Cooperation of Cytokine Signaling with Chimeric Transcription Factors in Leukemogenesis: PML-Retinoic Acid Receptor Alpha Blocks Growth Factor-Mediated Differentiation

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Received 13 September 2002/Returned for modification 8 November 2002/Accepted 19 March 2003

We utilized a mouse model of acute promyelocytic leukemia (APL) to investigate how aberrant activation of cytokine signaling pathways interacts with chimeric transcription factors to generate acute myeloid leukemia. Expression in mice of the APL-associated fusion, PML-RARA, initially has only modest effects on myelopoiesis. Whereas treatment of control animals with interleukin-3 (IL-3) resulted in expanded myelopoiesis without a block in differentiation, PML-RARA abrogated differentiation that normally characterizes the response to IL-3. Retroviral transduction of bone marrow with an IL-3-expressing retrovirus revealed that IL-3 and promyelocytic leukemia-retinoic acid receptor alpha (PML-RARα) combined to generate a lethal leukemia-like syndrome in <21 days. We also observed that a constitutively activated mutant IL-3 receptor, βv,449E, cooperated with PML-RARα in leukemogenesis, whereas a different activated mutant, βv,1374N, did not. Analysis of additional mutations introduced into βv,449E showed that, although tyrosine phosphorylation of βv is necessary for cooperation, the Src homology 2 domain-containing transforming protein binding site is dispensable. Our results indicate that chimeric transcription factors can block the differentiative effects of growth factors. This combination can be potently leukemogenic, but the particular manner in which these types of mutations interact determines the ability of such combinations to generate acute myeloid leukemia.

Numerous genetic abnormalities have been identified in human acute myeloid leukemia (AML) (3, 38). Alterations in transcription factors have been observed in particular morphological subtypes of AML. In addition, alterations in molecules that normally regulate cell behavior in response to external cues (hereafter referred to as “signaling molecules”) are common in AML, but these types of mutations are less closely associated with particular morphologies. In AML, chromosomal translocations commonly result in aberrant transcription factors, whereas small intragenic mutations can lead to abnormal transcription factors or mutant signaling molecules. Another type of alteration, chromosomal gain or loss, has been seen in some cases of AML, but the critical genes affected by these changes have not been definitively identified.

The number of genetic alterations required to cause human AML is not known and may vary with the type of leukemia. De novo leukemias with chromosomal translocations are believed to be genetically simpler than leukemias arising after myelodysplastic syndromes or cytotoxic chemotherapy, which are often karyotypically complex. Of note, the proportion of AMLs with simple translocations declines with age whereas, conversely, AMLs with complex karyotypes increase (49). Differences in karyotype, epidemiology, and response to therapy indicate that AMLs are heterogeneous diseases. Therefore, although there may be common elements and molecular pathways that result in conversion of normal hematopoietic cells to AML, the types of genetic changes that in combination cause leukemia are likely to be heterogeneous.

We sought to identify a simple combination of genetic changes that is sufficient to cause leukemic transformation and to examine the manner in which these changes exert their cooperative effects. For this purpose, we utilized a transgenic mouse model of acute promyelocytic leukemia (APL) in which the MRP8 promoter is used to express a PML-RARA fusion gene in myeloid cells. The t(15;17)(q22;q12) results in the expression of a chimeric promyelocytic leukemia-retinoic acid receptor alpha (PML-RARα) fusion protein that is responsible for nearly 99% of all human APLs (41, 50). There is evidence that, in fact, only a small number of genetic changes are required to cause APL. The t(15;17) is observed as the sole karyotypic change in 62% of cases (37), and the incidence of APL is constant over the human life span (56), suggesting one rate-limiting step. Although it is conceivable that the t(15;17) is fully sufficient to cause leukemia, there is evidence that additional mutations are required. In 38% of cases, the t(15;17) is accompanied by additional karyotypic changes (37), point mutations in genes...
encoding signaling molecules (such as FLT3) are common in APL (28, 33, 51, 58, 59), and expression of the PML-RARα fusion protein in mice initially has only a modest impact on myelopoesis (1, 15, 17).

