Acute promyelocytic leukemia (APL) is always associated with chromosomal translocations that disrupt the retinoic acid receptor α (RARα) gene. Whether these translocations relate to a role for endogenous RARα in normal granulopoiesis remains uncertain because most studies addressing this question have used non-physiological overexpression systems. Granulocyte differentiation in cells derived from RARα-deficient (RARα−/−) mice was studied and evaluated in the context of agonist-bound and ligand-free RARα. Our results demonstrate that RARα is dispensable for granulopoiesis, as RARα−/− mice have a normal granulocyte population despite an impaired ability to respond to retinoids. However, although it is not absolutely required, RARα can bidirectionally modulate granulopoiesis. RARα stimulates differentiation in response to exogenous retinoic acid. Furthermore, endogenous retinoids control granulopoiesis in vivo, as either vitamin A−/− deficient mice or animals treated with an RAR antagonist accumulate more immature granulocytes in their bone marrow. Conversely, RARα acts to limit differentiation in the absence of ligand because granulocyte precursors from RARα−/− mice differentiate earlier in culture. Thus, the block in granulopoiesis exerted by RARα fusion proteins expressed in APL cells may correspond to an amplification of a normal function of unliganded RARα. (Blood. 2001;97:1314-1320)

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Materials and methods

Mice
RARα−/− and RARγ-deficient mice were described previously. Mice were genotyped by polymerase chain reaction (PCR) (primers available upon request). All mice used in this study were between 2 and 6 months of age. Vitamin A deficiency (VAD) in cellular retinol-binding protein type I (CRBP1)−/− mice was produced by maintaining CRBP1−/− mice in vivo. The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked “advertisement” in accordance with 18 U.S.C. section 1734.

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on a VAD diet for at least 16 weeks after birth.27 VAD was confirmed by the observation of several VAD-related phenotypes: keratinization of the seminal vesicle and prostate epithelia, testis degeneration, and retinal defects.27 These symptoms were specifically observed in CRBPI−/− mice fed a VAD diet, not in control mice (ie, WT mice fed a VAD diet or CRBPI−/− mice fed a normal diet). All-trans-retinoic acid (ATRA) (Sigma Chemical Co, St Louis, MO) and the pan-RAR antagonist BMS493 were dissolved in ethanol and then placed in an emulsion with corn oil by vigorous vortexing. The emulsion was administered to test animals by gavage every 12 hours (BMS493) or every 36 hours (ATRA) for 3 days. Bone marrow (BM) cells were harvested by flushing femurs with medium. Fetal liver (FL) cell suspensions were prepared by sequential passage of dissected FLs through 18-, 22-, and 25-gauge needles.

Cell culture

Liquid myeloid cultures were performed by seeding 2 × 10^6 BM cells in 10 mL Iscoves modified Dulbecco medium supplemented with 20% fetal bovine serum (Gibco-BRL Life Technologies, Grand Island, NY), 5 ng/mL granulocyte colony-stimulating factor (G-CSF), and 5 ng/mL stem cell factor (SCF) (R&D Systems, Minneapolis, MN), penicillin/streptomycin, granulocyte colony-stimulating factor (G-CSF), and 5 ng/mL stem cell factor (R&D Systems, Minneapolis, MN). Liquid myeloid cultures were performed by seeding 2 × 10^6 BM cells in 10 mL Iscoves modified Dulbecco medium supplemented with 20% fetal bovine serum (Gibco-BRL Life Technologies, Grand Island, NY), 5 ng/mL granulocyte colony-stimulating factor (G-CSF), and 5 ng/mL stem cell factor (SCF) (R&D Systems, Minneapolis, MN), penicillin/streptomycin, granulocyte colony-stimulating factor (G-CSF), and 5 ng/mL stem cell factor (R&D Systems, Minneapolis, MN). Labelling with 5- and 6-carboxy fluorescein diacetate, succinimidyl ester (CFSE) (Molecular Probes, Eugene, OR) was performed by incubating BM cells for 10 minutes at 37°C at a concentration of 10^7 cells per mL in medium supplemented with 2 μM CFSE. Labeling with 5- and 6-carboxy fluorescein diacetate, succinimidyl ester (CFSE) (Molecular Probes, Eugene, OR) was performed by incubating BM cells for 10 minutes at 37°C at a concentration of 10^7 cells per mL in medium supplemented with 2 μM CFSE. The ratio of CFSE low cells, which correspond to cells that have arisen during the culture from proliferation of immature granulocytes. Note that CFSE high cells correspond exclusively to mature granulocytes with a Mac-1 high GR-1 high phenotype (data not shown). (B) Representative GR-1/Mac-1 profiles of CFSE low cells. Note that CFSE high cells correspond exclusively to mature granulocytes with a Mac-1 high GR-1 high phenotype (data not shown).

