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BRIEF NOTE

ARGININE DEPRIVATION IN HUMAN LYMPHOBLASTOID CELLS: MATURATION TO MYELOID CELL

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In many cases of myelocytic leukemia it is difficult to classify cells derived from the peripheral blood of patients as belonging to the myeloid series because the cells have lost chromosome marker, their azurophilic granules and peroxidase activity; consequently, such cell lines are regarded as a subpopulation of lymphocytes[1-4]. Recently the cell line from patient were cultured and established a chronic myelogenous leukemic cell line with ph[1] chromosome from a pleura effusion of a terminal case in blastic crisis[5]. As yet, very little is known about the differentiation and function of the long-term culture of human lymphoblastoid cell lines established from myelogenous leukemia, except of mouse myelogenous leukemic cells[7-16].

The authors[6] have previously reported that a lymphoblastoid cell line (Mono-1) established from the peripheral blood of a patient with acute myelomonocytic leukemia (AMML) comprised reticulum cells possessing properties that were more characteristics of monocytes or macrophage than those that are traditionally ascribed to lymphocytes. 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.

The peripheral white blood cell of a 33-year-old male patient with AMML reported in the previous investigation was cultured over a period of 1500 days. The cell suspension was maintained in 60 mm dishes containing 5 ml RPMI 1640 medium (20% FCS) in a 5% CO_2. Since the characteristics of this cell line have been reported previously[6], EBV was identified in a small number of cells in early stage of cultivation. EBNA could not be demonstrated. α-Naphthyl acetate esterase was present in all cells, but peroxidase was lost over the ensuing days of culture. Other features included the possession of receptors such as C_3, Fc and Ia as cell surface markers consistent with the characteristic of monocytes and B-cells. The cells are difficult to distinguish from B-cell of lymphoblastoid cells but are, however, thought to be different from lymphoid cells because of their lysosomal enzyme activity, marked

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cellular projection and development of mitochondria and lysosomal granules as will be discussed here. At the present after 1500 days of culture, a shift has occurred in the chromosome number from 46 to a 49, (hyperdiploid). No marker chromosome has been recognized. In less than four hours, the latex particles can be seen in the phagocytic vesicles.

Arginine was removed from the RPMI 1640 medium and Mono-1 cells were cultured in this arginine deficient medium with 20% non-dialysed FCS and dialysed FCS. The medium with non-dialysed FCS contained a small amount of arginine by comparative analysis of amino acids in medium. The cells (1×10^6/ml) were observed for two weeks and the medium changed, or fresh medium added, every two days to obviated cell damage. This cell line was the surviving cells in arginine starvation condition by changing or adding of fresh arginine deficient medium (Mono-1-207).

During the culture in arginine-deficient conditions, the decrease in the DNA synthesis is accompanied by the appearance, at 48 hours, of star-like, perinuclear pink cytoplasmic blushes close to the nuclear membrane. Nuclear lobulation had developed by approximately the fifth day (45%). At 12-14 days, the granules developing from blushes in the cytoplasm could not be distinguished from azurophilic granules which formed throughout the maturation to granulocytes (Figs. 1 and 2). Electron microscopy indicated that these granules were due to a marked development of lysosomes in which acid phosphatase was strongly present (Fig. 3).

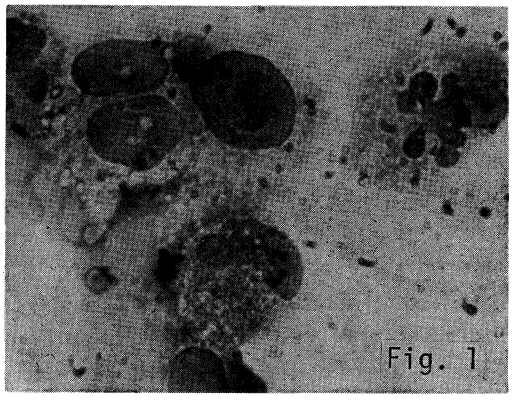


Fig. 1. Development of granules in the cytoplasma of Mono-1-207.
7 days of culture in arginine-deficient medium.
×400 Giemsa stain

