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Cell Biology

# A dominant negative retinoic acid receptor blocks neutrophil differentiation at the promyelocyte stage

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**ABSTRACT** **We have investigated the roles of retinoic acid receptors in the development of neutrophils by using an interleukin 3-dependent multipotent hematopoietic cell line (FDCP mix A4) as well as normal mouse bone marrow cells. Treatment of the FDCP mix A4 cells with murine granulocyte/macrophage-colony-stimulating factor (GM-CSF) induced these cells to differentiate into neutrophils and macrophages. When the endogenous retinoic acid receptor activity in FDCP mix A4 cells was suppressed by a dominant negative retinoic acid receptor construct, this GM-CSF-induced neutrophil differentiation was blocked at the promyelocyte stage. The blocked promyelocytes proliferated continuously as a GM-CSF-dependent cell line but could be induced to terminally differentiate into neutrophils with supraphysiological concentrations of all-*trans*-retinoic acid (1–10 μM). The ability of the dominant negative retinoic acid receptor to block neutrophil differentiation at the promyelocyte stage was also demonstrated in normal, primary mouse bone marrow cells. Our results indicate that retinoic acid receptors in conjunction with hematopoietic growth factors play a crucial role in the terminal differentiation of normal neutrophil precursors. The system described here may also serve as a model for studying the pathogenesis of human acute promyelocytic leukemia.**

The biologic effects of retinoic acid (RA) are mediated through RA receptors (RARs), which are members of the steroid/thyroid hormone receptor superfamily and function as ligand-inducible transcription factors (1). Several lines of evidence suggest that RARs may be involved in hematopoiesis. For instance, RAR genes (predominantly RARα) are transcribed in multiple hematopoietic lineages (2, 3). Moreover, in human acute promyelocytic leukemia (APL), the leukemic promyelocytes which harbor a *PML–RARA* fusion gene as a result of t(15;17) chromosomal translocation differentiate into mature neutrophils when treated with supraphysiological doses of RA (4–6). However, it is unknown whether RA and RARs play a role in the differentiation of normal neutrophils.

A difficulty intrinsic to the study of the possible roles of RA in normal neutrophil differentiation is that most cell lines or primary bone marrow cells suitable for such experiments require serum, which contains a substantial amount of RA (1–10 nM) (7). Although methods exist to remove RA from serum, such procedures unavoidably remove other serum constituents which may be important in the growth or differentiation of neutrophils. An alternative approach to study the role of RA in hematopoiesis is to use mutated RAR constructs with dominant negative activity to suppress the function of endogenous RARs in hematopoietic progenitors. A prototype dominant negative receptor is the v-*erbA* oncogene product, which represses the transcriptional function of the normal thyroid hormone receptor and contributes to the transformation of chicken erythroblasts (8, 9). A small deletion in the C-terminus of v-ErbA appears to be responsible for most of its dominant negative activity (8–10). Following this paradigm, a truncated RARα with dominant negative activity was constructed (11). This truncated RARα cDNA (RARα403) encodes a peptide of 403 amino acids retaining the N terminus, the DNA-binding domain, the dimerization domain, and part of the hormone-binding domain of RARα. In transient expression assays, the RARα403 construct exhibits dominant negative activity against endogenous RARs in mouse fibroblasts (11).

In a previous study (11), we utilized retrovirus-mediated gene transduction to introduce this dominant negative RARα403 construct into the multipotent murine hematopoietic cell line FDCP mix A4 (12). The retroviral vector, LRARα403SN (11), was constructed by inserting the RARα403 cDNA into the cloning site of the LXSN retroviral vector (13). FDCP mix A4 cells expressing this dominant negative construct switched from spontaneous neutrophil and macrophage differentiation to preferential basophil/mast cell development in the presence of interleukin 3 (IL-3) (11). The present study analyzes the differentiation process of LRARα403SN-infected FDCP mix A4 cells stimulated with granulocyte/macrophage-colony-stimulating factor (GM-CSF). During this GM-CSF-induced differentiation many cells appeared blocked at the promyelocyte stage, but this block was overcome with high concentrations of RA (1–10 μM). A similar phenotype was observed in primary mouse bone marrow cells transduced with this dominant negative construct. The blocked promyelocytes resemble human APL cells and may serve as an experimental model for studying the pathogenesis of this leukemia.

