CONCISE REPORT

Granulocytosis Associated With Tumor Cell Production of Colony-Stimulating Activity

By William Hocking, Jesse Goodman, and David Golde

A patient with metastatic soft tissue sarcoma presented with a leukemoid reaction. The elevated white blood cell count was due to an increase in bands and mature segmented neutrophils. The degree of granulocytosis correlated with the tumor burden. There was no evidence of superimposed infection and the degree of bone marrow involvement by metastatic tumor was minimal. A cell line derived from the sarcoma produces granulocyte-macrophage colony-stimulating activity (CSA) in vitro. Although CSA could not be detected in serum, the findings in this patient suggest that the leukemoid reaction was due to tumor production of CSA.

LEUKEMOID REACTIONS associated with malignancy are usually attributed to infiltration and replacement of the bone marrow by tumor cells or to stimulation of the bone marrow by an associated inflammatory process or infection. In two reported patients, however, there was evidence suggesting that the tumor was producing a granulopoietic substance. Colony-stimulating activity (CSA) is the term applied to a group of polypeptide factors capable of stimulating in vitro granulopoiesis. These factors are essential for the clonal growth of granulocytic precursors in vitro, but the role of CSA in vivo remains uncertain.

In this article we describe a patient with an undifferentiated soft tissue sarcoma and marked granulocytosis. A cell line derived from this tumor produced high concentrations of CSA, suggesting that paraneoplastic secretion of this substance caused the leukemoid reaction. These observations support an in vivo biologic role for CSA in the regulation of human granulopoiesis.

CASE REPORT

A 63-year-old woman was admitted to UCLA Medical Center because of a painful, enlarging mass on the right arm. She had a recent weight loss of 20 lbs associated with anorexia. Physical examination revealed a debilitated afebrile woman. A 10 × 12 cm ulcerated fungating mass extended from the middle third of the right arm. There was no jaundice, lymphadenopathy, or hepatosplenomegaly. Radiographic studies demonstrated a pathologic fracture of the right humerus. A bone scan showed abnormal uptake in the area of the mass but was otherwise normal. Initial chest radiograph was normal. There was no evidence of superimposed infection and the degree of bone marrow involvement by metastatic tumor was minimal. A cell line derived from the sarcoma produces granulocyte-macrophage colony-stimulating activity (CSA) in vitro. Although CSA could not be detected in serum, the findings in this patient suggest that the leukemoid reaction was due to tumor production of CSA.

Initial chest radiograph was normal. There was no jaundice, lymphadenopathy, or hepatosplenomegaly. There was no evidence of superimposed infection and the degree of bone marrow involvement by metastatic tumor was minimal. A cell line derived from the sarcoma produces granulocyte-macrophage colony-stimulating activity (CSA) in vitro. Although CSA could not be detected in serum, the findings in this patient suggest that the leukemoid reaction was due to tumor production of CSA.

Five days later, the right arm was disarticulated at the shoulder. The surgical specimen contained extensive necrotic tissue and an undifferentiated malignant neoplasm with frequent mitoses. Electron micrographs showed numerous aggregates of cytoplasmic filaments and abundant rough endoplasmic reticulum containing secretory products. These features were suggestive of a rhabdomyosarcoma.

Postoperatively, the serum calcium became normal and the white blood cell count decreased to 11,600/µl. During the next 10 days, a pathologic fracture of the left clavicle occurred and multiple bilateral pulmonary nodules and right hilar adenopathy were noted. The patient declined chemotherapy and over the next month progressive deterioration occurred. She was readmitted to UCLA with severe dyspnea. In the interval, numerous firm 1-2 cm skin nodules had developed. Serum calcium was 11.2 meq/liter (normal 1.5-2.1). Serum PTH by radioimmunoassay (Nichols Laboratory) was 83 deq/ml (normal 40-100).

The patient died 5 wk after her initial evaluation of respiratory failure. Autopsy revealed extensive neoplastic involvement of the lungs, lymph nodes, subcutaneous tissues, pericardium, myocardium, and focal tumors in the skull and left clavicle. The bone marrow of the vertebrae and ribs was involved focally with tumor but the remaining bone marrow showed myeloid hyperplasia. There was no evidence of an infectious complication.

