Structure and Expression of Genes of GM-CSF and G-CSF in Blast Cells From Patients With Acute Myeloblastic Leukemia


The hematopoietic growth factors granulocyte/macrophage colony-stimulating factor (GM-CSF) and G-CSF, available as recombinant products, stimulate the growth in culture of blasts from patients with acute myeloblastic leukemia (AML). We used cDNA probes for each gene to study the genomic organization in blast cells of 22 patients and expression in the blast cells of 18 patients. Alteration in the structure of G-CSF (two instances) and GM-CSF (two instances) was found. In two patients in whom it was possible to study DNA from bone marrow obtained at remission, the new bands detected in the leukemic cells were not found. Fifteen of 18 patients showed no RNA expression of either growth factor. Both patients with GM-CSF abnormalities as seen by Southern analysis expressed an abnormally large GM-CSF message but no G-CSF messages. One patient with an abnormal Southern pattern with GM-CSF expressed normal-sized G-CSF and GM-CSF messages. The biologic significance of these findings remains to be determined. Nonetheless, the abnormal Southern patterns may prove to be useful clonal markers in the study of AML.

© 1988 by Grune & Stratton, Inc.

Molecular Clones and recombinant products are now available for a number of the hematopoietic growth factors. Recombinant granulocyte/macrophage colony-stimulating factor (GM-CSF) acts principally on normal granulopoietic and macrophage precursors but also stimulates early stages in erythropoiesis. Recombinant G-CSF is a major stimulant of neutrophil production. Both recombinant factors have been shown to stimulate the growth of leukemic cells either as continuous cell lines or cells freshly obtained from patients with acute myeloblastic leukemia (AML); for the latter, patient-to-patient variation was found in responsiveness to each factor.

We used cDNA clones for GM-CSF and G-CSF to examine their structure and expression in leukemic cells. As for other biologic characteristics of such cells, patient-to-patient variation was seen. Usually the genes for GM-CSF and G-CSF were in the germline configuration; in samples from four patients abnormal patterns were observed. The blasts from most patients did not express either GM-CSF or G-CSF messages; however, abnormal genomic patterns of GM-CSF were associated with larger-than-normal messages in two patients. In one patient with an abnormal G-CSF pattern, normal-sized G-CSF and GM-CSF mRNA was detected.

Materials and Methods

Patient Population

Peripheral blood samples were collected with informed consent from 22 patients with a diagnosis of AML on the basis of conventional morphological and cytochemical criteria. Patients were included in the study when enough cells were available to prepare DNA and RNA (a mean peripheral blast count of 36,200 × 10⁹/L (median, 31,000; range, 500 to 250,000). Ten normal individuals donated blood cells for control experiments. The clinical characteristics of the patients were similar to those for other series from this institution. The morphological classification include French-American-British (FAB) classes 1 to 6 and a mean age of 51.6 years (median, 50; range, 18 to 82).

Southern Blot Analysis

DNA was extracted from a T cell-depleted, blast cell-enriched fraction from peripheral blood samples by using a two-step Ficoll-Hypaque separation procedure that usually yields cell populations consisting of greater than 95% blasts. The DNA was digested with one of the restriction enzymes BamH1, or HindIII, or EcoRI (according to the manufacturers recommendations), separated by agarose gel (0.8% agarose) electrophoresis, and transferred to Zeta-por filters (A.M.F. Cuno, Meridan, CT) as described by Southern. The filters were prehybridized, hybridized, washed, and autoradiographed as previously described.

Northern Blot Analysis

RNA from 18 patients was extracted by the guanidium thiocyanate/CsCl method. Ten micrograms of RNA were separated according to size in 1.1% agarose gels. The size-separated fragments were transferred to Zeta-por filters, prehybridized, hybridized, washed, and autoradiographed as previously described. The gels were stained with ethidium bromide to ensure that equal amounts of RNA were analyzed and that no degradation had occurred.

Preparation of Labeled DNA Probes

The GM-CSF probe was a 0.5-kilobase (kb) EcoRI-NcoI cDNA fragment. This probe detects an 8.0-kb BamH1 and a 5.2-kb HindIII germline fragment. Oligonucleotides to G-CSF were constructed from the published sequences and were used to screen an expression cDNA library prepared from RNA from TPA30-1, a simian virus (SV40)-transformed human trophoblast cell line. Positive clones were tested for biologic activity by expressing the clones in COS cells and testing the supernatants with cells sensitive to growth factors. The resulting G-CSF probe consists of a 0.7-kb EcoRI-AhAlIII cDNA fragment containing the coding sequence of G-CSF. This probe detects a 23-kb band in germline DNA digested with BamH1 or EcoRI. Sequence analysis showed that the probe was identical to previously published sequences of the corresponding region of G-CSF.