## MATERIALS AND METHODS

**Cell Lines.** FDCP mix A4 cells (generously provided by Elaine Spooncer and Michael Dexter) (12) were maintained in Iscove's modified Dulbecco's medium (IMDM; GIBCO) supplemented with 20% (vol/vol) horse serum (Flow Laboratories) and 10% (vol/vol) WEHI-3B conditioned medium as a source of IL-3.

**Mouse Bone Marrow.** Six-week-old male $BDF_1$ mice (The Jackson Laboratory) were injected intraperitoneally with 5-fluorouracil (100 mg/kg of body weight) 5 days prior to bone marrow harvest. The low-density marrow cell fraction containing hematopoietic progenitors was collected by density centrifugation through Nycodenz (specific gravity, 1.080; Robbins Scientific, Mountain View, CA).

**Retroviral Vectors, Producer Cell Lines, and Reporter Constructs.** The construction of retroviral vectors LXSN, LRARαSN, and LRARα403SN (all three contain the neomycin-resistance gene, *neo*) and the establishment of helper

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Abbreviations: APL, acute promyelocytic leukemia; CAT, chloramphenicol acetyltransferase; GM-CSF, granulocyte/macrophage-colony-stimulating factor; IL, interleukin; RA, all-*trans*-retinoic acid; RAR, RA receptor; RRE, RA response element.

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virus-free amphotropic retroviral producer cell lines PA317/LXSN, PA317/LRARαSN, and PA317/LRARα403SN have been reported (11, 13). pRRE$_4$-CAT is a reporter construct containing the RA response element (RRE) of the RARβ gene (14) and the chloramphenicol acetyltransferase (CAT) gene. pCMV-GH contains the human growth hormone gene driven by the promoter of the immediate early gene of cytomegalovirus (15) and was used to normalize transfection efficiency.

**Retroviral Infection and G418 Selection.** FDCP mix A4 cells were infected by a 24-hr cocultivation with irradiated (1100 rads; 1 rad = 0.01 Gy) retroviral producer cell lines in growth medium containing Polybrene (4 μg/ml), followed by G418 selection (300 μg/ml) for 8–10 days. Post-5-fluorouracil, low-density BDF$_1$ marrow cells were infected by cocultivation with unirradiated, subconfluent producer cells for 3 days in IMDM supplemented with 20% horse serum, 20% WEHI-3B conditioned medium, Polybrene (4 μg/ml), murine GM-CSF (2.5 ng/ml; Immunex), human IL-1β (10 ng/ml; Amgen), and human IL-6 (20 ng/ml; Amgen). At least 13% of LXSN-infected and 5% of LRARα403SN-infected marrow colony-forming cells were G418-resistant as determined by colony assays in soft agar.

**Transient Expression Assay.** Radioimmunoassay of human growth hormone and assay of CAT activity were performed as detailed (11). For electroporation of FDCP mix A4 and GMB cells, the following parameters were used: $10^7$ cells, 60 μg each of pCMV-GH and pRRE$_4$-CAT, 800 V, 25 μF. For MPRO cells the following parameters were used: $10^7$ cells, 75 μg each of pCMV-GH and pRRE$_4$-CAT, 250 V, 960 μF. Conditioned medium and cell lysate were collected 40 hr later for growth hormone quantitation and CAT assay (11).

**Southern and Northern Blots.** Genomic DNA samples obtained at different times in the establishment of MPRO cells were digested with *Eco*RI, electrophoresed (30 μg per lane), blotted onto nitrocellulose, and hybridized with a radiolabeled *neo* probe (13). For Northern analysis, samples (10 μg) of total RNA were electrophoresed, blotted, and hybridized with a radiolabeled human RARα probe (11).

