SUMMARY

Two human tissue culture cell lines, LI-i derived from a patient with acute myelogenous leukemia and IM-1 from a patient with lymphoblastic lymphoma, were compared with donor tissues. Morphologic and functional features were assessed to determine if there were significant differences between the culture lines and the malignant tumor cells of the corresponding patients.

The LI-i line contained mostly large primitive cells which did not resemble the myeloblasts of the donor. The IM-1 cell line was composed of similar primitive cells, lymphocytes, and a few macrophages, whereas the source of this line was a lymphoid tissue composed of a mixture of plasma and lymphoid cells.

The two cell lines produce electrophoretically discrete immunoglobulins in culture. Cell line LI-i synthesized IgG-κ and IgG-λ. Cell line IM-1 produces electrophoretically discrete IgM-κ, IgG-κ, and κ light polypeptide chains. Electrophoretically discrete immunoglobulins comparable to the immunoglobulins synthesized in culture, however, were not detected in the patients' serum or synthesized by their tissues.

These studies indicate that cell lines established in culture differ significantly from the major portion of malignant cells seen in vivo in these patients. Designation of such cell lines as leukemia cell lines seems inappropriate until further observations can be made on the relationship between the cells in culture and the normal and malignant cells of donor patients.

INTRODUCTION

Human lymphoid cell lines in continuous cell cultures were first established by Pulvertaft (19) and Epstein and Barr (5) with biopsy specimens obtained from patients with Burkitt's tumor. Soon after, Iwakata and Grace (14) and others (1, 11, 15, 17, 20, 22, 24) established similar cultures from the peripheral blood buffy coats of patients with leukemia and lymphoma. Many cell cultures from both these sources were subsequently shown to contain herpes-like virus particles (4, 17, 18, 20, 22, 26).

This laboratory (6, 10) and others (23–25) have shown that many of the lymphoid cell lines produce immunoglobulins. It remains unknown, however, whether the original cells in culture synthesized immunoglobulins or whether this capacity developed in the culture environment. The successful establishment of continuous white cell cultures from two patients who are still alive has given us an opportunity to investigate this question.

Morphologic, immunochemical, immunofluorescent, and biosynthetic methods were used to compare freshly obtained tissue with the cell lines established and growing in tissue culture.

MATERIALS AND METHODS

Cell Lines

The line LI-i was established from the peripheral blood of patient R. P., a 17-year-old white male with acute myelogenous leukemia. At the time the cell line was established, his blood contained 140,000 WBC/cu mm, 95% myeloblasts. The white cells were obtained by permitting the blood samples to stand for one hour and removing the white cell rich plasma. Initially, six cultures were established each containing 1 × 10⁶ cells in ten ml of Eagle's minimal essential medium (MEM) (3) supplemented with 20% fetal calf serum in 50-ml capped bottles. The culture fluid was changed biweekly or earlier if the pH was acid. After two weeks the cell concentration had fallen to 5 × 10⁵ cells/ml. Then four cultures were pooled yielding a concentration of 2 × 10⁶ cells/ml. After eight weeks the cells began to grow rapidly. Since then the cells have been maintained for 18 months with two to three medium changes per week in Eagle's MEM with fetal calf serum.

The second cell line (IM-1) was from a biopsy of lymphoid tissue (lingual tonsil) from a 54-year-old white female with diagnosis of lymphoblastic lymphoma and cutaneous lymphoma. The biopsy tissue was minced in Hanks' balanced salt solution and the supernatant fluid removed. This fluid was centrifuged and the pellet of cells transferred into four 4-oz glass bottles with 10 ml of Eagle's minimal essential medium (MEM) (3) supplemented with 20% fetal calf serum in 50-ml capped bottles. The culture fluid was changed biweekly or earlier if the pH was acid. After two weeks the cell concentration had fallen to 5 × 10⁶ cells/ml. Then four cultures were pooled yielding a concentration of 2 × 10⁶ cells/ml. After eight weeks the cells began to grow rapidly. Since then the cells have been maintained for 18 months with two to three medium changes per week in Eagle's MEM with fetal calf serum.