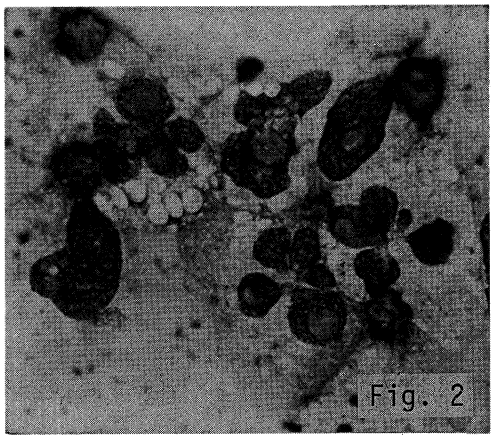


Fig. 2. Development of granules and nuclear lobulation.
10 days of culture in arginine-deficient medium.
×400 Giemsa stain

Even the cells cultured in arginine-deficient conditions showed a marked incorporation of ^{3}H-arginine by autoradiography under these conditions.

DNA synthesis (replicative nature) during culture in the arginine-deficient medium decreased gradually from approximately the 2nd day. RNA and protein synthesis, however, did not show any difference from that of the control cells in fully nourished culture (Fig. 4). Various metabolic agents, capable of affecting DNA synthesis (5-FuDR 1-10 μg/ml, 5-IdUR 1-10 μg/ml, 8-aza-guanine 1-10 μg/ml, hydroxyurea 10 mg/ml), RNA synthesis (actinomycin D 1 μg/ml), and protein synthesis (cyclohexamide 1 μg/ml) were investigated. By 48 hours, marked pink perinuclear blushes and a small amount of granules similar to those seen with DNA synthesis inhibition in arginine-deficient medium were evident. Nuclear lobulation, however, was not apparent by treatment with DNA synthetic inhibitors. Actinomycin D caused a marked degeneration of the cells with karyorrhexis and cyclohexamide, also induced vacuolation within the cells, but no blushes developed nor did the cells proceed to differentiate to granulocytes and macrophages.

Treatment with dimethyl sulfoxide[17] (DMSO 2.0-0.5%) and dexamethasone (0.1-1.0 μg/ml) resulted in cytoplasmic blushes and nuclear lobulation at the stage of metamyelocyte, but marked development of granules and maturation did not occur progressively.

The Mono-1-207 cell line was compared with other lymphoid cell lines (EB-3, Raji, P_3HRI-3, MOLT-4, B-cells derived from acute lymphocytic leuke-

