A mutated retinoic acid receptor- α exhibiting dominant-negative activity alters the lineage development of a multipotent hematopoietic cell line

Schickwann Tsai,¹ Stephen Bartelmez,¹ Richard Heyman,² Klaus Damm,³ Ronald Evans,³ and Steven J. Collins¹

¹Program in Molecular Medicine, Fred Hutchinson Cancer Research Center, Seattle, Washington 98104 USA; ²Ligand Pharmaceuticals, San Diego, California 92121 USA; ³The Salk Institute for Biological Studies, Howard Hughes Medical Institute, La Jolla, California 92037 USA

The retinoic acid receptor (RAR α) is expressed in virtually all hematopoietic lineages, but the role of this transcription factor in regulating the growth and differentiation of hematopoietic progenitors is unknown. We have constructed a mutant RAR α that both exhibits dominant-negative activity against the normal RAR α in transient expression assays in mouse fibroblasts and inhibits retinoic acid-induced neutrophilic differentiation of the HL-60 human promyelocytic leukemia cell line. When this dominant-negative RAR α construct is introduced into the multipotent interleukin-3-dependent FDCP mix A4 murine hematopoietic cell line, there is a rapid switch from spontaneous neutrophil/monocyte differentiation to basophil/mast cell development. Thus, in this multipotent hemopoietic cell line the normal RAR α transcription factor and/or related molecules appear to promote the differentiation of neutrophils and monocytes but suppress the development of basophils/mast cells.

[**Key Words:** RAR α ; hemopoietic differentiation; transcription factor; dominant-negative activity; transient expression assay]

Received May 18, 1992; revised version accepted September 17, 1992.

Retinoic acid (RA), the natural acidic derivative of vitamin A (retinol), is a critical molecule regulating growth and differentiation of a wide variety of cells. RA is centrally involved in epithelial differentiation (Lotan 1980), plays a critical role as a tissue-specific morphogen during embryogenesis (Thaller and Eichele 1987), and suppresses malignant transformation of epithelial cells both in vitro (Merriman and Bertam 1979) and in vivo (Hong et al. 1990). These diverse and complex biologic effects of RA are mediated through a number of closely related nuclear RA receptors (RARs) that are members of the steroid/thyroid hormone receptor superfamily and possess discrete DNA-binding and RA (ligand)-binding domains (Evans 1988).

The expression of RARs is widespread, with RAR mRNA noted in most fetal and adult tissue (Zelent et al. 1989). Unlike the steroid hormone receptors, the RA receptors, as well as thyroid hormone receptors, appear to be associated with chromatin and may be constitutively bound to *cis*-acting regulatory sequences in the absence of ligand (Lavin et al. 1988; Damm et al. 1989). Thus, these receptors may have different regulatory roles in the absence and presence of ligand.

Several lines of indirect evidence suggest that RA and

RARs may be involved in regulating the development of hematopoietic progenitors. For example, RAR mRNA (predominantly RAR α) is widely expressed in different hematopoietic cell types (Gallagher et al. 1989; de The et al. 1989; Largman et al. 1989). Treatment of a human myeloid leukemia cell line HL-60 with all-*trans* RA (ATRA) induces these cells to undergo terminal neutrophilic differentiation, and this induction is directly mediated through RAR α (Breitman et al. 1980; Collins et al. 1990). In addition, human acute promyelocytic leukemia (APL) exhibits a specific 15;17 chromosome translocation involving RAR α , which presumably results in the disruption of a normal, albeit unknown, regulatory function of this particular RAR (Borrow et al. 1990; de The et al. 1990b, 1991; Alcalay et al. 1991; Kakizuka et al. 1991). Furthermore, ATRA induces the leukemia cells from APL patients to differentiate into mature neutrophils both in vitro and in vivo (Huang et al. 1988; Castaigne et al. 1990; Chomienne et al. 1990; Warrell et al. 1991). However, it is currently unknown whether RA and RARs might also be involved in regulating normal hematopoietic differentiation.

Dominant-negative genes encode abnormal proteins that repress the function of their normal counterparts in

a dominant manner (Herskowitz 1987). A prototype example is the *v-erbA* oncogene, which represses the transcription-regulating function of normal thyroid hormone receptors (encoded by *c-erbA*), and this contributes to the transformation of erythroblasts (Zenke et al. 1988; Damm et al. 1989). A small deletion in the carboxyl terminus of *v-erbA* appears to be responsible for most of the dominant-negative activity of this oncogene in transient expression assays (Damm et al. 1989; Sap et al. 1989; Zenke et al. 1990). Thus, one way to examine the role of RAR α in hematopoiesis is to utilize dominant-negative receptor constructs that would suppress normal RAR function in hematopoietic precursors and then determine whether the expression of these constructs would alter the growth and differentiation of these blood cell progenitors.

In this study we have constructed a mutant RAR α , which exhibits dominant-negative activity in both mouse NIH-3T3 fibroblasts and in the HL-60 human promyelocytic leukemia cell line. This construct, when introduced into the multipotent interleukin-3 (IL-3)-dependent FDCP mix A4 murine hematopoietic cell line, induces a marked switch from spontaneous neutrophil/monocyte differentiation to the development of basophils/mast cells. These observations suggest that RARs play important roles in the development of myeloid progenitors.

Results

A truncated RAR α construct exhibits dominant-negative activity

As detailed in Materials and methods we created an RAR α cDNA with a truncation of the sequences coding for the carboxy-terminal 59 amino acids, as well as a portion of the 3'-untranslated region. This truncated cDNA (designated RAR α 403) encodes a peptide of 403 amino acids containing the amino terminus, the DNA-binding domain, and part of the hormone-binding domain of RAR α (Fig. 1A,B). This truncated receptor was inserted into the retroviral vector LXS α N (Fig. 1C) to generate the recombinant vector designated LRAR α 403SN (Fig. 1D). To determine whether the truncated RAR α 403 construct exhibits dominant-negative activity with respect to the normal RAR α , we performed *trans*-activation assays in mouse NIH-3T3 tk⁻ cells. When an expression vector harboring the normal RAR α gene (pEMSV-RAR α) was cotransfected with a chloramphenicol acetyl transferase (CAT) reporter construct, pTRE-CAT, containing a synthetic thyroid hormone/RA-responsive element (TRE) (Umesono et al. 1988), there was RA-induced *trans*-activation of the CAT reporter gene (Fig. 2A, lanes 5,6). In contrast, when both pEMSV-RAR α and the mutant LRAR α 403SN construct were cotransfected into the same target cells, there was a dose-dependent suppression of this RAR α -mediated *trans*-activation (Fig. 2A, lanes 6–10). An RAR α /RAR α 403 DNA ratio of 1 : 6 suppressed most of the activity of the normal RAR α . When this experiment was

Figure 1. Structures of RAR α 403 and the retroviral vector LRAR α 403SN. (A) Schematic representation of human RAR α cDNA (Giguere et al. 1987). The bar represents the open reading frame that encodes the normal human RAR α (462 amino acids). The solid region represents the DNA-binding domain of RAR α . (RA) The hormone-binding domain. (B) Structure of the truncated receptor RAR α 403 cDNA. Nucleotides 1311–1596 of human RAR α cDNA were deleted by digestion with *Sma*I, and a stop codon was inserted at amino acid position 404 using an *Nhe*I linker. The truncated receptor consists of 403 amino acids and is designated RAR α 403. (C) The retroviral vector LXS α N (from Miller and Rosman 1989). (LTR) Molony murine leukemia virus long terminal repeat. Arrows indicate the transcription initiation site. (SV) SV40 promoter; (NEO) the neomycin phosphotransferase gene; (pA) the polyadenylation site. (D) The structure of the retroviral vector LRAR α 403SN. RAR α 403 was inserted into the *Eco*RI–*Bam*HI cloning sites of LXS α N. This vector may be used both as an expression vector in transient expression assays and for producing retroviral particles.

performed using a different reporter, that is, pRRE-CAT, which harbors the natural RA-response element (RRE) of RAR β (de The et al. 1990a), there was prominent suppression of the endogenous RAR activities (Fig. 2B, lanes 3,4) and partial suppression of exogenous RAR α activity (Fig. 2B, lanes 6–9). These results indicate that the RAR α 403 construct exhibits dominant-negative activity with respect to the normal RAR α in mouse fibroblasts.

