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Original Investigations

Differentiation to Myeloid Cells of Lymphoblastoid Cells Established from Myelomonocytic Leukemia

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Summary. It has been previously reported that a lymphoblastoid cell line (Mono-1) established from the peripheral blood of a patient with acute myelomonocytic leukemia (AMML) consisted of reticulum cells, possessing properties that were more characteristic of monocytes and macrophages than those which are traditionally attributed to lymphocytes. These cells (Mono-1-207) exhibit myeloid cell properties when cultured in arginine-deficient medium and after treatment with DNA synthetic inhibitors. A comparison has been made with lymphoblastoid cell lines derived from other types of disease.

During culture in arginine-deficient condition, decreased DNA synthesis is accompanied by the appearance, at 48 h, of perinuclear pink cytoplasmic blushes; nuclear lobulation had developed by about the fifth day. At 12–14 days, the cytoplasmic granules developed from blushes could not be distinguished from azurophilic granules. Electron microscopy indicated that these granules were due to the development of lysosomes in which acid phosphatase was strongly present but which lacked any peroxidase activity. Mono-1-207 also has phagocytic activity. It is considered that the induction of differentiation of these cells in response to DNA synthesis by arginine-deficiency is related to possession of the characteristics described: they are hemopoietic precursor cells, and differentiate to myeloid cells.

Key words: Monocytic leukemia cells – lymphoblastoid cells – Arginine-starvation – Myeloid cell differentiation.

Introduction

Attempts to establish permanent cell-lines derived from the blood of patients with acute and chronic myelocytic leukemia have been consistently unsuccessful, and cultures showing myelocytic characteristics and the Philadelphia (Phl) chromosome have lost these characteristics after long-term cultivation. In all these

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cultures, the leukemic cells were replaced by immunoglobulin-producing lymphoid cells (Moore (1971), Nilsson, Pontén (1975), Sundström (1976), Klein (1977)). Recently, bone marrow and peripheral blood cells from patients with Phl chromosome were cultured and established a myelocytic leukemia cell line (C.B. Lozzio and B.B. Lozzio, 1975). In many cases of myelocytic leukemia, however, it is difficult to classify cells obtained from the peripheral blood of patients as belonging to the myeloid series because the cells have lost their azurophilic granules and peroxidase activity; consequently, such lymphoblastoid cells are regarded as a subpopulation of lymphocytes. As yet, very little is known about the differentiation and functions of lymphoblastoid cells established from peripheral blood cells.

The authors have previously reported that a lymphoblastoid cell line (Mono-1) established from the peripheral blood of a patient with myelomonocytic leukemia (MML) consisted of reticulum cells possessing properties that were more characteristic of monocytes or macrophages than those that are traditionally attributed to lymphoid cells. Because these cells exhibit myeloid cell properties when cultured in arginine deficient medium, the present paper presents a comparison made with lymphoblastoid cell-lines derived from other types of disease.

Materials and Methods

Cell Source of Human Myelomonocytic Leukemia Cell

Mono-1 Cell Line. The peripheral white blood cells of a 33 year old male patient with a AMML reported in the previous investigation was cultured over a period of 1480 days. The cell suspension (1×10^7/ml) was maintained in 60 mm dishes containing 5 ml RPMI 1640 medium supplemented with 20% inactivated fetal calf serum (FCS) in an atmosphere of 5% CO_2. The culture cells were supplied with 2 ml of fresh medium (V/V) every two days. After 1 week of culture the cells were subcultured by division of the cell into two groups of approximately equal numbers.

Mono-1-207 Cell Line. Arginine was removed from the RPMI 1640 medium and Mono-1-207 cells were cultured in this arginine deficient medium supplemented with 20% non-dialysed FCS. The medium contained a small amount of arginine (0.3 μmol/dl). The cells (1×10^6/ml) were observed for two weeks and the medium changed, or fresh medium added, every two days to prevent cell damage.

Human Lymphoid Cell Lines. Six cell lines have been established in our laboratory as shown in Table 1. They were compared with Mono-1-207 cell line. The suspension cells of EB-3, Raji and Jijoy Burkitt lymphoma cells were purchased from Flow Co., USA. Molt-4 cells were kindly provided by Dr. Minowada, Roswell Park Memorial Inst., USA and P_3-HRI cells were provided by Dr K. Kumagai, Tohoku Univ. Sendai, Japan.

Cell Growth

Cell proliferation of the Mono-1-207 cell line was studied using dialysed 20% FCS medium with arginine present in concentrations of 0 μg/ml, 20 μg/ml, and 25 μg/ml, supplemented with dialysed 20% FCS. The cell numbers, vitality, and degree of nuclear lobulation (resulting in metamyelocyte, stab and segmented forms) were estimated at two day intervals up to 12 days.

