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Establishment of a New Cell Line(MTT-95) Showing Basophilic Differentiation from the Bone Marrow of a Patient with Acute Myelogenous Leukemia (M7)[*]

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Abstract

A new myeloid cell line, MTT-95, was established from the bone marrow of a patient with acute myelogenous leukemia (AML, M7). MTT-95 cells differentiate into mature basophilic cells in culture medium with no chemical component or cytokine. Surface phenotypes were as follows: CD11b 79.3%, CD13 92.4%, CD33 99.8%, CD34 87.9%, CD41a 77.6% and HLA-DR 0.3%. MTT-95 cells were strongly positive for glycoprotein IIb/IIIa by immunohistochemical staining and revealed metachromatic granules. MTT-95 cells seem to possess characteristics of both megakaryocytes and basophils. These findings suggest that MTT-95 cells are basophil progenitors. MTT-95 cells might be useful in the study not only of the biological aspects of basophils, but also of the diversities of AML (M7).

KEYWORDS: myeloid cell line, acute myelogenous leukemia, basophil, megakaryocyte

Brief Note

Establishment of a New Cell Line (MTT-95) Showing Basophilic Differentiation from the Bone Marrow of a Patient with Acute Myelogenous Leukemia (M7)

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A new myeloid cell line, MTT-95, was established from the bone marrow of a patient with acute myelogenous leukemia (AML, M7). MTT-95 cells differentiate into mature basophilic cells in culture medium with no chemical component or cytokine. Surface phenotypes were as follows: CD11b 79.3%, CD13 92.4%, CD33 99.8%, CD34 87.9%, CD41a 77.6% and HLA-DR 0.3%. MTT-95 cells were strongly positive for glycoprotein IIb/IIIa by immunohistochemical staining and revealed metachromatic granules. MTT-95 cells seem to possess characteristics of both megakaryocytes and basophils. These findings suggest that MTT-95 cells are basophil progenitors. MTT-95 cells might be useful in the study not only of the biological aspects of basophils, but also of the diversities of AML (M7).

Key words: myeloid cell line, acute myelogenous leukemia, basophil, megakaryocyte

A cute myelogenous leukemia (AML) is known as a hematopoietic disease involving the cessation of differentiation of blastic cells in various stages of differentiation. Breton-Gorius et al. (1) classified M7 into two types. One is the pure type showing proliferation of megakaryoblasts only and another type is the mixed type showing proliferation of both megakaryoblasts and other myeloid lineages. A unique myeloid cell line, designated MTT-95, showing basophilic differentiation was newly established from the bone marrow of a patient with M7 (mixed type). A myeloid cell line (KU812), which is the first human basophil cell line, was established from a patient with blastic crisis of chronic myelogenous leukemia (2). It is already known that the human promyelocytic leukemia cell line, HL-60, differentiates into basophils in culture medium with various chemical components or cytokines (3, 4). In the present paper, some biological aspects of MTT-95 cell are described.

Materials and Methods

Patient profiles. The patient, a 52-year-old man, was referred to our hospital with a diagnosis of leukemia on December 13, 1995. The patient had suffered from fluminant hepatitis in 1994. Conjunctiva was anemic but not icteric. The liver was palpable 1.5 finger-breadths below the right costal margin. Examination of his blood showed a red blood cell count of $2.35 \times 10^6/\mu l$, hemoglobin of 7.2 g/dl, a platelet count of $2.9 \times 10^4/\mu l$ and a white blood cell count of $21.6 \times 10^3/\mu l$ with 27 % blasts and 0.5 % basophils. Bone marrow smears revealed 88.0 % blasts, 0.6 % promyelocytes, 0.4 % myelocytes, 1.6 % neutrophils, 2.0 % lymphocytes and 4.0 % erythroblasts, but no basophils. Blast cells were peroxidase negative, and some of them were positive for glycoprotein (GP) IIb/IIIa by immunohistochemical staining. Morphologically, some blast cells showed cytoplasmic blebs. The results of marker analysis of bone marrow cells were; CD2 79.9 %, CD7 43.2 %, CD33 53.2 %,

