Granulocyte Colony-stimulating Factor Receptors on Human Acute Leukemia: Biphenotypic Leukemic Cells Possess Granulocyte Colony-stimulating Factor Receptors

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ABSTRACT

Granulocyte colony-stimulating factor (G-CSF) receptors on the gated blast cells from newly diagnosed patients with acute leukemia or crisis of chronic myelogenous leukemia were investigated using flow cytometric detection. Surface marker analysis and cytochemical studies were conducted simultaneously to characterize the blast cells. Among 24 leukemia cases examined, G-CSF receptor-positive blast cells were detected in all 11 cases of acute myeloblastic leukemia even though the percentage range of positive cells was widely variable. On the other hand, they were not detected on the blast cells from patients with peroxidase-negative acute lymphoblastic leukemia with no myeloid surface antigens. However, G-CSF receptors were demonstrated in significant amounts on blast cells from 5 of 8 cases of peroxidase-negative acute leukemia expressing both myeloid and lymphoid surface antigens (biphenotypic leukemia). The percentage of blast cells positive for G-CSF receptors was significantly smaller in biphenotypic cases (33 ± 14% (SD)) than in acute myeloblastic leukemia cases (65 ± 22%) (P < 0.01). The percentage expression of CD13 antigen by blast cells was significantly related to their percentage positivity for G-CSF receptors (r² = 0.50, P < 0.05). These findings indicate that the distribution of flow cytometrically detectable G-CSF receptors on leukemic cells possessing myeloid characteristics may be related to the maturation process.

INTRODUCTION

G-CSF stimulates the proliferation and differentiation of human bone marrow hematopoietic precursor cells (1-3), and recombinant G-CSF is now used clinically for restoration of granulopoiesis (4). In vitro colony formation studies have shown that G-CSF can stimulate not only normal precursor cells but also some leukemic blast cells (1, 5-8). We and other investigators have demonstrated that blast cells from patients with AML have receptors specific for G-CSF (9-13), whereas lymphoblast cells are thought to have no G-CSF receptors (9, 10). Recently, a proportion of acute leukemias has been reported to show both myeloid and lymphoid characteristics and have been termed biphenotypic leukemia, hybrid leukemia, or mixed-lineage leukemia (14-18). G-CSF receptors on biphenotypic leukemia blast cells have not been well documented. In this article, we report the detection of G-CSF receptors on blast cells from biphenotypic leukemia in comparison with AML blast cells using a rapid flow cytometric detection method.

MATERIALS AND METHODS

Patients and Preparation of Blast Cells. From December 1990 to June 1991, peripheral blood, bone marrow, or lymph node samples from 24 patients with leukemia were presented to our institution for surface marker analysis. Acute leukemia was diagnosed according to the criteria of the French-American-British classification (19). Briefly, the presence of more than 3% MPO-positive leukemic blasts by light microscopy was considered diagnostic of AML, whereas the presence of less than 3% MPO-positive leukemic blast cells was considered diagnostic of acute lymphoblastic leukemia. Leukemic blast cells from patients with chronic myelocytic leukemia in crisis were also characterized in conformity with the French-American-British classification (19). Mononuclear cells obtained following density gradient centrifugation (20) were washed three times with phosphate-buffered saline, and then resuspended in a binding buffer consisting of phosphate-buffered saline, 0.1% bovine serum albumin, and 0.1% sodium azide.

Fluorescence Staining of Leukemic Blast Cells. We used the fluorescein method for detection of G-CSF receptors reported elsewhere. G-CSF was reacted with biotinyl N-hydroxysuccinimide ester (EOY Labs, San Mateo, CA) to yield b-G-CSF, and this b-G-CSF retained its ability to stimulate colony formation by normal bone marrow cells in methylcellulose. Mononuclear cells (1 × 10⁶) containing a blast population were incubated with 8 µl of b-G-CSF (3.5 µg/ml) in a volume of 100 µl of binding buffer. As controls, either the binding buffer alone or b-G-CSF in the presence of a 100-fold excess of unlabeled G-CSF was added instead of b-G-CSF. After incubation for 30 min at 4°C, the cells were washed twice with the ice-cold binding buffer and incubated with streptavidin-PE conjugate (Becton Dickinson, Mountain View, CA) for 30 min at 4°C. These treated cells were washed, and then the gated leukemic cells were analyzed using a FACScan (Becton Dickinson).

Immunophenotyping was also carried out by flow cytometry using the following antibodies: Leu-5b (anti-CD2), Leu-4 (anti-CD3), Leu-1 (anti-CD5), Leu-9 (anti-CD7), CALLA (anti-CD10), Leu-12 (anti-CD19), and Leu-16 (anti-CD20) (all from Becton Dickinson) as lymphoid markers; MY 7 (anti-CD13), MY 4 (anti-CD14), and MY 9 (anti-CD33) (all from Coulter Immunology, Hialeah, FL) as myeloid markers; and IOM 34 (anti-CD34; Immunotech, Marseille, France) and HLA-DR (Becton Dickinson) (10, 17, 18, 21). Positivity for any marker was considered to be expression in 30% or more of the gated leukemic blasts (17, 18, 21). According to cytochemical and immunophenotyping characteristics of leukemic blast cells, leukemia cases were classified into three groups: type A, in which blasts were MPO positive by light microscopy; type B, in which blasts were MPO negative by light microscopy but showed both myeloid and lymphoid surface antigens; and type C, in which blasts were MPO negative by light microscopy and showed lymphoid surface antigens only.