To determine whether this same RAR α 403 construct also exhibits dominant-negative activity in hematopoietic cells, we performed transient expression assays in the mouse hematopoietic cell line FDCP mix A4 (described below) by electroporation. Although the RAR α 403 construct suppressed some endogenous RAR activities in this cell line (data not shown), the extremely low efficiency of gene transfer achievable by electroporation in these target cells (estimated to be $1/10,000$ that of NIH-3T3) made them unsuitable for adequately assessing the suppressing potential of RAR α 403 by transient expression. To further examine the dominant-negative function of RAR α 403 in hematopoietic cells, we utilized retrovirus-mediated gene transduction to introduce this mutant re-

Figure 2. The truncated RAR α 403 exhibits dominant-negative function in transient expression assays. (A) The *trans*-activation activities of RAR α and RAR α 403 were studied in NIH-3T3 tk⁻ cells in the absence and presence of 1 μ M RA. An expression plasmid pTRE-CAT was used as the reporter. RAR α represents the expression plasmid pEMSV-RAR α ; RAR α 403 refers to pLRAR α 403SN (Fig. 1D). The volume of lysate used in CAT assay was normalized for transfection efficiency determined by expression of the cotransfected growth hormone reporter pCMV-GH. The DNA ratio of pEMSV-RAR α to pLRAR α 403SN varied from 1 : 1 to 1 : 6. pLXSN was used to equalize the total amount (23.5 μ g/100-mm dish) of DNA and LTRs transfected. (+) 2.5 μ g of plasmid DNA. Higher amounts (5 μ g) of transfected pLRAR α 403SN result in suppression of >60% of endogenous RAR activity (not shown). (B) Similar to A except a different reporter (pRRE-CAT) was used in place of pTRE-CAT. (+) 1 μ g of plasmid DNA. The total amount of DNA was 16 μ g/100-mm dish.

ceptor into the HL-60 human promyelocytic leukemia cell line (Collins et al. 1977). RA induces neutrophilic differentiation of HL-60 cells (Breitman et al. 1980), and this response is mediated directly through RAR α (Collins et al. 1990). As detailed in Materials and methods, we infected HL-60 cells with the amphotropic LRAR α 403SN retroviral vector (Fig. 1D) and utilized Northern blot analysis to identify retroviral vector-infected HL-60 clones that expressed the mutant RAR α 403 mRNA. As a control we also infected HL-60 with the LXSN retroviral vector, which confers G418 resistance but lacks a cDNA insert (Fig. 1C). We then compared the differentiative response of these different retroviral vector-infected HL-60 clones to RA. The control (LXSN)-infected HL-60 clones (D.1 and D.2) exhibited a similar response to RA as detailed previously for the parental HL-60 cells (Breitman et al. 1980), with 80–90% of the cultured cells undergoing morphologic differentiation to mature neutrophils after a 5-day exposure to 10^{-6} M RA (Fig. 3). In contrast, the LRAR α 403SN-infected HL-60 clones (D.2 and D.5) exhibited marked blunting of this differentiative response to RA with no more than 20–25% of the cells differentiating to neutrophils after exposure to concentrations of RA up to 10^{-5} M (Fig. 3).

Figure 3. Expression of RAR α 403 in HL-60 cells blunts RA-induced neutrophilic differentiation. HL-60/LXSN D.1 and D.2 are two randomly selected HL-60 clones infected with the control vector LXSN. HL-60/LRAR α 403SN D.2 and D.5 are two HL-60 clones infected with LRAR α 403SN that express high levels of the full-length 4.7-kb retroviral vector mRNA containing RAR α 403 sequence. Cells were induced with the indicated concentrations of RA for 5 days. Differential counts were then performed on Wright–Giemsa-stained cytospin preparations and represent the means of triplicate experiments.

These results indicate that the truncated RAR α 403 construct also exhibits dominant-negative activity in the HL-60 hematopoietic cell line, and its expression is not lethal in these particular neutrophil progenitors.

Characteristics of FDCP mix A4 cells

To examine the impact of the dominant-negative RAR α 403 on the commitment and differentiation of multipotent hematopoietic progenitors, we utilized the FDCP mix A4 cell line. This IL-3-dependent cell line was established from a murine long-term bone marrow culture and behaves like normal hematopoietic stem cells in many respects: it is nonleukemogenic, responds to several hematopoietic growth factors in a physiologically relevant manner, and spontaneously generates progenitors committed to different hematopoietic lineages (Spooncer et al. 1986; Just et al. 1991). When cultured in a medium containing horse serum and WEHI 3B cell line-conditioned medium (as a source of IL-3), the line maintained in our laboratory consists mainly of undifferentiated blasts (see Fig. 5A, below) but spontaneously differentiates into neutrophils and monocytes/macrophages (~10–30% combined) and, to a much lesser extent, into mast cells/basophils (1–2%). Northern blot analysis indicates that these cells express endogenous 3.6- and 2.6-kb RAR α mRNA transcripts (Fig. 4A, lane 1). Thus, this multipotent hematopoietic cell line appears to be well suited for investigating the impact of the dominant-negative RAR α 403 on hematopoietic differentiation.

Expression of RAR α 403 in FDCP mix A4 cells triggers the development of terminally differentiating basophils/mast cells

We infected the FDCP mix A4 cells with the amphotropic dominant-negative LRAR α 403SN retroviral vector, as well as the control vectors (LXSN and LRAR α SN; the latter harbors the cDNA of normal human RAR α) and selected the infected cells in a medium containing G418 and IL-3. Examination of Wright–Giemsa-stained cytopsin preparations of FDCP mix A4 cells infected with the control retroviral vectors (LXSN and LRAR α SN) revealed the predominant neutrophilic/monocytic differentiation characteristic of the uninfected parental FDCP mix A4 cells (Fig. 5A,B,D). In contrast, a very large number of differentiated cells with numerous, intensely basophilic granules appeared in the culture infected with the LRAR α 403SN retroviral vector within 10 days after the infection (Fig. 5C,E). These latter cells expressed high levels of the 4.7-kb retroviral mRNA harboring the dominant-negative RAR α 403 insert (Fig. 4A, lane 3). The granules of these cells stained metachromatically with toluidine blue (Fig. 5F) and are negative for nonspecific esterase (a monocyte marker) and alkaline phosphatase (a neutrophil marker). These morphological and cytochemical characteristics suggest that these basophilic granular cells are basophils/mast cells.