With a median latency of 8.5 months, MRP8 PML-RARA transgenic mice developed AML with features of human APL (1). The transition from modest abnormalities of neutrophil maturation to acute leukemia was accompanied by the appearance of karyotypic abnormalities. Thus, this mouse model can serve to identify genetic changes that cooperate with PML-RARα to cause acute leukemia.

We have previously reported that BCL-2 decreased the latency and increased the penetrance of leukemia in mice that express PML-RARA. However, the BCL-2–PML-RARα combination was not sufficient for the leukemic phenotype: there was still a latency of >3 months, and recurring chromosomal abnormalities and complex karyotypes were a constant feature of the doubly transgenic leukemias (29, 37). In considering events able to complete leukemic transformation, we noted that preleukemic PML–RARα/BL2 doubly transgenic mice exhibited a marked impairment of neutrophil differentiation but that, prior to the onset of acute leukemia, the immature cells were not disseminated into nonhematopoietic tissues. These results suggested that AML reflects not only impaired differentiation but also relative autonomy from external cues. We therefore hypothesized that providing continuous growth factor stimulation to PML-RARA-expressing cells would be sufficient to cause a rapidly fatal leukemia. We also hypothesized that this model system could be used to elucidate the potential of such activation to contribute to acute leukemia. These findings suggest a model of normal myelopoiesis in which growth factor receptor activation generates a mix of signals that balance increased proliferation and survival with maintenance of maturation. Abrogation of the molecular events that normally ensure differentiation in the face of a growth stimulus is one pathway to AML.

**MATERIALS AND METHODS**

**Mice.** Mice were bred and maintained at the University of California at San Francisco, and their care was in accordance with University of California at San Francisco guidelines. MRP8 PML–RARα transgenic mice in the FVB/N background and control FVB/N mice were used for all experiments.

**IL-3 injections.** Transgenic and control mice were injected subcutaneously with interleukin-3 (IL-3) at a dose of 200 ng (10 µg/kg) three times a day for 4 days. Mice were euthanized, and tissues were harvested 8 h after the last injection.

**Differentiation assays.** Bone marrow from FVB/N and MRP8 PML–RARα mice was harvested, depleted of red blood cells with Histopaque 1119 (Sigma) at 4°C according to the manufacturer’s instructions, and cultured in Myelocult 5300 (Stem Cell Technologies) with 10% X63Ag8–mIL-3 conditioned medium (25), without or with all-trans-retinoic acid (ATRA; Sigma) at a concentration of 10−7 or 10−5 M.

