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Granulocyte Colony-Stimulating Factor and Interleukin-6-Producing Lung Cancer Cell Line, LCAM

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Background and Objectives: We describe a case of granulocyte colony-stimulating factor (GCSF) and interleukin-6 (IL-6)-producing lung cancer.
Methods: A 53-year-old man underwent left upper lobectomy under diagnosis of lung cancer. The tumor obtained by a preoperative biopsy was analyzed.
Results: Preoperative data showed leukocytosis with left-shift of leukocytic morphology and thrombocytosis and an elevated serum GCSF level. Histological examination revealed poorly differentiated adenocarcinoma. A cell line, named LCAM, was established from the tumor and the cytokines in the culture medium were measured by enzyme immunoassay. GCSF and IL-6 were produced in large amounts by LCAM, but granulocyte-macrophage colony-stimulating factor (GMCSF) and interleukin-3 (IL-3) were not. A proportion of LCAM expressed GCSF receptor on the cell surface, but IL-6 receptor could not be detected. LCAM proliferation was inhibited in the culture with antihuman GCSF antibody in a dose-dependent manner.
Conclusions: We suggest that LCAM proliferation is positively regulated by GCSF. *J. Surg. Oncol. 64:347–350, 1997.*

KEY WORDS: GCSF; IL-6; carcinoma; leukocytosis; thrombocytosis

INTRODUCTION: LCAM CELL LINE

It has been reported that cancer cells occasionally produce some cytokines and that the proliferation may be regulated by the cytokines through an autocrine loop. We encountered a case of adenocarcinoma of the lung that showed leukocytosis and thrombocytosis. A cell line named LCAM was successfully established from the tissue obtained by a preoperative biopsy. The production of cytokines and the expression of their receptors were examined, and the proliferative regulation was studied.

CASE REPORT

The patient was a 53-year-old male who complained of left shoulder and back pain in February 1995. An abnormal shadow was present in the left upper field on a chest X-ray, and lung cancer was suspected on computed tomography of the chest. A fluoroscopy-guided needle biopsy revealed adenocarcinoma of the lung. The patient underwent left upper lobectomy of the lung on July 19, 1995. Postoperative histological examination showed a poorly differentiated adenocarcinoma of pathological stage 3A (T3: pericardium N0M0).

Leukocytosis (24,070/mm^3) with left-shift leukocytic maturation alteration (Neu 86.5%, Ly 9.8%, Mo 3.1%, Eo 0.4%, Ba 0.2%) and thrombocytosis (70.2 × 10^4/mm^3) were found, and serum granulocyte colony-stimulating factor (GCSF) level was increased to 65 pg/ml, as measured by an enzyme immunoassay (EIA). As obstructive pneumonia was suspected from computed to-

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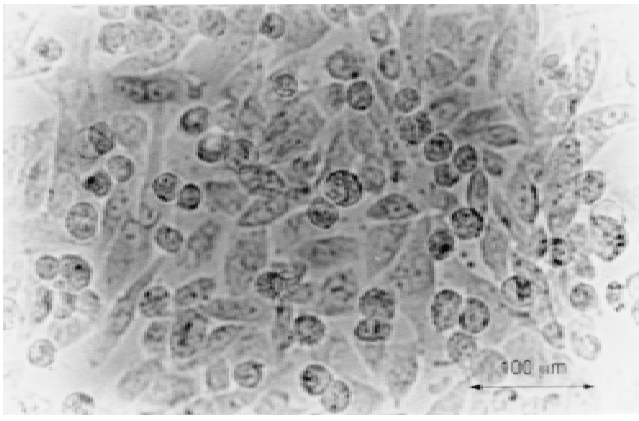


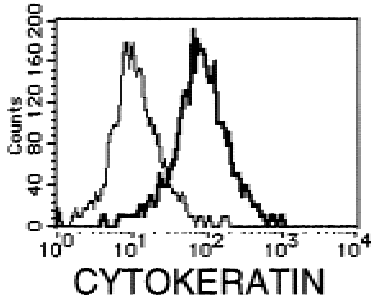
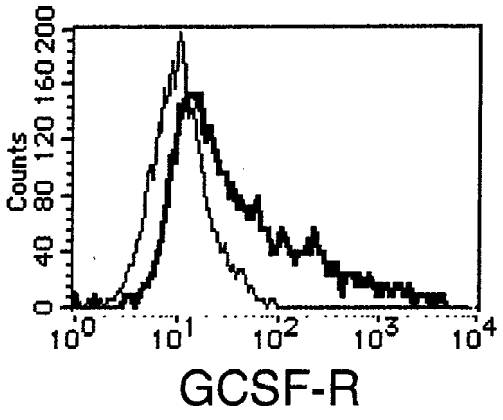