Table 1. Incidence of pink perinuclear blushes and cytoplasmic granules in established lymphoblastoid cells cultured with arginine deficient nutrition

Established cell lines	Cell origin (P) Peripheral blood (B) Bone marrow	Cell type	Pink cytoplasmic blushes	cytoplasmic granules
Mono-1-207	Acute myelomonocytic leukemia (P)	Reticulum	++	++
K-3	Hodgkin's disease (P)	Reticulum	+	−
K-5	Acute myelocytic leukemia (P)	Reticulum	+	−
KT-6	Acute lymphocytic leukemia (P)	Lymphoid, Reticulum	+	−
KS-7	Acute Myelocytic leukemia (P)	Reticulum	+	− ~ +
KS-9	Acute Myelocytic leukemia			
	(P)	Reticulum	+	− ~ +
	(B)	Reticulum	+	− ~ +
E·B#3	Burkitt lymphoma	Lymphoblastoid	−	−
P_3-HRI	Burkitt lymphoma	Lymphoblastoid	−	−
Raji	Burkitt lymphoma	Lymphoblastoid	−	−
Jijoy	Burkitt lymphoma	Lymphoblastoid	−	−
MOLT-4	Acute lymphocytic Leukemia (P)	Lymphoid	−	−

Comparative Analysis of Amino Acids in Culture Medium

The culture media (arginine deficient medium containing non-dialysed FCS, and the control complete medium) were subjected to amino acid analysis after 2, 4 and 10 days of culture. When a change of medium was not performed, the amino acid content fluctuated. Microscopic studies of cell differentiation were performed using Giemsa and May-Grünwald Giemsa staining techniques.

Cytochemical and Electron Microscopic Observation

Peroxidase Activity. Cultured cells were deposited on a glass slide by centrifugation. A benzidine reaction was performed by standard procedures.

Non-Specific Esterase Activity. A cell smear was prepared as above and fixed with glutaraldehyde as described below (acid phosphatase activity). The Burstone method, using α-naphthol acetate as the substrate, was used to introduce glycerine to the preparation. Non-specific esterase activity was then estimated.

Lysoplate Assay for Lysozyme. The lysoplate method of Osserman and Lowlor (1966) was used for assay of lysozyme activity in Mono-1 cells.

Acid Phosphatase Activity. The culture cells were concentrated in a cell pellet by centrifugation and the medium removed. Fixation was performed at 4° C for 1 h using 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.3) at a final concentration of 0.05 M sucrose. After overnight washing

with 0.1 M cacodylate buffer, the pellet was treated with Gomori reaction fluid for 20 min at 37° C, washed with 0.1 M cacodylate buffer, then fixed with 1% OsO_4 in 0.1 M cacodylate buffer for 1 h at 4° C. Next, washing with 0.1 M cacodylate buffer was followed by dehydration in an ethanol series, imbedded in Epon 812 and sectioned. The sections thus obtained were then either used unstained or stained with (a) uranylacetate or (b) uranylacetate and lead citrate. They were then examined with a HV-12A type (Hitachi) electron microscope.

General Electron Microscope Method

In a similar way to that described above for acid phosphatase study, the cell pellet was fixed with 2% glutaraldehyde in cacodylate buffer (pH 7.3) for 2 h at 4° C. This was followed, in order, by fixation with 1% OsO_4 in 0.1 M phosphate buffer for 1 h, at 4° C, washing with 0.1 M phosphate buffer, dehydration in an ethanol series, imbedding in Epon 812, and sectioning with a Porter-Blum MT-213 type microtome. The section was then double stained with uranylacetate and lead citrate.

Identification of Cell Surface Markers

C_3 Receptor and FC Receptor. Agglutination and rosette-forming tests were performed by a procedure similar to that used in the identification of T and B-cells and monocytes described previously (Ueki, 1975).

Detection of Immunoglobulines. Rabbit antisera against human IgG, IgM and IgA were purchased from Medical and Biological Co., Nagoya, Japan. Direct immunofluorescence technics were used.

Phagocytic Activity. As described in a previous report, O-type human red blood cells were coated with anti-IgD and used to estimate phagocytic activity and the presence of FC receptor with the scanning electron microscope. Phagocytic activity for latex 0.81 μ was seen. The cells (10^6/ml) were suspended in 1 ml RPMI 1640 using Leighton tubes, latex (2% added), and the preparation incubated for 1, 4, 6, and 24 h at 37° C. Giemsa staining and EM were then carried out.

EBNA

Human serum positive for P_3HRI cells were studied by indirect immunofluorescence method using Nilsson, Klein, and Henle's (1971) method and anti-human $C_3(\beta_1C/\beta_1A)$ rabbit globulin conjugated fluorescence.

DNA and RNA Synthesis of Mono-1-207 Cultivated with Arginine Deficient Medium

FCS in both non-dialysed and dialysed forms was compared. Results were similar for both forms. The cells (10^6/ml) were cultured as described previously. After 2, 4, 8, and 12 days, 1 μCi/ml of ^{3}H-thymidine and ^{3}H-uridine (Radiochemical center, Amersham, England) were added and the culture incubated for 1 h at 37° C. Thereafter, washing with cold PBS was performed three times, the cells were then left for 30 min with cold 5% TCA. They were pipetted

Table 2. Incidence of cytoplasmic blushes, granules and nuclear lobulation in Mono-1-207 cells by agents