To further confirm the basophil/mast cell lineage of

Figure 4. High-level expression of LRAR α 403SN and GATA-1 by infected FDCP mix A4 cells. RNAs were prepared from uninfected, control (LXSN)-infected, and LRAR α 403SN-infected FDCP mix A4 bulk cultures and from five clonal lines (labeled LRAR α 403SN c.1–c.5) and subjected to Northern blot hybridization. Ten micrograms of total RNA was applied to each lane and hybridized to the indicated probes. (A) Expression of LRAR α 403SN. The full-length message of LRAR α 403SN is 4.7-kb. The RAR α probe also detects the 3.6- and 2.6-kb endogenous mouse RAR α mRNA. The ratio of RAR α 403 to endogenous RAR α message in cells infected with LRAR α 403SN is >10 : 1. (B) Expression of GATA-1. The 1.8-kb GATA-1 mRNA is indicated.

the basophilic granular cells in the LRAR α 403SN-infected FDCP mix A4 cells, we utilized flow cytometry to examine the expression of cell-surface immunoglobulin E receptor (IgE R), another basophil/mast cell-specific marker (Metzger et al. 1986; Thompson et al. 1990). A high percentage (38.4%) of cells expressing surface IgE R was noted in LRAR α 403SN-infected cells, whereas only 0.4% of LXSN-infected cells were positive for IgE R (Table 1). We also phenotyped LRAR α 403SN as well as the control (LXSN and LRAR α SN)-infected cultures utilizing monoclonal antibodies specific for mouse neutrophils (antibody 7/4; Hirsch and Gordon 1983) and macrophages (MAC-1 antibody). Consistent with our morphological observations, the LXSN- or LRAR α SN-infected FDCP mix A4 cells contained 10–30% 7/4-positive cells, whereas the LRAR α 403SN-infected cultures exhibited significantly lower percentages (0–3.5%) of neutrophil antigen-positive cells (Table 1).

Histamine is a lineage-specific marker for basophils/mast cells (Riley and West 1953; Denburg 1992). Therefore, we measured both the histamine content within these infected cells and the concentration of histamine in the culture supernatant (Table 1). We found that the histamine content of the LRAR α 403SN-infected cells (2.17 pg/cell, assuming every cell contained equal amounts of histamine to allow comparison) was ~10-fold greater than the histamine content of the control LXSN-infected cells (0.25 pg/cell) and was similar to the histamine content of normal basophils/mast cells. Moreover, the histamine concentration in the supernatant of the LRAR α 403SN-infected cultures (3×10^{-4} M) was ~130-fold increased over the histamine concentration in the control (LXSN)-infected cells (2.3×10^{-6} M) (Table 1).

Figure 5. Morphology of uninfected and infected FDCP mix A4 cells. (A) Uninfected FDCP mix A4 cells. Most cells are undifferentiated blasts. The arrow points to a mature neutrophil. Arrowheads indicate differentiating cells. (B) FDCP mix A4 infected with control vector LXS. The arrow points to a neutrophil. (C) FDCP mix A4 infected with LRAR α 403SN. Most cells (arrows) are differentiated and contain numerous basophilic granules and oval nuclei. (D) A higher magnification of B. (E) A higher magnification of C. (F) Toluidine blue stain of FDCP mix A4 cells infected with LRAR α 403SN. The granules in many cells (arrows) stain metachromatically with toluidine blue, indicating that these cells are of the basophil/mast cell lineage. (A–E) Wright–Giemsa stain. Bars, 10 μ m. All except D and E have the same magnification.

We also quantitated the production of basophils/mast cells by FDCP mix A4 cells following retroviral infection and G418 selection. As shown in Figure 6, the production of basophils/mast cells in LRAR α 403SN-infected cultures was 20- to 70-fold higher than in LXS-infected

cultures in the first 2 weeks after the infection. These observations indicate that retrovirus-mediated gene transfer and expression of the dominant-negative RAR α 403 construct in FDCP mix A4 cells resulted in a switch from spontaneous neutrophil/monocyte differen-

Table 1. Cell-surface antigen expression and histamine production by LXSN- and LRAR α 403SN-infected FDCP mix A4 cells

Cell line	Expression ^a			Histamine ^b	
	7/4(+) (%)	MAC-1(+) (%)	IgE R(+) (%)	intracellular (pg/cell)	in supernatant (M)
Experiment 1					
LXSN infected	28.6	8.3	ND	ND	ND
LRAR α 403SN infected	1.4	1.0	ND	ND	ND
Experiment 2					
uninfected	11.1	2.0	ND	0.14	1.6×10^{-6}
LXSN infected	14.5	2.8	0.4	0.25	2.3×10^{-6}
LRAR α SN infected	11.4	1.0	ND ^c	ND	ND
LRAR α 403SN infected	3.6	1.0	38.4	2.17	3.0×10^{-4}

^aCell-surface antigen expression was analyzed using monoclonal antibodies specific for mouse neutrophils (7/4), monocytes/macrophages (MAC-1), or IgE receptor, as detailed in Materials and methods.

^bHistamine content was determined by radioimmunoassay. For quantification of intracellular histamine, cells were lysed in distilled water. Negative controls included NIH-3T3 fibroblast, which showed no measurable intracellular histamine, and complete culture medium, which contained $<10^{-9}$ M histamine.

^cExamination of Wright-stained cytospin preparations revealed very few basophils/mast cells. The LRAR α SN-infected culture was indistinguishable from uninfected or LXSN-infected FDCP mix A4 cells by morphology.

tiation to the development of basophils/mast cells. We have observed this same phenotype (i.e., markedly increased basophil/mast cell and decreased neutrophil/monocyte development) in the LRAR α 403SN-infected

FDCP mix A4 cells during continuous passaging for >9 months.

FDCP mix A4 cells infected with LRAR α 403SN exhibit diminished self-renewal

The LRAR α 403SN-infected FDCP mix A4 cells exhibited a marked decrease in growth rate (population doubling time of ~ 120 hr) compared with the control (LXSN)-infected cultures (population doubling time of ~ 30 hr) (Fig. 7). The prolongation of population doubling time of LRAR α 403SN-infected FDCP mix A4 cells occurred around passages 2–5 and coincided with the appearance of a large number of basophils/mast cells and a decreased number of undifferentiated blasts. These ob-

Figure 6. Net production of basophils/mast cells in infected FDCP mix A4 cells. FDCP mix A4 cells (1.4×10^6 cells) were infected with LXSN (hatched bars) or LRAR α 403SN (solid bars) vectors using the supernatant infection method and selected with G418 from days 2–8. Similar percentages (10–15%) of cells infected with either retroviral vector were G418 resistant. These G418-resistant cells were subsequently maintained in culture medium supplemented with WEHI-3B-conditioned medium (as a source of IL-3) and subcultured every 3 days. Production of basophils/mast cells was calculated from the total number of cells and the differential counts on Wright-stained cytospin preparations on each feeding. The total numbers of cells (corrected for subculturing ratios) for LXSN-infected cultures were 1.3×10^6 (day 8), 10.1×10^6 (day 11), and 93.8×10^6 (day 14). Corresponding numbers for LRAR α 403SN-infected cultures were 1.3×10^6 (day 8), 5.5×10^6 (day 11), and 16.8×10^6 (day 14). All numbers represent mean of duplicates.

