To the Editor:

Among patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ ALL), a considerable number carry myeloid surface markers. Bloomfield et al found that 40% of 19 adults with Ph+ ALL had these markers. Ph+ ALL and Ph+ chronic myelogenous leukemia can be differentiated by rearrangement of the classical bcr sequence (or M-BCR-1). The proteins resulting from each status also differ; p190protein and p210protein, respectively.

Recently, we found that adding granulocyte-colony stimulating factor (G-CSF) enhanced the availability of metaphase cells in chromosome analysis of myeloid leukemias. We considered that this effect was derived from activation of myeloblasts through G-CSF receptors located on the surface of these blasts. We wish to report here the in vitro responses to G-CSF and granulocyte-macrophage CSF (GM-CSF) in cells from a patient with Ph+ ALL expressing myeloid surface markers. In light of the response of the cells, we suggest that Ph+ ALL cells expressing myeloid surface markers have both myeloid phenotypes and myeloid functions.

A 35-year-old woman was referred to us because of muscle stiffness, chest pain, and splenomegaly (3 cm palpable). The peripheral blood white blood cell (WBC) count was 134,000/μL with 93.5% blasts. Surface marker analysis showed: Ia, 94.9%;...
with 89.8% blasts. Peroxidase, Sudan black B, α-naphthyl butyrate, and naphtol AS-D chloroacetate stainings were negative. Terminal deoxynucleotidyl transferase was positive. DNA extracted from the peripheral mononuclear cells showed evidence of rearrangement of the Ig heavy chain and T-cell receptor (TCR) δ chain genes with the germline configuration of the TCR β and γ chain genes. A standard chromosome analysis showed that the karyotype of the blast cells was 46,XX,t(9q+;22q−). Thus, the disease was diagnosed as a case of Ph′+ ALL (B-cell type) with myeloid surface markers. This patient was initially prescribed a combination of vincristine, prednisolone, and daunorubicin but there was no response. A protocol with intermediate administrations of cytosine arabinoside (ID-Ara-C) was commenced and blasts declined to 3.6% of bone marrow nuclear cells and 0% of the peripheral WBC.

Peripheral mononuclear cells at the initial presentation were separated on a density gradient and stored in liquid nitrogen until use. We used these cells for molecular analysis of the Ph′ translocation and for analyzing the effects of G-CSF or GM-CSF; G-CSF receptor assay, 3H-thymidine (3H-TdR) incorporation, morphologic examination, colony forming unit (CFU) assay and chromosome analysis.

No rearrangement of M-BCR-1 was observed when standard Southern hybridization techniques were used together with the large bcr probe (a kind gift from Dr J. Groffen).9 Analysis of the hybrid messenger RNA by polymerase chain reaction showed it to be of the p190 variety and no hybrid mRNA of p210 variety was observed. These results are compatible with the diagnosis of Ph′+ ALL expressing myeloid surface markers.

Binding of G-CSF to leukemic cells was examined using 125I-labeled modified G-CSF as described elsewhere.9 This radiolabeled G-CSF preserved the biologic activity, as assessed by MTT colorimetric assay.9 Radiolabeled G-CSF showed specific binding, and Scatchard analysis of the specific binding showed a single class of binding sites with an apparent dissociation constant (Kd) of 100 pmol/L and maximal binding sites of 660 sites per cell (Fig 1). Leukemic cells from a patient with acute myeloblastic leukemia (AML; MI by FAB classification) were used as the positive control and leukemic cells from another patient with ALL for the negative control (data not shown). The Kd value and the maximal binding sites for the former were 77 pmol/L and 660 sites per cell, respectively. Specific binding of 125I-G-CSF was not observed for the latter.