	Agents	Cytoplasmic pink blushes	Cytoplasmic granules	Lobulation of nucleus
DNA synthetic inhibitors	5-Fluorodeoxyuridine			
	0.8 μM/ml	++	++	±
	1.0 μM/ml	++	+	−
	10.0 μM/ml	++	−	−
	5-Iododeoxyuridine			
	1–10 μg/ml	++	−	−
	8-Azaguanine			
	1–10 μg/ml	++	−	−
	Hydroxyurea			
	1.0 μg/ml	++	++	−
	10.0 μg/ml	++	++	−
	10.0 mg/ml	++	±	−
RNA synthetic inhibitors	Actinomycin D			
	0.002 μg/ml	−	−	−
	1.0 μg/ml	Karyorrhexis		
Protein synthetic inhibitors	Cyclohexamide			
	0.001 μg/ml	−	−	−
	1.0 μg/ml	Vacuolation		
Hormones	Dexamethasone			
	10^{-7} M/ml	−	−	++
	10^{-6} M/ml	−	−	++
	Insulin			
	2.5 μg/ml	−	++	−
	5.0 μg/ml	−	++	−
Miscellaneous agents	Dimethyl Sulfoxide			
	0.1%	+	−	++
	1.0%	+	−	++
	2.0%	+	−	−

on to 25 mm glass filter paper (Whatman GF/c) and washed a further 3 times with 5% TCA.

After drying by filtration, the cells were placed in a vial, 5 ml of scintillator added, and the count taken with a scintillation counter (Parchard tricarb, Model-3385).

Incidence of Cytoplasmic Blushes and Granules in Mono-1-207 Cells by Agents

As shown in Table 2 Mono-1-207 cells were treated with various inhibitors of DNA, RNA and protein synthesis. Histological changes were observed and compared with changes in cells cultured in the arginine-deficient medium.

Autoradiography

After 0, 2, 4, and 5 days, the cells were incubated with 2.5 μCi/ml of ^{3}H-arginine for 2 h at 37° C. Next, they were washed 3 times with PBS, concentrated on a slide glass by centrifugation, fixed with methanol, dried, and placed on emulsion (SAKURA NR-M2). This was developed with condor X after exposure for 2 weeks in a 4° C dark box. The slides were fixed, stained with 2% Giemsa, and studied.

Karyotypic Analysis

The cells for chromosomal analysis were collected. The preparation was treated with 0.5% sodium citrate, fixed with ethanol, acetic acid (3:1), frame-dried, and stained with Giemsa. Fifty metaphase cells were studied.

Results

Characteristics of the Mono-1-207 Cell Line

Some of the characteristics of this cell-line have been reported previously. EBV was identified in a small number of cells in early stage of cultivation. EBNA

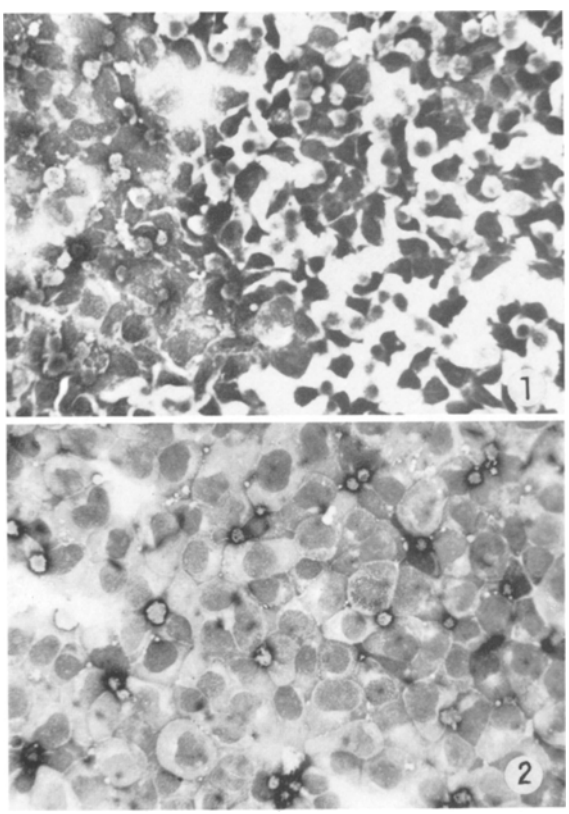


Fig. 1. Lymphoblastoid cells and reticulum cells (Mono-1). 1480 days ×100 Giemsa stain

Fig. 2. Lymphoblastoid cells (Mono-1-207). RPMI 1640+20% FCS 1480 days ×100 Giemsa stain

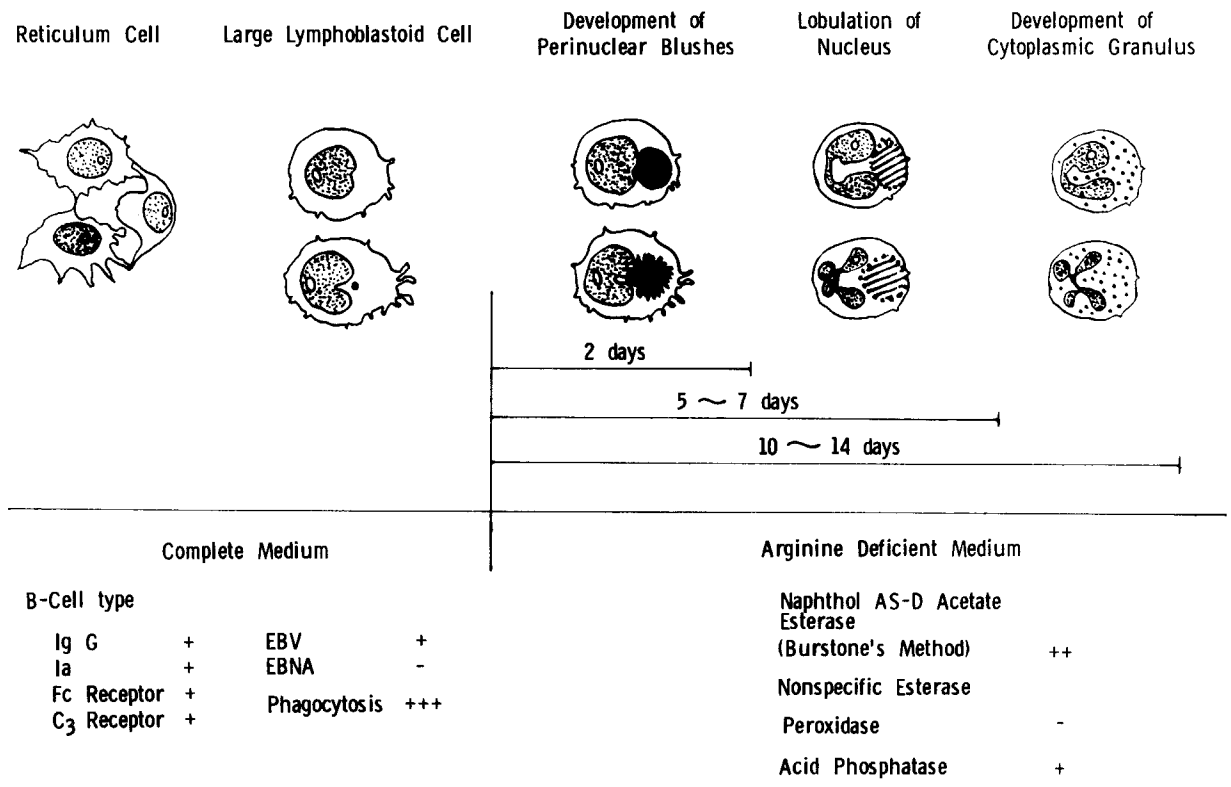
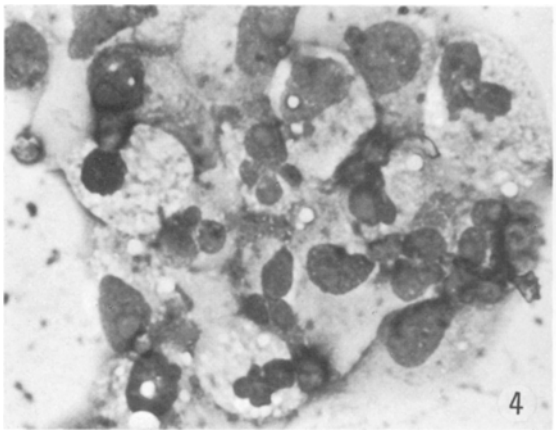
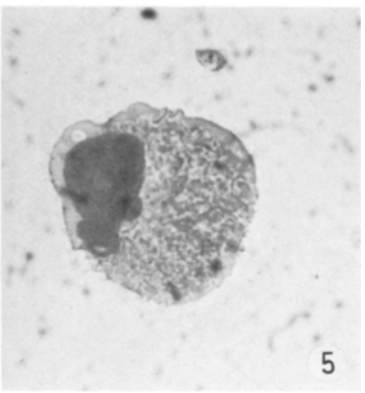
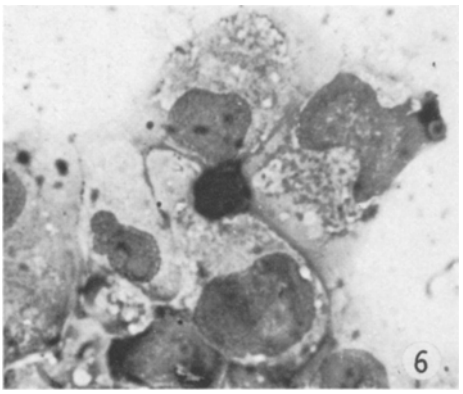


Fig. 3. Postulated sequence of development of cytoplasmic blushes and granules in Mono-1-207 cells by cultivation with arginine deficient RPMI 1640 medium







Figs. 4–7. Arginine-deficient cultivation of Mono-1-207

Fig. 4. Nuclear lobulation resembling granulocytes in Figure 8. 10 days in arginine-deficient medium. ×400 Giemsa stain

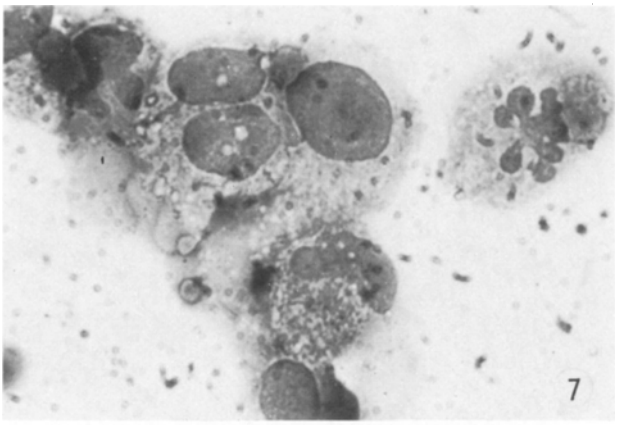
Fig. 5. Development of granules in the cytoplasma. 7 days in arginine-deficient medium. ×400 Giemsa stain

Fig. 6. Development of granules and lobulation. 10–12 days in arginine-deficient medium. ×400 Giemsa stain

could not be demonstrated. Nonspecific esterase, usually seen in monocytes, was present in almost all cells, but peroxidase activity although weakly positive early on, was lost over the ensuring days of culture. Acid phosphatase activity was marked in both the lysosomes and the Golgi apparatus. Lysozyme activity, however, was lost during culture.

Other features included the possession of receptors such as C_3, Fc and Ia (Dr. Greaves, personal communication) as cell surface markers consistent with the characteristics of monocytes or B-cells. A small quantity of surface antibody was present in the form of IgG, but neither IgM or IgA was identified.

At the present time, after 1480 days, the Mono-1-207 cells are in approximately the 225 doubling population and show hardly any morphological differences from the initial culture period. They possess round nuclei in which 1–2 small nucleolus are clearly visible. The cells are difficult to distinguish from B-cell lymphoblastoid cells but are thought to be different from lymphoid cells because of their lysosome enzyme activity, marked cellular projections from the cell surface, reticular structure, and marked development of mitochondria and lysosome (Figs. 9 and 10).



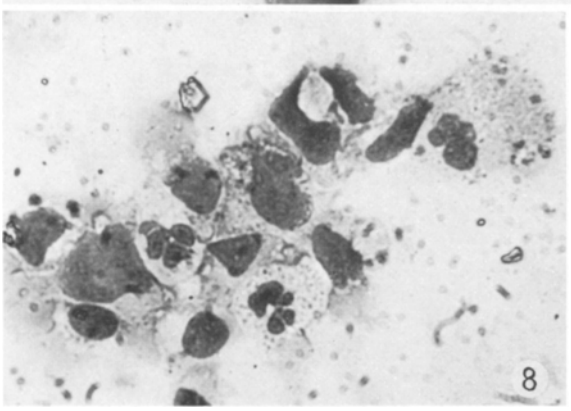


Fig. 7. Granulocyte like cells with cytoplasmic granules and lobulation. 12 days in arginine-deficient medium. ×400 Giemsa stain

Fig. 8. Granulocytes in a colony derived from AML (K-5-cell line) at 30 days of initial cultivation. The cells had peroxidase activity. ×200 Giemsa stain

Analysis of Chromosomes and Phagocytic Activity

At present after 1480 days of culture, a shift has occurred in the chromosome number from 46 to a hyperdiploid 49. No marker chromosome has been recognized. The cells are extremely active phagocytically and engulf not only colloid iron, formol-fixed erythrocytes but also latex particles. After less than four hours contact latex particles can be seen in phagocytic vesicles with the electron microscope.

Maturation to Myeloid Cells During Cultivation in Arginine-Deficient Medium

In RPMI 1640 complete medium (10%–20% FCS), immature Mono-1-207 cells, resemble reticulum or stem-type cells morphologically. They gradually become rounder and larger, and form lymphoblastoid cell colonies (Fig. 1). Repeated growth at this stage of differentiation makes it difficult to identify them, apart

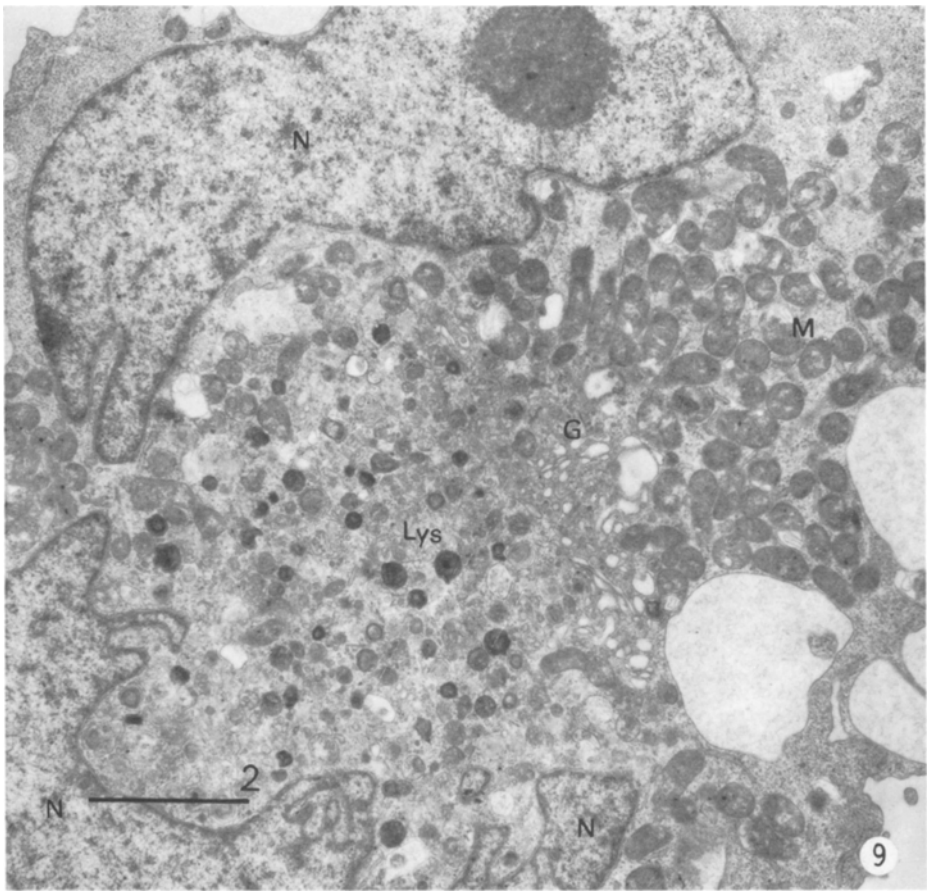


Fig. 9. Development of lysosomal granules and mitochondria in the cytoplasma of nuclear lobulated Mono-1-207 cell. *N* Nucleus, *M* Mitochondria, *G* Golgi, *Lys* Lysosome. 12 days in arginine-deficient medium

from definite lymphoblastoid cells (Fig. 2). During culture in arginine-deficient conditions, however, decreased DNA synthesis is accompanied by the appearance, at 48 h, of star-like, perinuclear pink cytoplasmic blushes close to the nuclear membrane (Fig. 3). Nuclear lobulation had developed by the fifth day (Fig. 4). At 12–14 days, the granules developed from blushes in the cell cytoplasm could not be distinguished from azurophilic granules which formed during cell maturation to granulocytes (Figs. 4–8). Electron microscopy indicated that these granules were due to the development of lysosomes in which acid phosphatase was strongly present (Figs. 9 and 10) but which lacked any peroxidase activity.

In both the arginine-deficient medium and complete medium supplemented with the 20% non-dialysed FCS, minute amounts of arginine were identified but were lost from the arginine-deficient medium within approximately three days of culture. By five days of culture, arginine was also lost from the complete medium.

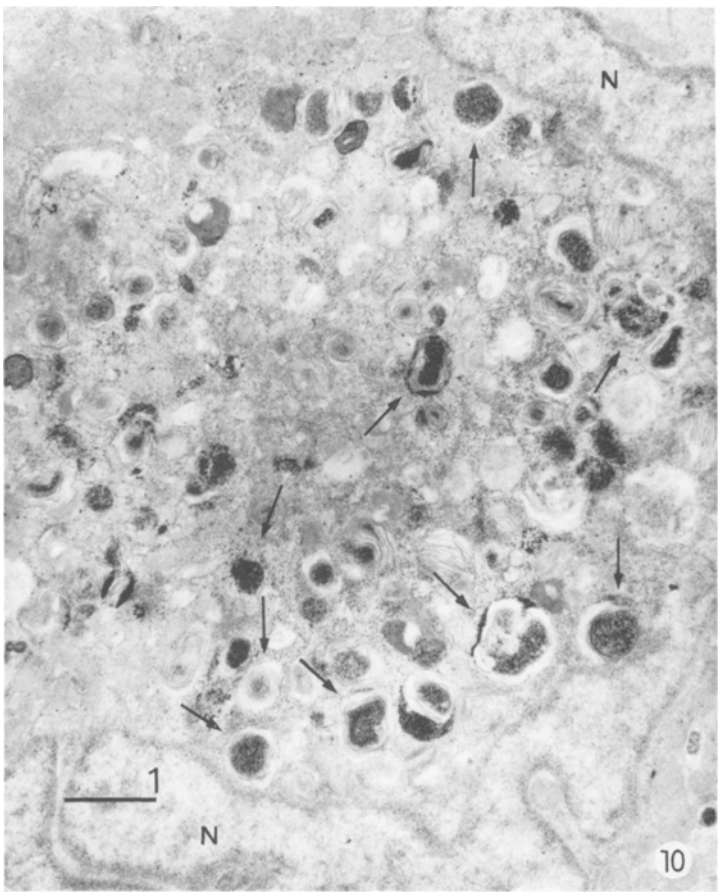


Fig. 10. Acid phosphatase activity is strong present in lysosomes (arrows). *N* Nucleus

In order to minimize any metabolic disturbances due to this fresh medium was added to, or substituted for, the culture medium every two days. Even the cells cultered in arginine-deficient conditions showed marked incorporation of ^{3}H-arginine (estimated by autoradiography) under these conditions.

Cell-Growth of Mono-1-207 in Arginine-Deficient Medium

As shown in Figure 11, when arginine-deficient RPMI 1640 supplemented with 20% non-dialysed FCS was used, the decrease in cell multiplication was less than when dialysed FCS containing a small amount of arginine was used. All proliferation had ceased by the tenth day. On the other hand, arginine in 3, 5, and 10 times the concentration in RPMI 1640 medium caused inhibition of all growth. The cell vitality decreased in line with decrease in all proliferative activity but observation of individual cells failed to show any change in their vitality; that is, there were no dead cells.

