Abnormalities of primitive myeloid progenitor cells expressing granulocyte colony-stimulating factor receptor in patients with severe congenital neutropenia

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Introduction

Severe congenital neutropenia (SCN) is characterized by onset in early childhood, recurrent life-threatening infections, and profound neutropenia of less than 200 absolute neutrophil count (ANC)/μL peripheral blood.1-3 The bone marrow usually shows a paucity of mature myeloid cells with a maturation arrest of neutrophil precursors at the promyelocyte-myelocyte stage of differentiation. To date, the underlying pathophysiology of SCN remains unclear, though the administration of recombinant human granulocyte colony-stimulating factor (G-CSF) has induced an increase in circulating neutrophil counts in most patients.2-8 Bone marrow cells from patients with SCN frequently show a markedly reduced or a complete lack of responsiveness to G-CSF in vitro culture.5,9-12 The role of G-CSF and G-CSF receptor (G-CSFR) in the stimulation of granulopoiesis has been documented through the analysis of G-CSF-deficient and G-CSFR-deficient mice.13-16 However, the deficiency of G-CSF, the lack of G-CSFR, or the G-CSFR mutation itself may not be a sufficient contributor to severe neutropenia and the SCN phenotype.15-18

We recently reported a defective proliferation of primitive myeloid progenitor cells from patients with SCN in response to hematopoietic factors including G-CSF.12 To define the role of G-CSFR in the growth of primitive myeloid progenitor cells in patients with SCN, we have analyzed myeloid progenitor cells expressing G-CSFR and studied their responsiveness to hematopoietic factors involved in myelopoiesis.

Study design

Patients

Five patients with SCN were enrolled in this study. The diagnosis of SCN or Kostmann syndrome was made according to accepted criteria, such as less than 200 ANC/μL peripheral blood, maturation arrest at the promyelocyte or myelocyte level in the bone marrow, absence of circulating antineutrophil antibodies as determined by granulocyte indirect immunofluorescence test, and onset of severe infections in early childhood.1,3 All patients had histories of recurrent life-threatening infection. Patient 1 continued to have recurrent skin abscesses and chronic gingivitis, and he has been maintained on daily subcutaneous administration of G-CSF for the last 7 years. The other 4 patients have received intermittent administration of G-CSF when infections were observed. None of the patients acquired myelodysplastic syndrome or acute myelogenous leukemia during the administration of G-CSF.

Separation and purification of bone marrow cells

In accordance with the institutional guidelines of the committee on human experimentation, bone marrow samples were obtained after informed consent was given for all participants (patients, their guardians, and healthy adult volunteers). Bone marrow cells used in this study were taken when G-CSF was administered to patient 1 but not to the other 4 patients with SCN. Separation and purification of bone marrow cells were performed according to methods reported previously.12,19 Flow cytometric analysis and cell sorting were carried out by a FACS Vantage (Becton Dickinson Immunocytometry Systems, San Jose, CA) equipped with a 4-W argon laser.
Results and discussion

Flow cytometric analysis of bone marrow cells

Figure 1 shows the results of the flow cytometric analysis of Kit and G-CSFR expression on CD34+ cells in 5 patients with SCN and in 2 representative subjects without SCN. The frequency of Kit+G-CSFR+ expression on CD34+ cells in patients was significantly decreased compared with that of subjects (P < .001). Some variations were seen in the frequencies of quadrant cells and in the staining pattern among patients with SCN. The total percentage of G-CSFR+ cells on CD34+ cells noted no significant difference between patients and subjects, resulting in a relative increase in the frequency of Kit+/G-CSFR+ cells in patients. The Kit+/G-CSFR+ on CD34+ cells yielded few granulocyte-macrophage colonies (GM) in response to hematopoietic factors, including G-CSF, in subjects and patients (data not shown). Thus, the significance of the inappropriate proportion of G-CSFR expression and the role of Kit+/G-CSFR+ cells on CD34+ cells in patients with SCN in developing the myeloid progenitor cells remains to be elucidated. To exclude the possibility that endogenous or exogenous G-CSF in vivo affects the binding of G-CSFR antibody to G-CSFR, CD34+ cells from subjects were incubated with G-CSF before staining for biotin-conjugated G-CSFR antibody. However, the preincubation of G-CSF did not affect the expression of G-CSFR on CD34+/Kit+ cells (data not shown). These results indicate that the decrease in the frequency of CD34+/Kit+/G-CSFR+ expression in patients with SCN is not due to either high serum concentrations of G-CSF or the administration of G-CSF.

The proportion of CD34+ cells in the bone marrow of patients with SCN was comparable to that in subjects, resulting in a reduction in the absolute number of CD34+/Kit+ cells expressing G-CSFR in patients. The CD34 antigen and Kit receptor identify cell populations that are enriched for pluripotent and lineage-restricted hematopoietic progenitor cells in vitro, and some of them are capable of bone marrow reconstitution in vivo.20,21 The decreased number of CD34+/Kit+/G-CSFR+ cells may be indicative of a defective origin of the myeloid progenitor for neutropenia in patients with SCN.