## RESULTS

**Characteristics of FDCP mix A4 Cells.** The IL-3-dependent FDCP mix A4 cells are a nonleukemogenic cell line established from a long-term culture of mouse bone marrow (12). When maintained in medium with 20% horse serum and IL-3, the FDCP mix A4 cells differentiate spontaneously into neutrophils and macrophages at low to moderate frequencies (10–20%). Removal of IL-3 and stimulation with GM-CSF in medium with horse serum induce these cells to differentiate into mature neutrophils and macrophages in 4–10 days (Table 1) (16, 17). This GM-CSF-induced neutrophil differentiation appears blunted when certain batches of fetal bovine serum were used in place of horse serum, but supplementation of such cultures with low concentrations of RA (1–10 nM) restores this GM-CSF-induced neutrophil differentiation (see Fig. 3*A*). Thus this GM-CSF-induced neutrophil differentiation of FDCP mix A4 cells appears to require the low concentrations of RA that are ordinarily present in most sera (7).

**Neutrophil Differentiation Is Interrupted in FDCP mix A4 Cells Expressing the Dominant Negative RARα403.** We infected the FDCP mix A4 cells with the LRARα403SN retroviral vector harboring the dominant negative RARα403 construct. In the presence of IL-3, the LRARα403SN-infected FDCP mix A4 cells remained multipotent but differentiated predominantly along the mast cell lineage (11). However, when these cells were transferred to medium containing GM-CSF (5 ng/ml) and only a trace (0.2%) of WEHI-3B conditioned medium, their behavior differed from that of the LXSN (control vector without insert)-infected cultures in two important ways. (*i*) Only the former produced significant numbers of basophils/mast cells (Table 1). (*ii*) While all the cells in LXSN-infected cultures differentiated and died after 2–3 weeks in GM-CSF, the LRARα403SN-infected FDCP mix A4 cultures continued to proliferate over an extended period (Fig. 1). Serial examination of GM-CSF-induced, LRARα403SN-infected FDCP mix A4 cells revealed numerous clusters of immature cells first appearing around days 8–14; such cells were not noted in the GM-CSF-induced control-infected cultures. These immature cells quickly became the predominant cell type in the culture and have proliferated continuously as a GM-CSF-dependent cell line for >1 year (referred to as the GMB cell line below).

**GMB Cells Are Neutrophilic Promyelocytes.** Morphologically, the GMB cells resemble promyelocytes or early myelocytes (Fig. 2*A*). Consistent with their morphology, these cells are strongly positive for the murine neutrophil-specific 7/4 antigen (18) and positive for chloroacetate esterase (specific for neutrophils) (data not shown). Northern blots show that these cells express high levels (at least 10-fold over endogenous RARα) of the 4.7-kb retroviral RNA containing the RARα403 message but do not express c-*fms* mRNA (specific for monocytic lineage) or mRNA encoding the GATA-1 transcription factor (hematopoietic expression restricted to erythroid, megakaryocytic, and mast cell lineages) (19). Furthermore, the GMB cells are negative for mast cell/basophil markers, including surface IgE receptors and toluidine blue metachromatic staining. These findings suggest that the GMB cells represent neutrophilic promyelocytes that fail to complete terminal differentiation. We have repeatedly established GMB-like cells from both low- and high-passage LRARα403SN-infected FDCP mix A4 cells