Fig. 1. The upper phase contrast photomicrograph with a 100 μm scale bar shows LCAM cells cultured in a flask. The lower histogram shows the expression of cytokeratin in LCAM cells. Narrow line indicates control. LCAM cells treated with 0.5% formalin and 0.025% saponin were incubated with fluorescein isothiocyanate-conjugated antihuman cytokeratin mouse antibody (DAKO, Glostrup, Denmark) for 30 minutes at 4°C. The cells were subjected to flow cytometric analysis. Data from 1×10^4 cells were collected and analyzed using the Cell Quest program.



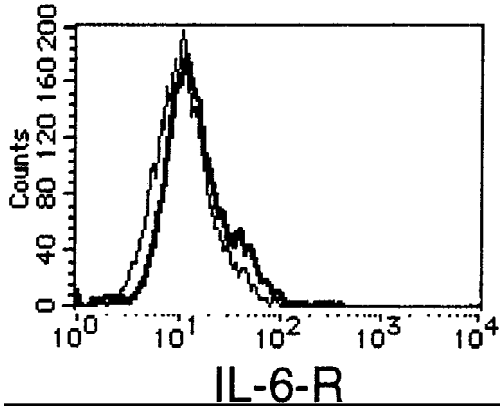


Fig. 2. The expression of granulocyte colony-stimulating factor (GCSF) receptor and IL-6 receptor on LCAM cells surface was examined with flow cytometry. Narrow line indicates control. LCAM expressed GCSF receptor weakly, although IL-6 receptor could not be detected. LCAM cells were incubated with antihuman GCSF receptor mouse antibody (Pharmingen, San Diego, CA) and with antihuman IL-6 receptor mouse antibody (Bender Medsystems, Vienna, Austria) for 45 minutes at 4°C. The cells were subjected to flow cytometric analysis. Data from 1×10^4 cells were collected and analyzed using the Cell Quest program.

mography of the chest and cross-reacting protein was elevated to 16.8 mg/dl, leukocytosis might have been partially due to the inflammation. The biopsy sample was torn with scissors and cultured in complete medium (RPMI 1640 supplemented with 10% fetal calf serum, 2 mM glutamine, and 50 μM 2-mercaptoethanol). A cell line named LCAM could be successfully established, and the cells were confirmed to be of epithelial origin using a flow cytometrical detection of cytokeratin (Fig. 1). The doubling time of LCAM calculated from the proliferation curve was 6.9 hours.

We next examined the production of cytokines by the cell line. GCSF, granulocyte-macrophage, colony-stimulating factor (GMCSF), interleukin-3 (IL-3), and interleukin-6 (IL-6) in the culture medium were measured with EIA after LCAM was cultured for 4 days in the complete medium. The results were GCSF: 95 pg/ml, GMCSF: <8 pg/ml, IL-3: <31 pg/ml, IL-6: 31,400 pg/ml, respectively. So LCAM produced GCSF and IL-6, which might have caused the leukocytosis and thrombocytosis. It is possible that the GCSF and IL-6 influenced the proliferation of LCAM in an autocrine fashion. We next analyzed the receptors on the surface of the cells using flow cytometry. GCSF receptor could be detected on 30.0% of LCAM, although IL-6 receptor was undetectable (Fig. 2). LCAM secreted IL-6, but it probably did not affect the proliferation of the cells. Next, we examined the effect on proliferation by neutralizing GCSF in the culture medium using an antihuman GCSF antibody. After 2.5×10^5/well LCAM cells were cultured for 2–4 days in 250 μl of complete medium with varying doses of antihuman GCSF antibody on a 48-well, flat-bottomed plate, the cell numbers were counted. The proliferation of LCAM was suppressed by anti-GCSF antibody at 100 μg/ml (Fig. 3). This result suggested that GCSF secreted by LCAM probably promoted the proliferation in an autocrine fashion.

