The granulocyte colony-stimulating factor (G-CSF) plays an important role in normal granulopoiesis. Its functions are mediated by specific receptors on the surface of responsive cells and, upon ligand binding, several cytoplasmic tyrosine kinases are activated. The cytoplasmic region proximal to the membrane of the G-CSF receptor (G-CSF-R) transduces proliferative and survival signals, whereas the distal carboxy-terminal region transduces maturation signals and suppresses the receptor’s proliferative signals. Mutations in the G-CSF-R gene resulting in truncation of the carboxy-terminal region have been detected in a subset of patients with severe congenital neutropenia who developed acute myelogenous leukemia (AML). In addition, the AML1-ETO fusion protein, expressed in leukemic cells harboring the t(8;21), disrupt the physiological function of transcription factors such as C/EBPα and C/EBPγ, which in turn deregulate G-CSF-R expression. The resulting high levels of G-CSF-R and G-CSF-dependent cell proliferation may be associated with pathogenesis of AML with t(8;21). Moreover, in vitro and in vivo studies demonstrated that G-CSF may act as a co-stimulus augmenting the response of PML-RARα acute promyelocytic leukemia cells to all-trans-retinoic acid treatment. Finally, in the PLZF-RARα acute promyelocytic leukemia transgenic model, G-CSF deficiency suppressed leukemia development. Altogether, these data suggest that the G-CSF signaling pathway may play a role in leukemogenesis.

Key words: Granulocyte-colony stimulating factor, Acute myelogenous leukemia, Leukemogenesis

Introduction

The term acute myelogenous leukemia (AML) refers to a group of neoplastic disorders characterized by the transformation of immature progenitors of the bone marrow, which exhibit morphological and immunophenotypic features common to the myeloid lineage. An important characteristic common to all subtypes of AML is the block of differentiation. Genetically, AML results from acquired mutations, frequently associated with chromosomal translocations, resulting in the generation of chimeric genes and fusion proteins.1 In most of the fusion genes associated with AML pathogenesis, one of the involved genes encodes for a transcription factor, which is physiologically involved in hematopoietic differentiation. In other cases, in the absence of chromosomal translocations, the transcription factors themselves are mutated. Therefore, the most common genetic mechanism that is associated with AML is the aberrant expression of a transcription factor or the production of an abnormal hybrid transcription factor.2

The granulocyte colony-stimulating factor (G-CSF) is a cytokine secreted by the stromal cells of the hematopoietic organs that influence the survival, proliferation and differentiation of myeloid progenitors.3 The responsiveness of AML cells to G-CSF has been reported extensively.4 The spontaneous in vitro proliferation of AML blasts and their growth response greatly vary and do not depend on the number or affinity of the growth factor. Particularly, AML cells harboring the t(15;17) or the t(8;21) may differentiate upon G-CSF administration.5–8 Moreover, mutations on the G-CSF receptor (G-CSF-R) detected in patients with severe congenital neutropenia (SCN) who developed AML suggest that the deregulation of this pathway may contribute to leukemogenesis.9–11 Finally, recent data demonstrated that transgenic mice expressing the t(11;17)/acute promyelocytic leukemia (APL)-associated fusion protein PLZF-RARα depend on G-CSF stimulus in order to develop
full-blown leukemia. This review discusses the current state of knowledge regarding the role of G-CSF in leukemogenesis.

Molecular mechanisms of G-CSF action

In vitro low concentrations of G-CSF stimulate the formation of granulocyte colonies exclusively, whereas higher concentrations can also stimulate small number of macrophage and mixed macrophage/granulocyte colonies. In addition, G-CSF enhances mature neutrophil function, including their antibody-dependent cellular cytotoxicity and their phagocytic capacities of these tyrosines to activate STAT3. However, at high G-CSF concentrations, other survival mechanisms also are activated such as PI3-kinase and mitogen-activated protein kinase.