Table 1. Serial differential counts of FDCP mix A4 cells infected with LXSN or LRARα403SN virus and induced by GM-CSF plus trace IL-3

| | % total cells | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | LXSN-infected | | | | | LRARα403SN-infected | | | | |
| Day | Blasts | Myelo. | Band + Seg. | Mϕ | Mast | Blasts | Myelo. | Band + Seg. | Mϕ | Mast |
| 0 | 75 | 13 | 7 | 4 | 1 | 48 | 5 | 4 | 6 | 37 |
| 6 | 2 | 14.5 | 49 | 34.5 | 0 | 3 | 35.5 | 45 | 8.5 | 8 |
| 12 | 0 | 0.5 | 25 | 74.5 | 0 | 0 | 51.5 | 32 | 8 | 8.5 |
| 18 | 0 | 0 | 1 | 99 | 0 | 0 | 66.5 | 16.5 | 4.5 | 12.5 |
| 24 | 0 | 0 | 0 | 0 | 0 | 0 | 69.5 | 20 | 2 | 8.5 |
| 30 | 0 | 0 | 0 | 0 | 0 | 0 | 78.5 | 15 | 3.5 | 3 |
| 150 | 0 | 0 | 0 | 0 | 0 | 0 | 96 | 4 | 0 | 0 |

Please refer to Fig. 1 legend for experimental details. The day-150 differential counts of the LRARα403SN-infected culture are those of the GMB cell line. Numbers represent mean percentages of duplicates. Myelo., promyelocytes, myelocytes, and metamyelocytes; Band, band-form neutrophils; Seg., segmented neutrophils; Mϕ, macrophages; Mast, mast cells.

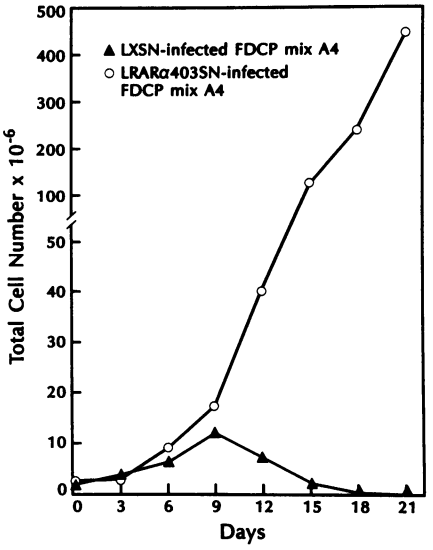


FIG. 1. Growth of LXSN-infected vs. LRARα403SN-infected FDCP mix A4 cells stimulated with GM-CSF. Two million G418-resistant LXSN (control)- or LRARα403SN-infected FDCP mix A4 cells were washed with buffered saline to remove excess IL-3 and reseeded at $2 \times 10^5$ cells per ml in IMDM with 20% horse serum, 0.2% WEHI-3B conditioned medium, and GM-CSF at 5 ng/ml. Cells were subcultured every 3 days. Cell numbers have been corrected for subculturing ratios. Note the decline after day 9 and the eventual extinction after day 21 of LXSN (control)-infected cultures, whereas the LRARα403SN-infected cultures continued to expand in GM-CSF. Values are means of duplicates.

under the conditions described above. In contrast, no such cell line could be isolated from uninfected or control vector (LXSN or LRARαSN, the latter harboring the cDNA of normal human RARα)-infected FDCP mix A4 cells under the same conditions, thus demonstrating the requisite role of the dominant negative RARα403 in the genesis of GMB cells.

**Expression of the Dominant Negative RARα403 in GMB Cells Reduces Their RA Responsiveness.** Perhaps the most compelling evidence that the GMB cells represent neutrophilic promyelocytes with blocked terminal differentiation is the finding that nearly all the GMB cells differentiate rapidly (72–96 hr) and synchronously into mature neutrophils without undue cytotoxicity when treated with supraphysiologic concentrations of RA (1–10 μM) in the presence of GM-CSF (Fig. 2*B*). The half-optimal concentration of RA that induces this terminal neutrophil differentiation of GMB cells is about 1 μM and the optimal concentration is 10 μM (Fig. 3*A*). This dose–response curve of RA-induced differentiation of GMB cells is comparable to the dose–response curve of RA-induced transactivation of a CAT reporter construct harboring the RARβ RREs (pRRE$_4$-CAT) (11) in GMB cells (Fig. 3*B*). In contrast, the dose–response curves of the effect of RA on GM-CSF-induced neutrophil differentiation and of the