**RESULTS**

PML-RARα has a modest impact on myelopoiesis in vivo. Although PML-RARα has been observed to inhibit the differentiation of cell lines and of primary cells in vitro (12–14, 48, 50),
we had observed that the expression of an MRP8 PML-RARA transgene in mice had only a modest effect on hematopoiesis. Careful analysis of myelopoiesis in these mice revealed subtle abnormalities (1, 29) (Fig. 1). Although peripheral blood counts are indistinguishable from control animals, in the bone marrow there is an increase in immature myeloid cells (Fig. 1A). Immunophenotypic analysis revealed that, in addition to the decrease in Ly-6G(Gr-1) expression we previously observed, the bone marrow of MRP8 PML-RARA transgenic mice showed a modest increase in immature cells because Ly-6G(Gr-1) expression increases when neutrophilic cells mature and CD31 expression decreases with myeloid maturation. Cytologic and histopathologic exami-
FIG. 2. PML-RARα blocks differentiation that accompanies IL-3 receptor activation. (A) Bone marrow differential cell counts are shown. Control or healthy preleukemic PML-RARA transgenic mice were injected with IL-3 for 4 days. Bars: littermate controls (□), n = 4; PML-RARA (■), n = 4, ✦, IL-3-injected PML-RARA transgenic animals had, compared to IL-3-injected controls, significantly more immature forms/blasts (P = 0.005) and significantly fewer lymphocytes (P = 0.02). Trends toward fewer mature neutrophilic cells and erythroid cells were also evident. (B) Differential cell counts of in vitro assays. Control or preleukemic PML-RARA transgenic bone marrow cells were harvested, depleted of erythroid cells, and cultured in IL-3 with or without ATRA for 24 h. Bars: littermate controls (□), n = 3; PML-RARA (■), n = 4. Abbreviations are as described in Fig. 1A with the following additions. In these in vitro assays, intermediate-stage cells that were clearly neutrophilic (Inter/Neu) were enumerated separately from those that appeared monocytic (Inter/Mo); mature monocytes and macrophages were enumerated together (Mo/Mac). (I) Control or PML-RARA transgenic bone marrow in IL-3 after 24 h; (II) control or PML-RARA transgenic bone marrow in IL-3 plus ATRA at 10⁻⁷ M; (III) control or PML-RARA transgenic bone marrow in IL-3 plus ATRA at 10⁻⁵ M. ✦, for PML-RARA cells, immature forms/blasts were markedly increased in IL-3 in culture after 24 h (compared to FVB/N, P < 0.0001), and mature neutrophils were markedly decreased (P = 0.03). In the presence of ATRA, PML-RARA marrow yielded significantly fewer immature forms/blasts and significantly more intermediate and mature neutrophilic cells (compared to PML-RARA without ATRA, P = 0.01 at 10⁻⁷ M and 10⁻⁵ M ATRA). Of note, at 10⁻⁵ M ATRA, the numbers of immature forms/blasts and mature neutrophilic cells were not significantly different between the FVB/N and PML-RARA cultures.
500,000 cells were injected into lethally irradiated nontransgenic histocompatible recipients. Recipients of control/IL-3-transduced mice showed a marked elevation in WBC counts, normal platelet numbers, moderate anemia, and marked thrombocytopenia (Fig. 3). Of note, the livers of both control/IL-3 and PML-RARα/IL-3 mice were heavily infiltrated with myeloid cells. Whereas spleens of control/IL-3 mice showed red pulp expansion with a mix of myeloid cells, erythroid cells, and megakaryocytes, the red pulp of PML-RARα/IL-3 mice was expanded predominantly with immature myeloid cells. The blood counts, marrow morphology, histopathology, and rapid clinical course of disease observed in PML-RARα/IL-3 recipients are characteristic of AML in mice.

To assess the response of the PML-RARα/IL-3 disease to ATRA, sublethally irradiated recipients of PML-RARα/IL-3 bone marrow were treated with ATRA 10 days after injection. The median survival of ATRA-treated mice was 45% longer than the survival of untreated or placebo-treated mice (un-treated/placebo treated, n = 20, 16 to 19 days; ATRA treated, n = 4, 25 to 26 days; P = 0.001).

Karyotypic analysis of six PML-RARα/IL-3 diseased recipients was performed (Table 1). Five cases lacked clonal abnormalities, whereas in one case, 2 of 11 metaphase cells exhibited a Robertsonian translocation (sample 740), which would not be expected to result in altered gene expression. These results contrast with the high frequency of clonal cytogenetic changes and the distinct pattern of recurring numerical abnormalities that we observed in PML-RARα (91%) and PML-RARα/BCL-2 (100%) leukemias (29, 37). These cytogenetic findings indicate that the combination of PML-RARα and IL-3 may be fully sufficient to generate the leukemia-like syndrome observed.

### TABLE 1. Karyotypic analysis of PML-RARα/IL-3 mice

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*a NCA, nonclonal abnormality; * Robertsonian translocation (centric fusion) noted in two cells.
investigate the ability of cytokine stimulation to cooperate with PML-RARα in leukemogenesis, as well as to establish a system in which to analyze the mechanisms underlying this cooperation, we utilized activated versions of the IL-3 receptor. The IL-3 receptor is part of a family of cytokine receptors that includes GM-CSF and IL-5. In humans, these three receptors have distinct alpha chains but share a beta chain ($\beta_c$). In mice there is an alternative chain ($\beta_l$) that combines with the IL-3 receptor alpha chain to generate a second class of IL-3 receptor. We have utilized activated versions of human $\beta_c$ that have been previously demonstrated to confer cytokine independence to mouse cells and have been used to enhance our understanding of GM-CSF/IL-3/IL-5 signal transduction (Fig. 5) (21, 22, 39). Although activating mutations in $\beta_c$ have not