Figure 2. RARα−/− myeloid progenitors are resistant to the inhibitory effects of 9C-RA. (A) Numbers of colonies developing from 5 × 10^4 BM cells from WT or RARα−/− mice in the presence or absence of 10−6 M 9C-RA. Each bar represents the average of duplicate samples from 3 mice. The cytokines used to stimulate growth of hematopoietic progenitors were SCF, IL-6, IL-3, and EPO. (B) Representative colonies (day 7) showing the reduction in size of WT granulocyte colonies grown in the presence of 9C-RA and the lack of such an effect in RARα−/− mutants.

Antibodies and flow cytometry

The following antibodies were used: fluorescein isothiocyanate (FITC)-labeled GR-1 (Caltag, South San Francisco, CA) or phycoerythrin-labeled GR-1 (PharMingen, San Diego, CA) and biotin-labeled Mac-1 (CD11b, PharMingen). Streptavidin-Cy5 was purchased from Jackson Immunoresearch Laboratories, West Grove, PA. Antibody stainings were performed of a 10 mg/mL CFSE solution in dimethyl sulfoxide followed by 2 washes with 10 mL medium. Clonogenic cultures were performed with commercial methylcellulose media (Methocult 3434; Stem Cell Technologies, Vancouver, BC, Canada). The media contained erythropoietin (EPO), interleukin-3 (IL-3), IL-6, and SCF for the experiments described in Figure 1C and Figure 2, and the media contained Methocult 3234 supplemented with granulocyte-macrophage (GM)–CSF (R&D Systems) at a final concentration of 50 ng/mL for the experiments described in Figure 4.
RESULTS

RARs are dispensable for granulopoiesis

Granulocyte populations appeared normal in RARα−/− mice at the level of blood neutrophil counts (data not shown) and in the BM (GR-1+ Mac-1+ population; Figure 5C, upper right panel, and data not shown). To investigate whether functional compensation by other RARs occurs in RARα−/− mutants, we examined RARα−/−RARγ−/− double mutants, as RARγ is also expressed in granulocytes. Because RARα−/−RARγ−/− mutants die in utero, granulopoiesis was studied in the FL. Granulocytes developed normally in RARα−/−RARγ−/− E14.5 FLs as tested by several criteria: double-mutant myeloid cells exhibited a normal GR-1+ Mac-1+ phenotype (Figure 1A); differentiated cells (containing ring-shaped or segmented nuclei) were detected at similar frequencies on cytospin preparations from mutant and WT FLs (data not shown); granulocyte-specific markers were expressed at similar levels (Figure 1B); and morphologically normal neutrophils were generated in myeloid colonies in vitro (Figure 1C). Because RARβ expression was not detected in freshly isolated or cultured FL cells (Figure 1D), granulopoiesis can thus proceed efficiently in the absence of any RAR.