Figure 7. Growth curves of FDCP mix A4 cells infected with LRAR α 403SN. Cultures were initiated with 2×10^5 low-passage (p.7) cells of bulk cultures of FDCP mix A4 cells infected with the indicated retroviral vectors and subcultured every 3–4 days. Each data point represents the mean of duplicates.

servations suggest that the decreased growth rate of LRAR α 403SN-infected FDCP mix A4 cells might be due in part to diminished self-renewal of undifferentiated blasts. Therefore, we measured the frequency of cells capable of forming macroscopic colonies in agar cultures in the presence of IL-3. (The macroscopic colonies are defined as colonies with a diameter >0.5 mm after a 14-day incubation and are formed by cells with greater self-renewal capacity.) The frequency of macroscopic colony-forming cells of FDCP mix A4 cells infected with LRAR α 403SN was 0.028%, which was 18-fold lower than that of LXSNI-infected cells (0.5%). Furthermore, even the macroscopic colony forming cells in the LRAR α 403SN-infected cultures demonstrated a relatively lower self-renewal capacity compared with those in the LXSNI-infected cultures because 80% of the individual macroscopic colonies of the latter, when transferred to liquid suspension cultures, were capable of long-term continuous proliferation in IL-3-containing media while only 20% of macroscopic colonies of LRAR α 403SN-infected FDCP mix A4 cells demonstrated this proliferative capacity.

Examination of cytospin preparations of individual macroscopic colonies showed that all such colonies of LRAR α 403SN-infected FDCP mix A4 cells contained high percentages (mean 47.9%) of basophils/mast cells and lower percentages of neutrophils, monocytoid cells,

and undifferentiated blasts when compared with LXSNI-infected cells (Table 2). Thus, the phenotypic change we observed in the bulk culture of LRAR α 403SN-infected FDCP mix A4 cells was recapitulated by all clonogenic cells.

All clonal lines of LRAR α 403SN-infected FDCP mix A4 cells exhibit predominant basophil/mast cell differentiation

The above analysis was performed on "bulk" cultures of G418-resistant retroviral vector-infected FDCP mix A4 cells. We also utilized soft agar cloning and limiting dilution to establish five continuously passaged clonal lines of the LRAR α 403SN-infected cells. All of these clones (c.1–c.5) expressed the appropriate 4.7-kb retroviral mRNA (Fig. 4A). We noted that the phenotype of these subclones was virtually identical to the bulk-infected cells. That is, all five subclones exhibited slower IL-3-dependent growth, and Wright–Giemsa-stained cytospin preparations revealed high percentages of basophils/mast cells and lower percentages of neutrophils and undifferentiated blasts in these subclones when compared with clones of LXSNI-infected FDCP mix A4 cells. This phenotype is further substantiated by flow cytometry using monoclonal antibodies 7/4, MAC-1, and IgE (Table 3).

Table 2. Differential counts of macroscopic colonies in agar culture of LXSNI- and LRAR α 403SN-infected FDCP mix A4 cells

Colony number	Basophil/mast cell (%)	Neutrophil (%)	Monocytoid (%)	Undifferentiated (%)
<i>LXSNI-infected cells</i>				
1	1	13	52	34
2	2	2	18	78
3	1	7	23	69
4	0	13	24	63
5	1	5	31	63
6	0	6	5	89
7	3	16	31	50
8	3	7	43	47
9	0	2	2	96
10	0	7	23	70
Mean	1.1	7.8	25.2	65.9
<i>LRARα403SN-infected cells</i>				
1	59	0	8	33
2	51	0	8	41
3	26	0	7	67
4	52	3	17	28
5	50	3	15	32
6	59	2	17	22
7	25	3	30	42
8	66	0	11	23
9	60	2	14	24
10	31	0	37	32
Mean	47.9	1.3	16.4	34.4

Colony assays were performed as described in Materials and methods. Individual macroscopic colonies (>0.5 mm diam.) were identified with a dissecting microscope, and cytospin preparations were made of individual colonies and stained with Wright–Giemsa stain. Differential counts were performed on 100 cells each.

Table 3. Cell-surface antigen expression by clonal lines of FDCP mix A4 cells infected with LXSN and LRAR α 403SN retroviral vectors

Clone	IgE R(+) (%)	7/4(+) (%)	MAC-1(+) (%)
<i>LXSN-infected cells</i>			
c.1	0.0	17.4	7.4
c.2	6.2	23.1	9.1
c.3	3.9	21.2	29.0
c.4	4.0	24.2	7.9
c.5	3.6	17.8	8.5
Mean	3.5	20.7	12.4
<i>LRARα403SN-infected cells</i>			
c.1	47.4	1.9	0.9
c.2	54.4	1.3	0.9
c.3	66.4	0	0
c.4	56.3	0	0
c.5	74.9	0	0
Mean	59.9	0.6	0.4

Clonal lines were established by limiting dilution subcloning in 96-well plates or in soft agar. Flow cytometry was performed as described in Materials and methods.

Enhanced GATA-1 expression in LRAR α 403SN-infected FDCP mix A4 cells

GATA-1 is a zinc finger-containing transcription factor that is preferentially expressed in hematopoietic cells of the erythroid, megakaryocyte, and mast cell lineages but not in neutrophilic or monocytic lineages (Tsai et al. 1989; Martin et al. 1990). Although GATA-1 was initially identified as a transcriptional regulator of globin gene expression, recent studies show that GATA-1 also binds to the promoter region of the gene of mast cell-specific carboxypeptidase A and activates its expression (Zon et al. 1991). We utilized Northern blot hybridization to compare GATA-1 mRNA levels in the LRAR α 403SN-infected FDCP mix A4 cells versus the control (LXSN)-infected cells. Relatively low levels of GATA-1 mRNA were detected in the LXSN-infected FDCP mix A4 cells. In contrast, a marked increase in steady-state GATA-1 mRNA was noted in the LRAR α 403SN-infected bulk cultures as well as the five individual infected subclones (Fig. 4B). This enhanced GATA-1 mRNA expression noted in the LRAR α 403SN-infected FDCP mix A4 cells further corroborates the other basophil/mast cell characteristics displayed by many of these cells.

Discussion

In this study we have observed that introducing a mutant RAR α exhibiting dominant-negative activity into the IL-3-dependent, multipotent FDCP mix A4 murine hematopoietic cell line results in a dramatic change in the lineage development of these cells. Although the FDCP mix A4 cell line is by no means the equivalent of

normal hematopoietic stem cells, it preserves many properties of multipotent hematopoietic progenitors and thus serves as a useful model for analyzing molecular events involved in myeloid differentiation. This cell line ordinarily commits spontaneously to neutrophilic and monocytic differentiation at moderate frequencies, but upon introduction of the dominant-negative RAR α 403 construct there is a rapid switch to the development of terminally differentiated cells with basophilic cytoplasmic granules (Fig. 5C,E). These differentiated cells exhibit many characteristics of basophils/mast cells, including metachromatic staining with toluidine blue (Fig. 5F), histamine production (Table 1), expression of cell-surface IgE receptor (Table 1), and enhanced GATA-1 mRNA levels (Fig. 4B).

The development of basophils/mast cells in the LRAR α 403SN-infected cultures is attributable to the expression of the dominant-negative RAR α 403 construct. None of the cultures infected with the control vectors (LXSN and LRAR α SN) preferentially differentiates into basophils/mast cells. In contrast, all of the LRAR α 403SN-infected subclones that we have analyzed exhibit predominantly basophil/mast cell phenotype (Tables 2 and 3). Moreover, the characteristic basophil/mast cell morphological and histochemical changes occur rapidly (within 10 days) after infection with this dominant-negative RAR α 403 retroviral construct. These observations exclude rare events such as retroviral vector-induced mutation as the cause of the prominent phenotypic changes in the cultures. In addition, we have observed that continuous passaging of control (LXSN)-infected FDCP mix A4 cells for 9 months does not result in the conversion to basophil/mast cell development, further excluding random mutation as the cause of the observed phenotypic changes.