DNA was labeled with ³²P deoxyctydinetriphosphate (dCTP) to a specific activity of 2 × 10⁶ cpm by nick translation.
**Blast Stem Cells in Culture**

Growth factors in crude 5637-CM or recombinant GM-CSF, and G-CSF were used to support the growth of leukemic blast cells either in a methylcellulose colony-forming assay or in a suspension assay. Both assays used growth medium consisting of α-minimum essential medium (α-MEM) supplemented with 20% fetal calf serum (FCS) to which growth factors were added. In the clonogenic assay cells were plated in 10-mm Linbro/Titerk multiwell plates (Flow Laboratories, McLean, VA) at a concentration of 10^5 cells/well. For the suspension assay, the cells were cultured at a concentration of 10^6/mL in 35-mm Lux culture dishes (Miles Scientific, Naperville, IL).

**RESULTS**

**G-CSF Analysis**

DNA samples from 20 of 22 patients and ten normal individuals were digested with BamHI or EcoRI and hybridized with ^32P-labeled G-CSF probe. The Southern blots showed a single germline 23.0-kb band. In DNA from the blasts of two patients abnormal patterns were seen (Fig 1).

**Patient 1.** Southern blots of BamHI-digested leukemic cell DNA showed a 23-kb germline band and a novel 6.5-kb band (Fig 1A, lane 4). EcoRl-digested DNA showed three bands of 23, 4, and 3 kb (Fig 1B, lane 4).

**Patient 2.** Blast cell DNA digested with BamHI and probed with the G-CSF probe showed a germline 23-kb fragment and a novel 8.0-kb band (Fig 1A, lane 2); after EcoRl digestion two bands were seen of 23.0 and 6.5 kb (Fig 1B, lane 2). In this case the new bands were of greater intensity than the 23-kb germline band. This difference was probably due to differences in the efficiency of transfer of DNA to the membrane rather than gene amplification because no differences in intensity were seen when the leukemic and control DNA were cut with other restriction enzymes and probed for G-CSF (data not shown).

**GM-CSF Analysis**

DNA samples from 22 AML patients were analyzed with the GM-CSF probe. DNA samples from 20 patients showed a single germline 8.0-kb band with BamHI digestion (see, for example, Fig 1C, lane 2) and a single 5.2-kb band on digestion with HindIII (data not shown). The abnormal patterns seen in DNA from the blasts of two patients were as follows (Fig 1C).

**Patient 3.** With BamHI digestion and GM-CSF cDNA probe analysis, there was an 8.0-kb germline band. In addition, there were two novel bands at 4.5 and 2.5 kb (Fig 1C, lane 1). DNA from fibroblasts of the same patient showed a single 8.0-kb fragment after BamHI digestion (Fig 1C, lane 2). With HindIII digestion, a single 5.2-kb band was detected in both the normal and leukemic DNA.

**Patient 4.** With BamHI digestion, there were two novel bands of 5 and 3 kb in addition to the 8.0-kb germline band (Fig 1C, lane 4). A single 5.2-kb band was detected with HindIII digestion.

**Serial Studies**

Repeat samples were obtained from patients no. 1 and 4 when they were in remission. DNA from three different samples (4, 7, and 9 weeks after diagnosis) from patient no. 1 and one sample from patient no. 4 (7 months after diagnosis) were examined by Southern analysis after digestion with BamHI, the restriction enzyme that was successful in detecting abnormalities at presentation. The DNA was in germline configuration for the three remission samples from patient 1 when the G-CSF probe was used (Fig 2A) and for the one...

---

**Fig 1.** Analysis of the genomic structure of G-CSF and GM-CSF. (A) DNA digested with BamHI and probed with a G-CSF cDNA fragment. Lanes 1 and 3 are DNAs from normal individuals, and lanes 2 and 4 are leukemic blast cell DNAs from patients no. 2 and 1, respectively. (B) DNA digested with EcoRl and probed with a G-CSF fragment. Lanes 1 and 3 are DNAs from normal individuals, and lanes 2 and 4 are leukemic blast cell DNAs from patients no. 2 and 1, respectively. (C) DNA digested with BamHI and probed with a GM-CSF cDNA fragment. Lane 1 is leukemic blast cell DNA of patient no. 3, lane 2 is DNA from skin fibroblasts of patient no. 3, lane 3 is DNA from the peripheral blood of a normal individual, and lane 4 is the blast cell DNA of patient no. 4. Molecular weights (mol wts) are indicated beside the lanes in kilobases.
remission sample from patient no. 4 when the GM-CSF probe was used (Fig 2B). These observations are compatible with the view that the abnormalities detected by Southern blot analysis using growth factor probes were characteristic of leukemic clones and that hematopoietic cells at remission belonged to clones other than the leukemic clones with abnormal growth factor genes.