The second cell line (IM-1) was from a biopsy of lymphoid tissue (lingual tonsil) from a 54-year-old white female with diagnosis of lymphoblastic lymphoma and cutaneous lymphoma. The biopsy tissue was minced in Hanks' balanced salt solution and the supernatant fluid removed. This fluid was centrifuged and the pellet of cells transferred into four 4-oz glass bottles with 10 ml of Eagle's MEM and 20% fetal calf serum with 100 units/ml penicillin, 50 μg/ml streptomycin. The fluid was changed every 24–72 hours depending on whether the medium was acid and the cells pooled to maintain a population of approximately 5 × 10⁶ cells/ml or greater.

After four weeks, free-floating cells began to increase in number. A fibroblast population was also present for the first six months of culture before gradually disappearing. The cells...
have been maintained in 10–20% fetal calf serum added to Eagle's MEM with antibiotics and the medium was changed biweekly. This line has been in continuous culture for ten months.

Immunoglobulin Biosynthesis

The technic used for studying immunoglobulin synthesis in the two cell lines has been reported in detail (10, 13). A modification of this method was used to detect immunoglobulin synthesis in the tissues of the two patients studied. Bone marrow aspiration was done with a Rosenthal needle and one ml of marrow delivered into a sterile 15-ml plastic tissue culture tube (Falcon) containing 200 units of sodium heparin.

The tube was rotated manually so that spicules of bone marrow adhered to the surface of the tube. The tube was allowed to stand upright for 10 minutes at room temperature and the uncotted blood removed with a sterile pipet. The tube was weighed before addition of heparin and after excess blood was removed. The 14C-labeled medium described by Finegold et al. (10) was added to the tubes containing the marrow. Two ml of medium were added to 50–100 mg of marrow, 3 ml for 100–150 mg, and 4 ml for 150–200 mg. The tubes were rotated at 3 rpm at 37°C for 48 hours and the reaction stopped with excess unlabeled isoleucine and lysine. The fluid was processed for immunoelectrophoresis in the same manner as the tissue culture growth fluid.

When lymphoid tissue was available, the tissue was cut with scissors into 5-cu mm fragments, washed in a medium without lysine and isoleucine, and minced between two scalpels until the fragments were about one cu mm. These fragments were placed along the sides of the tubes with a pipet, the fluid allowed to drain and then removed. The complete labeled medium was then added according to the same schedule used with the marrow and processed as described above.

Antiserums specific for IgG, IgA, IgM, IgD and kappa and lambda light chains (8) were used in immunochemical tests. They were rendered specific when necessary by appropriate absorption with myeloma proteins or bovine gamma globulin. Quantitative immunoglobulin studies were done at the NCI Immunoglobulin Reference Center utilizing ring diffusion in agar containing specific antiserum (7).

Preparation for Microscopy

Bone marrow was obtained from both patients from the posterior iliac crest, smeared, and Giemsa stained. Patient E. M. underwent a lingual tonsil biopsy. This material was fixed in formalin, stained with hematoxylin and eosin, and examined by the light microscope. Both cell lines were smeared, fixed in methanol, and Giemsa stained for conventional microscopy. Portions of each line were also taken for electron microscopy. Cells were sedimented by centrifugation at 2000 rpm for five minutes in an International PR2 centrifuge with #269 head. The pellet was fixed at 4°C for 1.5 hours in Dalton’s chrom-o-smium fixative (2), washed for 10 minutes in neutral 10% formalin, and postfixed overnight in 0.2% uranyl acetate solution in 10% formalin. After dehydration the cell pellets were embedded in epon and araldite (16). Sections 300–500 Å thick were cut using a LKB ultramicrotome with a diamond knife, and stained with uranyl acetate (12) followed by lead citrate (21). Micrographs were taken with an RCA-EMU-3G electron microscope.

Immunofluorescence

Immunofluorescent studies were carried out in both cell lines using direct immunofluorescent technics. These methods are described in detail by Finegold et al. (9). The cells were washed in Hanks’ balanced salts, suspended in 15% bovine albumin, smeared onto cover slips, air dried, and fixed for ten minutes in cold absolute methanol. Fluorescein-labeled anti-human IgG, IgA, IgM, and kappa and lambda light chain antiserums were used. The staining could be blocked by addition of unlabeled antiserum to the slides before staining or addition of purified antigen to the conjugate in vitro before staining. The fluorescein-labeled conjugates were tested and found to be specific by reactions with tissues from patients with myeloma and macroglobulinemia (9).