MATERIALS AND METHODS

A biopsy of a cutaneous tumor nodule was performed with informed consent of the patient. The biopsy was divided and a portion submitted for pathologic examination. The histologic features of this tumor were similar to the primary tumor. The remaining tissue was placed in a sterile plastic dish and teased apart. A single cell suspension was formed by sequential passage through needles and a single culture established by placing the cells into a T-75 flask (LUX) in 15 ml alpha medium containing 20% fetal calf serum and antibiotics. The flask was incubated at 37°C in 5% CO₂. In 3 wk, a confluent monolayer had formed. The cells were removed by exposure to 0.25% trypsin and split into 2 flasks. A continuous cell line (designated MB-I) was developed and has been maintained for more than 18 mo.

Cells were examined cytochemically for α-naphthyl butyrate esterase and peroxidase and periodic acid-Schiff and oil red 0 stains were performed. The presence of Fc receptors and surface membrane immunoglobulin was determined by standard techniques. The reactivity of the cell line with the OKT3 monoclonal anti-T-cell antibody and H4 monoclonal anti-DR antibody was determined.
Table 1. Hematologic Features

<table>
<thead>
<tr>
<th>Time</th>
<th>Hb (g/dl)</th>
<th>WBC (× 10^3/μl)</th>
<th>Differential (%)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>12.6</td>
<td>28.9</td>
<td>67 15 13 3</td>
<td>Disarticulation</td>
</tr>
<tr>
<td>5</td>
<td>11.3</td>
<td>24.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>10.9</td>
<td>18.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>10.3</td>
<td>13.9</td>
<td>76 3 14 7</td>
<td>Lung metastases</td>
</tr>
<tr>
<td>15</td>
<td>10.1</td>
<td>11.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>11.3</td>
<td>96.4</td>
<td>86 6 7 0</td>
<td>Skin metastases</td>
</tr>
<tr>
<td>46</td>
<td>9.4</td>
<td>72.8</td>
<td>69 23 4 2</td>
<td>Skin biopsy</td>
</tr>
<tr>
<td>53</td>
<td>7.9</td>
<td>117.6</td>
<td>36 58 1 2</td>
<td>Patient died</td>
</tr>
</tbody>
</table>

*Days after initial evaluation.

Conditioned medium was collected each time the culture medium was changed. The conditioned medium was stored at −20°C until tested. Conditioned medium was tested for colony-stimulating activity (CSA) after filtration through a 0.2-μm filter using normal human bone marrow cells as the target. Bone marrow was obtained by iliac crest aspiration from informed healthy volunteers. The bone marrow aspirate was centrifuged over Ficoll-Hypaque and the light density cells collected. The light density cells were incubated for 90 min on glass Petri dishes and the nonadherent suspended cells were harvested and used as the target cells in the assay.

The CFU-GM assay was performed in triplicate as previously described. Colonies of greater than 50 cells were counted on day 14. Control plates contained either no exogenous source of CSA, conditioned medium from the Mo T-lymphoblastoid cell line, or conditioned medium from cultures of normal skin fibroblasts. The serum of the patient obtained at the time her white blood cell count was 90,400/μl was also tested for CSA both before and after dialysis against phosphate-buffered saline or distilled water.

RESULTS

The MB-1 cell line has been in continuous culture for more than 18 mo. The cells grow to confluence and tend to pile up. Giemsa-stained smears showed cells with vacuolated, granular cytoplasm, 1–2 nuclei, and 1–4 nucleoli. Cytochemical stains for peroxidase and α-naphthyl butyrate esterase were negative. The periodic acid-Schiff stain was positive in cytoplasmic granules and the oil red 0 stain was negative. The cells did not have Fc receptors or surface membrane immunoglobulin and were not reactive with OKT3 or anti-DR monoclonal antibodies. Transmission electron micrographs of MB-1 cells demonstrated that the ultrastructural features of these cells were similar to those observed in the primary tumor and skin metastases.

Conditioned medium from the MB-1 cell line consistently contained high concentrations of CSA (Table 2). There was a clear dose–response effect of the CSA produced by MB-1 on normal human bone marrow light density nonadherent cells (Fig. 1). Media conditioned by continuous normal infant skin diploid fibroblast lines did not have detectable CSA (Table 2). The undialyzed serum from the patient had no detectable CSA. Serum dialyzed against phosphate-buffered saline or water stimulated formation of a few colonies and clusters (10–50 cells). Placing the serum in a 0.5% agar underlayer with bone marrow cells in 0.3% agar overlayer has been reported to increase CSA detected.

