) as each agent alone.

Mechanism of stimulation of GM-CFC by retinoids. We performed a series of experiments in an attempt to elucidate how the various retinoids might enhance clonal growth of normal human GM-CSF. One possibility is that the retinoids stimulate an accessory marrow cell to synthesize CSF. To test this hypothesis, we incubated bone marrow cells in liquid culture for 5 d with the potent retinoid No. 2 (10^{-7} M) and harvested the CM. The CM was exposed for 3 h to bright sunlight, which abolished the activity of the retinoid, and then was tested for CSF activity. No difference in number of GM-CFC occurred between culture plates containing CM of marrow cells exposed to retinoid No. 2 (mean 5.5±0.8 [±SD]) and control plates (mean 5.25±0.9 [±SD]) containing CM of marrow cells which were not exposed to a retinoid. Positive control plates, to which 1.5% CSF-rich CM was added, contained a mean 45.8±5 (±SD) GM-CFC.

We further studied the retinoid-mediated enhancement of myeloid clonal growth by performing CSF dose-response experiments in the presence of the maximally stimulating concentration of retinoid No. 4 (10^{-7} M) (Fig. 5). No GM-CFC formation could be observed in plates containing No. 4 alone, indicating that the compound is not by itself a growth factor for myeloid stem cells. However, in the presence of increasing concentrations of CSF, plates containing the retinoid had an ~1.8–2.0-fold enhancement of GM-CFC formation as compared with plates containing CSF alone. Even in the presence of maximally stimulating concentrations of CSF, the retinoid continued to enhance colony formation 1.8-fold as compared with plates containing CSF alone. (Total colony numbers were a mean 163±8 (±SD) per plate in those which contained no retinoid, and a mean 293±19 (±SD) per plate in those which contained retinoid No. 4). Similar experiments were done using maximally stimulating concentrations of both retinoid No. 2 (10^{-7} M) and either recombinant GM-CSF (dilution of 1:8,000) or partially purified GCT-CSF (20 μl/ml). Again, the retinoid continued to enhance colony formation. Mean values were 170%±22 (±SD) and 180%±25 (±SD) compared with control plates containing the maximally stimulating concentrations of CSF alone.

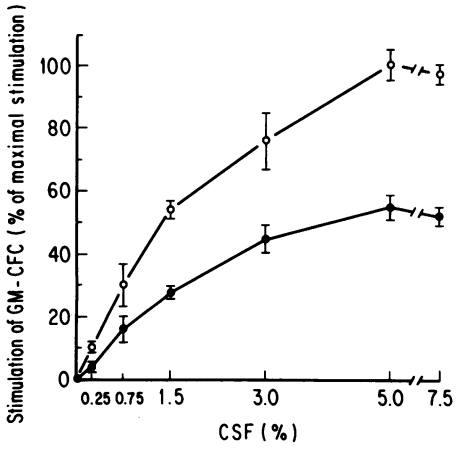


Figure 5. The effect of the 4,4-dimethyl analogue of retinoic acid (No. 4, 1 $\times 10^{-7}$ M) on the formation of GM-CFC in culture plates containing different concentrations of CSF. (●) control; (○) retinoid present in culture. Each point represents mean percentage±SD of two independent experiments carried out in triplicate plates.

Discussion

Our data defined the structure-activity relationship of 10 retinoids as measured by clonal growth inhibition of the HL-60 and KG-1 human myeloid cell lines. We found that retinoic acid derivatives with a carboxyl group were significantly more active than retinoids with a derivatized carboxyl moiety. Strickland et al. (15) recently emphasized the importance of the terminal carboxyl group in experiments that tested the ability of benzoic acid derivatives of retinoic acid to induce differentiation of HL-60 cells and murine F9 teratocarcinoma cells. Replacing the terminal acid group of TTNPB by hydrogen decreased activity with HL-60 cells by at least 10-fold. Increasing alkyl group substitution on the lipophilic head of the retinoid skeleton also enhanced activity. We found that the addition of two geminal methyl groups to the cyclohexenyl ring of all-*trans*-retinoic acid at position 3 or 4 increased the clonal leukemic cell inhibitory activity ~10-fold. Also, these retinoids were more potent than TTNPB, suggesting that the replacement of the 11*E*, 13*E*-double bond system by a benzene ring does not enhance biological activity in these systems. This finding is supported by the lower ED_{50} values found for retinoid No. 2 compared with No. 5.

We observed a similar structure-activity relationship between the retinoids in their growth inhibition of two human leukemic cell lines, HL-60 and KG-1. This suggests a common structural requirement for inhibition of human myeloid leukemic cells, even though the cells are blocked at different stages of differentiation and have different responsiveness to CSF (16). The potency of the retinoids to induce differentiation of HL-60 generally paralleled the ability of these compounds to inhibit clonal growth of both KG-1 and HL-60 cells. In contrast, the myeloblastic KG-1 cells could not be induced to undergo differentiation by the retinoids. Taken together, our data show that certain retinoids are potent inhibitors of leukemic cell proliferation without necessarily inducing differentiation of the cells.