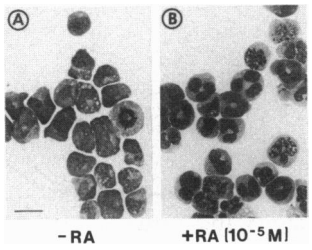


FIG. 2. (*A*) Morphology of uninduced GMB cells. Low percentages of mature neutrophils are normally present in GMB cultures. (*B*) GMB cells maintained in growth medium containing GM-CSF (5 ng/ml) were treated with 10 μM RA for 80 hr. (Bar = 20 μm.)

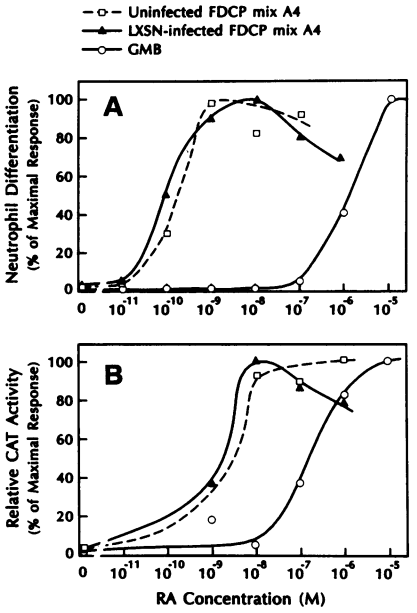


FIG. 3. (*A*) Dose–response curve of RA-induced neutrophil differentiation of GMB cells. GMB cells were treated with the indicated concentrations of RA for 72 hr. Differential cell counts were performed on Wright-stained Cytospin preparations. Over 95% of GMB cells differentiated into neutrophils 72–96 hr after exposure to 10 μM RA. For comparison, the uninfected and LXSN (control)-infected FDCP mix A4 cells were cultured in IMDM supplemented with 10% fetal bovine serum (Hyclone), GM-CSF (5.0 ng/ml), and the indicated concentrations of RA. (In these control experiments it was necessary to use selected batches of fetal bovine serum in which GM-CSF-induced neutrophil differentiation was relatively low in order to demonstrate the effect of exogenous RA on the differentiation process. Presumably these particular batches harbored relatively low concentrations of endogenous RA. All batches of horse serum seemed to have optimal concentrations of endogenous RA in our differentiation assays, and addition of RA resulted in toxicity. The dose–response curve of GMB cells was the same regardless of the types of serum used, due to the higher RA concentration required for differentiation.) Values are means of duplicates. (*B*) Dose–response curve of RA-induced transactivation of pRRE$_4$-CAT. The volumes of cell lysate used in CAT assays have been normalized for transfection efficiency as determined by growth hormone expression.

transactivation of pRRE$_4$-CAT in uninfected or LXSN (control)-infected FDCP mix A4 cells reveal a half-optimal RA concentration of 0.1–1 nM and an optimal concentration of about 10 nM (Fig. 3), concentrations reported to be present in most sera (7). This shifting of the RA dose–response curve by 2–3 orders of magnitude in GMB cells confirms that RARα403 exerts dominant negative effects against the endogenous RARs in GMB cells.

**The Dominant Negative RARα403 Also Blocks the Differentiation of Primary Mouse Neutrophil Precursors at the Promyelocyte Stage.** To determine whether the dominant negative RARα403 can also block the differentiation of normal mouse neutrophil precursors, we infected freshly harvested mouse bone marrow cells with the LRARα403SN or LXSN (control) retroviral vectors by cocultivation for 3 days. The infected cells were subsequently selected in medium containing G418 and GM-CSF. The LXSN (control)-infected mouse bone marrow cells proliferated and terminally differentiated into neutrophils, macrophages, and eosinophils, with all cells dying after 10–14 days (Fig. 4). In contrast, numerous cells in the LRARα403SN-infected marrow culture continued to proliferate after 14 days and stayed immature in morphology. These proliferating cells exhibited promyelocyte characteristics (Fig. 5*A*), including the presence of numerous azurophilic primary granules, cell surface