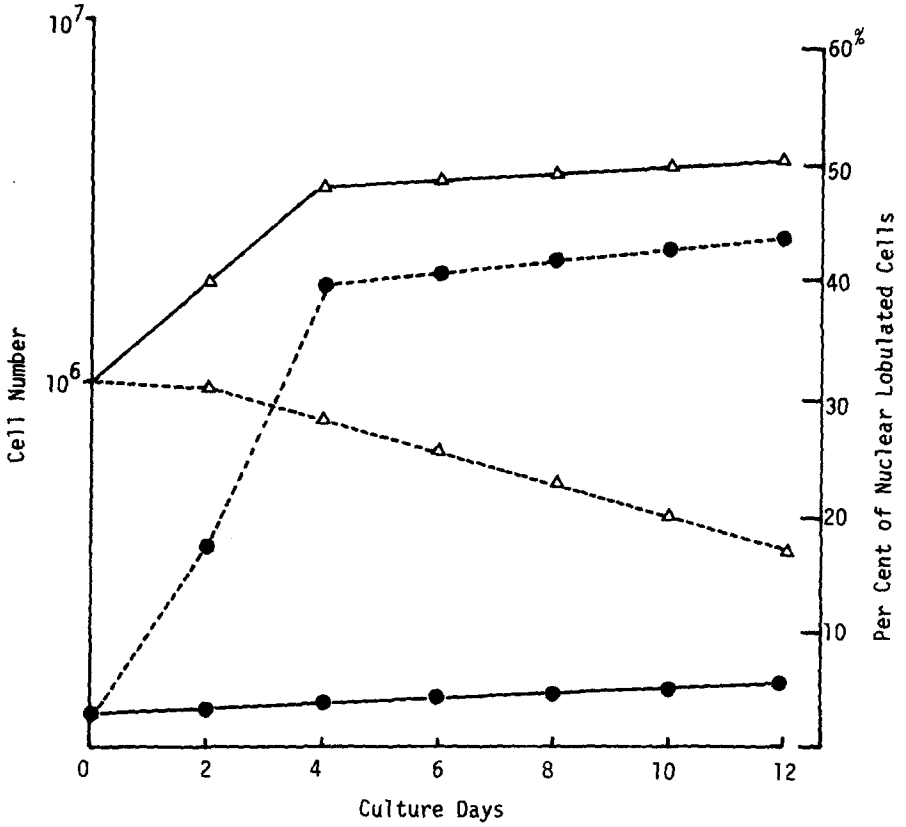


Fig. 11. Cell growth and nuclear lobulation of Mono-1-207 cell cultivated with arginine-deficient medium (20% non-dialysed FCS). Abscissa: △—△ control cell growth cultivated with complete medium △----△ Cell growth cultivated with arginine deficient medium ●—● Nuclear lobulation. Complete medium ●----● Nuclear lobulation. Arginine deficient medium

DNA, RNA Synthesis of Mono-1-207 in Arginine-Deficient Medium (20% Non-Dialysed FCS)

DNA synthesis during culture in arginine-deficient medium decreased gradually from the fourth day. RNA synthesis, however, did not show any difference from that of the control cells. Various metabolic agents, capable of affecting DNA synthesis (5-FuDr, 5-IdUR, 8-Azaguanine, Hydroxyurea), RNA synthesis (Actinomycin D), and protein synthesis (Cyclohexamide) were investigated. By 48 h, marked pink perinuclear blushes similar to those seen with DNA synthetic inhibition in arginine-deficient medium were evident. With the concentrations shown in Table 2, however, neither nuclear lobulation nor cytoplasmic granules was apparent microscopically.

The RNA synthetic inhibitor, Actinomycin D, caused marked degeneration of the cells with karyorrhexis and no cytoplasmic blushes clearly evident after 48 h. The protein synthetic inhibitor, cyclohexamide, also induced vacuolation

within the cells, but no blushes developed nor did the cells proceed to differentiate to granulocytes.

Treatment with dimethyl sulfoxide (DMSO) resulted in cytoplasmic blushes and nuclear lobulation, but development of granules did not occur.

Comparison with Lymphoblastoid Cells which Were Isolated from Various Diseases and Cultured in Arginine-Deficient Medium

As Table 1 shows, the Mono-1-207 cell line was compared with other cell lines established in our department. These other lines had been derived from leukemia and malignant lymphoma patients primarily, but also included Molt-4 cells, Burkitt lymphoma lymphoid cells, and lymphoblastoid cell lines established from bone marrow or peripheral blood.

The lymphoblastoid cell lines established from acute myelocytic leukemia, in particular, tended to show perinuclear cytoplasmic blushes by 48 h. These were not as marked as these of Mono-1-207, however, and the cells showed almost no differentiation. The other cell lines studied did not develop cytoplasmic blushes or granules.

Electron Microscopy Findings

In the arginine-deficient medium, the cytoplasmic blushes which appeared at 48 h and the blushes which were caused by DNA synthetic inhibitors were identical. Marked lysosome development occurred in the perinuclear area, especially around the Golgi apparatus. Lysosome autophagy and lysosome fusion with mitochondria were present, and the numbers of lysosomes and mitochondria were strikingly increased (Fig. 9). The activity of the lysosome enzyme, acid phosphatase, was particularly high (Fig. 10).

The decrease in DNA synthesis and the appearance of lysosomes in much greater numbers than in the control were noteworthy findings in this cell-line.