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CD34 48.6 % and CD41a 21.4 % by flow cytometry. The cells expressed both the marker for megakaryoblasts and the marker for myeloid. On the basis of hematological findings the patient was diagnosed as having 'leukemia' to 'M7' (mixed type). Histological examination of a bone marrow clot showed hypercellular marrow. From December 14, 1995, cytosine arabinoside (60 mg/day, iv drip) was administered for 7 days. On January 4, 1996, the bone marrow biopsy showed fibrous changes, and bone marrow biopsy stamps revealed 86.6 % blasts. From January 6, 1996, idarubicin was administered in combination with cytosine arabinoside. From February 19, 1996, cytosine arabinoside and mitoxantrone were administered, but this yielded no improvement. Cytogenetic analysis by Giemsa-banding showed 45, XY, add (3) (q27), del (4) (q21), − 5, add (10) (p15), add (12) (p13), add (17) (p11), add (18) (p11), add (19) (p13), i (21) (q10), + mar (in 20 cells examined). The patient died of pneumonia on March 9, 1996.

Cell culture method.   On December 13, 1995, a mononuclear cell fraction was obtained from the heparinized bone marrow aspirates by Ficoll-Conray fractionation (Pharmacia Biotech AB, Uppsala, Sweden) and cells $(3.0 \times 10^6$ cells/ml) were cultured in suspension in 35 mm petri dishes (Falcon, Lincoln Park, NJ, USA) containing RPMI 1640 medium (Gibco, Grand Island, NY, USA), 20 % fetal calf serum (ICN Biomedicals Japan Co., Osaka, Japan) and antibiotics at 37°C with 5 % CO_2 and 100 % humidity. Culture medium was changed twice weekly.

Karyotype and surface phenotype analyses.   Chromosome studies were performed by the G-banding technique. Surface phenotypes of MTT-95 cells were analyzed by a flow cytometer using fluoresceinisothiocyanate (FITC)-or phycoerythrin (PE)-labeled monoclonal antibodies (MoAbs). The MoAbs used were as follows: CD2, CD3, CD4, CD5, CD7, CD10, CD13, CD14, CD19, CD20, CD33, HLA-DR (MBL, Nagoya, Japan), Mo1, CD41a, Glycophorin A (Coulter Immunology, Hialeah, FL, USA) and CD34 (DAKO, Glostrup, Denmark). FITC-and PE-labeled mouse IgG and FITC-labeled mouse IgM (Coulter Immunology) were used as negative controls.

Morphology and Cytochemistry.   Smears were stained with May-Giemsa stain. Peroxidase, dual esterase, alkaline-phosphatase, periodic acid-Schiff (PAS), acid phosphatase, Sudan black B and alcian blue staining were also performed. The metachromatic nature

of the granules in basophils was confirmed using 0.1 % toluidine blue staining.

Detection of GP IIb/IIIa.   Immunohistochemical staining was performed using a streptoavidin-biotin- peroxidase complex technique on acetone-fixed MTT-95 cells using mouse anti-GP IIb/IIIa monoclonal antibody (DAKO).

Electron microscopy.   The cell pellets of the MTT-95 cells were doubly fixed in 3 % glutaraldehyde and 1 % osmium tetroxide, dehydrated in a graded series of ethanol and embedded in Epon 812 (Nacalai Tesque, Tokyo, Japan). Ultrathin sections were stained with uranyl acetate-lead citrate and examined under a JEM-100S electron microscope (JEOL Ltd., Tokyo, Japan). For the ultrastructural platelet peroxidase (PPO) method, the cell pellets were fixed in Tannic acid fixative, then washed and incubated in a PPO reaction mixture (5). The specimen was post-fixed in osmium tetroxide and embedded in Epon 812. Ultrathin sections without staining were also examined.

Results and Discussion

Five months after cultivation, cell growth became marked and stable. The cell line continues to reproduce itself 2 years after its initial establishment. The number of chromosomes, examined on the 314th cultivation day, was 46 (16/20 metaphases), and 47 (4/20 metaphases). The karyotype of MTT-95 cells was 46–47, XY, add (3) (q27), − 4, − 5, − 11, add (12) (p13), − 17, − 18, add (19) (p13), + 20, i (21) (q10), + mar. Hutt-Taylor et al. (3) reported that sodium butyrate induced basophilic differentiation of HL-60 cells. Denburg et al. (4) also described how IL-5 could foster basophilic differentiation in the HL-60 cells, as evidenced by the induction of metachromatic cells and histamine synthesis. The MTT-95 cells described in this paper showed differentiation into mature basophilic cells in culture medium with no chemical component or cytokine (Fig. 1-A). Cytochemical stainings of mature cells differentiated from MTT-95 revealed uniform negativity with the peroxidase and Sudan black B and positivity with PAS, alkaline-phosphatase, acid phosphatase and naphthol AS-D-chloroacetate esterase staining. MTT-95 cells were further investigated with toluidine blue (Fig. 1-B) and alcian blue stain. The granules were metachromatic with toluidine blue staining and stained blue with alcian blue staining. The frequencies of these cells were 10–40 %. By immunohistochemical

staining, MTT-95 cells were strongly positive for GP IIb/IIIa, which is an indicator of megakaryocyte-lineage (6) (Fig. 1-C). The megakaryocytes originating from

MTT-95 did not differentiate into platelets. MTT-95 cells seem to differentiate into basophils, which results in their showing the characteristics of both megakaryocytes and
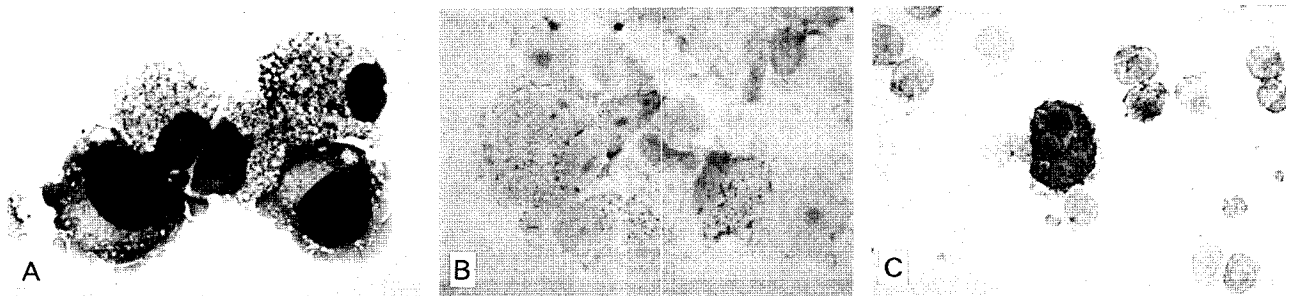