FIG. 4. PML-RARα and IL-3 disease has features of AML. (A to C) Blood and bone marrow analyses of diseased recipients of IL-3-transduced control and PML-RARα transgenic bone marrow are shown. The results are compared to normal FVB/N mice and leukemias that arose spontaneously in MRP8 PML-RARα transgenic animals. (A) Peripheral blood: WBC, 10,000/µL; hemoglobin (HGB), grams/deciliter, platelet count (PLT), 100,000/µL. *, control/IL-3 mice exhibited leukocytosis ($P = 0.01$), displayed a trend toward anemia, and were not thrombocytopenic. PML-RARα/IL-3 mice exhibited leukocytosis, anemia, and thrombocytopenia ($P = 0.004$). Bars: control FVB/N (□), n = 14; leukemias from PML-RARα mice (○), n = 10; control/IL-3 (●), n = 6; PML-RARα/IL-3 (■), n = 10. (B) Peripheral blood: percentage of nucleated WBCs. *, compared to control/IL-3 mice, PML-RARα/IL-3 mice had increased immature forms/blasts ($P = 0.01$), increased intermediate forms ($P = 0.01$), and decreased mature neutrophils ($P = 0.0001$). Not shown are mast cells that comprised 4% of cells in control/IL-3 marrow, 0.7% of cells in PML-RARα/IL-3 marrow, and <0.25% of cells in control and PML-RARα leukemic marrows. Bars: control FVB/N (□), n = 8; leukemias from PML-RARα mice (○), n = 6; control/IL-3 (●), n = 5; PML-RARα/IL-3 (■), n = 5. (C) Bone marrow: percentage of nucleated cells. *, compared to control/IL-3 mice, PML-RARα/IL-3 mice had increased immature forms/blasts ($P = 0.01$) and decreased mature neutrophils ($P = 0.0003$). Not shown are mast cells that comprised 4% of cells in control/IL-3 marrow, 0.7% of cells in PML-RARα/IL-3 marrow, and <0.25% of cells in control and PML-RARα leukemic marrows. Bars: control FVB/N (□), n = 16; leukemias from PML-RARα mice (○), n = 6; control/IL-3 (●), n = 5; PML-RARα/IL-3 (■), n = 5. (D) Flow cytometric immunophenotyping of control/IL-3 or PML-RARα/IL-3 bone marrow. Subpanels: a to d, control/IL-3; e, f, g, and h, PML-RARα/IL-3; a and e, bone marrow smears stained with Wright's Giemsa (magnification, ×400); b to d and f to h, histopathology sections stained with H&E; b and f, bone marrow (magnification, ×400); c and g, spleen (magnification, ×400); d and h, liver (magnification, ×100).
PML-RARα/β, V449E leukemias were responsive to retinoic acid. Sublethally irradiated recipients of 10⁶ cells from a PML-RARα/β, V449E animal were treated with placebo or 5-mg ATRA pellets on day 14 after transplantation of the leukemia. The median survival of ATRA-treated mice was 33% longer than the median survival of placebo-treated mice (placebo treated, n = 6, 19 to 22 days; ATRA treated, n = 6, 27 to 32 days; P = 0.001). Interestingly, this effect on survival was similar to the 45% prolongation observed in PML-RARα/IL-3 mice treated with ATRA (see above) but appeared to be less than the twofold median prolongation of survival caused by ATRA when PML-RARα leukemias without activation of the IL-3 receptor were treated (29, 36).