**Northern Blot Analysis**

Northern blot analysis of RNA from the factor-producing cell line 5637 showed a 1.2-kb band hybridizing to GM-CSF (Fig 3A, lanes 3 and 4) and a 1.6-kb band hybridizing to G-CSF (Fig 3B, lane 3). RNA samples from 15 blast samples did not show expression of a message for either GM-CSF or G-CSF. In contrast, in patients no. 3 and 4 in whom the structure of the GM-CSF genes were abnormal, mRNA of 4.5 kb was detected on Northern blots (Fig 3A, lanes 1 and 6). RNA from the blasts of patient 2 contained a 1.2-kb GM-CSF (Fig 3A, lane 2) and a 1.6-kb G-CSF message (Fig 3B, lane 2). In common with most of the patients, no message was detected in blast RNA from patient no. 1 with either probe (Fig 3A, lane 5; Fig 3B, lane 1).

**Cell Culture Studies**

Growth in culture of blast cells from patients no. 1, 3, and 4 required the addition of either 5637-CM or recombinant growth factors. Blasts from patient no. 3 responded to both recombinant GM-CSF and G-CSF; synergism was detected when the two recombinant factors were added together. Cells from patient no. 4 required 5637-CM for growth, but cells were not available to analyze the response to each of the recombinant factors. Cells from patient no. 2 were independent of added factors for growth.

**DISCUSSION**

In this paper we describe the use of cDNA clones for GM-CSF and G-CSF as probes to examine the structure and expression of these genes in blast cells from AML patients. In most instances the genes were in the germline configuration, and no mRNA was detected. Four exceptions were
encountered in which the genes for either G-CSF or GM-CSF were altered. The bases for the changes were unlikely to be gross rearrangements or deletions because abnormalities of chromosomes 5 or 17 (where GM-CSF and G-CSF have been mapped) were not seen (data now shown). We consider polymorphism an unlikely explanation of these findings because DNA, obtained either from fibroblasts or remission samples, was in the germline configuration in three (nos. 1, 3, 4) of the four patients. In patient no. 2 where normal DNA was not available, the GM-CSF gene abnormality was detected with two restriction enzymes and was not seen in DNA from ten normal individuals or other AML patients.

In three of the four cases in which the structure of the gene was altered, mRNA was also found. For the instances where the GM-CSF gene was abnormal, the detected mRNA was 4.5 kb; this is larger than GM-CSF mRNA found in cell lines or activated T cells that secrete GM-CSF protein. However, leukemic cells of these two patients required the addition of GM-CSF for growth, and maximum effect was seen with a combination of GM-CSF and G-CSF. By contrast, in one patient found to have a rearrangement of the GM-CSF gene, GM-CSF and G-CSF mRNA of normal size was detected in the patient’s leukemic cells. The blast cells from this patient grew optimally without added growth factor; secretion of the factor into the media was not detected when using factor-sensitive leukemic blasts as assay targets as described previously. Recently Young et al. reported expression of GM-CSF genes in 11 of 22 AML blast populations. Six of these secreted growth factor, three of which formed colonies without added factor. Their assay for factor was different and perhaps more sensitive than ours; it is possible, therefore, that the discrepancy between their data and that reported here may be a consequence of the small numbers of patients included in both studies. Together, our findings and those of Young et al. provide further evidence of blast cell heterogeneity. Such variation may reflect a variety of mechanisms for the regulation of blast cell growth. If such mechanisms are to be exploited therapeutically, it may be necessary to identify which is involved in the leukemic cells of each patient.

To our knowledge, alteration of the genomic structure for GM-CSF and G-CSF in human AML are described here for the first time. Regardless of the molecular mechanisms leading to their generation, the abnormalities are clonal markers with leukemia specificity. They may have a wider application to the study of AML clones than markers restricted to females with X-linked polymorphisms. Our findings that marrow cells from two patients obtained at remission showed a germline configuration are examples of a potential application; if we are correct in interpreting our findings as evidence for the return of normal clones after chemotherapy, abnormal patterns detected with growth factor probes might be used to detect the reappearance of leukemic clones or study the cellular basis for remission.

REFERENCES

9. Minden MD, Buick RN, McCulloch EA: Separation of blast


Structure and expression of genes of GM-CSF and G-CSF in blast cells from patients with acute myeloblastic leukemia

GY Cheng, CA Kelleher, J Miyauchi, C Wang, G Wong, SC Clark, EA McCulloch and MD Minden

Updated information and services can be found at:
http://www.bloodjournal.org/content/71/1/204.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml