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Characteristics of Hematopoietic Cell Line Established from Human Myelomonocytic Leukemia

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Summary. The peripheral blood of an acute myelomonocytic leukemia patient has been cultured for 16 months. The culture is at present at the 140th population doupling level. The cultured cells have the characteristics of so-called lymphoblastoid cells and proliferate actively as individual cells in small clusters, or in large clumps consisting of large mononuclear cells. Some of these cells appeare to be lymphoid, but the majority are immature mononuclear cells with a tendency to lobulate. They gave a weakly positive peroxidase reaction at the beginning of cultivation, and have given a strongly positive esterase reaction persistently. The cytoplasm shows ciliary or tail-like projections as the cell matures. Complement (C_3) receptor and IgG receptors are found on the cell surface, and active phagocytosis is mannifest. Colloidal iron particles or viable red blood cells attached to the cell membrane suggesting possible differentiation to reticulum cells or macrophages. The cultured cells are mostly diploid but some cells show chromosome abnormality.

Herpes type virus was found in the nucleus, cytoplasma and on the cell membrane. The transplantation of cultured cells to the cheek pouch of hamsters produced small tumors with histological findings resembling reticulum cell sarcoma.

Key words: Myelomonocytic leukemia — Cell line — Reticulum cell — EB virus.

Introduction

The classification of myelomonocytic leukemia has been a problem since the classic dispute between Naegeli (monocytic leukemia) and Schilling (histiocytic leukemia). A recent review by Bennett (1971) on myelomonocytic leukemia continues the discussion of whether monocytes should be considered as an independent cell line or part of the myelogranulocytes. At present most hematologists classify myelomonocytic leukemias by morphologically differentiating the monoblasts and mononuclear cells, both of which are found in a high percentage of so-called myelogranulocytic leukemias. However, classifying blast type mononuclear cells in peripheral blood is difficult, and the relationships between such undifferentiated mononuclear cells and stem cells remain unclear. In the present study the cytologic properties of hematopoietic cells cultured from peripheral blood of a patient with monocytic leukemia were successfully defined kinetically and the presence of E.B. virus was evident in the established cells. These findings may provide important information concerning the onset of myelomonocytic leukemia.

Materials and Methods

Cell Source of Human Myelomonocytic Leukemic Cells

Five ml of peripheral blood from a 33-year old male patient with acute myelomonocytic leukemia was provided by Prof. Susumu Shibata, Department of Internal Medicine, Ka-

wasaki Medical School. The diagnosis was established by bone marrow biopsy and the subtype of acute leukemia was determined by peripheral blood and bone marrow cell morphology. The patient did not receive medication or transfusion prior to the collection of cells for culture.

Classification of peripheral white blood cells indicated about 25% monocytic leukemic cells and 60% lymphocytes. Bone marrow classification of nucleated blood cells indicated 41% monocytic leukemic cells and 30% lymphocytes (Fig. 1).

During a remission 16 months after the initial sampling the peripheral blood white cells were found to be exclusively lymphocytic. These lymphocytes were cultured by the same procedures as cells from the acute stage.

Culture of Cells

Freshly drawn blood (10 ml) was transferred to centrifuge tubes and incubated for one hour at 37° C. During this period, the red cells settled, leaving a clear layer of plasma and leukocytes. The pure leukocyte suspension was subjected to the one step sodium metrizoate/Ficoll (19.6/5.6%, W/V) procedure of Bøyum (1968). The collected leukocytes were washed in the medium, and the cell suspension (1×10^7/ml cells) was prepared in 50 mm-glass dishes containing R.P.M.I. 1640 supplemented with 30% inactivated (56° C for 30 min) fetal calf serum. The use of a CO_2 incubator was helpful for maintaining the pH at an appropriate level. The cultures were fed every other day with 50% fresh medium (V/V). Under this schedule the mononuclear cells grew vigorously as clusters or single cells.

Identification of Complement Receptors

Sheep red cells were commercially obtained from Japan Biotest Laboratory, Tokyo, and stored at 4° C in Alsever's solution. The cells were washed three times in phosphate buffer saline solution (PBS) (pH 7.2) and adjusted to a concentration of 1×10^9 cells/ml PBS before use.

The mononuclear cells were washed three times in PBS, and 5×10^6/ml cells were suspended in PBS. Agglutination tests were performed by the same procedure as described in the previous report (Ueki et al., 1975).

Detection of Receptor of IgG

Red cells of Type 0 antigen from D-positive donors were coated with anti-D for one hour at 37° C as described by Huber et al. (1969). Red cells coated with anti-D were incubated with mononuclear cells in PBS suspension (pH 7.2) at 37° C for one hour and then at room temperature for one hour. These red cells attached to the mononuclear cells and formed rosettes. The reaction was not dependent on the presence of serum or inhibited by EDTA. These mononuclear cells were observed under light and scanning electron microscopy.

Detection of Immunoglobulins

Rabbit antisera against human IgG, IgM and IgA were obtained from commercial sources (Medical and Biological Laboratory Ltd., Japan). An immunodiffusion test was performed.