We also examined the karyotypes of the PML-RARα/β, V449E leukemias. As shown in Table 2, seven of nine (78%) leukemias had clonal abnormalities, which included the gain of chromosome 15 in two cases and gain of chromosome 8 in seven cases. Four cases showed loss of an X chromosome.
In light of these clonal karyotypic abnormalities, we examined whether splenic DNA of PML-RARα/H9251/H9252cV449E leukemias also demonstrated a monoclonal (or oligoclonal) pattern of retroviral insertion sites. Interestingly, Southern blot analyses of four leukemias, including two leukemias for which we had cytogenetic data (samples 108 and 363), demonstrated that proviral DNA was present in the leukemias (Fig. 7A) and that there was more than one site of integration in each sample, with various band intensities indicative of multiple clones (Fig. 7B). A number of explanations for the contrasting clonality
results obtained by karyotypic and Southern blot analyses are considered below (see Discussion).

Tyrosine phosphorylation of activated beta common is essential for cooperation with PML-RARα. Mutations in the juxtamembrane region (extracellular) and transmembrane region of βc can activate the receptor and confer cytokine independence onto factor-dependent cell lines and primary hematopoietic cells (22, 39). Whereas βcV449E exemplifies the transmembrane mutations, βcI374N (βcI358N, signal sequence excluded) exemplifies the juxtamembrane class of mutations.

Because these classes of mutations differ in their biological and biochemical effects (21, 40), we compared the combination of βcI374N and PML-RARα with what we had observed with PML-RARα plus βcV449E. In contrast to the latter, βcI374N did not cooperate with PML-RARα to induce leukemia (Fig. 8).

Previous studies had demonstrated that βcV449E exhibited ligand-independent phosphorylation of the intracellular tyrosine residues of βc, whereas βcI374N did not exhibit detectable ligand-independent tyrosine phosphorylation (21). We therefore hypothesized that signals derived from the phosphorylated tyrosines of βc were responsible for the ability of this activated cytokine receptor to cooperate with PML-RARα in leukemogenesis. We tested this hypothesis by utilizing βcV449E/Y6xF in which tyrosines 593, 628, 711, 766, 822, and 882 have all been mutated to phenylalanines. Although βcV449E/Y6xF retains two tyrosines located near the transmembrane-intracellular junction, βcV449E/Y6xF did not cooperate to induce acute leukemia (Fig. 8).

Particular tyrosines of βc have been implicated in specific biochemical functions. Y593F (Y577F, signal sequence excluded) is the binding site for Src homology 2 domain-containing transforming protein (SHC) and may also contribute to phosphorylation of protein tyrosine phosphatase, non-receptor type 11 (SHP-2) (10, 20, 43). Y766F (Y750F, signal sequence excluded) can contribute to STAT activation (although it is not necessary for such activation) and may enhance tyrosine phosphorylation on βc (10, 19, 55). Interestingly, both βcV449E/Y593F and βcV449E/Y766F were able to cooperate with PML-RARα to induce leukemia (Fig. 8), indicating that these individual tyrosines were not essential for cooperative leukemogenesis. These leukemias were phenotypically similar to those arising in PML-RARα/βcV449E recipients (data not shown). There was a trend toward prolonged survival in the presence of these point mutations (P < 0.05). Nevertheless, we
cannot definitively conclude that these point mutations influenced latency because (i) there are small numbers of mice per group, (ii) at least one animal in each group became ill in <50 days, and (iii) it is possible that differences in viral titers influenced survival.

**DISCUSSION**

We have utilized MRP8 PML-RARA mice to investigate how genetic changes cooperate in myeloid leukemogenesis. We observed that, whereas PML-RARα had only modest effects on myelopoiesis and cytokine stimulation alone resulted in expanded myelopoiesis with retained differentiation, PML-RARα blocked the differentiation that normally accompanies growth factor stimulus. IL-3 combined with PML-RARα to rapidly generate a lethal disease with pathological features of AML. An activated βc chain similarly cooperated with PML-RARα to generate a transplantable AML. Analysis of various activated βc chains showed that (i) the ability to confer cytokine independence, per se, is not sufficient for cooperative leukemogenesis; (ii) signals induced by phosphoryrosines are critical for transformation in this system; and (iii) a tyrosine residue critical for SHC activation is not required for cooperation with PML-RARα.