The most revealing approach to define the in vivo role of hematopoietic growth factors is to study the phenotype of mice genetically deficient in these factors. Murine models of factor deficiency have been reported for monocyte-CSF, granulocyte–monocyte (GM)-CSF and G-CSF. The biologic activities of the hematopoietic growth factors G-CSF and GM-CSF overlap significantly both in vitro and after pharmacologic administration. However, mice deficient in each of these factors have characteristic features. G-CSF-deficient mice have reduced hematopoietic progenitors in the bone marrow and spleen and neutropenia. These animals are markedly predisposed to spontaneous infections, have a reduced long-term survival and a high incidence of reactive type AA amyloidosis. GM-CSF-deficient mice manifest no detectable deficiency in steady-state hematopoiesis, but have increased splenic hematopoietic progenitors and impaired pulmonary surfactant clearance resulting in abnormalities resembling human alveolar proteinosis. They also have a modest impairment of reproductive capacity, a propensity to develop lung and soft tissue infections, and a similarly reduced survival as in G-CSF-deficient animals. These contrasting phenotypes do not exclude the occurrence of additional redundant functions. The residual granulopoiesis in G-CSF-deficient mice, for example, may be attributed to GM-CSF. Moreover, the normal neutrophil level in GM-CSF-deficient animals does not mean that GM-CSF has no physiologic function in steady-state granulopoiesis, but indicates that in the presence of regulators such as G-CSF any such role is dispensable. Later studies generated mice lacking both G-CSF and GM-CSF, which demonstrated phenotypic and pathologic features consistent with the additive effects of the constituent genotypes. Thus, these animals manifest a high incidence of soft-tissue and pulmonary infections, and a mortality rate significantly greater than either of the singly deficient strains. Some additional features may also be observed: a greater degree of neutropenia among newborn mice than in those lacking G-CSF alone,
an increased neonatal mortality rate, and a dominant influence of the lack of G-CSF on splenic hematopoiesis resulting in reduced numbers of splenic progenitors. These data provide an opportunity to assess the role of these growth factors in normal hematopoiesis and help to understand the consequences of a single factor deficiency.

**Acquired G-CSF receptor mutations in SCN**

A number of studies suggest that an abnormal response of granulocytic progenitor cells to G-CSF may be involved in the pathogenesis of SCN. This disorder, also named Kostmann’s syndrome, is characterized by persistent neutropenia and bone marrow maturation arrest of neutrophil precursors at the promyelocytic or myelocytic stage. It occurs as maturation arrest of neutrophil precursors at the characteristized by persistent neutropenia and bone marrow disorder, also named Kostmann’s syndrome, is char-

This effect may induce proliferative stress to myeloid progenitors, and additional oncogenic events could underlie the development of AML in patients with antecedent SCN.

Aprykian et al. described cellular and molecular studies of 12 SCN patients, including five patients that evolved to develop AML, that revealed impaired proliferative characteristics and accelerated apoptosis of bone marrow progenitors cells in SCN compared with 11 healthy controls as demonstrated by flow cytometry analysis. This study also revealed heterozygous deletion or substitution mutations in the neutrophil elastase (NE) gene in nine of 12 patients comparing with controls. The authors conclude that the impaired survival of myeloid progenitor cells is probably driven by the expression of mutant NE, which may be the cellular mechanism responsible for neutropenia in SCN. Finally, they suggest that acquired G-CSF mutations may initiate signaling events that override the pro-apoptotic effect of mutant NE in primitive progenitor cells, resulting in an expansion of the abnormal AML clone. This statement is in accordance with Hunter and Avalos, who have showed that mutations in the G-CSF receptor in SCN/AML confer resistance to apoptosis and enhance cell survival, events mediated by activation of PI3-kinase and its downstream targets Akt and Bad, in response to G-CSF. This extension of cell survival would permit the acquisition of additional oncogenic events that could lead to development of AML.