To determine whether granulocyte progenitors from RARα−/− mice could still respond to retinoids, we compared the response of WT and RARα−/− BM cells to 9-cis retinoic acid (9C-RA, a pan-RAR and pan-RXR [retinoid X receptor] agonist) in a myeloid colony-forming assay. As previously reported, the total number of colonies developing from WT BM cells, as well as the size of pure granulocyte colonies, were markedly reduced in the presence of 9C-RA (Figure 2A). Neither of these effects were observed in cultures of RARα−/− BM cells (Figure 2) (P < .01), indicating that RARα−/− granulocyte progenitors are impaired in their response to retinoids. These results, together with the apparent normal granulopoiesis in RARα−/−RARγ−/− double mutants, demonstrate that RARs and retinoids are dispensable for granulopoiesis.

Accelerated granulocyte differentiation in the absence of RARα

Although the RA/RAR pathway is not required, it can clearly modulate granulocyte differentiation, as shown by the response of WT cells to retinoids. Consequently, we analyzed the effects of RAR agonists and antagonists on granulocyte differentiation in

Figure 4. Accelerated differentiation of RARα−/− granulocytes in GM-CSF-dependent clonogenic cultures. We plated 5 x 10⁴ BM cells in methylcellulose medium containing 50 ng/mL GM-CSF. (A–D) Pooled cells from all the colonies developing on a dish (about 70 colonies) were cytocentrifuged onto a glass slide and stained with May-Grünwald-Giemsa. Arrowheads in panel A indicate promyelocytes that are numerous on slides from day-5 WT cultures, but are less frequent in mutant samples. The inset in panels A and B corresponds to the scoring of approximately 400 cells of the granulocyte lineage for each case. (E,F) Typical colonies from culture dishes at day 8; note the reduction in the number and size of dense cellular aggregates (arrows), which correspond to granulocytes.
metamyelocytes, whereas Mac-1^low/GR-1^high cells correspond mainly to band and segmented neutrophils (data not shown). (A) WT mice were treated for 3 days with 10 mg/kg BMS493 or vehicle. In the right panels the GR-1 profile of the granulocyte population (Mac-1^low cells, gated in the left panels) is shown, and the percentage of GR-1^high immature granulocytes within that population is indicated. (B) Granulocyte populations in VAD mice. Note the overall increase of the granulocyte population (gated in the top panels) and the increase, within that population, of the immature granulocyte population (GR-1^low cells, bottom panels) that occurs specifically in the VAD mice (ie, CRBPI^-/- mice fed a VAD diet); (C) Effect of ATRA administration. Mice were treated with 100 mg/kg ATRA or vehicle for 72 hours. Note the shift to the right of GR-1 expression within the immature granulocyte population (gated) in ATRA-treated WT mice, producing a comet-like profile, which is not seen in the ATRA-treated RARalpha^/-/ mice. Note that a reduction in the number of the mature (GR-1^high) BM granulocytes was also observed in ATRA-treated WT mice, which probably reflects an enhanced efflux of neutrophils from the BM, as peripheral blood neutrophil numbers were increased 1.5- to 2-fold (data not shown).

**Figure 5. Effect of retinoid excess and VAD on granulopoiesis in vivo.** BM granulocyte populations were analyzed by flow cytometry with the Mac-1 and GR-1 markers. Granulocyte differentiation proceeds along a curved path in which increasing levels of Mac-1 and GR-1 are acquired as the cells mature. Thus, Mac-1^low/GR-1^low cells correspond mainly to promyelocytes, myelocytes, and metamyelocytes, whereas Mac-1^high/GR-1^high cells correspond mainly to band and segmented neutrophils (data not shown). (A) WT mice were treated for 3 days with 10 mg/kg BMS493 or vehicle. In the right panels the GR-1 profile of the granulocyte population (Mac-1^low cells, gated in the left panels) is shown, and the percentage of GR-1^high immature granulocytes within that population is indicated. (B) Granulocyte populations in VAD mice. Note the overall increase of the granulocyte population (gated in the top panels) and the increase, within that population, of the immature granulocyte population (GR-1^low cells, bottom panels) that occurs specifically in the VAD mice (ie, CRBPI^-/- mice fed a VAD diet); (C) Effect of ATRA administration. Mice were treated with 100 mg/kg ATRA or vehicle for 72 hours. Note the shift to the right of GR-1 expression within the immature granulocyte population (gated) in ATRA-treated WT mice, producing a comet-like profile, which is not seen in the ATRA-treated RARalpha^/-/ mice. Note that a reduction in the number of the mature (GR-1^high) BM granulocytes was also observed in ATRA-treated WT mice, which probably reflects an enhanced efflux of neutrophils from the BM, as peripheral blood neutrophil numbers were increased 1.5- to 2-fold (data not shown).

**Liquid cultures of WT BM cells stimulated with G-CSF and SCF.** To monitor cells that had undergone division during the culture period, freshly isolated BM cells were cytoplasmically labeled with the vital dye CFSE (a fluorescent compound that is gradually diluted upon cell division) prior to culture, and analysis was confined to CFSE^-/low cells (Figure 3A). Increased levels of GR-1 and Mac-1 expression were used to measure differentiation. 9C-RA induced an increase in the proportion of de novo GR-1^high cells in cultures of WT cells (Figure 3B, upper right panel, gate C, and Figure 3C) as well as a decrease in the proportion of CFSE^-/low cells (data not shown), which is consistent with the premise that RA limits the proliferation of granulocyte precursors and induces their differentiation.

In contrast, the pan-RAR antagonist BMS493 (H. Gronemeyer and P.C., unpublished observation, February 1996) induced an increase in the proportion of immature granulocytes (Figure 3B, upper middle panel, gate B, and Figure 3C), a finding corroborated by the higher frequency of promyelocytes/myelocytes on cytoospin preparations from these cultures (data not shown). Interestingly, the magnitude of the response to BMS493 greatly exceeded that to 9C-RA, suggesting that retinoids in the serum might play an important part in controlling the differentiation that occurs in the absence of exogenous ligands. When RARalpha^/-/ BM cells were grown under identical culture conditions, the ratio of immature versus mature cells generated was unaffected by treatment with either 9C-RA or BMS493 (Figure 3B,C), confirming the resistance of RARalpha^/-/ granulocytes to the effects of RAR ligands. In addition, the proportions of mature cells were consistently increased when compared with WT samples in the control and BMS493-treated cultures to levels usually observed in 9C-RA–treated WT cultures (Figure 3B,C). Thus granulocyte differentiation appears to be accelerated in the absence of RARalpha.

This apparent acceleration of maturation was also seen in clonogenic methylcellulose cultures stimulated with GM-CSF (Figure 4). After 5 days of culture, granulocytes from pooled RARalpha^/-/ colonies were more mature than those from WT colonies (compare Figure 4A with B and inset). By day 8, there was a marked underrepresentation of granulocytes in the mutant cultures, which now consisted mainly of macrophages (Figure 4C,D), suggesting that the prematurely differentiated neutrophils seen on day 5 of culture had died off over time. At the colony level, dense granulocyte clusters were less abundant and smaller in RARalpha^/-/ cultures after 8 days (Figure 4E,F), a phenotype that reliably allowed for the blind separation of dishes containing WT or RARalpha^/-/ cells. It is noteworthy that both types of experimental cultures used different cytokines (G-CSF and SCF for the liquid cultures and GM-CSF for the methylcellulose cultures), thus excluding a cytokine-specific effect. These observations provide evidence that lack of RARalpha relieves a physiological restraint on granulocyte differentiation normally exerted by this receptor in the absence of ligand.

**Retinooids control granulopoiesis in vivo**

To understand how retinoids may modulate granulopoiesis in vivo, we evaluated granulocyte differentiation in WT mice treated with BMS493. Of 11 treated WT mice, 7 mice exhibited a clear increase in the proportion of immature granulocytes in the BM (Mac-1^low/GR-1^low, Figure 5A). As this increase was not seen in vehicle-treated or untreated animals, its occurrence in BMS493-treated mice most likely reflects a role for endogenous retinoids in regulating granulocyte differentiation. To investigate further, we analyzed VAD mice using CRBPI-deficient mice that, owing to low vitamin A stores, become readily VAD when starved for vitamin A. In all VAD CRBPI^/-/ mice tested (n = 6), there was a marked expansion in the size of the entire granulocyte population (Figure 5B, upper right panel) when compared with control animals (ie, CRBPI^/-/ mice on a normal diet or CRBPI^/-/ mice on a VAD diet) as well as an increase in the proportion of immature Mac-1^low/GR-1^low cells (Figure 5B, lower right panel). This effect was accompanied by an increase of neutrophils in the spleen and blood (data not shown), which is consistent with a previous report showing enhanced neutrophil numbers in VAD rats. Importantly, the VAD-induced increase in the immature granulocyte compartment of the BM mirrors that seen following BMS493 administration (Figure 5A), underscoring a role for endogenous retinoids in regulating granulocyte differentiation.

In contrast, ATRA treatment of WT mice (n = 7) elicited an increase in the levels of GR-1 and Mac-1 expressed by the Mac-1^low/GR-1^low immature granulocyte population (Figure 5C, gated region, compare upper and lower left panels), an effect that did not occur in RARalpha^/-/ mice (n = 4) (Figure 5C, right panels). This indicates that RARalpha is the direct mediator of exogenous (and, by inference, endogenous) retinoid-induced differentiation. Significantly, the fact that all 7 ATRA-treated WT mice responded in a similar manner also suggests that under normal dietary conditions,
RARα proteins are not fully liganded in vivo, thus placing them in an appropriate state to negatively regulate differentiation.

Discussion

RARα: a bidirectional modulator of granulopoiesis

We have shown here that (1) endogenous RARα is a key mediator of the effects of retinoids on granulopoiesis, and (2) the RARα receptor can bidirectionally modulate granulopoiesis as a differentiation factor when liganded to RA or as an inhibitor in the absence of ligand (or when liganded to an antagonist), probably by acting as a transcriptional repressor.32-34 The fact that RA is a differentiating agent for granulocytes in culture has been well established for both normal and leukemic cells.19,20,35 However, the role of retinoids during normal granulocyte differentiation in vivo has so far not been documented. The increase in the immature granulocyte populations, seen both in the VAD and BMS493-treated mice here, show for the first time that retinoid-controlled differentiation actively contributes to the regulation of granulocyte numbers in vivo. Our VAD results are in agreement with those of Kuwata et al., who recently reported a collective increase in granulocyte numbers during murine VAD as well as with an earlier report documenting elevated neutrophils in the peripheral blood of VAD rats.31 Although Kuwata et al. did not study the relative contributions of immature versus mature cells in their granulocyte population, their VAD mice also seemed to accumulate more cells with a GR-1low phenotype, as seen in the Kuwata et al Figure 3A.36 and may therefore exhibit a phenotype similar to that of our VAD mice. We have noted that optimal resolution of the GR-1low and GR-1high populations appears to depend on the fluorochrome to which the anti–GR-1 antibody is conjugated; FITC-conjugated anti–GR-1 (as used in our study) provided the best resolution, while other conjugated forms of this antibody appeared to give more compact stainings similar to those shown by Kuwata et al.36 These authors also found less apoptosis within the granulocyte population of their VAD mice, an observation that they used to explain their increase in granulocyte numbers. Although we cannot exclude that retinoids directly regulate cell survival, it is possible that the decrease in apoptosis seen by Kuwata and colleagues is an indirect consequence of a shift toward the more immature stages because apoptosis is the ultimate endpoint of differentiation.

The finding that endogenous unliganded RARα negatively regulates differentiation is consistent with results from previous studies showing that overexpression of WT RARα blocks promyelocyte differentiation in vitro.22,23 However, the non-physiological levels of receptor expressed in these systems have prevented any clear conclusion about the role of the endogenous molecule. In fact, these latter studies often claimed that the overexpressed receptors functioned in a dominant-negative manner by titrating out retinoids present in the medium, thereby countering the differentiating role of these compounds. Our present results suggest otherwise; the differentiation block exerted by overexpressed RARα corresponds to an amplification of an intrinsic inhibitory activity of the unliganded receptor. It is important to stress that in contrast to the overexpression studies, blocking the activation of the endogenous receptor in vivo or in vitro (with VAD or BMS493) never resulted in a complete arrest in differentiation such as the arrest achieved by overexpressed receptors, thereby reflecting the possibility that RARα is expressed in limiting amounts.

Interestingly, our results appear to contradict those of Labrecque et al., who reported that granulocytes from myeloid colonies derived from RARα1−/−RARγ−/− neonatal BM cells tend to be more immature than those derived from WT cells (RARα1 being one of the 2 major isoforms produced from the RARα gene).37 As we never observed such a block in colonies derived from RARα2−/−RARγ−/− FL cells, the apparent difference in the behavior of the RARα1−/−RARγ−/− cells might be due to the persistent expression of RARα2, an isoform not present in our double mutants. It should be noted that we used FL cells in our study, while Labrecque and colleagues used BM cells. However, we observed a similar premature differentiation in GM-CSF–dependent colonies derived from either FL or adult BM RARα−/− cells (unpublished observations, February 1998). Thus, it appears unlikely that RARs control granulopoiesis differently in FL and BM cells. With respect to RARγ, its role appears to be minor at best, as cells lacking only RARα are already resistant to the effects of retinoids.

The present results demonstrate that granulocyte differentiation can proceed in the absence of RARs. The RA/RARα pathway is therefore not an obligatory regulator of granulopoiesis, but rather a modulator of this differentiation. Both retinoid excess and deficiency affect granulopoiesis, suggesting that a balance between liganded and unliganded receptor molecules exists in vivo under normal conditions. The question therefore arises as to which physiological or pathological conditions modulate retinoid levels. Dietary VAD, a condition that is associated with higher susceptibility to bacterial infections, is an obvious candidate. The negative regulation of granulopoiesis by unliganded RARα (which ultimately translates into an elevated granulocyte pool) might thus represent an innate response used to off-set VAD-associated infections. In this view, an important role of RARα during granulopoiesis could paradoxically be to mediate the effect of ligand deficiency. Analysis of the VAD response in RARα−/− mice should help to clarify this issue.

At the molecular level, several downstream candidates might mediate the actions of RARα. A prime candidate is C-EBPα, an RA-target gene induced by RA in myeloid cell lines38,39 and shown to be essential for terminal granulocyte differentiation.40 However, because granulocytes can freely differentiate in the absence of RARs or during retinoid deficiency, it is unlikely that retinoids are the only regulators of C-EBPα expression in vivo. In this respect we have found that overall, C-EBPα transcript levels were not altered in the BM of RARα−/− mice (unpublished data, March 1998). Other interesting candidates are the cdk inhibitors p21 and p27, whose increases have been shown to be associated with the transition between immature, proliferating cells and post-mitotic differentiated cells for several cell types including myeloid cells.41-45 Interestingly, p21 has been proposed to be a target gene for nuclear receptors, RARs included.41,46 How these proteins are regulated in cells lacking RARα thus merits further investigation. Hox genes, which are regulated by retinoic acid in the embryo, might also contribute to mediating RAR function during hematopoiesis47; their precise role during granulopoiesis, however, and their possible regulation by RARs remain unknown. Finally, Mad1 has been shown to positively regulate cell cycle exit during granulopoiesis, possibly by antagonizing the effects of myc proteins.48 Whether the Myc/Mad and the RAR pathways are parallel or convergent modulators of granulopoiesis remains to be seen.

The role of retinoid signaling during hematopoiesis is likely to extend beyond its role of regulating granulocyte differentiation described here. RA has also been suggested to act as a commitment factor for the granulocyte lineage, as exogenous RA is able to
redirect erythroid-, monocyte-, or eosinophil-committed progenitors toward a granulocyte fate. Although our study does not directly address this issue, it is clear that other factors besides RA can influence granulocyte lineage commitment, as commitment was not impaired in RARα−/−RARγ−/− double mutants or upon VAD. However, the proportion of granulocytes was often reduced in myeloid colonies derived from RARα−/− BM cells, and the frequency of granulocyte-only colonies was diminished (unpublished data, October 1998), phenotypes that could indicate defective granulocyte commitment in the mutants. The present effects of RAR agonists and antagonists during terminal granulocyte differentiation also contrast with those observed when similar compounds were used on hematopoietic stem cells, where RAR agonists and antagonists antagonized and promoted their differentiation, respectively. These differences suggest a complex pattern of retinoid receptor function during hematopoiesis.

Implications for APL pathogenesis

RARα chromosomal rearrangements represent the genetic hallmark of APL. The tight association between RARα alterations and APL strongly suggests that a normal RARα-dependent function must be altered to transform promyelocytes. Generally, it has been assumed that the APL RARα fusion proteins act as dominant-negative inhibitors of retinoid-dependent differentiation. However, because granulocyte differentiation is only partially blocked when retinoid signaling through RAR is prevented, dominant-negative inhibition of retinoid-induced differentiation cannot account for the complete differentiation block seen in APL cells. Our data suggest an alternative mechanism of action for the RAR fusion proteins: enhancement of the normal inhibitory function of unliganded RARα. This enhancement may be due to an overexpression of the fusion proteins vis-à-vis WT RARα, as is often observed in APL cells, and/or to the more potent repressive properties of the fusion proteins themselves. In this respect, PML-RARα and PLZF-RARα are powerful transcriptional repressors that efficiently recruit histone deacetylases to target promoters. Interestingly, overexpression of thyroid hormone receptor α (TRα) in erythroid precursors blocks their differentiation, suggesting that the differentiation arrest mediated by v-erbA, the oncogenic form of TRα, during erythropoiesis might also correspond to an enhancement of a normal property of TRα. Different nuclear receptors may therefore control differentiation and leukemogenesis in distinct hematopoietic lineages via similar mechanisms.

If interference with RARα-regulated differentiation appears important for APL pathogenesis, it probably represents only one facet of the action of RARα fusion proteins in APL. Fusion of RARα with PML or PLZF has been shown to be required to provoke APL. Dominant interference with other functions, such as those of PML (a tumor suppressor gene that controls apoptosis and p53 acetylation), is likely to be important. In this respect the coiled coil moiety of PML appears to mediate the formation of high molecular weight oligomeric complexes containing both PML-RARα and PML, complexes which could disrupt normal PML function. Simultaneous targeting of several cellular functions by the RARα fusion proteins might therefore cooperate to transform promyelocytes.

Acknowledgments

We thank P. Reczek (Bristol-Myers Squibb, Princeton, NJ) for providing BMS493 and M. Sporn for 9cis-RA; C. Bronn, B. Bondeau, and B. Feret for excellent technical assistance; C. Ebel for help with flow cytometry; and A. Paillieux, F. Fischer, and P. Michel for maintaining the mice.
45. Paul CC, Mahrer S, Tolbert M, et al. Changing the differentiation program of hematopoietic cells: retinoic acid-induced shift of eosinophil-commit-
49. Kastner P, Perez A, Lutz Y, et al. Structure, localization and transcriptional properties of two classes of retinoic acid receptor alpha fusion proteins in acute promyelocytic leukemia (APL); structural similarities with a new family of orico-
50. Pandolfi PP, Alcalay M, Fagioli M, et al. Genomic variability and alternative splicing generate multiple PML/RAR alpha transcripts that encode ab-
errant PML proteins and PML/RAR alpha iso-
51. Bauer A, Mikulits W, Lagger G, Sengst A, Brosch G, Beug H. The thyroid hormone receptor func-
tions as a ligand-operated developmental switch between proliferation and differentiation of ery-
germization of RAR and AML1 transcription fac-
tors as a novel mechanism of oncogenic activa-
Positive and negative regulation of granulopoiesis by endogenous RAR α

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