The combination of PML-RARα and IL-3 resulted in a disease with features of murine AML: accumulation of numerous immature forms/blasts, anemia and thrombocytopenia, dissemination of immature cells outside of the bone marrow and spleen, and rapid lethality. Injections of small numbers of cells immediately after transduction into sublethally irradiated mice was fatal in <21 days. In addition, in contrast to previous studies of leukemias in MRP8 PML-RARA transgenic mice, clonal cytogenetic changes were observed in only one of six leukemias studied, and in the one case, only a minority of cells showed a nonpathogenic chromosomal abnormality. These results strongly suggest that PML-RARα plus IL-3 are sufficient to cause a leukemia-like syndrome.

We wanted to demonstrate that cytokine receptor activation would cooperate with PML-RARα in a cell autonomous manner in order to develop a system in which we could delineate the mechanisms contributing to leukemic transformation. For this reason, we made use of an activated βc chain of the GM-CSF/IL-3/IL-5 receptor family. Like IL-3, βcV449E cooperated with PML-RARα and in fact gave rise to a transplantable AML with features of APL.

Differences between our results with IL-3 and βcV449E retroviruses are likely due in part to cell nonautonomous effects of the IL-3 retrovirus. Mice reconstituted with bone marrow transduced with the IL-3 retrovirus have markedly elevated IL-3 levels in serum (46). IL-3 is therefore able to influence not

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**TABLE 2. Karyotypic analysis of PML-RARα/βc V449E leukemias**

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* NCA, nonclonal abnormality.

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**FIG. 7. PML-RARα/βc V449E leukemias have multiple proviral insertion sites.** Genomic DNAs derived from spleen samples of PML-RARα/βc V449E leukemic mice were used to assess retroviral integration. Filters were probed with a 1.1-kb Neo probe derived from pRufNeo. The sizes of molecular weight markers are shown in kilobases. (A and B) Genomic DNAs were digested with KpnI (which cuts in each viral long terminal repeat) to assess the presence of provirus (A) or digested with BamHI to assess the number of integration sites (B). BamHI cleaves once within the retroviral sequence; therefore, each band represents a site of retroviral integration.

**FIG. 8. Tyrosine phosphorylation of hβc is essential for cooperation with PML-RARα.** Compared to PML-RARα/βc V449E leukemic recipients, mice that received PML-RARA bone marrow transduced with βc 1374N (n = 17) and βcV449E-Y6xF (n = 8) did not become ill in the first 200 days. Both βcV449E-Y593F (n = 7) and βcV449E/Y766F (n = 3) mice developed leukemia. The phenotype of these leukemias was similar to that observed in PML-RARα/βc V449E mice (data not shown).
only the transduced cells but also nontransduced cells in the transplanted marrow and in host cells as well. The shorter latency of disease in PML-RARα/IL-3 mice compared to PML-RARα/β, V449E recipients is therefore probably due, at least partially, to the impact of IL-3 on nontransduced cells. Supporting this possibility is the fact that, whereas injection of 50,000 PML-RARA cells transduced with IL-3 was fatal to sublethally irradiated recipients in <20 days (Fig. 3), injection of 1,000,000 cells from PML-RARα/β, V449E leukemic mice took >20 days to cause lethality (Fig. 6E, P < 0.001) and the latency of leukemia after injection of 50,000 cells from PML-RARα/β, V449E leukemic mice into sublethally irradiated recipients was 67 days (data not shown). Cell nonautonomous effects of IL-3 might also contribute to the lack of transplantability of PML-RARA/IL-3 disease to secondary recipients. In addition, the fact that five of six karyotypes of PML-RARα/IL-3 disease were normal, whereas seven of nine PML-RARα/β, V449E leukemias were abnormal could similarly reflect the ability of secreted IL-3 to expand both transduced and untransduced PML-RARA-expressing cells.