The inhibition of growth of leukemic cells by the retinoids is unlikely to be merely a "toxic" effect for several reasons: (*a*) Very low concentrations of the potent retinoids (Nos. 1–6) markedly inhibited clonal growth (ED_{50} 3×10^{-10}–2.0×10^{-8} M). (*b*) These concentrations of the retinoids had little effect on the clonal growth of the murine myeloid leukemia cell line, M1. (*c*) The same concentrations of these retinoids that inhibited leukemic colony formation markedly stimulated normal human myeloid colony formation.

Retinamides, the amide derivatives of all-*trans*-retinoic acid, are of interest because they have reduced toxicity in animal

models, but nevertheless retain their high biological activity in these models (17–19). Retinamides inhibit chemically-induced carcinogenesis in the mammary gland and urinary bladder of experimental animals (19–21) and reverse epithelial keratinization of hamster trachea (22, 23). We found that retinamides had no activity on HL-60, KG-1, and normal human GM-CFC. This inactivity could be explained by the absence of the necessary metabolizing systems in normal and leukemic human hematopoietic cells. Probably, as a class, retinamides must be converted to retinoic acid to be active, and perhaps human hematopoietic cells cannot metabolize retinamides to retinoic acid.

Previously, we showed that all-*trans*-retinoic acid enhanced the in vitro growth of normal myeloid progenitor cells (2). Our present study shows a relationship between retinoid structure and the ability of the compound to stimulate normal myeloid colony formation. Furthermore, we observed a parallel potency of the various retinoids both in stimulating normal myeloid clonal growth and in inhibiting leukemic myeloid clonal growth. These findings suggest, but do not prove, that certain common structural moieties of the retinoids are important in the modulation of both normal and abnormal hematopoiesis. For example, the terminal carboxyl moiety is the most important structure for both the inhibitory and the stimulatory activities. Changes in ring structure (substitution of the β-cyclogeranylidene group with a 1,1,3,3-5-indanyl ring system) and side-chain (addition of two methyl groups to the cyclohexenyl ring) also contribute to the stimulation of normal GM-CFC and inhibition of leukemic clonogenic cells.

In a further attempt to understand how retinoids modulate hematopoietic clonal proliferation, we added in combination two potent retinoids (Nos. 2 and 4) having different ring and side-chain structures to culture plates containing either HL-60 or normal human marrow cells. The combinations of the retinoids had an additive, but not synergistic, effect on the inhibition of HL-60 clonal growth. A subadditive effect on the stimulation of normal human GM-CFC occurred when each retinoid, at its maximally stimulating concentration, was added in combination to the culture plates. Neither experimental result allows us to determine unequivocally whether structurally dissimilar retinoids act by a similar cellular pathway. A synergistic response by the combination of retinoids with HL-60 cells would have suggested that different retinoids mediate their effects by different cellular mechanisms. Likewise, a clearly additive enhancement of GM-CFC by maximally stimulating concentrations of both retinoids would again have suggested that structurally different retinoids might mediate their actions by alternate cellular pathways. In contrast, although not conclusive, our data are consistent with the hypothesis that structurally dissimilar retinoids can effect hematopoietic proliferation through a common cellular pathway.

One potential common pathway of retinoid action is a binding to a cytoplasmic retinoic acid binding protein (cRABP). A positive correlation occurs between binding of some retinoids to cRABP and their biological potency in various tissues (23, 24). The terminal carboxyl group seems to be essential for the binding ability of the retinoids to cRABP (24). We found in previous studies that KG-1, HL-60, and acute myeloid leukemic cells harvested from patients do not possess detectable cRABP (3). Nevertheless, the rank-order of potency of retinoid action on hematopoietic cells parallels the rank-order of potency of retinoid action on other tissues that do contain cRABP. These data may suggest that retinoids mediate their biological effects through different mechanisms in different tissue; or, also likely, that they may mediate through the same mechanism and that this mechanism is independent of cRABP.

We found that retinoids do not act by themselves as a myeloid growth factor (CSF), nor do the retinoids stimulate cells in culture to produce additional CSF. Our CSF–dose-response experiments showed that, even in the presence of maximally stimulating concentrations of CSF, the new retinoids enhanced normal human myeloid colony formation. These findings suggest that retinoids may either recruit earlier progenitors to become GM-CFC or that retinoids might enhance the sensitivity of normal human GM-CFC to the action of CSF, perhaps by increasing CSF receptor number or affinity on the surface of the cell. Further studies are in progress in an effort to understand the difference in response of normal and leukemic colony-forming cells to retinoids.

Acknowledgments

We would like to thank Suzanne Bookstaver and Regina Simon for their excellent secretarial help; Daniel Heitjan for superb statistical analysis; Dr. Richard Moon and Dr. Michael Sherman for generously providing retinoids used in this study; Dr. Judy Gasson and Dr. David Golde for kindly providing recombinant human CSF, and Dr. Camille Abboud for generously providing GCT-CSF.

This work was supported in part by US Public Health Service grants CA26038, CA32737, CA33936, CA30512 and CA32428 (Dr. Dawson); and by The Dr. Murray Geisler Memorial Fund and The Louis Fagin Leukemia Research Foundation. Dr. Tobler is a Fellow of the Swiss and Bernese Cancer Ligues.

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