RESULTS

Immunobiosynthetic Studies

In each cell line several immunoglobulins were synthesized (IgG-κ and IgG-λ by LI-i cells and IgM-κ and IgG-κ by IM-1 cells) (Table 1). The immunoglobulins synthesized by the IM-1 culture line were electrophoretically discrete (Fig. 1), and similar discrete IgG components were formed by LI-i cells (10).

A bone marrow aspirate from the first patient, R. P., contained many malignant myeloblasts (Fig. 4). This marrow synthesized IgG, IgA, and IgM (Fig. 2). These immunoglobulins were electrophoretically diffuse and resembled those formed in normal marrow. Marrow from the second patient, E. M., showed a similar diffuse biosynthetic pattern as did the lymphoid tumor (source of IM-1 cell line) from patient E. M. The IgG, IgA, and IgM formed by these tissues were electrophoretically heterogeneous and thus differed from the discrete components formed in the culture cell line.

Immunofluorescent studies of both cell lines were in agreement with the immunobiosynthetic studies. The cells were tested with fluorescein-labeled antiserums for γ, α, and μ heavy chains and κ and λ light chains. Fifteen percent of the LI-i cells stained for γ, 2% for κ, and 18% for λ chains; the IM-1

<table>
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<th>Sample</th>
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<th>Light chains</th>
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<tbody>
<tr>
<td></td>
<td>γ</td>
<td>α</td>
</tr>
<tr>
<td>R. P. (marrow)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>LI-i (derived from R. P.)</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>LI-i (peripheral blood)</td>
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<td>0</td>
</tr>
<tr>
<td>E. M. (marrow)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>(lymphoid tissue)</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IM-1 (derived from E. M.)</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>IM-1* (lymphoid tissue)</td>
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<td>0</td>
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Immunoglobulins synthesized by R. P., E. M., and the two cell lines. All results are from biosynthetic studies except that marked with an asterisk, indicating immunofluorescence testing. +, positive; 0, negative.
cells were positive for m (18%), γ (26%), and k (17%) chains. Table 1 summarizes results of all these tests compared with the donor tissues.

Differences between the immunoglobulins of the patients and the immunoglobulins synthesized by the culture cells were sought by Ouchterlony tests. The IgG synthesized by LI-i formed a line of identity with R. P. serum and a normal control serum (Fig. 3). Similarly a line of identity was formed with anti-lambda antiserum (Fig. 3). A faint line with anti-kappa antiserum (Fig. 3) indicated smaller amounts of kappa light chains were present, but identity could not be evaluated. LI-i IgG was identical with R. P. IgG and normal IgG when tested with three additional antisera. Similar studies with IM-1 immunoglobulins revealed lines of identity with four anti-IgM antisera and one anti-kappa antiserum. The IgG formed by this culture gave a faint reaction.

From these studies it is clear that the IgG and k made by LI-1 cells and the IgM and k made by IM-1 cells are clearly not antigenically deficient. The other immunoglobulin chains synthesized by these lines cannot be accurately evaluated. These studies rule out a major loss of antigens.

Serums were obtained from both patients at the time the original cultures were initiated and at later dates. Serum immunoelectrophoresis did not reveal any of the characteristic abnormalities such as seen in myeloma or macroglobulinemia. Quantitative immunoglobulin levels also were determined (Table 2). The serum immunoglobulin levels of patient E. M. were normal initially and decreased after therapy. The serum of R. P. at the time when LI-i culture was initiated, however, had increased serum immunoglobulin levels. Coincident with chemotherapy and clinical improvement these levels returned to normal.

**Morphologic Studies**

The appearance of LI-i cells are shown in Figs. 4d and 4e. A mixture of cell types is present. Most cells are 20 μm in diameter with a large nucleus containing several distinct nucleoli and a moderate amount of dark blue cytoplasm. An occasional cell is two to three times larger with a reticular nuclear pattern and abundant cytoplasm. There are also cells present resembling small lymphocytes.

Precise morphologic classification of these cells is difficult. They are generally too immature to be identified firmly as members of the lymphoid or myeloid series. The cells present in the peripheral blood of R. P. on the day the LI-i culture was initiated are shown in Fig. 4b. The predominant cell present (94%) is a myeloblast. Similar cells were present in the bone marrow (Fig. 4a). The other cells are 4% lymphocytes, 1% polymorphonuclear cells, and one plasma cell (Fig. 4c).

