Granulocyte Colony-Stimulating Factor-Producing Large Cell Undifferentiated Carcinoma of the Lung

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We report a case of granulocyte colony-stimulating factor (G-CSF) producing lung cancer. A 38-year-old Japanese woman had a large cell undifferentiated carcinoma of the left lung with severe granulocytosis without any evidence of infection. A specimen was taken from a metastatic cervical lymph node and a tumor cell line was established. The culture supernatant of the line as well as patient's serum exhibited a high level of G-CSF by sandwich enzyme immunoassay. Immunohistochemical analysis demonstrated that tumor cells from transplanted nude mice were stained granularly in the cytoplasma by anti-human G-CSF monoclonal antibody, 4A6. (Internal Medicine 31: 277-280, 1992)

Key words: G-CSF, granulocytosis, enzyme immunoassay

Introduction

The stimulation of hematopoiesis by colony-stimulating factors (CSFs) is well known (1) and four growth factors thus far have been identified as human CSFs, i.e. interleukin-3 (IL3), granulocyte/macrophage-CSF (GM-CSF), granulocyte-CSF (G-CSF) and macrophage-CSF (M-CSF) (2). Each cDNA has already been cloned (3-6) and recombinant products are available. Patients with malignant tumors sometimes reveal varying degrees of leukocytosis without any apparent infection (7). Some of these cases might be caused by unregulated production of CSFs or CSF-like substances by the tumor cells. However, there have been few cases from which CSF-production has been proved (8-15). In this paper, we report on a lung cancer patient with leukocytosis from which G-CSF-producing cell line was established.

Case Report

A 38-year-old Japanese woman was admitted to Sapporo Medical College Hospital on February 14, 1989, because of low grade fever and hemosputa. Physical examination revealed the swelling of left supraclavicular lymph node. A roentgenogram of the chest disclosed a nodular shadow in the left lung field (Fig. 1). The cytological findings of sputa and the aspiration of the swollen cervical lymph node revealed the presence of malignant cells. The white blood cell count was 25,700/mm³ with a differential count of 78% neutrophils, 2% eosinophils, 3% monocytes and 17% lymphocytes. The red cell count was 393 x 10⁴/mm³. The hemoglobin was 12.2 g/dl and hematocrit was 35.8%. The platelet count was 44.4 x 10⁴/mm³. With exception of the increased erythrocyte sedimentation rate (81mm/h) and C-reactive protein (4+), all laboratory analyses (serum glucose, urea nitrogen, total protein, LDH, ALP, GOT, GPT, Na, K, Cl, Ca, tumor markers and urinary analysis) showed data within normal limits. The serum G-CSF level as detected by sandwich enzyme immunoassay (EIA) was high (351pg/ml) on admission, though the serum GM-CSF detected by EIA of human GM-CSF (Genzyme Corp., Boston) was a trace level (4pg/ml). Bacteriological examinations of peripheral blood, sputa and urine were negative. The reduction in the tumor size by anticancer chemotherapy was paralleled by an improvement of the peripheral blood granulocytosis, but the white cell count reincreased after discontinuation of chemotherapy, reaching 72,000/mm³ (98% neutrophils and 2% lymphocytes) shortly before death. However, differentiation of peripheral white blood cells did not...
demonstrate any atypical cells in her clinical course, and bone marrow aspiration examinations showed hyperplasia of myeloid series, but not atypical cells (nucleated cell count, \(44.0 \times 10^4/\text{mm}^3\); myeloblasts, 1.2%; promyelocytes, 6.2%; myelocytes, 18.3%; metamyelocytes, 5.1%; band neutrophils, 15.2%; segmented neutrophils, 24.9%; erythroblasts, 23.7%; lymphocytes, 2.5%; plasma cells, 1.9%), when bone marrow aspiration was performed at the zenith of granulocytosis. The serum G-CSF level was also high (430 pg/ml), when a sample was taken at almost the same time of peak of granulocytosis. The histopathological findings of the biopsied and necropsied tumor showed a large cell undifferentiated carcinoma of the lung (Fig. 2). A tumor cell line was established from cells from a metastatic cervical lymph node. The established line was shown to markedly produce G-CSF, because the line’s culture supernatant exhibited a high level of G-CSF (20836 pg/ml) detected by sandwich EIA. However, there was a trace level of GM-CSF in the culture supernatant (15 pg/ml) measured with EIA.

Immunohistochemical analysis was performed using cryostat sections of tumor tissues from transplanted nude mice and employing the avidin-biotin-peroxidase technique with monoclonal anti-human G-CSF antibody, 4A6 (18) and polyclonal anti-human GM-CSF antibody (Genzyme Corp.). In conjunction with massive granulocyte infiltration into the tumor tissue from transplanted nude mice, tumor cells were stained granularly in the cytoplasm by anti-G-CSF antibody, 4A6, whereas tumor cells did not react with anti-human GM-CSF antibody, control mouse sera or control rabbit sera (data not shown).

Mouse bone marrow proliferation assay was also performed using the culture supernatant and the patient’s serum as well as human recombinant G-CSF (a generous gift from Chugai Pharmaceutical Company, Tokyo) because human G-CSF is known to support the proliferation of mouse bone marrow cells (10, 16). The results of this assay demonstrated that the culture supernatant of the cell line greatly enhanced mouse bone marrow cell proliferation, whereas it was significantly inhibited by the patient’s serum as shown in Table 1.