Our observation that many clones of LRAR α 403SN-infected FDCP mix A4 cells continue to produce a small number of neutrophils and monocytes in addition to the large number of basophils/mast cells (Tables 2 and 3) indicates that the individual clonogenic cells are at least tripotent [i.e., capable of differentiating into (1) basophils/mast cells, (2) neutrophils, and (3) monocytes]. Therefore, the observed phenotypic change does not result from immortalization of any rare committed, unipotent basophil/mast cell progenitors. (In fact, the basophils/mast cells in LRAR α 403SN-infected cultures have a limited life span of 6–12 days and degenerate thereafter.) Rather, this phenotype is the result of a shift by the multipotent FDCP mix A4 cells from the spontaneous production of primarily neutrophils and monocytes to the continuous, preferential production of basophil/mast cells. It should be emphasized that the net production (absolute number) of basophils/mast cells produced by LRAR α 403SN-infected FDCP mix A4 cells is increased by 20- to 70-fold within the first 2 weeks of infection. (Fig. 6). This finding makes it unlikely that the altered FDCP mix A4 phenotype is the result of selective elimination of neutrophils and monocytes in the culture resulting in the progressive accumulation of basophils/mast cells. In this latter scenario, the percentage of ba-

sophils/mast cells would increase in the infected cultures, but the absolute number would not.

Concomitant with the switch from spontaneous neutrophil/monocyte differentiation to the development of basophils/mast cells, the LRAR α 403SN-infected cells exhibit lower percentages of morphologically undifferentiated blasts (Table 2), display an 18-fold reduction in the frequency of macroscopic colony-forming cells, and show a markedly reduced growth rate (Fig. 7). These three parameters suggest that the self-renewal of LRAR α 403SN-infected cells has decreased, either owing to the prolongation of the cell cycle of undifferentiated blast cells, to the increased recruitment of these cells into the terminal differentiation pathway, or both. The effects of the dominant-negative RAR α 403 on the development of different myeloid lineages and the self-renewal of FDCP mix A4 cells are schematically summarized in Figure 8.

Our observation that introducing a dominant-negative RAR α construct into the FDCP mix A4 cells results in a dramatic change from spontaneous neutrophil and monocyte differentiation to basophil/mast cell develop-

ment indicates that the normal RAR α plays important roles in regulating the development of myeloid progenitors. It appears that normal RAR α promotes the development of neutrophils and monocytes but represses the development of basophils/mast cells. One possible model is that the normal RAR α blocks the development of basophils/mast cells by uninfected FDCP mix A4 cells. Removal of this blockade by the dominant-negative RAR α 403 allows the development along the basophil/mast cell lineage under the strong influence of IL-3 used to maintain the culture. (IL-3 is a potent inducer of basophil/mast cell differentiation by normal hematopoietic stem cells.) In this model, the normal RAR α does not necessarily play the role of a "master switch" that commits cells to different specific hematopoietic lineages. More likely, this transcription factor plays some important role or roles in the complex cascade of molecular events leading to or subsequent to the lineage commitment process. It is important to note that this apparent hematopoietic regulatory activity of RAR α occurs in the relatively low concentrations of RA that are endogenous to serum ($<10^{-8}$ M; DeRuyter et al. 1979; Cullum and Zile 1986). (There is 20% horse serum in our complete culture media.) This suggests that RAR α exerts its hematopoietic regulatory activity in FDCP mix A4 cells either in the absence of or in relatively low concentrations of ligand. In contrast, significantly higher concentrations of RA (10^{-7} to 10^{-5} M) are required to induce the differentiation of promyelocytic leukemia cells (Breitman et al. 1980; Chomienne et al. 1990) or to activate genes at the 5' end of the Hox-2 cluster in human embryonal carcinoma cells (Simeone et al. 1990).

Although it is clear from cotransfection studies in NIH-3T3 fibroblasts (Fig. 2) that the RAR α 403 construct exhibits dominant-negative activity against the normal RAR α , the full spectrum of its biological activity is yet to be defined. The prototype dominant-negative nuclear receptor is the *v-erbA* oncogene, which exhibits deletions in the carboxy-terminal ligand-binding domain compared with its homolog *c-erbA*, the thyroid hormone receptor. These carboxy-terminal deletions abolish T_3 binding to the *v-erbA* protein, and as a result *v-erbA* protein may interfere with normal thyroid hormone function by forming heterodimers with *c-erbA* that cannot be activated by T_3 (Damm et al. 1989; Desbois et al. 1991). Our RAR α 403 construct involves a 59-amino-acid truncation in the carboxy-terminal RA binding domain, which most likely also interferes with ligand (RA) binding. Thus, RAR α 403 may interfere with normal RAR α function by forming inactive heterodimers with RAR α . Alternatively, the mutated RAR α may compete for interactions with other transcription factors that may be essential for RAR α activity. For example, members of the RXR family of RA receptors can dimerize with RARs, and these heterodimers display greater affinity for specific DNA target sequences than do RAR homodimers (Kliwer et al. 1992; Leid et al. 1992; Yu et al. 1992; Zhang et al. 1992). The RAR α 403 gene product potentially may form inactive heterodimers with RXRs, and this sequestration of RXRs may prevent the latter

A Normal RAR α

B RAR α Suppressed

Figure 8. Schematic summary of the effects of the dominant-negative RAR α 403 on the lineage development of FDCP mix A4 cells. (A) When RAR α is presumably functioning normally, the IL-3-dependent FDCP mix A4 cells exhibit prominent self-renewal and a predominance of spontaneous differentiation along the neutrophil and monocyte lineages (solid arrows). Spontaneous differentiation to basophil/mast cell lineage is suppressed (broken arrows). (B) In FDCP mix A4 cells infected with the dominant-negative LRAR α 403SN retroviral vector, the normal RAR α function is presumably suppressed. These cells now exhibit prominent spontaneous differentiation to basophils/mast cells, and differentiation to neutrophils and monocytes is suppressed. The infected cells remain IL-3 dependent, but self-renewal is markedly diminished.

from potentiating the activity of other RA receptors. Of relevance is the finding that a truncated construct containing the first 404 amino acids of RAR α (differing from our RAR α 403 construct by only a single amino acid) can dimerize with RXRs (Yu et al. 1992). Thus, it is possible that the dominant-negative RAR α 403 exerts its biologic effects not only by directly inhibiting the normal RAR α but also by indirectly inhibiting related receptors whose full function depends on dimerization with RXRs.

The data reported here indicate that in the multipotent IL-3-dependent FDCP mix A4 murine hematopoietic cell line, RAR α and/or related molecules positively influence the development of neutrophils and monocytes, as well as the self-renewal pathway, but repress the development of basophils/mast cells. We do not know whether RAR α directly participates in this hematopoietic lineage commitment process or, alternatively, is involved in regulating the differentiation after lineage commitment has occurred. In either case, the alteration in the lineage development of the multipotent FDCP mix A4 hematopoietic cell line following the transduction of the dominant-negative RAR α construct indicates that RAR α and/or closely related molecules play important roles in the development of neutrophils, monocytes, and basophils/mast cells.