The patient followed a good clinical course after operation and was discharged 26 days after operation. The

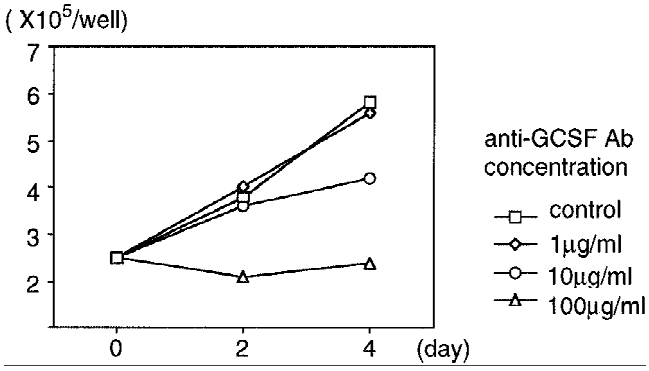


Fig. 3. The effect of antihuman granulocyte colony-stimulating factor (GCSF) antibody on proliferation. The proliferation of LCAM cells was suppressed by 100 μg/ml of antihuman GCSF rabbit antibody (Genzyme, Cambridge, MA), which is an antibody for culture system not including NaN_3. After LCAM cells (2.5×10^5/well) were cultured with anti-GCSF antibody for 2–4 days, the cells were collected using trypsin and counted. Each number is the average of three culture wells.

leukocytes normalized to 5,950/mm^3 (Neu 56.2%, Ly 27.7%, Mo 6.1%, Eo 9.2%, Ba 0.9%) and the platelets decreased to 42.9×10^4/mm^3 23 days after operation. The patient is healthy at the time of writing without a sign of recurrence and the serum GCSF level is within normal limits (<10 pg/ml).

DISCUSSION

Previous reports have indicated that some cancer cells secrete cytokines, including GCSF, GMSCF, IL-1α, IL-1β, TNFα, and IL-6 [1–7]. These cytokines may promote or suppress proliferation in an autocrine fashion [1,8–10]. We encountered a case of adenocarcinoma of the lung who showed leukocytosis and thrombocytosis. A cell line (LCAM) was successfully established from a sample that had been obtained by needle biopsy before the operation, and the production of cytokines by LCAM was analyzed. LCAM produced GCSF, which may promote proliferation by an autocrine mechanism.

GCSF is one of the glycoprotein hormones that stimulate granulocyte generation and functional activation of mature granulocytes, and the chromosomal location for the GCSF is assigned to the q21-q22 region of chromosome 17 [11–13]. A few cases of GCSF producing lung cancer have been reported, and this is the first case in which an autocrine loop is established by detecting the expression of GCSF receptor and by showing an inhibitory effect on the proliferation of cells by neutralizing GCSF in the culture medium using an antibody [2,11,14]. GCSF secreted by lung cancer cells can affect the proliferation of the cancer cells. Additionally, since the serum GCSF level was elevated preoperatively and normalized after operation, it may be useful as a tumor marker in the follow-up the patient.

IL-6 is a 19–30 kD protein, which is a stimulating factor of B cell differentiation and megakaryocytes proliferation. It is produced by T cells, monocytes, fibroblasts, endothelial cells, and keratinocytes. However, IL-6 has been recognized as an inhibitor of some solid tumors or leukemia cells. The clinical trials using recombinant human IL-6 have been used for cancer patients [15]. Some IL-6-producing lung cancer cell lines expressed IL-6 receptors and the growth was suppressed by IL-6 [8]. In our case, although LCAM secreted IL-6 measured by EIA, IL-6 receptor on the cells could not be detected. The significance of IL-6 produced by LCAM was therefore unclear. As the elevated platelet count became normal postoperatively, we deduced that IL-6 had caused the thrombocytosis.

The cytokine production by tumor cells was due to the gene amplification or the turning on of transcriptional factors [16]. The gene structures of GCSF and IL-6 are reported to be alike and share a common 5′ consensus sequence, named CK1, in the promoter region [16–18]. It is thus possible that the transcription of both genes may have been activated through a common pathway in LCAM.

Clinically, recombinant human GCSF has been widely used for myelosuppression, which is one of the side effects after chemotherapy in lung cancer patients. The GCSF may, however, promote proliferation of lung cancer cells that bear GCSF receptors, as shown in our case. When leukocytosis is, therefore, found before operation, especially without inflammation, the serum GCSF level should be measured to examine whether the tumor cells secrete GCSF or not. If GCSF-producing tumor is suspected, the administration of GCSF may be contraindicated.

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