Heterotransplantation of a Hematopoietic Cell Line Derived from a Patient with Multiple Myeloma

Tatsuo Imamura, Chester C. Huang, Yuji Matsuoka, Jun Minowada, and George E. Moore

SUMMARY

A cell line has been established from the buffy coat of a patient with multiple myeloma. This cell line, which was designated RPMI 8226, has a triploid chromosome constitution and synthesizes only \( \lambda \) light-chain protein. When \( 10^7 \) or \( 2.5 \times 10^7 \) viable cells were inoculated s.c. in sublethally irradiated mice, all of them developed subcutaneous tumors. Nonirradiated mice developed fewer and much smaller tumors, and histological preparations of the latter tumors revealed cell degeneration. Inoculation i.p. of \( 10^7 \) viable cells resulted in milky, cell-rich ascites tumors at the site of inoculation and small intraperitoneal tumors at incidences of 50, 37, and 30%, respectively. The total number of ascites cells amounted to approximately 2 to 10 times the number of injected cells 10 days after inoculation. Chromosomal, immunological, and cloning examinations of the cultured cells derived from induced tumors and ascites fluid showed that these cells were identical to the inoculated cells.

INTRODUCTION

A number of cell lines have been established from leukocytes from the peripheral blood of patients with leukemia or lymphoma (11, 19) and nonmalignant diseases (8, 25) and from normal individuals (7, 18). These cell lines show a broad spectrum of immature lymphoblastoid forms (21).

In June 1966, a unique cell line was established in our laboratory from the buffy coat of a patient with multiple myeloma. This cell line synthesizes \( \lambda \)-type light-chain immunoglobulin, which was found to be similar to the Bence-Jones protein of the donor patient, and the cultured cells are thus considered to be identical to the original myeloma cells (15, 20).

Successful heterotransplantation of established cells of human malignant tumors has been reported by many investigators (4, 9, 12, 13, 16, 22, 26). Nevertheless, only a few reports concerning heterotransplantation of cultured cells derived from the peripheral blood of patients with leukemia or other diseases have been published. Adams et al. (1-3) implanted human leukemic cells and cultured leukemic cells or lymphoblasts into newborn or X-irradiated Syrian hamsters. Southam et al. (27) reported successful heterotransplantation of cells from Burkitt tumor cell lines, leukemia cell lines, an infectious mononucleosis cell line, and a cell line from a normal lymph node into newborn rats by i.v. inoculation.

The present paper deals with the heterotransplantation of cultured cells derived from a patient with multiple myeloma.

MATERIALS AND METHODS

Cell Line. On June 16, 1966, leukaphoresis was performed on a 61-year-old man with multiple myeloma. A leukocyte culture was initiated; 66 days later, the culture became self-sustaining and was designated RPMI 8226. The general methods used for establishing the cell line, as well as the case history of the patient, have been reported previously (15, 19).

The morphological features of this cell line have also been reported previously (20). The cells are almost the same as the lymphoblastoid cells of other lines derived from leukocytes of the peripheral blood. Some immature plasma cells are present, as well as lymphoblast-like cells with an unusual amount of endoplasmic reticulum. Herpes-types virus particles (leukovirus), often found in other hematopoietic cells, were not observed in this cell line. Shortly after establishment of the cell line, a hypodiploid mode was dominant; but after some months in culture, a triploid mode appeared. When the present study was carried out, the chromosome pattern was triploid.

RPMI 8226 released only \( \lambda \)-type light chains of immunoglobulin into the culture medium (15). This light-chain protein gave a reaction of identity with Bence-Jones protein of the original patient's urine upon immunodiffusion, and showed similar molecular size upon gel filtration. In contrast to other human hematopoietic cell lines, this 1 adhered to glass surfaces. Cells in media showed a mixture of viable and dead cells of varying cytological maturity. The doubling time in static cultures in RPMI Medium 1640 supplemented with

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10% fetal calf serum ranged from 40 to 80 hr (20). These cells were able to grow in RPMI Medium 1640 in the absence of serum (20) for several weeks. Even so, some serum proteins were detectable for many weeks after serumless media were used. The doubling times of such cultures was greatly prolonged. Cell viability was maintained at 85 to 90% and the cells grew slowly but steadily in suspension culture.

Preparation of the Cells for Transplantation. Cells were maintained and harvested in static culture in 16-oz Brockway bottles containing RPMI Medium 1640 supplemented with 10% heat-inactivated fetal calf serum (18). The cells adhering to the surface of the glass were collected by scraping with a rubber scraper. Cells were caused to settle by slow centrifugation (approximately 800 to 1000 rpm) for 5 min, and the supernatant was discarded. After cell population and cell viability were checked, cell concentration was adjusted to 5 \times 10^7 viable cells/ml of phosphate-buffered saline. In every experiment, cell viability was found to be more than 80% on the basis of exclusion of 0.1% trypan blue (17).