Materials and methods

Plasmid constructions

The sequence of full-length cDNA of human RAR α has been published (Giguere et al. 1987). The plasmid pGEM3Z-hRAR α D5' harbors human RAR α cDNA sequences from the initiator ATG to the *Bam*HI site at position 2103. This plasmid was digested with *Sma*I, which cut at position 1311 and 1597 of RAR α cDNA (Fig. 1A). The larger fragment was isolated, ligated with an *Nhe*I linker (which inserted a stop codon), and recircularized. The new construct was designated pGEM3Z-RAR α 403 (Fig. 1B). An *Eco*RI site was present in the pGEM3Z polylinker immediately 5' to the initiator ATG of the RAR α 403 insert. The 1.8-kb *Eco*RI-*Bam*HI fragment of pGEM3Z-RAR α 403 was then cloned into the *Eco*RI-*Bam*HI site of the retroviral vector LXS α N (Fig. 1C) (Miller and Rosman 1989), and the recombinant vector was designated pLRAR α 403SN (Fig. 1D). The plasmid pLRAR α SN was constructed by subcloning a 2.8-kb *Eco*RI fragment of human RAR α cDNA clone (Giguere et al. 1987) into the *Eco*RI site of pLXS α N as detailed previously (Collins et al. 1990). The expression plasmid pEMSV-RAR α was constructed by cloning the 2.8-kb human RAR α cDNA into the *Eco*RI site of the expression vector pEMSVscribe (Davis et al. 1987). Plasmids pTRE-CAT and pRRE-CAT consist of a single copy of either a synthetic palindromic TRE (5'-TCAGGTCATGACCTGA-3') (Umesono et al. 1988) or the RRE identified in the RAR β promoter (5'-GTAGGGTTCACCGAAAGTTCACCTC-3') (de The et al. 1990a) inserted upstream of the thymidine kinase promoter (-109 to +5) and CAT reporter gene and was obtained from Jeannette Bigler (Fred Hutchinson Cancer Research Center). Plasmid pCMV-GH was constructed by inserting a 1.1-kb *Pst*I-*Xba*I fragment containing the cytomegalovirus immediate early gene promoter from pON249 (Geballe et al. 1986) into the human growth hormone reporter construct p ϕ GH (Nichols Institute, San Juan Capistrano, CA) and was provided by Ed Agura (Fred Hutchinson Cancer Research Center).

Transient expression assays

NIH-3T3 tk⁻ cells were maintained in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS) and plated 24 hr before transfection. The medium was replaced with fresh DMEM supplemented with 10% charcoal-resin-stripped FCS 4 hr before transfection. Cells were transfected by calcium phosphate precipitation with 2.5 μ g of pEMSV-RAR α , varied amounts of pLRAR α 403SN, 2.5 μ g of pTRE-CAT, 1 μ g of pCMV-GH as an internal control for transfection efficiency, and pLXS α N to equalize the total amount (23.5 μ g/100-mm dish) of DNA and long terminal repeats (LTRs) transfected. For transient expression assays using pRRE-CAT as the reporter, the following conditions were employed: 1 μ g of pEMSV-RAR α , varied amounts of pLRAR α 403SN, 4 μ g of pRRE-CAT, 1 μ g of pCMV-GH, and pLXS α N to equalize the total amount (16 μ g/100-mm dish) of DNA and LTRs transfected. Sixteen hours after transfection, the cells were washed with PBS and refed with DMEM supplemented with 10% charcoal-resin-stripped FCS and incubated for another 32 hr with or without 10^{-6} M ATRA. Electroporation of FDCP mix A4 cells was performed in 0.4-mm cuvettes using a Bio-Rad gene pulser (Richmond, CA) with the following parameters: 2×10^7 cells, 25 μ F/800 V, 75 μ g each of pCMV-GH, pRRE $_4$ -CAT (containing four copies of RRE instead of one), pEMSV-RAR α and pLRAR α 403SN. Culture supernatants were collected for determination of growth hormone concentration using a radioimmunoassay kit (Nichols Institute). Preparation of cell lysates and CAT assay were performed according to published methods (Sambrook et al. 1989). The volume of cell lysate used in the CAT assay was normalized for transfection efficiency as determined by the growth hormone reporter (pCMV-GH) internal control. Following thin layer chromatography on silica gel, the nonacetylated and acetylated 14 C-labeled chloramphenicol was quantitated by a PhosphorImager and ImageQuant software (Molecular Dynamics).

Retroviral producer cell lines and retroviral infection

Amphotropic viral producer cell lines were established according to published procedures (Miller and Rosman 1989). Briefly, pLRAR α 403SN plasmid was transfected into the ecotropic viral packaging cell line PE501, and the transiently expressed viral particles were rescued to infect the amphotropic viral packaging cell line PA317 (Miller 1990). Infected PA317 cells were selected in G418-containing medium, followed by subcloning. One clone, PA317/LRAR α 403SN c.10, expressing high levels of retroviral mRNA of the predicted size by Northern blot analysis and producing supernatants with a titer of 4×10^6 to 6×10^6 colony-forming units (CFU)/ml, as assayed on NIH-3T3 tk⁻ cells was chosen as the producer of retroviral vector particles for this study. A cell line, PA317/LRAR α SN producing a retroviral vector (LRAR α SN) harboring the cDNA of the normal human RAR α was established similarly as described previously (Collins et al. 1990).

HL-60 and FDCP mix A4 cells were infected with retroviral vectors by either a 24-hr cocultivation with irradiated (1200 rads) viral producers or infection with viral supernatants in the presence of 4 μ g/ml of polybrene. Infected cells were then selected in complete culture medium containing 1 mg/ml (for HL-60) or 300 μ g/ml (for FDCP mix A4) of G418 for 8–10 days. Clonal lines were generated by limiting dilution in 96-well plates or cloning in 0.3% agar.

Cell cultures

HL-60 cells were maintained in RPMI 1640 supplemented with 5% FCS. FDCP mix A4 cells were maintained in Iscove's mod-

ified Dulbecco's medium (IMDM) supplemented with 20% (vol/vol) horse serum and 10% (vol/vol) WEHI 3B cell line-conditioned medium (as a source of IL-3) and subcultured every 3 days. Colony assays of A4/LXSN and A4/LRAR α 403SN were done in IMDM supplemented with 25% horse serum, 10% WEHI 3B-conditioned medium, and 0.3% agar. These agar cultures were incubated at 37°C in 5% O₂, 5% CO₂ and 90% N₂ for 14 days.

Northern blots

Total RNAs were extracted with guanidine hydrochloride and subjected to Northern blotting in formaldehyde gels as described previously (Collins et al. 1987). Molecular probes used in Northern hybridization included the following: hRAR α , the 1.3-kb *EcoRI*–*SmaI* fragment of the pHK1 RAR α cDNA clone (Giguere et al. 1987), and the mouse GATA-1 cDNA probe (Tsai et al. 1989), which was obtained from David Martin (Fred Hutchinson Cancer Research Center). All probes were labeled with ³²P by nick translation before hybridization.

Cell phenotyping

Cytospin preparations of cell suspensions were stained with 1% toluidine blue for 15 min and washed with tap water. For quantitation of intracellular histamine, 2×10^5 cells were washed with PBS and resuspended in 1 ml of distilled water and lysed by three cycles of freezing and thawing. Radioimmunoassay of histamine in cell lysates or culture supernatants was performed according to the manufacturer's instructions (Immunotech International).