Fig. I   May-Giemsa (**A**), toluidine blue (**B**) and immunohistochemical staining (**C**) of the MTT-95 cell line. Basophils are visible in **A** and granules are metachromatic with toluidine blue staining (**B**). Cells positively stained with glycoprotein (GP) IIb/IIIa are visible in **C**.
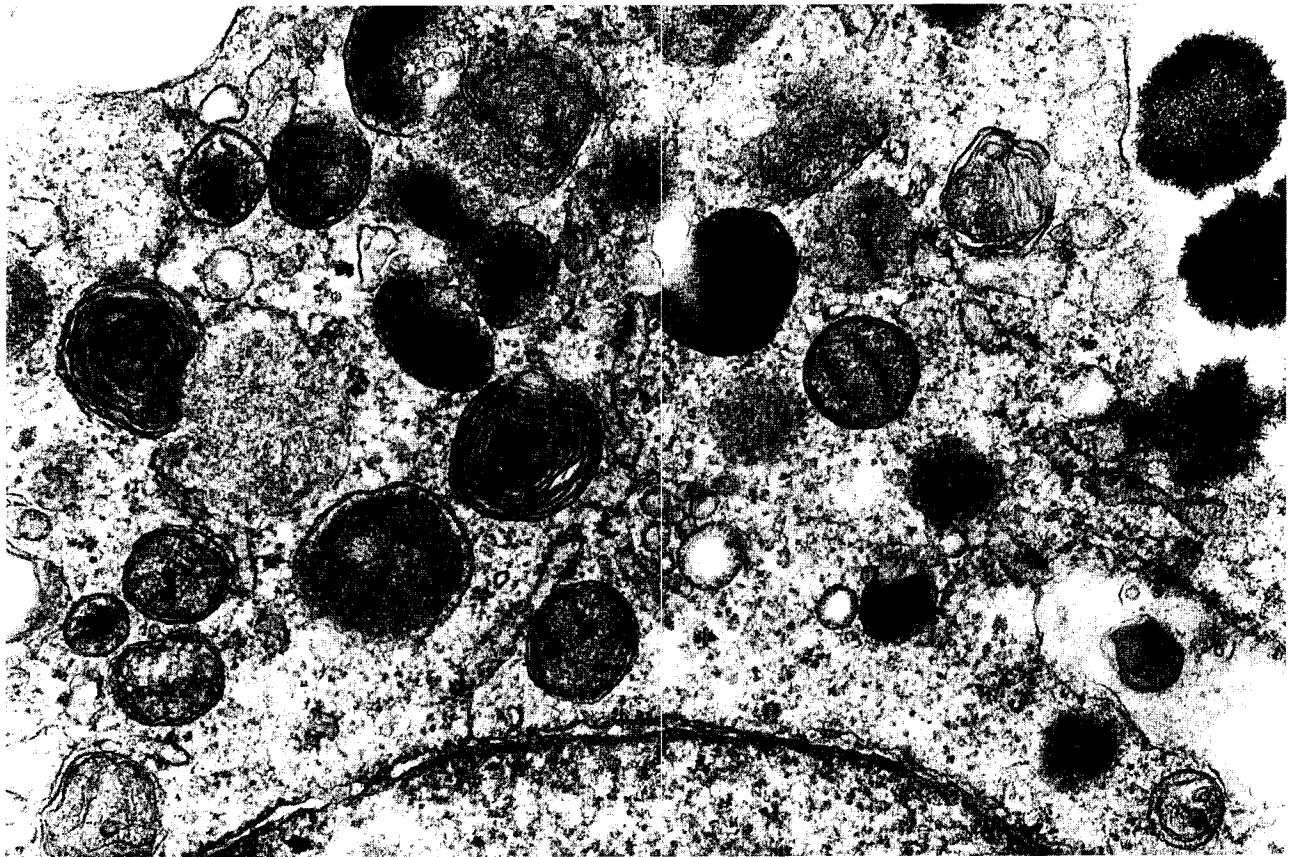

Fig. 2   Electron micrograph of MTT-95 cells. The granules exhibit reduplication of membranes and contain fine particles, occasionally with scroll-like or layer-like figures. × 20,000.

basophils. Ultrastructurally, the MTT-95 cells were negative for PPO. In the cytoplasm, a number of granules with reduplication of membranes were observed. The granules usually contained fine particles. Granules with scroll-like and layer-like figures, which are characteristics of tissue mast cells, were also observed (Fig. 2). Surface phenotypes of MTT-95 cells were as follows: CD11b 79.3 %, CD13 92.4 %, CD33 99.8 %, CD34 87.9 %, CD41a 77.6 % and HLA-DR 0.3 %. Fukuda *et al.* (7) demonstrated that leukemic basophil precursors expressed My7 (CD13), My9 (CD33), and OKM1 (CD11b), but no HLA-DR or Ia- like antigens, even in blastic configuration. The early myeloid antigens CD13 and CD33 were expressed on a majority of MTT-95 cells. These findings suggest that MTT-95 cells possess the properties of basophil progenitors. Differentiation may be occurring along two pathways, to basophil/mast cells and to megakaryocytes but not to biphenotypic cells. The situation of basophil/mast cells in the hematopoietic cell system is not well enough understood. MTT-95 cells might be useful in the study not only of the biological aspects of basophil/mast cells, but also of the diversities of AML (M7).

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