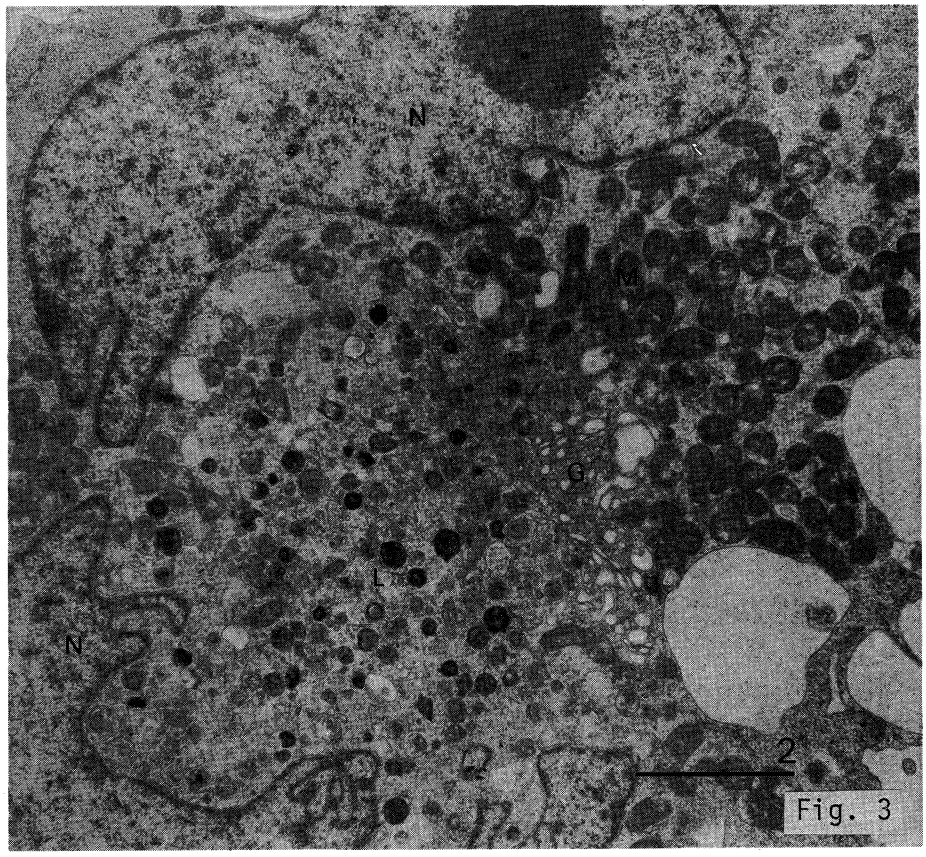


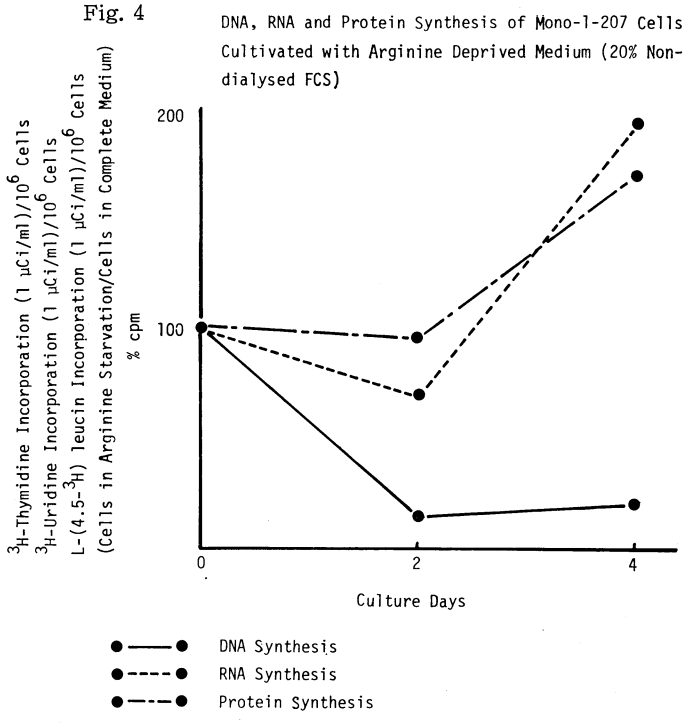
Fig. 3. Development of lysosomal granules and mitochondria in the cytoplasma of nuclear lobulated Mono-1-207.
12 days of culture in arginine-deficient medium.
N: Nucleus M: Mitochondria G: Golgi L: Lysosome

mia). These lymphoid cells were not so marked as these of Mono-1-207, however, and the cells did not develop cytoplasmic blushes or granules.

In the arginine-deficient medium, the cytoplasmic blushes which appeared at 48 hours and the blushes which were caused by DNA synthetic inhibitors were essentially identical and blushes and granules were consisted of lysosomes developing from Golgi apparatus.

The Mono-1-207 cells with treatment of insulin (0.5 μg/ml) showed marked lysosomal granules in many mitotic cells and it seems that blushes and granules did not develop as products following of degraduation of cells. Weissfeld *et al.* (1977)[18-20] also reported that arginine-deprived Chinese hamster

Fig. 4



ovary cells ceased all multiplication after 12 hours of arginine deprivation, but continued DNA synthesis. From the above it is considered that in the absence of exogenous arginine, the majority of the Mono-1-207 in an exponentially growing precursor cells (lymphoblastoid cells type) are arrested during the S-stage of the cell cycle. Further, when DNA synthesis is continued at a reduced rate, RNA and protein synthesis are not reduced after transfer to arginine deficient condition and precursor cells are able to initiate maturation to myeloid cells in response to moderate inhibition of replicative DNA synthesis.

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