Typical cells from IM-1 cultures are shown in Fig. 5b. Primitive lymphoid cells and cells similar to small lymphocytes are present. In addition, macrophages have been observed in this cell line (Hirshaut and Finegold, unpublished observations).

The lymphoma tissue from which this line was established is shown in Fig. 5a. A variety of plasma cells are present but no pathologic process could be diagnosed from this tissue specimen. A lymph node biopsy one month later was diagnostic of lymphoblastic lymphoma. In contrast to the overwhelming numbers of myeloblasts in the peripheral blood of R. P., it is obvious that there were many more cell types in the node tissue from which IM-1 was isolated.

Electron microscopic studies of the cells of the two cell lines are shown in Figs. 6–8. Most of the cells in both cultures have a roughly circular outline with a large nucleus containing one or more nucleoli, surrounded by a moderate amount of cytoplasm. The cytoplasm contains a small number of mitochondria and relatively sparse endoplasmic reticulum. A few larger cells are seen which have a more ovoid outline with abundant cytoplasm and many mitochondria. There are also a group of cells with a large nucleus and a scant rim of cytoplasm. Their nucleus is of greater density and usually does not contain a nucleolus. The IM-1 cell line is unique in having some cells which have been observed to contain cell fragments and in one instance a whole cell. No herpes-like or other virus particles were seen in either culture.

**DISCUSSION**

Human leukocytes in long-term cultures are often capable of synthesizing immunoglobulins in vitro. This finding has raised new questions about the identity of the cells which survive transfer into tissue culture and give rise to established cell lines.

In attempting to find a relationship between the cells in culture and the original donor tissues, we hoped that the immunoglobulins produced in such cultures might provide a label by which the origin of the cells could be traced. For this study two cell lines and the patients from which they were derived were available. LI-i cells produced IgG-K (γ2 k2) and IgG (γ2 k2), IM-1 cells synthesized IgM-K (μ2 k2)5,

<table>
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<tr>
<th>Sample</th>
<th>IgG</th>
<th>IgA</th>
<th>IgM</th>
<th>IgD</th>
<th>Kappa</th>
<th>Lambda</th>
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<td>Normal adult</td>
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<td>2.8 ± 0.7</td>
<td>1.2 ± 0.35</td>
<td>0.0-0.3</td>
<td>8.0 ± 2.0</td>
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<td></td>
<td></td>
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<tr>
<td>10/13/66</td>
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<td>5.6</td>
<td>1.6</td>
<td>0.23</td>
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<tr>
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<td>4.4</td>
<td>0.80</td>
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<td>1.1</td>
<td>0.79</td>
<td>0.02</td>
<td>5.7</td>
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Serum immunoglobulin levels, mg/ml.

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IgG-K (γ2 k2) and free K light chains. With this knowledge of the immunoglobulins synthesized by the cultures we then looked for these proteins in the serum and hematopoietic and/or lymphoid tissues of both patients.

The serum of the two patients, obtained at the time the cultures were initiated, did not reveal an increased electrophoretically discrete immunoglobulin as is seen in myeloma or macroglobulinemia. By Ouchterlony tests the cell line immunoglobulins appeared to be antigenically identical with patient and normal serum globulins. Further, immunochemical studies revealed that the cell lines produced proteins that were grossly of the same size, polypeptide chain, and antigenic composition as normal immunoglobulins.

Immunobiological studies undertaken with the bone marrow and lymphoid tissues of patient E. M. demonstrated active synthesis of three heavy chain and two light chain types of normal immunoglobulins. Diseased marrow from patient R. P. was also synthesizing normal immunoglobulins. Similar observations of a discrepancy between the immunoglobulins formed in the malignant peripheral blood leukemic cells and the immunoglobulin formed in a derivative culture were described by Wakefield et al. (25).

Since the discrete immunoglobulins produced by the cell lines cannot be distinguished from normal immunoglobulins produced by the patient, it would only be possible to determine their source if they were found in excess in the serum or were produced in excess by a particular tissue. Such excess was not found in either patient. Whether the immunoglobulin-forming culture cells are derived from malignant or normal cells, therefore, remains unknown.

Morphologic studies raised additional difficulties. The predominant cell in both culture lines is a large primitive form. Its appearance is different from the myeloblasts of patient R. P. from whom the LI-1 cell line was isolated. Also, this primitive cell was not seen among the lymphocytes and plasma cells in the lymphoid tissue of E. M., from which the IM-i cell line was established. At the same time it does not resemble any normal cell type found in bone marrow, peripheral blood, or lymph node. We cannot distinguish, therefore, if the cell in culture is an altered malignant cell or a dedifferentiated normal form.