Granulocyte-macrophage colony formation of CD34+/Kit+ cells

According to the expression of CD34, Kit, and G-CSFR, the CD34+/Kit+/G-CSFR+ and CD34+/Kit+/G-CSFR- cells were...
GM indicates granulocyte-macrophage, G-CSF, granulocyte colony-stimulating factor receptor; SCF, stem cell factor; FL, flk2/flt3 ligand; IL-3, interleukin-3; SCN, response to G-CSF alone and to the combination of SCF, FL, and G-CSFR on CD34 cells, as shown in Figure 1.

Cultures were grown under serum-deprived conditions. One milliliter of the culture mixture contained 250 purified cells, 1% deionized crystallized bovine serum albumin, 300 μg/mL fully iron-saturated human transferrin, 10 μg/mL soybean lecithin, 6 μg/mL cholesterol, 1 × 10^{-7} mol/L sodium selenite, 10 μg/mL insulin, 4.5 mmol/L L-glutamine, 1.5 mmol/L glycine, 5 × 10^{-5} mol/L 2-mercaptoethanol, 1.2% 1500 cP methylcellulose, and designated factors. Cultures were incubated for 14 days at 37°C in a humidified atmosphere with 5% CO2/95% air. The concentrations of SCF, FL, and IL-3 were 100 ng/mL, 50 ng/mL, and 100 U/mL, respectively. GM colonies included pure granulocyte colonies consisting primarily of neutrophils and their precursors and of mixed GM colonies consisting mainly of neutrophils, macrophages-monocytes, and their precursors. All assays were carried out in triplicate cultures. The CD34+/Kit+/G-CSFR+ and CD34+/Kit+/G-CSFR− cells were purified according to flow cytometric analysis based on the expression of Kit and G-CSFR on CD34 cells, as shown in Figure 1.

<table>
<thead>
<tr>
<th>Factors</th>
<th>No. of GM colonies</th>
<th>Mean ± SD Range</th>
<th>Mean ± SD Range</th>
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<tr>
<td>CD34+/Kit+/G-CSFR+ cells</td>
<td>G-CSF (1 ng/mL)</td>
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<td>0-1</td>
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<tr>
<td>CD34+/Kit+/G-CSFR+ cells</td>
<td>SCF, FL, IL-3</td>
<td>14 ± 3</td>
<td>11-16</td>
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<td>23-30</td>
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<tr>
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<td>41 ± 8</td>
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GM indicates granulocyte-macrophage, G-CSF, granulocyte colony-stimulating factor receptor; SCF, stem cell factor; FL, flk2/flt3 ligand; IL-3, interleukin-3; SCN, severe congenital neutropenia.

Purified. Results of GM colony formation of purified cells are presented in Table 1. The number of GM colonies of CD34+/Kit+/G-CSFR+ cells in patients with SCN was significantly reduced in response to G-CSF alone and to the combination of SCF, FL, and IL-3 with or without G-CSF, which are primarily involved in myelopoiesis, compared with the number of colonies in subjects. The CD34+/Kit+/G-CSFR− cells failed to respond to G-CSF in both subjects and patients. There was no difference in the number of GM colonies of CD34+/Kit+/G-CSFR− cells supported with SCF, FL, and IL-3 with or without G-CSF between subjects and patients. Similarly, the CD34+/Kit+/G-CSFR−, but not CD34+/Kit+/G-CSFR−, cells showed a decreased proliferation in response to SCF, FL, and IL-3 with or without G-CSF in liquid suspension culture (data not shown). We recently reported direct evidence, using a single-cell proliferation assay, of the defective proliferation of CD34+/Kit− cells in patients with SCN. Taken together, the faulty granulopoiesis of CD34+/Kit+/G-CSFR+ cells in patients suggests that abnormal responsiveness to hematopoietic factors in patients with SCN lies in primitive myeloid progenitor cells expressing G-CSFR.

G-CSFR-deficient mice show a modest but significant reduction in the total number of hematopoietic colonies formed in response to pokeweed mitogen-stimulated conditioned media, IL-3, GM-CSF, or SCF. These data demonstrate that G-CSFR is required for the maintenance of a normal number of hematopoietic progenitor cells. In the current study, CD34+/Kit+/G-CSFR+ cells of patients with SCN showed a reduced number of GM colony formations in response to a combination of SCF, FL, and IL-3, irrespective of the presence or absence of G-CSF. This evidence also suggests that functional G-CSFR is necessary for the full stimulation of hematopoietic cells in response to hematopoietic factors. Alternatively, the decrease in the responsiveness of CD34+/Kit+/G-CSFR+ cells might reflect the functional abnormality of the G-CSFR or G-CSFR–mediated signal pathway in patients with SCN.

On the basis of abnormalities in CD34+/Kit+/G-CSFR+ cells in patients with SCN, the key to detecting the underlying pathophysiology of SCN is to clarify the significance of G-CSF expression on primitive myeloid progenitor cells in the development of hematopoietic progenitor cells associated with cell survival. It is likely that the quantitative abnormality of CD34+/Kit+/G-CSFR+ cells might be a consequence of an underlying primary cellular defect of patients with SCN. Further studies are required to search for the origin of the quantitative and qualitative abnormalities of primitive myeloid progenitor cells expressing G-CSFR in patients with SCN.

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References


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