For flow cytometry, cells were exposed to saturating concentrations of primary rat IgG2a monoclonal antibodies 7/4 (generously provided by S. Gordon, Oxford, UK) or MAC-1 (PharMingen) at 4°C for 10 min, washed with PBS/1% FCS, and stained with an FITC-labeled goat anti-rat IgG (H + L) for 10 min at 4°C. After final washing with PBS/1% FCS, fluorescence was measured with a Becton–Dickinson FACScan flow cytometer and analyzed by the Reptoman software (Kirkegaard & Perry Labs, MD). An irrelevant rat IgG2a monoclonal antibody (PharMingen) was used as the isotype control. For IgE R staining, cells were incubated with culture medium containing 15 μ g/ml of mouse IgE (PharMingen) at 37°C overnight, washed three times with PBS/1% FCS, stained with FITC-goat anti-mouse IgE (Nordic Immunologicals) at 4°C for 10 min, followed by washing with PBS/1% FCS.

Acknowledgments

We are most grateful to Drs. Elaine Spooner and Michael Dexter for making the FDCP mix A4 cell line available to us and to Hal Weintraub for his critical reading of the manuscript. We also thank Dusty Miller for the gift of the retroviral vector LXSN and the retroviral packaging cell lines, and LeMoyne Mueller and Babak Emami for excellent technical assistance. This work was supported by American Cancer Society research grant MV-455 to S.J.C.

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

Alcalay, M., D. Zangrilli, P. Pandolfi, L. Longo, A. Mencarelli, A. Giacomucci, M. Rocchi, A. Biondi, A. Rambaldi, F. Lo Coco, D. Diverio, E. Donti, F. Grignani, and P. Pelicci. 1991. Translocation breakpoint of acute promyelocytic leukemia

lies within the retinoic acid receptor α locus. *Proc. Natl. Acad. Sci.* **88**: 1977–1981.

Borrow, J., A.D. Goddard, D. Sheer, and E. Solomon. 1990. Molecular analysis of acute promyelocytic leukemia breakpoints cluster region on chromosome 17. *Science* **249**: 1577–1580.

Breitman, T.R., S.E. Selonick, and S.J. Collins. 1980. Induction of differentiation of the human promyelocytic leukemia cell line (HL-60) by retinoic acid. *Proc. Natl. Acad. Sci.* **77**: 2936–2940.

Castaigne, S., C. Chomienne, M.T. Daniel, P. Ballerini, R. Berger, P. Fenaux, and L. Degos. 1990. All-trans retinoic acid as a differentiation therapy for acute promyelocytic leukemia. I. Clinical results. *Blood* **76**: 1704–1710.

Chomienne, C., P. Ballerini, N. Balitrand, M. Daniel, P. Fenaux, S. Castaigne, and L. Degos. 1990. All-trans retinoic acid in acute promyelocytic leukemias. II. In vitro studies: Structure-function relationship. *Blood* **76**: 1710–1717.

Collins, S.J., R.C. Gallo, and R.E. Gallagher. 1977. Continuous growth and differentiation of human myeloid leukaemic cells in suspension culture. *Nature* **270**: 347–349.

Collins, S., H. Coleman, and M. Groudine. 1987. Expression of *bcr* and *bcr-abl* fusion transcripts in normal and leukemic cells. *Mol. Cell. Biol.* **7**: 2870–2876.

Collins, S.J., K. Robertson, and L. Mueller. 1990. Retinoic acid-induced granulocytic differentiation of HL-60 myeloid leukemia cells is mediated directly through the retinoic acid receptor (RAR α). *Mol. Cell. Biol.* **10**: 2154–2161.

Cullum, M. and M. Zile. 1986. Quantitation of biological retinoids by high-pressure liquid chromatography: Primary internal standardization using tritiated retinoids. *Anal. Biochem.* **153**: 23–32.

Damm, K., C.C. Thompson, and R.M. Evans. 1989. Protein encoded by *v-erbA* functions as a thyroid-hormone receptor antagonist. *Nature* **339**: 593–597.

Davis, R., H. Weintraub, and A. Lassar. 1987. Expression of a single transfected cDNA converts fibroblasts to myoblasts. *Cell* **51**: 987–1000.

Denburg, J.A. 1992. Basophil and mast cell lineages in vitro and in vivo (review). *Blood* **79**: 846–860.

DeRuyter, M.G., W. Lambert, and P. DeLunheer. 1979. Retinoic acid: An endogenous compound of human blood. Unequivocal demonstration of endogenous retinoic acid in normal physiological conditions. *Anal. Biochem.* **98**: 402–409.

Desbois, C., D. Aubert, C. Legrand, B. Pain, and J. Samarut. 1991. A novel mechanism of action for *v-erbA*: Abrogation of the inactivation of transcription factor AP-1 by retinoic acid and thyroid hormone receptors. *Cell* **67**: 731–740.

de The, H., A. Marchio, P. Tiollais, and A. Dejean. 1989. Differential expression and ligand regulation of the retinoic acid receptor α and β genes. *EMBO J.* **8**: 429–433.

de The, H., M. Vivanco-Ruiz, P. Tiollais, H. Stunnenberg, and A. Dejean. 1990a. Identification of a retinoic acid responsive element in the retinoic acid receptor β gene. *Nature* **343**: 177–180.

de The, H., C. Chomienne, M. Lanotte, L. Degos, and A. Dejean. 1990b. The t(15;17) translocation of acute promyelocytic leukemia fuses the retinoic acid receptor α gene to a novel transcribed locus. *Nature* **347**: 558–561.

de The, H., C. Lavau, A. Marchio, C. Chomienne, L. Degos, and A. Dejean. 1991. The PML-RAR α fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell* **66**: 675–684.

Evans, R. 1988. The steroid and thyroid hormone receptor superfamily. *Science* **240**: 889–895.

Gallagher, R., F. Said, I. Pua, P. Papenhausen, E. Paietta, and P.