**G-CSF in AML with t(8;21)/AML1-ETO**

Approximately 5–12% of AML cases harbor translocations between chromosomes 8 and 21 [t(8;21)(q22; q22)] that generate the AML1-ETO chimeric gene. The AML1-ETO fusion protein blocks normal granulocytic differentiation through the disruption of the function of a transcription factor named CCAAT enhancer binding protein α (C/EBPα). C/EBPα function is essential to normal granulocytopenia as demonstrated by the fact that the non-conditional inactivation of the C/EBPα gene in mice (knockout) led to the complete absence of mature granulocytes, without affecting other lineages, including monocytes. Conversely, the enforced expression of C/EBPα in bipotential myeloid progenitors resulted in induction of granulocytic differentiation and inhibition of monocytic development. There are two hypothetical models of the mechanism through...
which AML1-ETO can disrupt normal C/EBPα function: (a) AML1-ETO fusion protein can recognize and bind to the AML1 binding site on DNA and recruit co-repressors resulting in the inhibition of the physiological activation of promoters of granulocytic specific genes by C/EBPα; or (b) AML1-ETO can inhibit a positive feedback loop exerted by C/EBPα on its own expression (positive autoregulation) (Fig. 1).2 Corroborating with the second hypothesis is the detection of decreased C/EBPα RNA levels in t(8;21) patients.27 Among the target genes of C/EBPα are G-CSF-R, IL-6, E2F, c-MYC and defensin.2 Interestingly, G-CSF treatment can lead to in vitro and in vivo differentiation of t(8,21) AML cell samples. Shimizu et al.29 showed that AML1-ETO might modulate cell responses to G-CSF since cells transfected with this fusion protein showed increased proliferation in response to G-CSF without differentiating into mature granulocytes. Moreover, the ectopic expression of AML1-ETO was found to induce the expression of G-CSF receptor, an effect that does not seem to depend on the AML1-binding sequence, but on the C/EBP binding site.

In addition to C/EBPα, another C/EBP isoform that is deregulated by AML1-ETO is the C/EBPβ, which is almost exclusively expressed in the granulocytic lineage and preferentially up-regulated during granulocytic differentiation. The expression of C/EBPβ was significantly up-regulated in the cells ectopically expressing AML1-ETO (Fig. 1). The overexpression of C/EBPβ induced expression of G-CSF receptor and G-CSF-dependent cell proliferation. High levels of G-CSF receptor expression might provide leukemia cells a growth advantage, and may also suppress apoptosis.29 However, this up-regulation of G-CSF receptor does not completely explain all the effects of AML1-ETO in leukemogenesis. It is possible that additional factors, whose expression would be deregulated by AML1-ETO, are required to fully express its transforming activity.

G-CSF and APL

APL is a distinct-subtype of AML that corresponds to the M3 and M3 variant of the French American British classification and to AML subtype with t(15;17)(q22;q12) and variants according to World Health Organization. APL is characterized by: (a) its invariable association with reciprocal and balanced translocations always involving the retinoic receptor α (RARα) gene locus on chromosome 17q21; (b) the clonal expansion of myeloid precursors presenting a block of differentiation at the promyelocytic stage; and (c) its unique sensitivity to the differentiating action of the all-trans-retinoic acid (ATRA). At the molecular level, the RARα gene on chromosome 17 can be fused to the promyelocytic leukaemia gene (PML), to the promyelocytic leukaemia zinc finger (PLZF) gene, to the nucleophosmin (NPM) gene, to the nuclear mitotic apparatus (NuMA) gene, or to the signal transducer and activator of transcription 5B (STAT 5B) gene located on chromosome 15, chromosome 11, chromosome 5, chromosome 11, or chromosome 17, respectively. In the vast majority of APL cases, the t(15;17)/PML-RARα is involved and ATRA treatment induces long-term remissions. However, the rare APL cases harboring the t(11;17)/PLZF-RARα

FIG. 1. Hypothetical models of the mechanisms through which AML1-ETO fusion protein disrupt CEBPα function. AML1-ETO can recruit repressor complexes to AML1 binding sites on the promoter regions of granulocytic specific genes and/or inhibit a positive feedback loop exerted by C/EBPα on its own promoter. Both mechanisms lead to the decrease of C/EBPα transcriptional activity and block of granulocytic differentiation. In addition, AML1-ETO may induce up-regulation of C/EBPβ which by its turn lead to overexpression of the G-CSF receptor and provide the leukemic cells with proliferative advantage.
do not respond to ATRA and the prognosis is dismaying.\(^3\)

The expression of the G-CSF-R, as well as the proliferative response induced by G-CSF, is higher in APL cells compared with other AML subtypes.\(^5\) In vitro studies have shown that induction of differentiation of PML-RAR\(^\alpha\)-positive cells by ATRA can be enhanced when G-CSF is applied as a co-stimulus.\(^6\) Cassinat et al.\(^3\) reported that G-CSF in combination with retinoic acid enhanced the terminal differentiation of fresh t(15;17)-positive APL blasts. Katayama et al.\(^3\) described the achievement of complete remission with G-CSF treatment in an APL patient resistant to both cytotoxic agents and ATRA. The finding that APL cells are exquisitely responsive to G-CSF supports the view that G-CSF is useful for augmentation of their vulnerability to cell-cycle specific agents.\(^5\)