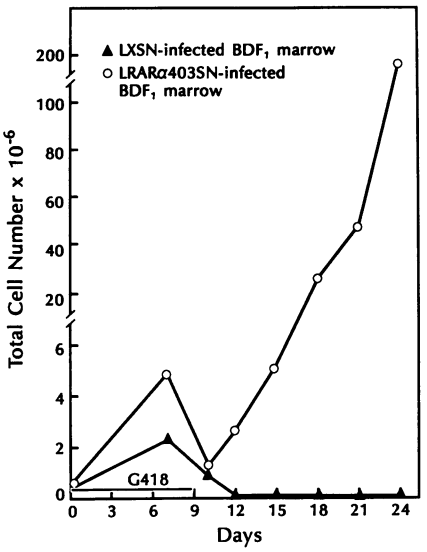


FIG. 4. Growth pattern of LXSN (control)- and LRARα403SN-infected $BDF_1$ bone marrow cells. Half a million infected cells were cultured in IMDM supplemented with 20% horse serum and GM-CSF at 5 ng/ml. G418 (400 μg/ml) selection was applied from day 0 to day 9. Values are means of duplicates.

expression of mouse neutrophil-specific antigen 7/4 (18), and positive staining for chloroacetate esterase. They have proliferated continuously as a GM-CSF-dependent cell line for >8 months and are referred to as the MPRO (for mouse promyelocyte) cell line. Northern blot analysis indicates that the MPRO cells express high levels of retroviral mRNA harboring the truncated RARα403 sequence (Fig. 6*A*). No cell lines could be isolated from LXSN-infected cultures.

**MPRO Cells Exhibit Diminished RA Responsiveness But Differentiate into Mature Neutrophils with High Concentrations of RA.** Like GMB cells, MPRO cells also have greatly reduced RA responsiveness in transient expression assays using the $pRRE_4$-CAT reporter: the half-optimal concentration of RA is about 2 μM and the optimal concentration is 10 μM (Fig. 7). Similar to the GMB cells derived from the FDCP mix A4 cell line, when MPRO cells are treated with 10 μM RA they terminally differentiate into mature neutrophils (Fig. 5*B*). As with the GMB cells, the dose–response curve of RA-induced neutrophil differentiation of the MPRO cells is almost identical to that of the transactivation of $pRRE_4$-CAT in these target cells (Fig. 7).

**Multiclonal Origin of MPRO Cells.** The rapid emergence of large numbers of promyelocytes in LRARα403SN-infected,

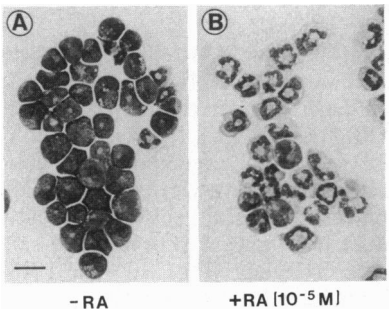


FIG. 5. (*A*) Morphology of uninduced MPRO cells derived from LRARα403SN-infected $BDF_1$ bone marrow. Note the presence of few mature neutrophils. The MPRO cells have a tendency to form aggregates. (*B*) MPRO cells treated with 10 μM RA for 4 days. Cells with doughnut-shaped nuclei and pale cytoplasm are differentiated neutrophils. Two metamyelocytes are seen in the center field. (Bar = 20 μm.)