Mice. Male DBA/2 mice obtained from the Aurora Farm, Buffalo, N. Y., were used. Their age ranged from 25 to 40 days, with an average of 32. Two to 5 mice were placed in the same cage, and they were given commercial food and water without any antibiotics.

X-irradiation. Each mouse was irradiated with 600 R at a rate of 166 R/min with a 250-kV X-ray machine (Maxitron two-fifty, General Electric, Schenectady, N. Y.) at 30 mA with 0.25 mm Cu and 1 mm Al filters.

Cell Inoculation. Twenty-four hr after 600 R of whole-body irradiation, mice were inoculated i.p. or s.c. with 10^7 or 2.5 \times 10^7 viable cells suspended in 0.2 or 0.5 ml of phosphate-buffered saline, respectively. Injection s.c. was done in the flank. Nonirradiated mice were inoculated with the same numbers of cells. As a control group, some mice were given only phosphate-buffered saline.

Evaluation of Tumor Growth. Ten days after cell inoculation, the mice were killed and autopsied. Approximately 10% of the irradiated mice died before 10 days and were discarded. Tumorous masses from the inoculated mice were fixed with formalin, and histological preparations were made by staining with hematoxylin and eosin. Ascites cells were fixed with a modified Carnoy’s solution (methyl alcohol: acetic acid, 3:1) and were stained with Wright-Giemsa solution. Tumor “takes” were recorded as positive only if the histological studies revealed viable tumor cells and some cells in mitosis.

Reculture of Tumor and Ascites Cells. Subcutaneous or peritoneal tumor fragments and ascites fluid were removed under aseptic conditions and placed on plastic Petri dishes with 5 ml of RPMI Medium 1640 supplemented with 10% fetal calf serum, 100 units of penicillin, and 100 µg of streptomycin/ml. The dishes were incubated at 37° in an incubator with 100% humidity and a continuous flow of 5% CO_2 in air.

Characterization of Recultured Cells. Successfully recultured cells from tumors or ascites cells were characterized and compared with the original RPMI 8226 cells. Their chromosome constitutions were studied according to the procedure described by Moorehead et al. (23). The determination of immunoglobulin production by the cells was previously reported by Matsuoka et al. (15). The cloning efficiency of the cells was also determined by methods reported previously (10).

RESULTS

Induction of Tumors by s.c. Inoculation of Cells. Subcutaneous tumor masses developed in the irradiated mice inoculated with 10^7 or 2.5 \times 10^7 viable cells. The incidence of tumors induced in irradiated and nonirradiated mice by s.c. inoculation of RPMI 8226 cells is summarized in Table 1. The tumors ranged from 5 to 10 mm in diameter, from 2 to 3 mm in thickness, and were soft, flat, and white (Fig. 1). Some tumors invaded the skin and chest or abdominal wall and were adherent. No metastases were found in the abdominal or thoracic organs. The nonirradiated mice developed subcutaneous tumors that were fewer and much smaller than those in irradiated mice.

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of cells inoculated and surviving 10 days</th>
<th>No. of mice with tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^6</td>
<td>5</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>10^7</td>
<td>18</td>
<td>18 (100%)</td>
</tr>
<tr>
<td>2.5 \times 10^7</td>
<td>4</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>Nonirradiated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10^6</td>
<td>5</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>10^7</td>
<td>8</td>
<td>5 (63%)</td>
</tr>
</tbody>
</table>

Histological preparation of these subcutaneous tumors following inoculation of RPMI 8226 cells revealed them to be similar in appearance to human lymphosarcoma. The cells were arranged densely and some were undergoing active mitosis. Histological preparations of the tumors of nonirradiated mice revealed both cell growth and cell degeneration (Fig. 2).

All of the mice irradiated with 600 R developed several nodules in the spleen, even when only phosphate-buffered saline was injected. These nodules were 1 to 2 mm in diameter and could be seen with the naked eye. It is supposed that they originated from stem cells in the mice that received sublethal doses of X-irradiation. They are not correlated with inoculation of human cells (14).