Jansen et al.\(^3\) studied a patient with a t(11;17)-positive APL and determined that the combined use of ATRA and G-CSF could overcome the maturation block of the leukemic cells. With retinoic acid and G-CSF treatment alone, complete granulocytic maturation of the leukemic cells occurred in vivo, followed by a complete cytogenetical and hematological remission. Bone marrow and blood became negative in fluorescence in vivo hybridization analysis, and semi-quantitative polymerase chain reaction showed a profound reduction of PLZF-RAR\(^\alpha\). The response was transient, as fluorescence in situ hybridization-positive cells reappeared in the bone marrow after 7 weeks of treatment. This study suggests that t(11;17)-positive leukemia cells are not intrinsically resistant to retinoic acid and that the G-CSF co-stimulus may be sufficient to render the cells ATRA sensitive.

TM expressing the PML-RAR\(^\alpha\) fusion gene under the control of the human cathepsin G promoter (bCG-PML-RAR\(^\alpha\)) develop a form of leukemia that resembles human APL, presenting the characteristic block of myeloid differentiation at the promyelocytic stage and the sensitivity to ATRA treatment. In contrast to leukemia in bCG-PML-RAR\(^\alpha\) TM, leukemia in bCG-PLZF-RAR\(^\alpha\) TM lacks the distinctive block of differentiation at the promyelocytic stage of myelopoiesis and is characterized by leukocytosis and infiltration of all organs by terminally differentiated myeloid cells (a picture that resembles human chronic myeloid leukemia). In both TM models, leukemia develops after a long latency. Moreover, bCG-PLZF-RAR\(^\alpha\) TM do not respond to RA treatment.\(^5\)

Lieschke et al.\(^5\) analyzed the relevance of G-CSF and GM-CSF stimuli for leukemia development in bCG-PLZF-RAR\(^\alpha\) TM. By crossing these TM with knockout mice for either G-CSF (G-CSF\(^{-/-}\)) or GM-CSF (GM-CSF\(^{-/-}\)), mutants deficient in either growth factor were obtained. The survival data suggested that while wild-type bCG-PLZF-RAR\(^\alpha\) and GM-CSF\(^{-/-}\) bCG-PLZF-RAR\(^\alpha\) mice developed the characteristic transgene-induced leukemia, G-CSF\(^{-/-}\) bCG-PLZF-RAR\(^\alpha\) mice did not succumb to leukemia and survived indistinguishably from G-CSF\(^{-/-}\) controls.\(^3\) This experiment has demonstrated that aberrant endogenous G-CSF signals seem to be essential for modifying the leukemogenic propensity of fusion genes like PLZF-RAR\(^\alpha\). This suggests that therapeutic approaches exploring G-CSF as a co-stimulus may be useful for the treatment of some leukemias.

In fact, Lowenberg et al.\(^3\) recently published a study including 640 adult AML patients who were randomized to receive or not receive G-CSF before and during their first two courses of chemotherapy. Although the remission rates were similar in both groups, increased overall survival, disease-free survival as well as event-free survival were observed in the G-CSF-treated group. The benefit of chemotherapy-sensitization by G-CSF was particularly evident among the intermediate-risk subset of patients. Unfortunately, G-CSF priming did not improve the outcome among patients with chemotherapy-refractory, unfavorable-risk AML.\(^3\) Nevertheless, these results encourage the strategy of using G-CSF priming to extinguish subpopulations of leukemic cells relatively insensitive to chemotherapy and reduce the risk of relapse.

**Conclusion**

Although the relevance of G-CSF in the regulation of granulopoiesis is well established, and much of the molecular mechanisms underlying its function has been elucidated, only recently scientific evidence has been gathered to suggest that acquired mutations in G-CSF-R, or the disruption of the G-CSF function by leukemogenic fusion proteins, may contribute to leukemogenesis. In addition, therapeutic approaches using G-CSF to mobilize subpopulations of leukemic cells, rendering them sensitive to chemotherapy, may represent an important progress in leukemia treatment.

**References**


15. Mediators of Inflammation


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