Immunodiffusion

The medium was harvested after 4 days cultivation, concentrated about 30-fold by refrigeration and dialysed against veronal buffer (μ=0.025, pH 8.6) overnight in a cold room. The spent medium was placed in a well in 1.2% agarose layer prepared with veronal buffer on a glass slide. Specimens were allowed to react with antiserum overnight.

Phagocytosis of Colloidal Iron

Iron particles were added to the culture as iron chondroitin sulfate (Cs-Fe). The Cs-Fe (Dainippon Seiyaku Co., Osaka, Japan) used was in colloidal solution (pH 7.2) with 1 ml of solution containing 4 mg of iron. The size of the iron colloidal particles estimated by electron

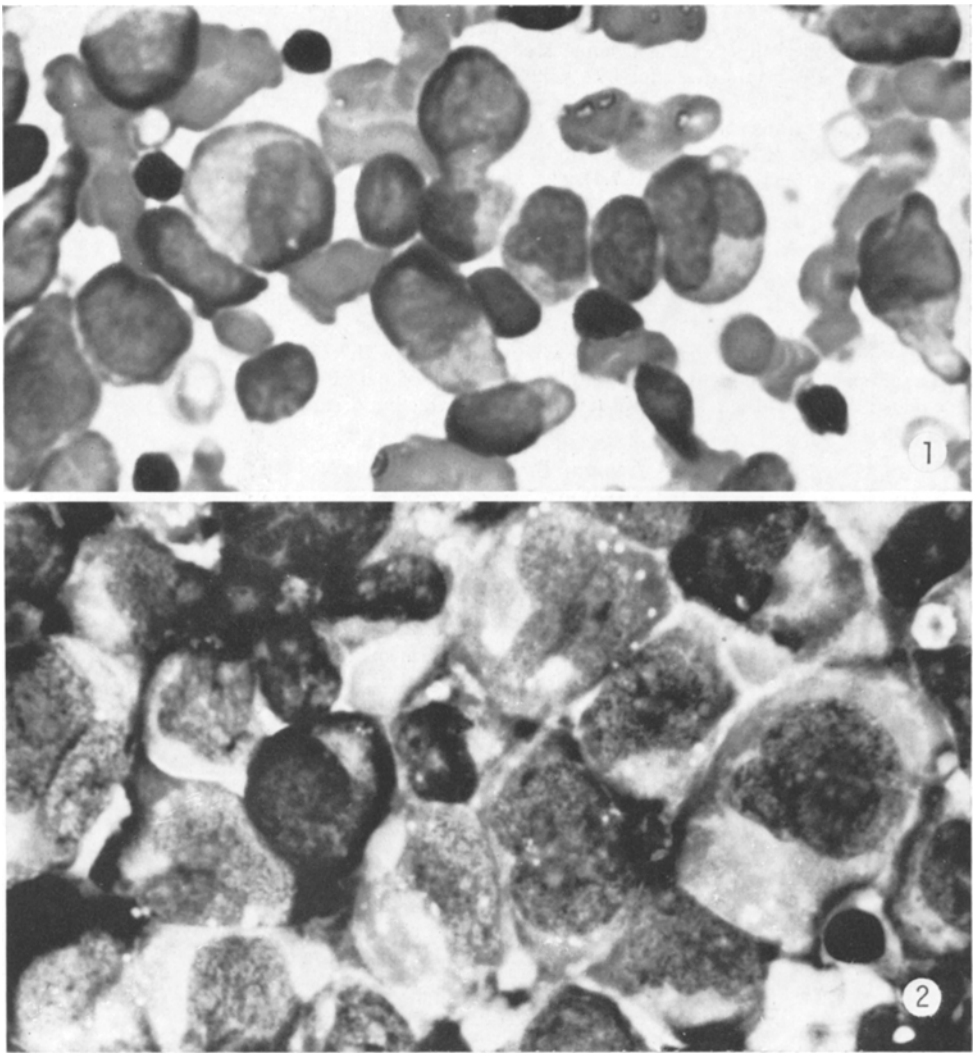


Fig. 1. Smear of bone marrow at the time of culture onset. Many large myelomononuclear cells were observed and they were positive to peroxidase activity. Giemsa stain. 10×20

Fig. 2. In vitro lymphoblastoid cells in small clusters at the 100th population doubling level after a culture period of 13 months. They resemble myelomonocytic leukemic cells in marrow (Fig. 1). Giemsa stain. 10×40

microscopy from about 30 Å in aggregated size. The colloidal particles in Hank's solution were negatively charged as revealed by paper electrophoresis in veronal-acetate buffer (pH 7.4). The iron was combined so firmly with chondroitin sulfate that the two components were not easily separated by electrophoresis (Kimoto et al., 1971). Iron colloid solution was added to the culture media in a 1:3 volume in Leighton tubes, in which cells were suspended. At 2, 4, 6 and 24 h after the addition of iron colloid, cells were centrifuged, washed by Hank's solution, dried, fixed with ethanol, treated with Perl's reaction for iron, stained by neutral fast red and observed by light microscopy.

Esterase Activity in Culture Cells

Using α-naphthyl acetate as substrate, air dried fresh cell smears were fixed in formalin vapour for 4 min and dried for 30 s at 25° C; the cells were subsequently rinsed under tap water for 5 min and air dried; the fixed smears were incubated for 20 min at 25° C in freshly prepared substrate solution which was filtered before use. The substrate solution was prepared as follows: 20 mg of α-naphthyl acetate was dissolved in 0.5 ml acetone; 20 ml of Söresen's phosphate buffer (pH 7.4), and 20 mg of fast blue B were added; after incubation the smears were rinsed under tap water, air-dried, and protected with a coverglass mounted on glycerin jelly of pH 7.2.

Karyologic Analysis

The culture was used on the third day after resuspension in fresh medium. The cells for chromosomal analysis were collected after treatment with colchicine (1 r/ml) for 2 or 4 h. The preparation were treated with 0.5% or 1.0% sodium citrate, fixed with ethanol/acetic acid (3:1), frame-dried, and stained with Giemsa. One hundred metaphase cells were studied.

Human Lymphoblastoid Cells

Suspension culture cells of MOLT-4 cells were kindly provided by Dr. Jun Minowada, Roswell Park Memorial Institute, Buffalo, N.Y., U.S.A., and the Burkitt lymphoma cells (E.B.-3 and Jijoye) were purchased from Flow Co., U.S-A-

Transplantation of Cultured Cells

A population of 1×10^8 mononuclear cells were transplanted into the cheek pouch of hamsters conditioned by a subcutaneous injection of 0.1 ml of Decadoron (Merck, U.S.A.) before and after the day of transplantation. The tumor induced by the cell transplantation was removed, fixed with 10% formalin solution, embedded in paraffin, sectioned, aud stained by hematoxylin-eosin.

Electron Microscopy and Scanning Electron Microscopy (SEM)

For electron microscopy, the cells were fixed with 2.5% glutaraldehyde in 0.1M phosphate buffer at 4° C for 30 min and post-fixed with 1% OsO_4 in phosphate buffer (pH 7.2) for 30 min. After fixation, the cells were washed with cold water, dehydrated through ethanol series, and embedded in Epon 812. Sections were stained with 5% uranylacetate in 7% aqueous ethanol and observed by a Hitachi HU-IIA electron microscope.

For SEM, the suspended cells were fixed with 1% glutaraldehyde, rinsed twice in PBS (pH 7.2), and dehydrated in series of ethanol and in gradient series of amyl acetate/absolute ethanol and absolute amyl acetate. The cells were then dried in a Hitachi critical point apparatus (HCP-1) and coated with a thin layer of carbon and gold in a rotary stage (Hitachi vacuum apparatus, HUS-4GB). The specimes were examined by a Hitachi SSM-2 scanning electron microscope at an accelerating voltage of 20 KV.

Results

A lymphoblastoid cell line was established in suspension cultures of mononcytic leukemic cells isolated from the peripheral blood of an untreated myelomonocytic leukemia patient. At present, the culture has been growing for 16 months and is at about the 140th generation of population doubling.

Figure 2 shows the characteristic features of the cultured cells. The cells revealed a chromatin pattern and cytoplasm unique to immature mononuclear cells of the patient's peripheral blood and bone marrow at the onest of the sus-

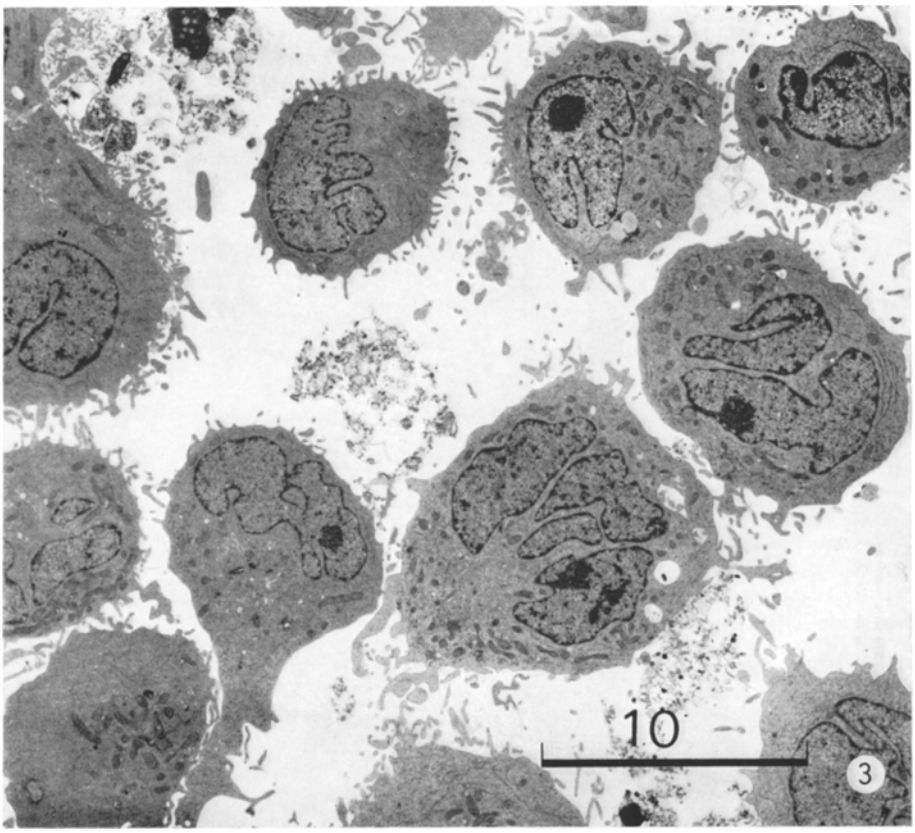
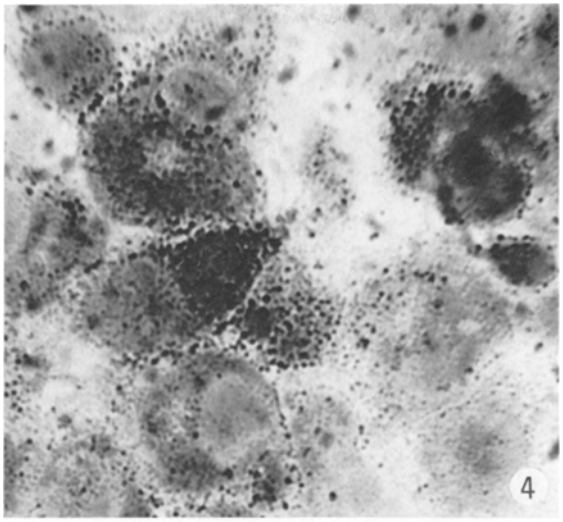


Fig. 3. Electron microscopy of cultured cells at 13 months of cultivation. Nuclear lobulations differ in the lymphoid cells

pension culture (Fig. 1). The cultured cells were weakly positive in the peroxidase reaction in contrast to the strongly positive reaction in the peripheral blood and bone marrow at the beginning of the culture.

As reported in other lymphoblastoid cell cultivations (Iwakata et al., 1964; Moore et al., 1970; Paron et al., 1970; Robinson et al., 1971; Nilsson et al., 1975), the cells proliferated and divided quite actively and formed clusters. When examined at various culture intervals some mononuclear cells showed little morphological change and lymphocytes were present. Immature mononuclear cells had a tendency to slight lobulation and most were classified as cells with a lobulated nucleus or reticulum cells. (Fig. 3). These latter cells were large, round monocytes with features identical to the leukemic mononuclear cells infiltrating the peripheral blood and the bone marrow at the start of culture (Figs. 1 and 2). The relevant features included: the presence of one or two large nucleoli; the absence of azur granules in many cells; and the same reactions for α-naphthyl acetate esterase. Although the significance of cellular nonspecific esterases is poorly understood, Rozenszajn et al (1968) reported that strong α-naphthyl acetate esterase activity



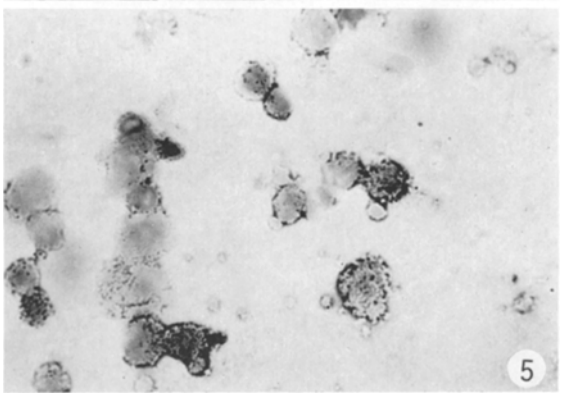


Fig. 4. Esterase reaction of cultured cells. 10×40

Fig. 5. Uptake of Cs-Fe particles by cultured cells (perl's reaction). 10×20

was found in monocytes, reticulum cells, megakaryocytes, and in monoblasts in monocytic leukemia. Weak activity was shown in thrombocytes and lymphocytes. The cultured mononuclear cells exhibited strong esterase activity as indicated by diffuse dark staining and by brown granules scattered in the cytoplasm (Fig. 4). In so-called lymphoid cells, such as Molt-4 and EB cells, the presence of fine granules was noted and one to three brown aggregates were seen. The cytoplasm of cells which wandered and proliferated were quite unique, with flagellar projections at one or more places on the cell surface. These flagellar projections become distinct under E.M. and SEM (Figs. 3 and 6) as the cells matured, some times resembling the microvilli of macrophages. After two hours of culture in the medium containing chondroitin sulfate colloidal iron particles, the mononuclear cells showed marked phagocytosis by Peal's reaction (Fig. 5). The cultured mononuclear cells had complement (C_3) receptors on the cell surfaces. When IgM (19 S)-complement conjugated sheep red cells were incubated with the culture they

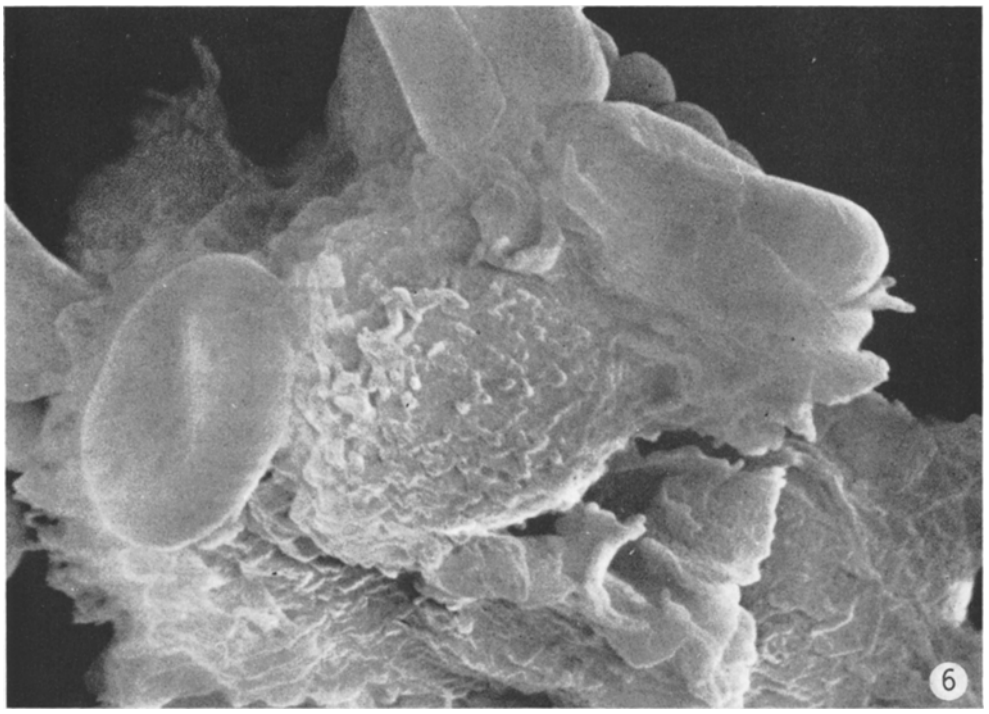


Fig. 6. Marked phagocytic activity of culture cells by SEM. Cytoplasmic projections of a mononuclear cell enveloping an erythrocyte completely. The erythrocyte shows a circular shape initially and is later completely embedded in the cell membrane of the mononuclear cell. ×7,500

became arranged in rosette formation on the cell surfaces of the cultured mononuclear cells.

Healthy human 0-group red cells coated with incomplete Rh (anti-D) antibodies formed rosettes in vitro by adhering tenaciously to mononuclear cells. Within two hours of their attachment, the cell membranes showed active flagellar projections, and these grew into pseudopodia of membrane-like structures which surrounded and later ingested erythrocytes (Fig. 6).

These findings clearly reflected the phagocytic activity of macrophages and suggested that these cultured cells were functionally hematopoietic reticulum cells. By electron microscopy well-developed smooth endoplasmic reticulum, Golgi apparatus, and secretory granules were present in the cytoplasm.

At 16 months after the initial cell collection, when the patient was in a remission, lymphocyte fractions of peripheral blood were cultured but a lymphoblastoid cell line was not successfully established.

At 321 days after the start of culture (77th generation), 77.4% of the cultured cells had a chromosome number of 2 n (Figs. 7 and 8). The aneuploid cells showed changes at 41, XY, C−(3X), D−(2X), G−, dic+; 45, XY, A−; 45, XY, F−, 45, XY, A−(2X), dic+; and 47, XY, A+. Neither chromosome mutations nor marker chromosomes were found.

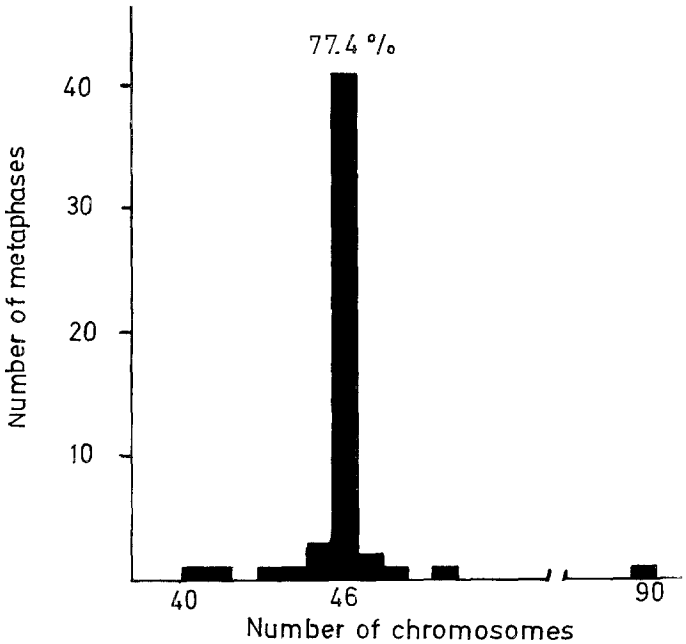


Fig. 7. The distribution of chromosome numbers of the cell line at the 70th population doubling level (321 culture days)