**Discussion**

Without any evidence of infection, leukocytosis is sometimes observed in patients with malignant tumors (7). In very few cases, unregulated production of CSFs and CSF-like substances from tumor cells has been
In this lung cancer patient, severe granulocytosis was observed without any sign of infection and no atypical hematopoietic cells were recognized in her bone marrow. Therefore, it was likely that some CSF was produced by the tumor cells. To date, a bioassay has been used to measure CSF activity using human bone marrow cells as well as mouse bone marrow cells (1). Several different CSFs have been distinguished through careful analysis of the cell types found in hematopoietic colonies grown with various sources of growth factor activity. In the present case, mouse bone marrow cell proliferation assay was performed using the patient’s serum and the culture supernatant, since some human CSFs are known to support the proliferation of mouse bone marrow cells (10, 16). The results of the mouse proliferation assay clearly demonstrated that the culture supernatant obtained from the established tumor line as well as human recombinant G-CSF supported the proliferation of mouse bone marrow cells, however mouse bone marrow cell proliferation was strongly inhibited by this lung cancer patient’s serum (Table 1). These results suggested the following: a) the established cell line can produce some CSF which supports mouse bone marrow proliferation, and b) immunosuppressive substances might be contained in the patient’s serum by which mouse bone marrow proliferation is inhibited. Although colony stimulating activity of the patient’s serum probably would have been detected by colonogenic assays using human bone marrow cells, we could not perform the colonogenic assays since all the preserved patient’s serum had been used already.

Recently, growth factors which stimulate hemopoiesis have been identified through a structural approach and each cDNA has been cloned (3–6). Four growth factors (IL3, GM-CSF, G-CSF and M-CSF) are well known to stimulate hematopoiesis, and these CSFs have considerable overlap in their proliferative actions on granulocyte-macrophage progenitor cells (2). IL3 and GM-CSF can stimulate the formation of both granulocytes and macrophages. G-CSF tends to stimulate only granulocyte formation and M-CSF only macrophage formation (2). Moreover, very recently it became possible to measure the levels of G-CSF in serum and culture supernatant by sandwich EIA employing anti-recombinant human G-CSF polyclonal antibody (16, 17). Since EIA assay does not influence immunosuppressive substances, the G-CSF level was high in the patient’s sera as well as in the culture supernatant of the established cell line. Futhermore, as a result of the measurement of GM-CSF content using EIA, a trace level of GM-CSF was detected in the patient’s serum and in the culture supernatant of the established cell line. In order to demonstrate directly the production of G-CSF by tumor cells, we performed immunohistochemical analysis by anti-human G-CSF monoclonal antibody, 4A6. However, by immunohistochemical analysis using specimens from biopsy and necropsy, G-CSF production was difficult to detect in the present case, because these specimens consisted of massive necrosis. Therefore, immunohistochemical

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### Table 1. Mouse Bone Marrow Proliferation Assay*

<table>
<thead>
<tr>
<th>medium content</th>
<th>G-CSF concentration</th>
<th>(^3\text{H} \text{ thymidine uptake (cpm)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>patient’s serum</td>
<td>85.0 pg/ml</td>
<td>286 ± 120</td>
</tr>
<tr>
<td></td>
<td>42.5 pg/ml</td>
<td>824 ± 375</td>
</tr>
<tr>
<td>healthy volunteer serum</td>
<td>&lt;7.5 pg/ml</td>
<td>4,980 ± 184</td>
</tr>
<tr>
<td>culture supernatant of cell line</td>
<td>5.2 ng/ml</td>
<td>14,989 ± 924</td>
</tr>
<tr>
<td>human recombinant G-CSF</td>
<td>5.0 ng/ml</td>
<td>14,569 ± 791</td>
</tr>
<tr>
<td></td>
<td>1.0 ng/ml</td>
<td>10,817 ± 1136</td>
</tr>
<tr>
<td></td>
<td>100.0 pg/ml</td>
<td>9,841 ± 1021</td>
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<tr>
<td></td>
<td>10.0 pg/ml</td>
<td>5,280 ± 647</td>
</tr>
<tr>
<td></td>
<td>1.0 pg/ml</td>
<td>5,109 ± 352</td>
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</tbody>
</table>

* The assay was carried out in triplicate. The data are expressed as the mean of counts per minute (cpm) ± SEM.
analysis was performed using tumor tissues from nude mice transplanted with tumor cells of the established line. This analysis clearly demonstrated that the tumor cells produced G-CSF, since tumor cells were stained granularly in their cytoplasm by anti-G-CSF monoclonal antibody, 4A6, but not by anti-GM-CSF antibody.

Lung cancer is known to be one of the most frequent human cancers. Sometimes granulocytosis without infection is observed in lung cancers and CSFs may be produced in these lung cancers. However, there are very few cases in which CSF production by lung cancer cells has been proven (8, 9), since it has only been recently that techniques to easily prove production of CSFs by tumor cells have been developed. Therefore, new diagnostic approaches were necessary for such cases. In order to detect G-CSF, enzyme immunoassay and immunohistochemical analysis were developed (16–18). The results here clearly demonstrated that enzyme immunoassay and immunohistochemical analysis by anti-human G-CSF antibodies are very useful and important for the proving G-CSF production by tumor cells. The recent advances in molecular biology will greatly contribute to the determination of numerous biological factors from patients that have specific clinical signs including paraneoplastic syndrome, because many cytokines are known to be related to various specific biological phenomena.

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References