Our findings that the PML-RARA/β, V449E leukemias exhibited clonal karyotypic abnormalities, whereas the patterns of proviral insertion suggested oligo- or polyclonality, raise interesting questions regarding the sufficiency of the combination of PML-RARα and β, V449E to fully transform normal blood cells into acute leukemia. One possibility is that PML-RARα plus β, V449E is sufficient for transformation, that this is reflected in the multiple insertion sites present in the spleens of leukemic mice, and that the clonal karyotypic abnormalities reflect the rapid acquisition of cytogenetic changes, with clonal selection, taking place within already-transformed cells. Alternatively, PML-RARα plus β, V449E may induce a preleukemic state characterized by expanded myelopoiesis that is not initially acute leukemia. The proviral insertion patterns could reflect persistence of multiple preleukemic clones in the spleens of leukemic mice. If this is the case, then additional genetic changes, reflected as clonal karyotypic abnormalities, are necessary and, in this system, inevitable. Inducible systems represent a powerful approach for assessing sufficiency in malignant transformation (45). Studies of mice carrying a PML-RARA transgene in combination with an inducible activated cytokine receptor could clarify the number of steps required for leukemogenesis.

An important finding from this work was that growth factor independence does not necessarily permit cooperation with PML-RARA in leukemogenesis, that is, differences among events able to confer factor independence can influence the potency of cooperation. We observed that although β, I374N, like β, V449E, is able to confer growth factor independence and activate mitogen-activated protein kinase, JAK2, and STAT5 (21), β, I374N did not readily cooperate with PML-RARα to generate AML. What then underlies this difference between these two versions of an activated receptor? At the cellular level, β, I374N and β, V449E have different effects on the murine factor-dependent FDB cell lines (40). Both confer factor independence, but FDB cells that grow and survive due to the presence of β, I374N undergo differentiation, whereas FDB cells dependent on β, V449E remain undifferentiated. Therefore, when these activated receptors are expressed in vivo, the signals generated by β, I374N might foster greater maturation than those caused by β, V449E. Of note, activated FLT3, which like β, V449E has been reported to favor continued proliferation while impairing maturation (60), does cooperate with PML-RARA to cause leukemia (27, 53). At the molecular level, known qualitative differences between the β, I374N and β, V449E mutants that might underlie their different effects include the following: (i) β, I374N results in ligand-independent association with the α chain of mouse GM-CSF receptor; (ii) β, V449E but not β, I374N results in phosphorylation of the tyrosines in the intracytoplasmic region of β, and (iii) such phosphorylation results in SHC activation in cells that express β, V449E but not β, I374N (21, 23; T. Blake and T. Gonda, unpublished observations). A lack of SHC activation in PML-RARA cells transduced with β, I374N was a possible explanation for the lack of cooperative leukemogenesis we observed with β, I374N, but the β, V449E/Y693F construct, which lacks the SHC binding site, still cooperated with PML-RARα to cause leukemia. The fact that mutations of six tyrosines of β, in the β, V449E/Y693F construct blocked cooperation indicates that signals generated by phosphorylation of these tyrosines are critical, and the fact that β, V449E/Y766F did cooperate highlights the redundancy of the signals generated by interactions with these tyrosines (10, 19, 43, 55). The use of additional β, mutations, as well as complementary approaches to assessing the importance of downstream effectors, should permit our model system to be further utilized in identifying critical mediators through which activated cytokine receptors contribute to AML.

Given the subtle impact of PML-RARA on myelopoiesis in vivo, what is the role of this protein in APL? The transcriptional effects of PML-RARA, including the ability of PML-RARα to repress transcription through recruitment of histone deacetylases (41) and DNA methyltransferases (8), likely underlies the ability of this protein to cause a subtle increase in immature myeloid cells. In addition, by inhibiting PML’s ability to induce apoptosis, PML-RARA may foster the acquisition of additional pathogenic mutations in this expanded immature cellular compartment (47). Finally, as we have demonstrated, PML-RARA contributes to a profound block in differentiation in the presence of additional genetic lesions. PML-RARA exemplifies a principle that seems to apply to transcription factor mutations that contribute to human AML: it has multiple effects that each individually contribute to transformation.