The presence of macrophages in IM-1 line demonstrates that some cells may retain their functional specialization in culture. The immunoglobulin-synthesizing culture cells might well derive from immunocytes whose morphology has changed without affecting their ability to produce immunoglobulin. Alternate, but less likely, the ability to undertake specialized functions such as phagocytosis and immunoglobulin synthesis could develop in vitro following cellular dedifferentiation.

ACKNOWLEDGMENTS

The authors wish to express their gratitude to Miss Joyce Ellis, Mrs. Helen Grimes, Mrs. Oveilla E. Ayers, Mrs. Irene E. Clark, and Mr. Benjamin F. Elliott for their expert technical assistance.

REFERENCES


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Fig. 1. Radioimmunoelectrophoresis of amino acid-14C-labeled tissue culture fluid from IM-1 cells. Normal human serum added as carrier for immunoelectrophoresis. Specificity of the antisera is indicated on the right side of the figure.

Fig. 2. Radioimmunoelectrophoresis of 14C-labeled fluids from R. P. bone marrow and E. M. lymphoid tissue. Normal human serum added as carrier for immunoelectrophoresis. Specificity of the antisera is indicated on the right side of the figure.

Fig. 3. Ouchterlony tests comparing LI-i and IM-1 concentrated growth fluids with R. P., E. M., and normal serum. 1, LI-1 fluid; 2, normal serum; 3, E. M. serum; 4, IM-1 fluid; 5, normal serum; 6, R. P. serum. Antiserums in center walls are against: above left, γ heavy chains; above right, μ heavy chains; below left, κ light chains; below right, λ light chains.

Fig. 4a. Bone marrow aspirate smear from R. P. (10/66). Many large myeloblasts are present. Giemsa, X 1084.

Fig. 4b. Peripheral white cells of patient R. P. Smear was done on the day LI-1 cultures were initiated. Field shows four myeloblasts and a mature polymorphonuclear leukocyte. X 1084.

Fig. 4c. Same smear as in Fig. 6 showing a plasma cell. X 1084.

Fig. 4d. Most common cell type in LI-1 line. These cells have large nuclei, one or more nucleoli, and a moderate amount of cytoplasm often filled, as here, with vacuoles.

Fig. 4e. Small cell resembles lymphocyte in LI-1 line. Similar cells can be seen in IM-1 cultures. X 1084.

Fig. 5a. H & E stained section taken from E. M. biopsy specimen used to start line IM-1. Mature lymphoid elements and plasma cells are seen.

Fig. 5b. Typical IM-1 cells with large nuclei and moderate rim of cytoplasm. X 1084.

Fig. 6. Large ovoid cell from LI-1 cell line with well-developed endoplasmic reticulum, golgi apparatus, and many mitochondria. Similar cells are seen in IM-1, x 13,000.

Fig. 7a. Predominant cell type in LI-1 and IM-1. Large nucleus with prominent nucleolus is surrounded by moderate amounts of cytoplasm with poorly developed cytoplasmic organelles. Few mitochondria are seen, x 13,000.

Fig. 7b. Cell resembling small lymphocyte in IM-1 line. Note uniform density of nucleus with scant rim of cytoplasm. X 13,000.

Fig. 8. Macrophage which has engulfed cellular debris, IM-1 line. X 13,000.
RADIOIMMUNOELECTROPHORESIS OF IM-1 CULTURE FLUID*

IMMUNOGLOBULINS

AUTORADIOGRAPHY

ANTISERUM AGAINST:

K

γ

α

μ

μ

λ

(-) (+) (-) (+)

BIOSYNTHESIS OF IMMUNOGLOBULINS BY R.P. MYELOBLASTIC LEUKEMIA BONE MARROW

Immunoelectrophoresis*  Autoradiograph

(-)  (+)  (-)  (+)

*Normal human serum added as carrier to concentrated incubation medium.
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Human Immunoglobulin-producing Tissues

![Cell Images]

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Human Immunoglobulin-producing Tissues
Immuonochemical and Morphologic Comparison of Donor Tissues with Immunoglobulin-producing Tissue Culture Lines from Two Patients with Malignancies

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