Statistical Analysis. The percentage of G-CSF receptor-positive cells among the gated leukemic blast cells were compared by Student’s t test. The correlation between the percentage of cells positive for G-CSF recepte

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tors and the surface immunophenotype of gated populations was evaluated using Spearman's rank correlation coefficient analysis.

RESULTS

Fig. 1A illustrates a typical case (Table 1, Case 1) which expressed G-CSF receptors. The gated blast cells were subjected to a single-color histogram analysis. Abscissas indicate fluorescence intensity, which reflects the amount of labeled G-CSF and streptavidin-PE. A high fluorescence signal is shown by a solid line, whereas a dotted line is shown when a 100-fold excess molar unlabeled G-CSF was added to the assay. This shift indicates the specific binding of labeled G-CSF to the blast cells.

Table 1 summarizes the positivity ratio for G-CSF receptors as well as the surface immunophenotype of the gated blast cell population from the 24 patients studied. These included 11 cases of type A, 8 cases of type B, and 5 cases of type C. All cases of type A (Cases 1–11) demonstrated the presence of 34–91% G-CSF receptor-positive cells among the gated blast populations with a mean positive percentage of 65 ± 22% (SD). On the other hand, all cases of type C (Cases 20–24) had no blast cells with significant G-CSF receptors (Fig. 1C). Type B cases (Cases 12–19), however, showed the presence of 12–57% G-CSF receptor-positive blast cells with a mean percentage positivity of 33 ± 14% (Fig. 1B). The percentage positivity for G-CSF receptors in the gated blast cell population from type A patients (65 ± 22%) was significantly higher than that from type B cases (33 ± 14%) (P < 0.01).

The relationship between the percentage of cells positive for G-CSF receptors and the surface immunophenotype was also studied. The correlation between the percentage of cells positive for G-CSF receptors and that for CD13 antigens was found to be statistically significant (r = 0.50, P < 0.05; Fig. 2). However, no significant relationship between the percentage of cells positive for G-CSF receptors and that for other surface markers was established.

DISCUSSION

Flow cytometric detection of cytokine receptors has been widely conducted using either monoclonal antibodies (22) or labeled ligands (23, 24). However, flow cytometric detection of G-CSF receptors has not been reported previously. We have developed a flow cytometric detection method for G-CSF receptors using biotinylated G-CSF and streptavidin-PE. The labeling of cells with b-G-CSF and streptavidin-PE resulted in a fluorescence signal sufficiently high for detection of surface G-CSF receptors on leukemic blast cells. The specificity of the method was demonstrated by blocking the staining of fluorescence-positive cells by coincubation of the cells with a 100-fold molar excess of unlabeled G-CSF (Fig. 1). Traditionally, 125I-labeled G-CSF (9–13) or murine G-CSF (25) has been used to detect surface G-CSF receptors on enriched populations of leukemic cells. Our flow cytometric detection method has several advantages, which make it superior to the traditional radioreceptor assay for the study of clinical samples composed of heterogeneous cell populations, since it can analyze only the gated cells. The most striking advantage of flow cytometric detection is the short time required for the assay: only 1 h for completion of analysis. Furthermore, only a small number of cells is required, as few as 2 x 10^3. These advantages make it useful for clinical investigations.

We and other investigators have already shown that G-CSF receptors are expressed on blast cells from patients with AML using radioreceptor assay (9–13). The number of G-CSF receptors was found to vary from patient to patient. In the present study, blast cells from all AML cases possessed G-CSF receptors, even though the percentage of G-CSF receptor-positive AML blast cells ranged widely from 35 to 91%. The coexistence of leukemic blast cells without G-CSF receptors and blast cells with G-CSF receptors in each AML case indicates probable blast heterogeneity in cytochemically and immunophenotypically defined AML. Similar to our findings, Motoji et al. (12) observed considerable variation in the number of grains on blast cells from AML patients when G-CSF receptors were evaluated by autoradiography (12).

In order to analyze the distribution of G-CSF receptors on leukemic blast cells, we examined cell surface markers together. Among the surface markers used, the percentage of cells posi-
Table 1 Clinical characteristics and G-CSF receptors in leukemia

<table>
<thead>
<tr>
<th>Case</th>
<th>FAB</th>
<th>Surface phenotype (%)</th>
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<tbody>
<tr>
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* Type A, shows myeloid surface antigens only; type B has MPO-negative blasts by light microscopy but shows both myeloid and lymphoid surface antigens; Type C shows lymphoid surface antigens only.

G-CSF-R, G-CSF receptor; S, sample. Leukemic blast cell sources: B, bone marrow; P, peripheral blood; L, lymph node. NE, not examined; FAB, French-American-British classification.

Fig. 2. Relationship between the percentage of cells positive for G-CSF receptors and those positive for CD13 antigen. A clear correlation between the two parameters was demonstrated statistically ($r_s = 0.50$, $P < 0.05$).

Percentage positive for CD13 (%)  
Percentage positive for G-CSF receptors (%)  

Cases retained a significant number of G-CSF receptor-positive cells, although the percentage of G-CSF receptor-positive cells was lower than that of G-CSF receptor-positive AML cells ($P < 0.01$). Since biphenotypic leukemia is reported to occur at a relatively immature stage in comparison with committed lymphoid or myeloid cells and to differentiate partially in both directions (28, 29), the fact that biphenotypic leukemia blast cells possessed less G-CSF receptors than AML blast cells also suggested the above possibility that relatively mature blast cells may express more G-CSF receptors than immature ones.

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