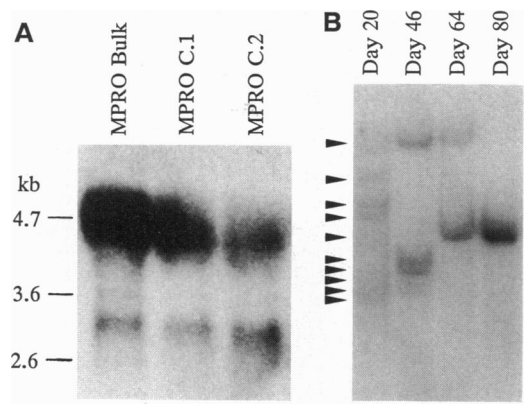


FIG. 6. (*A*) Northern analysis of the expression of RARα403 in MPRO cells. Samples (10 μg) of total RNA from bulk culture of MPRO cells as well as two clonal lines (C.1 and C.2) were applied to each lane and hybridized with a RARα probe. The 4.7-kb full-length viral RNA containing the RARα403 sequence and the 3.6- and 2.6-kb endogenous RARα are indicated. (*B*) Southern analysis of the LRARα403SN-infected $BDF_1$ bone marrow cells. Genomic DNAs were collected at the indicated times after infection and GM-CSF stimulation (same experiment as described in Fig. 4), digested with *Eco*RI, and subjected to Southern hybridization with a *neo* probe. Multiple bands (at least seven) are visible on day 20 (by then all the growing cells are blocked promyelocytes), with at least five bands visible on day 46. One clone eventually dominated the culture by day 80. Arrowheads point to all visible bands.

GM-CSF-stimulated primary mouse bone marrow cells by day 20 suggests a multiclonal origin of these MPRO cells. Indeed, when genomic DNA from the emerging promyelocytes was digested with *Eco*RI (which cuts only once within the provirus) and subjected to Southern blot analysis with a provirus-specific probe (*Neo*), multiple bands were noted at days 20 and 46 after infection, confirming the polyclonal origin of the MPRO promyelocytes (Fig. 6*B*). The multiclonal origin of these promyelocytes indicates that it is the transduction of the dominant negative RARα403 construct *per se* rather than a rare genetic event that accounts for the block in neutrophil differentiation.

## DISCUSSION

Research in the past 20 years has demonstrated the importance of hematopoietic growth factors for the survival, proliferation, and differentiation of hematopoietic progenitors (20). In this and a previous report (11), we provide strong evidence that members (RARs in this case) of the steroid/thyroid hormone receptor superfamily also play significant roles in the differentiation of normal neutrophils. Previous

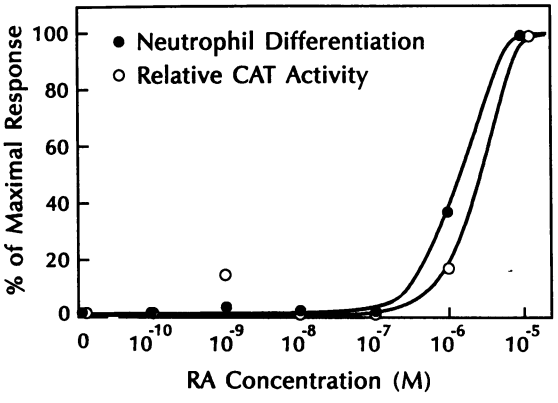


FIG. 7. Dose–response curves of RA-induced neutrophil differentiation and transactivation of $pRRE_4$-CAT in MPRO cells. Values are means of duplicates.

work demonstrated that introducing a dominant negative RARα403 construct into the IL-3-dependent, multipotent FDCP mix A4 cells resulted in a shift from spontaneous neutrophil/macrophage differentiation to the preferential development along the basophil/mast cell lineage in the presence of IL-3 (11). Thus it appears that in the multipotent FDCP mix A4 cells, the differentiation along the neutrophil lineage is permitted or even promoted by normal RARs whereas the development along the mast cell lineage is inhibited. Suppression of endogenous RAR activity with the dominant negative RARα403 reverses this pattern (11).