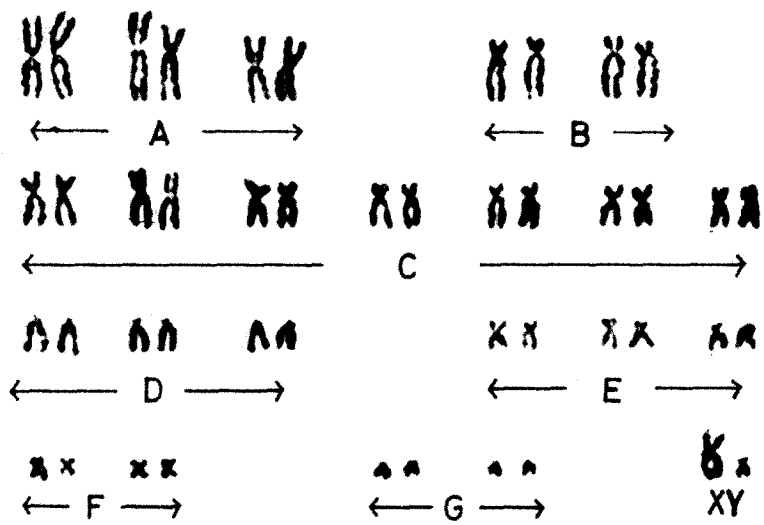


Fig. 8. Chromosome alignment of the culture cell line at the 70th population doubling level (321 culture days)

After incubation at 37° C in a 5% CO_2 gas chamber, a small amount of herpes type virus was observed in the nucleus and on cell membranes, developing to complete viral particles in some instances (Fig. 9).

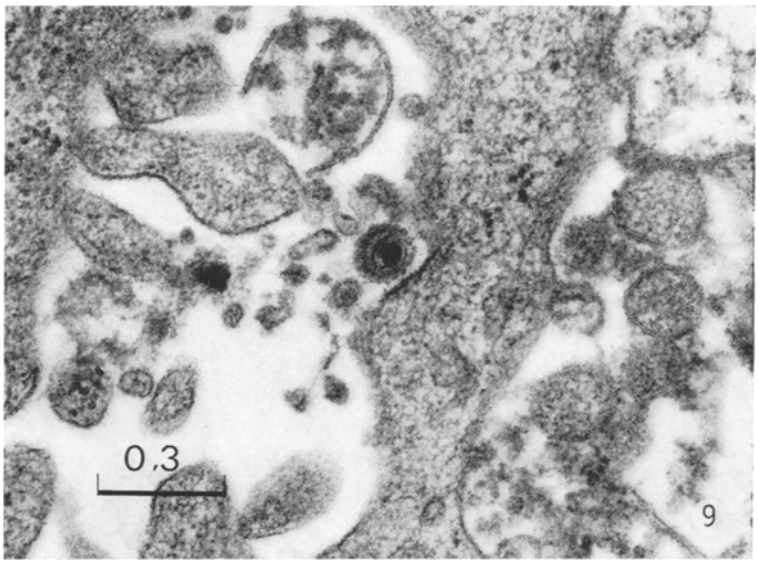


Fig. 9. Viral particles (herpes-like virus) on the cell membrane of lobulating leukemic cells

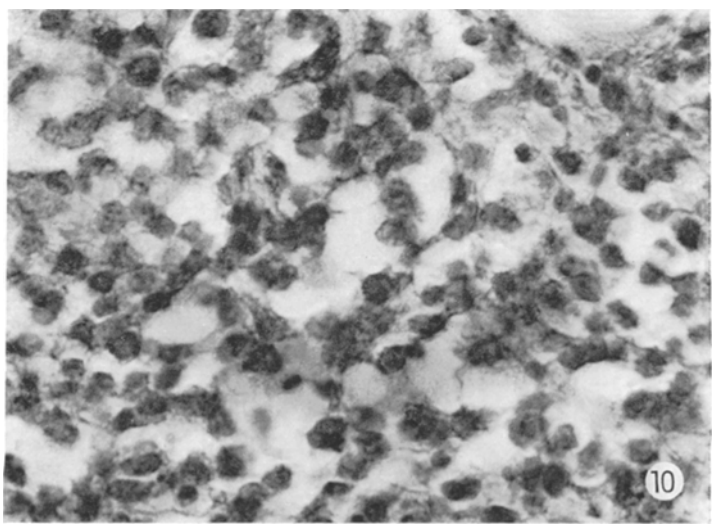


Fig. 10. Cultured tumor cells planted in the hamster cheek pouch. Reticulum cell sarcoma. H-E stain. 10×20

When the cultured cells were transplanted into the cheek pouch of adult hamsters previously conditioned by Decadron, a small tumor was formed within one week. The majority of these tumor cells were in a necrotic state. Some cells, however, began to infiltrate and proliferate the surrounding areas, and showed protruding cellular projections, presenting a histological picture of reticulum cell sarcoma (Fig. 10).

The immunodiffusion method was used for detection of immunoglobulin production by cultured cells. A small amount of IgG was detected but IgM was not detected in 30-fold concentrated spent medium.