Induction of Tumors by i.p. Inoculation of Cells. The results of i.p. inoculation of cultured 8226 cells are summarized in Table 2. Some tumors were found at the site of injection; i.e., in the track of the needle through the abdominal wall and the parietal membrane of the peritoneum. The size of the tumors in this experiment (3 to 6 mm) was smaller that that of the subcutaneous tumors. The tumors often invaded the abdominal musculature and sometimes adhered to the skin. Their histological features were the same as those of the subcutaneous tumors.
Table 2

Induction of ascites and peritoneal tumors after i.p. inoculation of RPMI 8226 cells into irradiated and control mice

<table>
<thead>
<tr>
<th>Animals</th>
<th>No. of cells inoculated and surviving 10 days</th>
<th>No. of mice with ascites</th>
<th>No. of mice with tumor membrane</th>
<th>No. of mice with intraperitoneal tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irradiated mice</td>
<td>10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>15 (50%)</td>
<td>11 (37%)</td>
<td>9 (30%)</td>
</tr>
<tr>
<td></td>
<td>2.5 X 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>16 (59%)</td>
<td>22 (82%)</td>
<td>23 (85%)</td>
</tr>
<tr>
<td>Nonirradiated mice</td>
<td>1.0&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td></td>
<td>2.5 X 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>0 (0%)</td>
<td>2 (33%)</td>
<td>1 (7%)</td>
</tr>
</tbody>
</table>

Some irradiated mice developed ascites after i.p. inoculation of 10<sup>7</sup> or 2.5 X 10<sup>7</sup> viable cells. Every such mouse had approximately 1 to 3 ml of milky ascites with a high cell concentration. The abdominal cavity was washed 2 or 3 times and the cells collected by centrifugation. The number of ascites cells per mouse ranged from 1.2 to 15.8 X 10<sup>7</sup> and from 2.9 to 26.6 X 10<sup>7</sup> in mice inoculated with 10<sup>7</sup> and 2.5 X 10<sup>7</sup> viable cells, respectively (Table 3).

Morphologically, the ascites cells were the same as the original RPMI 8226 cells in culture (Fig. 3). Binucleate and trinucleate cells were seen in the ascites, as is characteristic of this cell line. Intra- and retroperitoneal tumor masses were found in both irradiated and nonirradiated mice inoculated with the cells. The intraperitoneal tumors were small, white, firm, and less than 2 mm in diameter. They were located mostly in the mesentery. There were a few retroperitoneal tumors, but no metastases were found in other organs. The histological appearance of these tumors was the same as that of the subcutaneous tumors.

Reculture of Tumor and Ascites Cells and Characterization of Recultured Cells. Two or 3 days after reculture of tumor minces, cells began to migrate from small fragments of tumor into the medium and multiplied rapidly. By the 6th or 7th day, the bottom of the Petri dish was covered with round cells. Most of the cells adhered to the plastic wall, but the attachment was loose. Some fibroblast-like cells, which probably were mouse cells, were attached to the plastic wall very firmly. The loosely attached and floating lymphoblastoid cells continued to grow after transference from the Petri dish to 8- or 16-oz Brockway bottles. Ascites cells also began to grow and divide immediately and rapidly on reculture. No fibroblast-like cells were seen in the culture. The morphological appearance of these recultured cells was the same as that of the original cells in culture.

Characterization of these recultured cells is summarized in Table 4. Chromosome studies revealed these cells to be identical to the original cells. It was reported previously that the RPMI 8226 cell line had a high cloning efficiency in soft agar and that this might reflect its malignant nature (10). The recultured cells also had high cloning efficiencies, and they produced macroscopic colonies in the agar medium. The recultured cells produced no heavy chains, but only λ-type light-chain immunoglobulin, as the original cells did (Fig. 4). Thus it is reasonably certain that these cells were identical to the original RPMI 8226 cells.

DISCUSSION

All of the tumors and ascites obtained from the mice inoculated with RPMI 8226 cells were studied histologically, and cells were recultured from some of them. Species identification analyses indicated that these cells were the progeny of human cells. The recultured cells had the same chromosome constitution as the original cells and synthesized only λ light-chain immunoglobulin.

In general, tumors and ascites can often be produced only in newborn or young animals (3, 13, 26). Irradiation with 600 R is considered to be enough to destroy the immunologically responsive cells of mice of this age. RPMI 8226 cells were introduced while the mice were immunologically unrespon-
The condition of the host and the viability and nature of the inoculated cells eventually determine whether or not a tumor will result from the cells inoculated into the animal, whether the tumor will grow initially and regress later, or whether it will continue to grow and invade adjacent tissues or metastasize to other organs.

Heterologous tumors produced by inoculation of tumor cells from surgical specimens or from effusions are histologically similar to the original human tumors (12, 22). In contrast, the heterologous tumors produced by inoculation of tumor cells from tissue culture may be histologically quite different from the original tumors (22).