Discussion

At present, many cell lines have been established from the peripheral blood of individuals with myelocytic leukemia and of healthy individuals, Many of these cell lines are thought of as lymphocyte subpopulations. Moreover, a possible causal relationship between EBV and lymphocyte leukemia has been clarified by the EBV and EBNA reactions.

However, prolonged culture of myeloid cells from myelocytic leukemia patients results in the loss of peroxidase and azurophilic granules, so that the cellular characteristics of a granulocyte are lost. All surface markers of these established cells have many points in common with lymphocytes, thus the two groups are difficult to differentiate morphologically, except by immunoglobulin production. At present, they are thought of as lymphoblastoid cells.

The literature indicates that culture of cell lines from patients with AMML and myelocytic leukemia results in the loss of their granulocyte characteristics if culture exceeds three months in length. Diploid cell differentiation of human lymphoblastoid cells is, however, poorly understood.

Paran (1970), Fibach (1972–1975), Sachs (1970–1975), Hayashi (1974), Ichikawa (1976), Honma and Hozumi (1977) studying granulocytes cultured over a long period, have suggested that they resemble mouse myelocytic leukemia cell lines. It must be remembered, however, that these cells are unique stem cells resembling lymphoid cells but which show differentiation into granulocyte cells or macrophages in response to agents such as conditioning factor, steroid hormone, DNA synthetic inhibitors and Actinomycin D. Our study has investigated the nature and interrelationships of these lymphoblastoid cells as stem cells, since human hemopoietic cells are diploid, can be established as cell-lines in vitro and do not show the changes of aging.

The cells considered to be lymphoblastoid cells in the present study developed nuclear lobulation, perinuclear cytoplasmic blushes, intracytoplasmic granules and showed inhibition of DNA synthesis when cultured in arginine-deficient medium. They were, therefore, seen to undergo differentiation to myeloid cells or to macrophages with fully developed lysosomes (Fig. 11).

The Mono-1-207 cells, as described above, possess the characteristics of B-cells. Morphologically, the immature cells are like reticulum or stem cells, being either fissured or star-like with small projections (Fig. 1). With culture and the development of cell colonies, their cytoplasm becomes larger and rounder, and they show an increase in their number of cytoplasmic projections. They are different from so-called lymphoid cells in both size and morphology. Moreover, all cells contained non-specific esterase and performed marked phagocytosis of latex.

In arginine-deficient conditions, nuclear lobulation occurred and granule formation was evident; it was then difficult to distinguish the cells from neutrophils, morphologically. The granules which developed were lysosomes containing acid phosphatase and esterase, but lacking peroxidase activity. Our observation of the development of granules following the appearance of cytoplasmic blushes agrees with the differentiation of myeloid cells to granulocytes reported by Williams (1972).

As shown in Table 1, we have established cell lines from various types of leukemia, Hodgkin's disease, and Burkitt lymphoma cells. In arginine-deficient medium, Mono-1-207 cells developed nuclear lobulation and cytoplasmic granules. Lymphoid cells definitely derived from lymphocytes, however, did not show any signs of differentiation to granulocyte-like cells.

Another interesting point (also mentioned by Sachs et al., 1974) was that in contrast with the beginning of differentiation following inhibition of DNA synthesis, inhibition of either RNA or protein synthesis caused metabolic disturbances, which culminated in marked degeneration, karyorrhexis, and vacuolation. Nuclear lobulation and granule formation were not seen. Hozumi et al. (1976), however, has reported the induction of differentiation to macrophages in mice granulocytes using a non-toxic 0.2 μg/ml dose of Actinomycin D. We propose to investigate this induction further using Mono-1-207 cells.

It was also interesting that the stem cells of erythroblasts from tumor-inducing Friends virus (Friend, 1971; Sugano et al., 1974), induced synthesis of hemoglobin and maturation to erythrocytes in response to DMSO. Mono-1-207 treated with the same concentration of DMSO, however, developed perinuclear cytoplasmic blushes after 48 h that were identical with those produced by inhibition of DNA synthesis. With 2% concentration, no induction of cell differentiation occurred, but it cannot be denied that the appearance of these blushes may be a sign of the onset of differentiation with 0.1–1% DMSO nuclear lobulation could be induced.

With EM observation, these star-like blushes were seen after 48 h (that is, an early stage) during culture in arginine-deficient medium, after inhibition of DNA synthesis, and with DMSO treatment. Lysosomes containing strong acid phosphatase activity were present in much greater numbers than in the control. Intra-cytoplasmically, autophagy and clustering of the increased numbers of mitochondria were evident. Taken in conjunction with the nuclear lobulation, these findings suggested that the cells were different from the lymphocyte series of cells (Figs. 4–8). The moderate inhibition of DNA synthesis with marked development of lysosomes in the cytoplasm suggested a causal relationship between these two. Moreover, development of lysosomes could be observed in mitotic cells with treatment of insulin.

By autoradiography continued arginine synthesis (40% cells) was observed in Mono-1-207 cells cultivated in arginine deficient medium as reported by Weissfeld (1977), so the role of arginine in the induction of differentiation of hemopoietic cells is very important.

In *summary* we consider that the induction of differentiation of Mono-1-207 cells in response to stimulation of DNA sythesis is related to possession of the following special characteristics: they are B-cells with differentiation blocked by gene repression, they are hemopoietic precursor cells, and represent myeloid cells.

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