One type of cooperating event, activation of cytokine receptors on its own fosters proliferation and survival and maintained differentiation. An appropriate balance in the cellular response to growth factors permits expansion of the immature or mitotic compartment while ensuring the generation of large numbers of mature effector cells. Recent data suggest that growth factor-induced activation of RARs may in fact be the basis for the differentiation response. For example, both GM-CSF and IL-3 enhance the activity of RARs (24, 52). Although under steady-state conditions loss ofRAR activity has, like the expression of PML-RARA, mild effects on myelopoiesis (26, 35), it is plausible that PML-RARA blocks the increased RAR activity induced by growth factor stimulation. This block would thus abrogate a differentiation signal required to maintain neutrophil production in the presence of increased cytokine receptor activation.

It is notable that specific expression of a number of translo-
cation proteins in myeloid cells, including PML-RARα, AML1-ETO, and CBβ-SMMHC, can have modest in vivo effects (1, 15, 17, 18, 31). It therefore appears that the genetic changes of AML central to impaired differentiation are able to block maturation only when accompanied by changes that promote autonomous growth. This idea is supported by the observations that activated FLT3 cooperates with PML-RARα (27, 53) and that BCR-ABL cooperates with AML1-DS1-EVI1 or NUP98-HOXA9 (4, 6) to cause acute leukemia. By understanding the critical events that underlie cooperative effects, we should be able to therapeutically tip the cells of human AMLs back toward normal behavior, as has been done successfully for APL. Our findings explore one pathway for leukemia transformation, i.e., cooperation of abnormal transcription factors with activated cytokine receptors, and provide a system for identifying the particular signals that are critical for this process. Other pathways for leukemic transformation almost certainly exist, including overexpression of complement-regulating transcription factors (e.g., Hoxa9 plus Meis1 [34]), as well as unidentified events in the karyotypically complex leukemias seen after myelodysplasia, cytotoxic chemotherapy, and in the elderly. Further work is needed to reveal whether these different types of AML share fundamental molecular abnormalities.

ACKNOWLEDGMENTS

We thank Warren Pear, Richard Van Etten, and Yosef Refaeli for their assistance with protocols for retroviral transduction of bone marrow; Sheila Bitts, Elizabeth M. Davis, and Bhumi Patel for assistance with cytogenetic analysis; Suzanne Cory for pMPZen-IL-3; H. Jeffrey Lawrence for critical comments on the manuscript and mentoring; and J. Michael Bishop, Kevin M. Shannon, and Daphne A. Haas-Kogan for helpful discussions and support.

S.K. is a recipient of a Burroughs Wellcome Fund Career Award and is the 32nd Edward Mallinckrodt Junior Scholar. Funding was also provided by grants CA75986 and CA95274 (S.K.) and CA84221 (S.K. and M.M.L.) from the National Institutes of Health and by the National Health and Medical Research Council of Australia (T.J.G.).

REFERENCES


AUTHOR’S CORRECTION

Cooperation of Cytokine Signaling with Chimeric Transcription Factors in Leukemogenesis: PML-Retinoic Acid Receptor Alpha Blocks Growth Factor-Mediated Differentiation

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Volume 23, no. 13, p. 4573–4585, 2003. We regret that the construct labeled β1,1374N in Fig. 5 and 8 and in the text has been found to be β1,1374A, a construct that is not constitutively activated. Following the discovery of this unfortunate error, we have transduced PML-RARA transgenic bone marrow with the correct β1,1374N construct. In contrast to what we reported, β1,1374N can, like β1,V449E, cooperate with PML-RARA to induce leukemias with features of acute promyelocytic leukemia. Hence, although we believe that the other results and conclusions of the work are correct (including the central point of the paper, the cooperation of cytokine signaling with PML-RARα in blocking differentiation and inducing leukemia), the results that we presented and discussed for β1,1374N (Discussion, paragraph 6) are incorrect. We apologize for any difficulties or confusion that this error may have caused for our colleagues.