Discussion

Iwakata and Grace (1964) reported the first long term culture of peripheral white blood cells of a myelogenic leukemia patient. Later, Moore (1970) and Minowada et al. (1972) established cell cultures of human lymphocytic leukemia cells (Molt-4) and normal human lymphocytes, as well as Burkitt lymphoma cells. Other reports on relatively long culturing of myelogenic leukemia cells include those of Paron et al. (1970), Pike and Robinson (1970), Iscove et al. (1971), Robinson et al. (1971), Moore and Metcalf (1973) and Aye et al. (1974). However, no investigation describes successful cultures of human myelomonocytic leukemic cells for over a year. The reports by Paran et al. (1970), Clarkson et al. (1974) and Moore et al. (1974) were on cultures of less than three months duration and no concise cytological or cytochemical data is available on cell lines established for extended periods.

Our cell cultures reached the 140th population doubling level in rather more than 1 year. They proliferated actively by forming clusters that resembled the normal proliferation of lymphoblastoid cells. The mononuclear cells had extensive cytoplasm, and with maturation showed unique flagella projections and marked phagocytic capacity. The presence of IgG receptors on these cells is an indication of immunological activity (Huber et al., 1969; Abramson et al., 1970). Once erythrocytes were attached to the cell surface, contact was quite firm. Even erythrocytes arranged as rosettes were ingested by active movements of the mononuclear cell surface membrane, indicating that substances coming in contact with the membrane were engulfed by the cell (Fig. 6). The histological appearance of these cultured cells was quite similar to malignant mononuclear cells infiltrating the bone marrow (Figs. 1 and 2). Transformation of granulocytes to macrophages or monocytes to reticulum cells and macrophages has been reported by Florey and Gowans (1962), Bennett (1966), Ichikawa et al. (1966) and Sutton and Weiss (1966).

In the present study, it has been possible to demonstrate, by kinetic observations of reticulum cells, that most macrophages in peripheral blood might be transformed from mononuclear cells derived from hematopoietic tissues. Mononuclear cells derived from bone marrow appeared to play a role similar to lymphocytes in responding to EB virus and other chemical agents. Mononuclear cells mixed with lymphoid cells might be exposed to and be target cells for various oncogenic viruses. The in vitro transformed cell lines might show reticulum cells as a lymphoblastoid cell.

Nilson and Pontén (1975) investigated the morphological and functional parameters of hematopoietic cells and showed that the lymphoblastoid cell lines derived from normal and neoplastic hematopoietic tissues were characterized by morphologic flexibility of individual lymphoblastoid cells. These lymphoblastoid cells were in constant association with EB virus, had polyclonal origins, were differentiated for immunoglobulin production, and were diploid. However, the free-floating myelomonocytic cells and myelogranulocytes resembled lymphoblast-

oid cells in a tendency to form clumps with the uropods pointing away from the center. These free-floating cells differed in phagocytic activity, showed a weak positive peroxidase reaction, specific secondary granules in the cytoplasm, and had elongated villous surfaces. They also showed a strongly positive esterase reaction. Such morphology characterized the Schilling monocytic leukemia cells (histiocytic type) but it is possible that if they were lymphoblastoid cells as reported by Nilsson and the others, some might be derived from mononuclear or reticulum cells as a subpopulation of hematopoietic cells.

Synthesis and secretion of immunoglobulins by this cell line has been investigated by immunodiffusion analysis. A small amount of IgG was detected in the medium but IgM was not found and it is possible that IgG may have been secreted from lymphoid cells in the culture. Monocytes and reticulum cells did not clone on the semi-solid agar cloning cultures.

Phl and C10 chromosome markers (Nowell and Hungerford, 1960) are not always seen in myelocytic leukemia and Burkitt lymphoma, but the present cultured cells seemed to differ from typical granulocytic leukemia cells and had no chromosome markers.

In the present study, growth of herpes virus was detected in the nucleus, cytoplasma and on the cell surfaces of the cultured mononuclear cells as reported in myelogranulocytic and lymphocytic leukemia (Dmochowski, 1965; Henle et al. 1968; Pope et al., 1969; Falk et al., 1974; Katsuki et al., 1975). The discovery of EB virus in myelomonocytic leukemia raises the possibility of viral oncogenesis in human myelomonocytic leukemia. In viral infection, mononuclear cells in hematopoietic tissues may be target cells, and the possibility of oncogene or malignant cell transformations cannot be ruled out. We are at present conducting studies on the induction of EB virus and examining the causal relationships between these viral particles and myelomonocytic leukemia.