Table 2. CSA Production by a Human Tumor Cell Line

<table>
<thead>
<tr>
<th>CM Source</th>
<th>Concentration (μl/ml)</th>
<th>CFU-GM × 10^-3</th>
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</thead>
<tbody>
<tr>
<td>Normal diploid fibroblast</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Patient serum</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Mo T-cell line</td>
<td>50</td>
<td>76.0 ± 2.0</td>
</tr>
<tr>
<td>MB-1</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Passage day 40</td>
<td></td>
<td>23.7 ± 3.3</td>
</tr>
<tr>
<td>Passage day 46</td>
<td></td>
<td>86.0 ± 10.8</td>
</tr>
<tr>
<td>Passage day 49</td>
<td></td>
<td>73.7 ± 6.4</td>
</tr>
</tbody>
</table>

*CFU-GM, colony-forming unit-granulocyte, macrophage, from normal human bone marrow light density, nonadherent cells. The results are the mean ± SEM for 3 replicate plates.
in the serum, presumably by inactivating inhibitory substances.\textsuperscript{11} This approach failed to augment colony formation stimulated by serum from our patient. Addition of serum from the patient to agar cultures using conditioned medium from the Mo or MB-1 cell line resulted in 60\%–100\% inhibition of colony formation.

DISCUSSION

This patient had leukocytosis and a soft tissue sarcoma that was shown to produce CSA in vitro. A striking feature of the leukocytosis was the presence of predominantly mature granulocytosis. On only two occasions was a granulocyte precursor earlier than a band observed on a peripheral blood smear. Nucleated erythroid precursors and poikilocytosis were not observed. The degree of leukocytosis correlated clinically with the tumor burden (Table 1). At her initial presentation there was a modest granulocytosis that resolved when the primary tumor was removed. When she was seen 1 mo later with extensive metastases, the white blood count was dramatically elevated.

The continuous cell line derived from a cutaneous metastasis produced CSA comparable in potency to that produced by the Mo T-lymphoblastoid cell line.\textsuperscript{10} We hypothesize that the leukemoid reaction in this patient was due to the secretion of CSA by the tumor in vivo. A single serum sample from the patient had a low CSA level that was detected after the serum was dialyzed. Human serum contains inhibitors of CFU-GM colony formation\textsuperscript{12} that may not be completely removed by dialysis. Furthermore, the role of circulating CSA in stimulating granulopoiesis is unclear. A murine granulocytosis-inducing mammary tumor has been described in which tumor-derived cell lines produce CSA.\textsuperscript{13} CSA is not detectable in the serum of these mice.\textsuperscript{14} This murine model may be analogous to our patient. We cannot completely exclude the possibility that the physical presence of tumor within the bone marrow contributed to the granulocytosis, although the findings on the peripheral blood smear suggest that this was not the case. In addition, the areas of bone marrow adjacent to the tumor showed myeloid hyperplasia, suggesting that the bone marrow was being stimulated.

Robinson studied 12 patients with granulocytosis and neoplasia and found elevated levels of CSA in the serum and urine of these patients, although tumor tissue from 2 of these patients had no detectable CSA.\textsuperscript{2} Evidence that granulocytosis may be secondary to a CSA-producing tumor was reported in a patient with squamous carcinoma of the oral cavity with pleural metastases. Pleural fluid and extracts of the tumor from that patient contained CSA.\textsuperscript{3} In that patient, and in another patient with squamous cell carcinoma of the lung and mild leukocytosis, transplantation of tumor tissue into nude mice provoked a leukocytosis in the recipient animals.\textsuperscript{3,4}

A number of human cell lines have been reported to produce CSA,\textsuperscript{10,15,16} but these lines were derived from patients without granulocytosis. In contrast, the MB-1 cell line was derived from a patient with marked granulocytosis. The hypothesis that the leukemoid reaction in this patient was due to CSA production by the tumor is supported by CSA production by the continuous tumor cell line, correlation of the degree of granulocytosis with the tumor burden and the absence of other causes for granulocytosis. These observations provide further evidence for the biologic activity of CSA and the pathophysiologic role of CSA in some leukemoid reactions.

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REFERENCES


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