Many investigators have reported that malignant cells could be transplanted into newborn or immunosuppressed heterologous hosts and that cells that could not be transplanted might be normal cells (5, 6). Certain cells that were derived from normal tissue and subsequently might have undergone malignant transformation in vitro could also be transplanted successfully (27). RPMI 8226 cells were derived from the peripheral blood of a patient with myeloma and continued to synthesize the same incomplete immunoglobulin. The cultured cells had had an abnormal chromosome constitution ever since they were first established (15, 20) and they had a high cloning efficiency in soft agar (10). The behavior of these cells in heterotransplantation is likewise indirect evidence that they are malignant (Fig. 5).

There are only a few reports concerned with the comparative heterotransplantation of cultured hematopoietic cells or leukocytes from the peripheral blood of patients with leukemia or other cancers. In 1965, Nielsen et al. (24) reported that the transplantation of bone marrow cells from patients with plasmacytic, lymphocytic, and monocytic leukemia into axenic mice was followed by the induction of abnormal changes in the thymus or spleen. Zlotnick and Robinson (28) reported that 2 out of 40 irradiated hamsters developed tumor growth in the cheek pouch following inoculation with $10^8$ viable leukocytes from a patient with chronic lymphocytic leukemia.

Adams et al. (1-3) were the first to use cultured lymphoblasts derived from the peripheral blood of a patient with leukemia for heterotransplantation. They reported that the lymphoblasts repopulated the bone marrow and were detected in the peripheral blood, the lymph nodes, or in both in lethally irradiated Syrian hamsters following i.v. inoculation. They also succeeded in transplanting $2 \times 10^6$ lymphoblasts from a child with lymphosarcoma directly into newborn hamsters, which later progressed to leukemia. They recovered a serially transplanta
table lymphosarcoma which was proved antigenically to consist of human cells. Southam et al. (27) found transplanted cells growing in the brain, eye, and kidney when cultured Burkitt cells and cells derived from patients with leukemia and infectious mononucleosis and from a normal lymph node were given by inoculation i.v. into newborn rats.

Previous workers have reported that approximately $10^6$ tumor cells are needed in order to produce intraperitoneal tumors from cultured cells in laboratory animals conditioned with X-irradiation or cortisone (4, 16). Adams et al. (2) used approximately $10^6$ viable hematopoietic cells. In the present study, subcutaneous tumors were obtained in 100% of the animals by inoculation with $10^7$ cells; $10^6$ viable cells produced tumors much smaller than those in mice inoculated with $10^7$ cells. Inoculation i.p. of $10^7$ or $2.5 \times 10^7$ viable cells produced ascites and intraperitoneal tumors. The total number of ascites cells collected from the mice following 10 days was approximately 10 times the number of cells inoculated; thus evidence of replication of the cells in the heterologous host was attained.

X-irradiated mice inoculated with RPMI 8226 cells for temporary heterologous growth can be regarded as a kind of cell culture in vivo; that is, as living culture vessels. Heterotransplantability is not an absolute criterion of cancer, but cell lines that are presumably cancerous, such as RPMI 8226, are often characterized by vigorous growth when heterotransplanted.

### Table 4

**Characterization of cells recultured from tumors and ascites in mice given inoculations of RPMI 8226 cells**

<table>
<thead>
<tr>
<th>Experiment and animal nos.</th>
<th>Origin</th>
<th>No. of cells counted</th>
<th>No. of cells with chromosome numbers of</th>
<th>Cloning efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(RPMI 8226)</td>
<td></td>
<td></td>
<td>≤63</td>
<td>64</td>
</tr>
<tr>
<td>30-3</td>
<td>Tumor</td>
<td>20</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>34-2</td>
<td>Ascites</td>
<td>20</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>34-4</td>
<td>Ascites</td>
<td>(not counted)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

REFERENCES


Fig. 1. Subcutaneous tumor produced in irradiated mouse inoculated s.c. with $10^7$ RPMI 8226 cells.
Fig. 2. Subcutaneous tumor in irradiated mouse. High magnification. X 400.
Fig. 3. Ascites produced in irradiated mouse inoculated i.p. with $2.5 \times 10^7$ RPMI 8226 cells.
Fig. 4. Ascites cells. High magnification. X 400.
Fig. 5. Immunodiffusion with cells recultured from tumor or ascites in mouse inoculated with RPMI 8226 cells. A, anti-8226-λ; B, anti-8226-λ absorbed with λ-Bence-Jones protein; C, anti-λ. 1 and 4, RPMI 8226; 2, recultured cells (Experiment and Animal No. 30-3); 3, recultured cells (Experiment and Animal No. 34-4); 5, recultured cells (Experiment and Animal No. 34-2); 6, λ-type Bence-Jones protein (absorbing antigen).
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