3H-TdR incorporation into the cells was examined as follows. Leukemic cells (2 – 105) were cultured for 72 hours in 200 μL of RPMI 1640 supplemented with 10% fetal calf serum (FCS) with or without G-CSF (20,000 U) or GM-CSF (5,000 U). Then 1 μCi of 3H-TdR (methyl-3H-TdR; Amersham, Buckinghamshire, England) was added to each well. All experiments were performed in triplicate and the results were expressed as the stimulation index (mean counts per minute [CPM] with G-CSF or GM-CSF/mean cpm without a factor). Stimulation indices were 4.5 and 5.8 for G-CSF and GM-CSF, respectively. In the AML patient serving as the positive control, the indices were 6.1 and 1.6; in the ALL patient for a negative control, the indices were 0.9 and 0.4.

Liquid culture in RPMI 1640 supplemented with 10% FCS was performed with G-CSF (2,000 U; Kirin Brewery, Tokyo, Japan) or GM-CSF (10,000 U; Hoechst, Tokyo, Japan) for 72 hours and cytospin specimens were prepared.4 Although myeloid maturation was not observed in either condition, cells with a monocytic morphology appeared. α-Naphthyl butyrate stained cells accounted for 0%, 4.0%, 5.0% and 11.0%, respectively, before the culture, cultured without factors, and cultured with G-CSF and with GM-CSF. These observations suggest part of the blast cells differentiated to monocyte lineage with the stimulation of GM-CSF.

For the CFU assay, 2 × 105 blasts were plated per dish containing 30% human plasma, erythropoietin, 2-mercaptoethanol, and 0.7% methylcellulose with 100 U/mL of G-CSF or GM-CSF. No colony was observed under this condition after 14 days.

For chromosome analysis with G-CSF, 20,000 U of the factor were added to 3.5 × 105 cells. The control cells were prepared in the same manner except that G-CSF was not added. Standard chromosome analysis and G-banding analysis were performed as described.2 The karyotype of all of 14 metaphases with G-CSF was 46,XX,t(9q+;22q−); however, no metaphase was obtained in the absence of G-CSF.

Our study showed: (1) the diagnosis is to be Ph′+ ALL expressing both B-lineage lymphoid and myeloid surface markers; (2) leukemic cells from this patient had G-CSF receptors with a Kd...
of 100 pmol/L and maximal binding sites of 40 sites per cell; (3) enhanced uptake of $^3$H-TdR was observed in these cells stimulated by G-CSF and GM-CSF; (4) part of the cells differentiated to monocyte lineage with the stimulation of GM-CSF; (5) no colony was observed with either G-CSF or GM-CSF; and (6) Ph$^+$ metaphases were observed with G-CSF. These results suggest that cells from this patient with Ph$^+$ ALL expressing myeloid markers can respond to G-CSF and GM-CSF via receptors to these factors. Thus, Ph$^+$ ALL cells expressing myeloid surface markers have both myeloid phenotypes and myeloid functions. Motoji et al$^9$ have recently found the G-CSF receptor to be expressed on AML blasts in 18 of 20 patients. The Kd values of these patients ranged from 15 to 130 pmol/L (mean, 79 pmol/L) and the number of binding sites for G-CSF varied from 55 to 1,200 per cell (mean, 278). Although the Kd of the present patient was comparable, the number of binding sites was smaller, a finding which may explain why no colony was observed. They also suggested a correlation between the number of G-CSF receptors expressed and colony number produced by G-CSF.$^8$ Finally, the good response to ID-Ara C in our patient may relate to the myeloid-specific characteristics of her leukemic cells.

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HIROYUKI TSUCHIYA
NAOTO ADACHI
NORIO ASOU
KIYOSHI TAKATSUKI
ICHIRO MATSUDA
Kumamoto University Medical School
FUMIO KAWANO
TETSUJI MURAKAMI
Kumamoto National Hospital
Kumamoto City
SHUKI MIZUTANI
National Children’s Medical Research Center
Tokyo
MASAHIKO WATANABE
Pharmaceutical Research Laboratory
Kirin Brewery Co, Ltd
Takasaki, Japan

REFERENCES


Responses to granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage CSF in Ph1-positive acute lymphoblastic leukemia with myeloid surface markers [letter] [see comments]

H Tsuchiya, N Adachi, N Asou, K Takatsuki, I Matsuda, F Kawano, T Murakami, S Mizutani and M Watanabe