In the present study, we first induced the LRARα403SN-infected FDCP mix A4 cells with GM-CSF, which greatly increased the frequency of commitment/differentiation along the neutrophil lineage. Under such growth conditions, many differentiating cells are blocked at the promyelocyte stage (Table 1; Figs. 1 and 2*A*). Since we have observed that GM-CSF-induced neutrophil differentiation of the uninfected FDCP mix A4 cells requires physiological concentrations of RA (1–10 nM; Fig. 3*A*), the differentiation block exhibited by the LRARα403SN-transduced cells is probably due to inability of these cells to respond to the physiological concentrations of RA present in the culture medium (which contains 20% horse serum). Nevertheless, this differentiation block can be overcome by supraphysiological concentrations of RA (1–10 μM), which induce virtually all cells to differentiate to mature neutrophils (Fig. 2*B*). This contrasts with the inability of 10 μM RA to induce LRARα403SN-transduced HL-60 promyelocytic leukemia cells to differentiate (11). This may be due to the fact that the HL-60 cell line was originally derived from leukemia cells and may harbor mutations that decrease their RA responsiveness. Indeed, relatively high concentrations of RA (0.1–1 μM) are required to induce untransduced HL-60 cells to differentiate.

The mechanism by which the RARα403 construct exerts its dominant negative activity remains to be elucidated. RARα must dimerize with retinoid X receptors (RXRs) in order to function optimally (21, 22) and a truncated RARα (RARα404, which differs from RARα403 by one amino acid at the C terminus) can dimerize with RXRα (21). Thus it is very likely that the overexpressed RARα403 dimerizes with available RXRs in LRARα403SN-infected cells and thereby prevents the formation of functional RAR-RXR dimers. The differentiation response of GMB cells to supraphysiological concentrations of RA (1–10 μM) may be mediated by residual intact RAR–RXR heterodimers or via alternative pathways that are less affected by RARα403 and have a higher RA requirement.

Infection of primary, normal mouse bone marrow cells with the LRARα403SN retroviral vector carrying the dominant negative receptor construct led to a differentiation block at the promyelocyte stage with establishment of GM-CSF-dependent promyelocyte cell lines (MPRO). Several considerations support the notion that the expression of the dominant negative RARα403 by itself is sufficient for this differentiation block: (*i*) the viral producer cell lines are free of helper viruses that may induce additional mutations; (*ii*) the rapid emergence (within 14 days) of MPRO cells argues against accumulated spontaneous mutations that may contribute to the differentiation block; (*iii*) Southern blot analysis of the proviral integration sites indicates multiclonal nature of MPRO cells in the early stages of establishment (days 20–46; Fig. 6*B*), thus making it unlikely that a common proviral insertional mutation or a second genetic event plays a role in generating the blocked promyelocyte phenotype. The capacity of the dominant negative construct alone to inhibit GM-CSF-induced neutrophil differentiation of normal mouse bone marrow cells provides strong evidence that RAR activity is essential for normal neutrophil differentiation.

It is intriguing that the arrested promyelocyte phenotype that characterizes the GM-CSF-dependent GMB and MPRO cells (expressing the dominant negative RARα403 construct) is similar to the phenotype of human APL cells (which express the aberrant *PML–RARA* fusion gene). Both the infected mouse cell lines and human APL cells are blocked at the same developmental stage and can be induced to terminally differentiate with supraphysiological concentrations of RA. Since the differentiation block in the GMB and MPRO cells is attributable to the dominant negative activity of the transduced RARα403 construct, this phenotypic similarity among GMB, MPRO, and APL cells suggests that the aberrant PML–RARα fusion protein (or the reciprocal translocation product RARα–PML) that characterizes most cases of human APL might also exert dominant negative activity in neutrophil precursors. However, PML–RARα does not appear to consistently repress RAR activity in transactivation assays using various target cells (23, 24). It is possible that the dominant negative activity of PML–RARα (or RARα–PML) may be cell type-, developmental stage-, and promoter-specific. If so, the system described here may be suitable for examining these possibilities.

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