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References

Abramson, N. A., Lo Buglio, A. F., Jandl, J. H., Cortan, R. S.: The interaction between human monocytes and red cells: Binding characteristics. J. exp. Med. **132**, 1191–1215 (1970)

Aye, M. T., Niho, Y., Till, J. E., McCulloch, E. A.: Studies of leukemic cell populations in culture. Blood **44**, 205–219 (1974)

Bennet, J. M.: Myelomonocytic leukemia: A historical review and perspectives. Cancer (Philad.) **27**, 1218–1220 (1971)

Bøyum, A.: Separation of leukocytes from blood and bone marrow. Scand. J. clin. Lab. Invest. **21**, Suppl. 97 (1968)

Clarkson, B., Strife, A., De Harven, E.: Continuous culture of new cell lines (SK-LITO 7) from patients with acute leukemia. Cancer (Philad.) **20**, 926–947 (1967)

Dmochowski, L.: Electron microscope studies in leukemia in animals and man. In: F. G. J. Hayhoe (ed.), Current research in leukemia. London: Cambridge Univ. Press 1965

Epstein, M. A., Barr, Y. M.: Cultivation in vitro of human lymphoblasts from Burkitt's malignant lymphoma. Lancet **1964** I, 252–253

Falk, L. A., Nigida, S. M., Deinherdt, F., Wolfe, L. G., Cooper, R. W., Hernandez-Camacho, J. I.: Herpesvirus ateles: Properties of an oncogenic herpesvirus isolated from circulating lymphocytes of spider monkeys (ateles SP). Int. J. Cancer **14**, 473–482 (1974)

Florey, H. W., Gowans, J. L.: The reticulo-endothelial system. In: H. W. Florey (ed.), General pathology, p. 178. Philadelphia: W. B. Saunders Company 1962

Henle, G., Henle, W., Diehl, V.: Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. Proc. nat. Acad. Sci. (Wash.) **59**, 94–101 (1968)

Huber, H., Douglas, S. D., Fudenberg, H. H.: The IgG receptor: An immunological marker for the characterization of mononuclear cells. Immunology **17**, 7–21 (1969)

Ichikawa,Y., Pluznic, D. H., Sachs, L.: In vitro control of the development of macrophage and granulocyte colonies. Proc. nat. Acad. Sci. (Wash.) **56**, 488–495 (1966)

Isocove, N. N., Seum, J. S., Till, J. E., McCulloch, E. A.: Colony formation by normal and leukemic human marrow cells in culture: Effect of conditioned medium from human leukocytes. Blood **37**, 1–5 (1971)

Itoh, U., Minowada, J., Moore, G. E., Pressman, D.: Rosette-forming human lymphoid cell line (T-cell line MOLT). II. Ability for clonal growth. J. nat. Cancer Inst. **52**, 1403–1407 (1974)

Iwakata, S., Grace, J. T.: Cultivation in vitro of myeloblasts from human leukemia. N.Y. St. J. Med. **64**, 2279–2282 (1946)

Katsuki, K., Hinuma, Y.: Characteristics of cell lines derived from human leukocytes transformed by different strains of Epstein-Barr virus. Int. J. Cancer **15**, 203–210 (1975)

Kimoto, T., Yokomura, E., Shimizu, Y., Yamakawa, M., Seno, S.: Malignant transformation of human cell line in vitro by the SV40 DNA and related alteration in biological activity of cell membranes. Acta med. (Okayama) **25**, 77–86 (1971)

Minowada, J., Ohnuma, J., Moore, G. E.: Rosette-forming human lymphoid cell lines. I. Establishment and evidence for origin of thymus derived lymphocytes. J. nat. Cancer Inst. **49**, 891–895 (1972)

Moore, G. E.: The culture of human lymphocytoid cell lines. In: H. Busch (ed.), Method in cancer research, Vol. V, pp. 423–453. New York, London: Academic Press 1970

Moore, M. A. S., Williams, N., Metcalf, D.: In vitro colony formation by normal and leukemic human hematopoietic cell: Characterization of the colony-forming cells. J. nat. Cancer Inst. **50**, 603–623 (1973)

Nilsson, K., Pontén, J.: Classification and biological nature of established human hematopoietic cell lines. Int. J. Cancer **15**, 321–341 (1975)

Nowell, P. C., Hungerford, D. A.: Chromosome studies on normal and leukemic human leukocytes. J. nat. Cancer Inst. **25**, 85–109 (1960)

Paran, M., Sachs, L., Barak, Y., Resnitzky, P.: In vitro induction of granulocytes differentiation in hematopoietic cells from leukemic and non-leukemic patients. Proc. nat. Acad. Sci. (Wash.) **67**, 1542–1549 (1970)

Pike, B. L., Robinson, W. A.: Human bone marrow colony growth in agar-gel. J. Cell Physiol. **76**, 77–84 (1970)

Pope, J. H., Horne, M. K., Scott, W.: Transformation of foetal human leukocytes in vitro by filtrates of human leukaemic cell line containing herpes-like virus. Int. J. Cancer **3**, 857–866 (1968)

Robinson, W. A., Kurnick, J. E., Pike, B. L.: Colony growth of human leukemic peripheral blood cells in vitro. Blood **38**, 500–508 (1971)

Rosenszajn, L., Leibovich, M., Shoham, D., Epstein, J.: The esterase activity in megaloblasts, leukemic and normal haemopoietic cells. Brit. J. Haemat. **14**, 605–610 (1968)

Sutton, J. S., Weiss, L.: Transformation of monocytes in tissue culture into macrophages, epitheloid cells and multinucleated giant cells. J. Cell Biol. **28**, 303–332 (1966)

Ueki, A., Itaguchi, Y., Hyodoh, F., Kimoto, T.: The receptor sites for complement (C_3) on human diploid fibroblasts. Virchows Arch. Abt. B **18**, 101–107 (1975)

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