CONCISE REPORT

Two Cases of Carcinoma of the Lung Characterized by a Bone Marrow Agar Culture Pattern Resembling Acute Myeloid Leukemia

By J. H. McCarthy, J. R. Sullivan, B. Ungar, and D. Metcalf

In vitro agar culture patterns of bone marrow cells in acute myeloid leukemia may show several growth patterns, including cultures where no colonies or clusters develop, cultures with varying numbers of clusters and no colonies, or colony and cluster formation with an extremely high ratio of clusters to colonies. Twelve cases of carcinoma of the lung are described, of which two show an in vitro growth pattern of cluster formation alone, characteristic of that seen in acute myeloid leukemia. The remaining ten patients showed slightly reduced colony numbers compared to normal.

THE IN VITRO GROWTH patterns in acute myeloid leukemia, using the bone marrow assay system devised by Pike and Robinson, have been described. There have been few studies of the in vitro culture characteristics of bone marrow from patients with solid tumours. The in vitro cloning of hemopoietic progenitor cells is dependent on the presence in the cultures of a glycoprotein colony-stimulating factor (CSF). Human or monkey peripheral blood leukocytes and human placenta are potent sources of CSF. In nonhematologic disease states, culture of 10⁵ marrow cells produces 20–100 colonies (containing more than 40 cells) and 5–10 times that number of clusters (aggregates from 3 to 40 cells).

We describe 12 patients with carcinoma of the lung, of which 2 showed cluster formation typical of acute myeloid leukemia following culture of their bone marrow. Both patients had small cell carcinoma of the lung, 1 with morphological evidence of bone marrow infiltration.

MATERIALS AND METHODS

Bone marrow samples were obtained from 12 patients over a period of 12 mo. Nine patients had small cell carcinoma of the lung, two large cell anaplastic, and one squamous cell carcinoma. All bone marrow aspirates were performed on initial presentation to hospital prior to the commencement of treatment. Morphological evidence of bone marrow infiltration was demonstrated in three of the nine patients with small cell carcinoma of the lung. For each bone marrow specimen cultured, suitable control subjects were cultured concurrently.

Culture Technique

Bone marrow samples were collected in a single strength Dulbecco's modified Eagle's medium. Following centrifugation at 1500 rpm for 7 min, the buffy coat was removed and transferred to fresh medium. A dispersed cell suspension was prepared by repeated gentle pipetting. A viable cell count using

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eosin dye exclusion was performed. The cells were cultured in a mixture containing equal volumes of double strength Dulbecco's modified Eagle's medium and an 0.6% agar. The agar medium mixture containing either 50,000 or 100,000 bone marrow cells was pipetted in 1-ml volumes into 35-mm plastic Petri dishes and allowed to gel. Colony-stimulating factor was provided by 0.1 ml of human placental conditioned medium or a peripheral blood underlayer. Following incubation for 14 days in a fully humidified atmosphere of 10% CO₂ in air, colony counts were performed using an Olympus dissection microscope (35 ×). Individual colonies or clusters were removed using a fine Pasteur pipette placed on albumin-coated slides, allowed to dry, and then stained with luxol fast blue. Following staining, the colonies were classified as granulocyte-macrophage or eosinophil.

CASE REPORTS

Case 1

A 59-yr-old woman with rheumatic heart disease, right hemiplegia, and aphasia was admitted in March 1978 with weight loss, malaise, and depression. She was a five-cigarette per day smoker. On examination, there was a small right pleural effusion and hepatomegaly. The hemoglobin was 7.9 g/100 ml, the white cell count 12,500/cu mm, and the platelet count 144,000/cu mm. The smear was typical of a leukoerythroblastic anemia with anisopoikilocytosis, 2% normoblasts, and 4% reticulocytes and a neutrophilia with left shift. A liver scan showed multiple space-occupying lesions. A liver biopsy showed extramedullary erythropoiesis with hepatic siderosis and infiltration by small clumps of malignant cells of uncertain origin. A bone marrow aspirate and trephine biopsy were obtained from the right posterior iliac crest. The aspirate contained no marrow particles and very few marrow elements, but was extremely hypercellular and densely packed with pleomorphic immature neoplastic cells. These occurred in large sheets, clumps or singly; they exhibited frequent mitotic figures. The neoplastic cells ranged from 12 to 35 μ in diameter and had a variable amount of moderately pale, basophilic cytoplasm that often contained a few azurophilic granules and sometimes one or several large basophilic inclusion bodies, which were faintly PAS positive, as well as some coarse PAS positive granules. The nuclei of these neoplastic cells were large and pleomorphic with a fairly dense chromatin pattern and one or more indistinct nucleoli. Some of the neoplastic cells exhibited prominent erythrophagocytosis, having ingested some 1–20 red cells. Section of the marrow trephine specimen confirmed virtually total marrow replacement by masses of immature foreign cells consistent with metastatic spread from an anaplastic carcinoma. The agar culture result is shown in Table 1. The patient was treated with blood transfusion alone and died 9 days later.