- Wiernik. 1989. Expression of retinoic acid receptor- α in human leukemia cells with variable responsiveness to retinoic acid. *Leukemia* **3**: 789–795.
- Geballe, A., R. Spate, and E. Mocarski. 1986. A cis-acting element within the 5' leader of a cytomegalovirus beta transcript determines kinetic class. *Cell* **46**: 865–876.
- Giguere, V., E. Ong, P. Segui, and R. Evans. 1987. Identification of a receptor for the morphogen retinoic acid. *Nature* **330**: 624–629.
- Herskowitz, I. 1987. Functional inactivation of genes by dominant negative mutations. *Nature* **329**: 219–222.
- Hirsch, S. and S. Gordon. 1983. Polymorphic expression of a neutrophil differentiation antigen revealed by monoclonal antibody 7/4. *Immunogenetics* **18**: 229–239.
- Hong, W.K., S.M. Lippman, L. Itri, D.D. Karp, J.S. Lee, R.M. Byers, S.P. Schatz, A.M. Kramer, R. Lotan, L.J. Peters, I.W. Dimery, B.W. Brown, and H. Goepfert. 1990. Prevention of second primary tumors with isotretinoin in squamous cell carcinoma of the head and neck. *New Engl. J. Med.* **323**: 795–801.
- Huang, M.-E., Y.-C. Ye, S.-R. Chen, J.-R. Chai, J.-X. Lu, L. Zhao, H.-T. Gu, and Z.-Y. Wang. 1988. Use of all-trans retinoic acid in the treatment of acute promyelocytic leukemia. *Blood* **72**: 567–572.
- Just, U., C. Stocking, E. Spooncer, T.M. Dexter, and W. Oestergaard. 1991. Expression of the GM-CSF gene after retroviral transfer in hematopoietic stem cell line induces synchronous granulocyte-macrophage differentiation. *Cell* **64**: 1163–1173.
- Kakizuka, A., W. Miller, K. Umehono, R. Warrell, S. Frankel, V. Murty, E. Dmitrovsky, and R. Evans. 1991. Chromosomal translocation t(15;17) in human acute promyelocytic leukemia fuses RAR α with a novel putative transcription factor, PML. *Cell* **66**: 663–674.
- Kliwer, S., K. Umehono, D.J. Mangelsdorf, and R.M. Evans. 1992. Retinoid X receptor interacts with nuclear receptors in retinoic acid, thyroid hormone and vitamin D3 signalling. *Nature* **355**: 446–449.
- Largman, C., K. Detmer, J. Corral, F. Hack, and H. Lawrence. 1989. Expression of retinoic acid receptor alpha mRNA in human leukemic cells. *Blood* **74**: 99–102.
- Lavin, T. N., J.D. Baxter, and S. Horita. 1988. The thyroid hormone receptor binds to multiple domains of the rat growth hormone 5'-flanking sequence. *J. Biol. Chem.* **263**: 9418–9426.
- Leid, M., P. Kastner, R. Lyons, H. Nakshatri, M. Saunders, T. Zacharewski, J.Y. Chen, A. Staub, and P. Chambon. 1992. Purification, cloning, and RXR identity of the HeLa cell factor with which RAR or TR heterodimerizes to bind target sequences efficiently. *Cell* **68**: 377–395.
- Lotan, R. 1980. Effects of vitamin A and its analogs (retinoids) on normal and neoplastic cells. *Biochim. Biophys. Acta* **605**: 33–91.
- Martin, D.I.K., L.I. Zon, G. Mutter, and S.H. Orkin. 1990. Expression of an erythroid transcription factor in megakaryocytic and mast cell lineages. *Nature* **344**: 444–447.
- Merriman, R. and J. Bertram. 1979. Reversible inhibition by retinoids of 3-methylcholanthrene-induced neoplastic transformation in C3H/10T clone 8 cells. *Cancer Res.* **39**: 1661–1666.
- Metzger, H., G. Alcaraz, R. Hohman, J. Kinet, V. Pribula, and R. Ovario. 1986. The receptor with high affinity for immunoglobulin IgE. *Annu. Rev. Immunol.* **4**: 419–470.
- Miller, A.D. 1990. Retrovirus packaging cells. *Hum. Gene Ther.* **1**: 5–14.
- Miller, A.D. and G.J. Rosman. 1989. Improved retroviral vectors for gene transfer and expression. *Biotechniques* **7**: 980–990.
- Riley, J.F. and G.B. West. 1953. The presence of histamine in tissue mast cells. *J. Physiol.* **120**: 528–532.
- Sambrook, J., E. Fritsch, and T. Maniatis. 1989. *Molecular cloning: A laboratory manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- Sap, J.A., A. Munoz, J. Schmitt, H. Stunnenberg, and B. Vennstrom. 1989. Repression of transcription mediated by a thyroid hormone response element by the v-erbA oncogene product. *Nature* **340**: 242–244.
- Simeone, A., D. Acampora, L. Arcioni, P. Andrews, E. Boncinelli, and F. Mavilio. 1990. Sequential activation of HOX2 homeobox genes by retinoic acid in human embryonal carcinoma cells. *Nature* **346**: 763–766.
- Spooncer, E., C. Heyworth, A. Dunn, and T.M. Dexter. 1986. Self-renewal and differentiation of interleukin-3-dependent multipotent stem cells are modulated by stromal cells and serum factors. *Differentiation* **31**: 111–118.
- Thaller, C. and G. Eichele. 1987. Identification and spatial distribution of retinoids in the developing chick limb buds. *Nature* **327**: 625–628.
- Thompson, H.L., D.D. Metcalfe, and J.-P. Kinet. 1990. Early expression of high affinity receptor for immunoglobulin E during differentiation of mouse mast cells and human basophils. *J. Clin. Invest.* **85**: 1227–1233.
- Tsai, S.-F., D. Martin, L. Zon, A. D'Andrea, G. Wong, and S. Orkin. 1989. Cloning of cDNA for the major DNA-binding protein of the erythroid lineage through expression in mammalian cells. *Nature* **339**: 446–451.
- Umehono, K., V. Giguere, C. Glass, M. Rosenfeld, and R. Evans. 1988. Retinoic acid and thyroid hormone induce gene expression through a common responsive element. *Nature* **336**: 262–265.
- Warrell, R., S. Frankel, W. Miller, D. Scheinberg, L. Itri, W. Hittelman, R. Vyas, M. Andreef, A. Tafuri, A. Jakubowski, J. Gabrilove, M. Gordon, and E. Dmitrovsky. 1991. Differentiation therapy of acute promyelocytic leukemia with tretinoin (all trans retinoic acid). *New Engl. J. Med.* **324**: 1385–1393.
- Yu, V.C., C. Delsert, B. Anderson, J.M. Holloway, O.V. Devary, A.M. Naar, S.Y. Kim, J.-M. Boutin, C.K. Glass, and M.G. Rosenfeld. 1992. RXR β : A coregulator that enhances binding of retinoic acid, thyroid hormone, and vitamin D receptors to their cognate response elements. *Cell* **67**: 1251–1266.
- Zelent, A., A. Krust, M. Petkovich, P. Kastner, and P. Chambon. 1989. Cloning of murine α and β retinoic acid receptors and a novel receptor predominantly expressed in skin. *Nature* **339**: 714–717.
- Zenke, M., P. Kahn, C. Disela, B. Vennstrom, A. Leutz, K. Keegan, M. Hayman, H.-R. Choi, N. Yew, J.D. Engel, and H. Beug. 1988. v-erbA specifically suppresses transcription of the avian erythrocyte anion transporter (band 3) gene. *Cell* **52**: 107–119.
- Zenke, M., A. Munoz, J. Sap, B. Vennstrom, and H. Beug. 1990. v-erbA oncogene activation entails the loss of hormone-dependent regulator activity of c-erbA. *Cell* **61**: 1035–1049.
- Zhang, X.-K. B. Hoffman, P. B.-V. Tran, G. Graupner, and M. Pfahl. 1992. Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature* **355**: 441–446.
- Zon, I.L., M.F. Gurish, R. Stevens, C. Mather, D.S. Reynolds, K.F. Austen, and S.H. Orkin. 1991. GATA-binding transcription factors in mast cells regulate the promoter of the mast cell carboxypeptidase A gene. *J. Biol. Chem.* **266**: 22948–22953.

A mutated retinoic acid receptor-alpha exhibiting dominant-negative activity alters the lineage development of a multipotent hematopoietic cell line.

S Tsai, S Bartelmez, R Heyman, et al.

Genes Dev. 1992, **6**:

Access the most recent version at doi:[10.1101/gad.6.12a.2258](https://doi.org/10.1101/gad.6.12a.2258)

References

This article cites 53 articles, 13 of which can be accessed free at:
<http://genesdev.cshlp.org/content/6/12a/2258.full.html#ref-list-1>

License

Email Alerting Service

Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or [click here](#).