### Table 1. Bone Marrow Culture Results From 12 Cases of Carcinoma of the Lung

<table>
<thead>
<tr>
<th>Patient</th>
<th>Colonies</th>
<th>Clusters</th>
<th>Percent Eosinophil</th>
<th>No Stimulus</th>
<th>Clusters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td>0</td>
<td>&gt;1,800</td>
<td>0</td>
<td>0</td>
<td>&gt;1,000</td>
</tr>
<tr>
<td>Case 2</td>
<td>0</td>
<td>157 ± 6</td>
<td>0</td>
<td>0</td>
<td>140 ± 5</td>
</tr>
<tr>
<td>Case 3</td>
<td>36 ± 6</td>
<td>79 ± 10</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Case 4</td>
<td>45 ± 4</td>
<td>115 ± 15</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Case 5</td>
<td>29 ± 3</td>
<td>68 ± 5</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Case 6</td>
<td>37 ± 5</td>
<td>113 ± 8</td>
<td>13</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Case 7</td>
<td>12 ± 1</td>
<td>47 ± 2</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Case 8</td>
<td>18 ± 3</td>
<td>89 ± 20</td>
<td>7</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Case 9</td>
<td>39 ± 2</td>
<td>144 ± 17</td>
<td>18</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Case 10</td>
<td>29 ± 2</td>
<td>68 ± 4</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Case 11</td>
<td>17 ± 1</td>
<td>38 ± 5</td>
<td>21</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Case 12</td>
<td>12 ± 1</td>
<td>36 ± 7</td>
<td>17</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Normal range 20–100 200–1,000

Mean ± standard deviation of four replicate cultures. Stimulated cultures contained 0.2 ml of human placental conditioned medium. Similar results were obtained when 10⁵ rhesus monkey peripheral blood white cells were used as a stimulus.
Post-mortem examination showed a tumor arising from the right lower lobe bronchus, extending to adjacent hilar nodes, anterior pericardium, and the lung substance on the right. The enlarged liver was infiltrated with metastatic tumor. There was infiltration of lymph nodes in both the chest and abdomen and bone marrow. A hypostatic pneumonia was present.

**Case 2**

A 51-yr-old man presented with chest pain 1 yr after coronary artery bypass surgery for chronic angina. He smoked 60 cigarettes per day. Chest x-ray showed a right hilar mass, which, on bronchoscopy, was found to be due to widening of the carina with an intrinsic lesion of the left upper lobe bronchus. The left vocal chord was paralyzed. Cytology showed a small cell carcinoma of the lung. The hemoglobin was 13.9 g/100 ml, the white cell count 3100/cu mm, with 1612 neutrophils/cu mm. The platelet count was 375,000/cu mm. The smear was normal apart from neutropenia. A bone marrow aspirate and trephine biopsy were obtained from the right posterior iliac crest. These showed a cellular, normoblastic marrow with active erythropoiesis, normal granulopoiesis, slightly increased numbers of megakaryocytes, prominent platelet clumps in the aspirate, and slightly prominent plasma cells. There was no evidence of malignant infiltration in the aspirate or trephine biopsy specimens. Liver and bone scans were normal. Treatment was given with methotrexate, cyclophosphamide, and vincristine. The patient died 2 mo later. An autopsy was not performed.

**RESULTS**

Analysis of the growth characteristics of bone marrow from the 12 patients showed that in the 2 cases described there was failure of normal colony formation in culture. In these cases, only clusters containing less than 10 cells developed (Table 1). The results were similar whether human placental conditioned medium or monkey peripheral blood underlayers were used as a source of colony-stimulating factor. In cultures without added colony-stimulating factor, there was significant cluster formation. Microscopic examination of 576 clusters revealed that they were all macrophage in type. In control cultures prepared at the same time as case 1 and case 2, respectively, there was significant colony formation (103 ± 7 colonies and 508 ± 27 clusters, control 1; 60 ± 5 colonies and 130 ± 5 clusters, control 2). Morphological examination of the colonies revealed that in control culture 1, 98% of the colonies were granulocyte–macrophage, while 2% were eosinophils; in control culture 2, 15% of the colonies were eosinophils.

The 10 remaining patients showed slightly reduced colony formation with a normal cluster to colony ratio. Microscopic examination showed normal maturation of granulocyte and macrophage cells in the colonies and the presence of less than 30% eosinophil colonies (Table 1).

**DISCUSSION**

The observation of cluster formation alone in in vitro agar marrow culture in carcinoma of the lung is a new finding. Previously, this in vitro growth pattern has only been described in acute leukemia, preleukemia states, and chronic myeloid leukemia in terminal blast transformation. Both normal and leukemic bone marrow require the continuous presence of colony-stimulating factor in order to produce colonies and clusters in vitro. The fact that cultures from both patients with carcinoma of the lung formed clusters in the absence of colony-stimulating factor may be interpreted in several ways. As unfractionated bone marrow cells were cultured, granulocyte–macrophage colony-stimulating factor production by normal bone marrow cells in a culture containing 100,000 cells is quite possible. However, this is unlikely to explain the presence of such a large number of clusters and total
absence of colonies in the cultures. It has been reported that human lung cancer can produce colony-stimulating factor in vitro; therefore, it could be postulated that in both these patients the in vitro culture pattern was due to the production of hemopoietic humoral regulators by neoplastic cells. Finally, the cluster-forming cells in these cultures may be the progeny of neoplastic lung cells that have infiltrated the bone marrow. Further studies are being undertaken to clarify the mechanisms responsible for these culture results.

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REFERENCES

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