An Epstein-Barr Virus-Negative B Lymphoma Cell Line (THP-2) from a Burkitt's Lymphoma of a Japanese Patient

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TSUCHIYA, S., YAMAGUCHI, Y., KOBAYASHI, Y., MINEGISHI, M., IMAIZUMI, M., SUZUKI, H., KONNO, T. and TADA, K. *An Epstein-Barr Virus-Negative B Lymphoma Cell Line (THP-2) from a Burkitt's Lymphoma of a Japanese Patient.* Tohoku J. exp. Med., 1983, 140 (4), 429-434 — An Epstein-Barr virus-negative lymphoma cell line, THP-2, was established from a Burkitt's lymphoma taken from a Japanese patient. THP-2 cells grew in single cell suspension with a doubling time of 24 hr; the cells carried surface-bound μ - λ immunoglobulins and formed rosettes with IgG antibody-coated ox erythrocytes. THP-2 cells expressed B1, common acute lymphoblastic leukemia (ALL) (J5) and Ia-like antigens of their surface as defined by monoclonal antibodies. Epstein-Barr virus-associated nuclear antigen (EBNA) was not detected. The cell line THP-2 has been maintained for over 5 years. Among the markers examined, only common ALL antigen has been present on the cell surface of THP-2 for these 5 years. ——— B cell line; Burkitt's lymphoma

Establishment of permanent cell lines derived from patients with leukemia or lymphoma has provided useful tools for studies of malignant hematopoietic cells. Such studies have brought important information concerning differentiation sequences of cells in lymphatic series, the origins of leukemia-lymphoma, or precise mechanisms of the differentiation of myeloid cells (Rovera et al. 1980; Minowada et al. 1981; Magrath 1981; Tsuchiya et al. 1982)

The present paper describes the establishment of a new EBNA negative Burkitt's lymphoma cell line (THP-2) with B cell properties and its marker profile.

MATERIALS AND METHODS

Case report

A ten-year-old boy who had had a large mass anterior to the sacrum for 5 months was admitted to the Tohoku University Hospital in July 1977. The diagnosis of Burkitt's lymphoma had already been made by biopsy findings of the tumor, showing infiltration of poorly differentiated malignant lymphoma cells with a typical "starry sky" pattern. On his admission the patient was severely ill and the peripheral blood picture

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Fig. 1. Wright-Giemsa stain of THP-2 cells.

revealed 18% of lymphoblasts indicating leukemic transformation of the malignant lymphoma. The patient died of sepsis and intestinal bleeding in August 1977 without any remission despite chemotherapy.

Cell cultures

Lymphoblasts from the peripheral blood isolated by Ficoll-Isopaque gradient centrifugation were distributed on a Microplate (Falcon, No. 3040, Oxnard, Calif.) at a density of 6×10^5 per 0.2 ml per well in RPMI-1640 medium containing 20% fetal calf serum and kanamycin. They were cultured at 37°C in an atmosphere of 5% CO₂ in air. The medium was changed twice a week. After being established, the cell line, THP-2, was maintained in 8-ml cultures in glass-stoppered plaque bottles.

Morphological studies

Coverslip smears of the cultured cells were stained with Wright-Giemsa solution for light microscopy. THP-2 was prepared for transmission electron microscopy (TEM) and scanning electron microscopy (SEM) as described previously (Tsuchiya et al. 1982).

TABLE 1. *Marker profiles of THP-2*

Period of in vitro culture	Surface immunoglobulin positive cells (%)					
	μ	δ	γ	α	κ	λ
0*	ND	ND	ND	ND	ND	ND
2 months	97.6	0	0	0	0	98.2
6 months	98.5	0	0	0	0	98.2
1 year	96.9	0	0	0	0	99.2
2 years	98.8	0	0	0	0	98.3
3 years	96.8	0	0	0	0	97.2
5 years	0	0	0	0	0	0

* Peripheral lymphocytes before cultivation.

Rosette formation with sheep erythrocyte (EN), with ox erythrocyte (EAC^{mo}). TdT, terminal deoxynucleotidyl transferase; ND, not de-

Characterization of cell-surface antigens

The Fc receptor for IgG (EAox), receptors for complements, C3b (human serum) and C3d (mouse serum) (EAC^{hu} and EAC^{mo}, respectively) and spontaneous rosette formation with sheep erythrocytes (EN) were examined as described previously (Tsuchiya et al. 1980). Cell-surface immunoglobulins and cytoplasmic immunoglobulins were detected by direct immunofluorescence. Common ALL (J5) antigen, Ia-like antigen, B1 antigen, and T3, T4, T6, T8 antigens were detected by indirect immunofluorescence with respective monoclonal antibodies (Reinherz and Schlossman 1980; Ritz et al. 1980; Stashenko et al. 1980). Monoclonal antibodies used were purchased from Coulter Corporation (Hialeah, Fla.) and Ortho Pharmaceutical Corporation (Raritan, NJ). Terminal deoxynucleotidyl transferase (TdT) was determined by indirect immunofluorescence using a terminal transferase assay kit (Bethesda Research Lab., Inc., Gaithersburg, MD). Detection of EBNA was done by the method of Reedman and Klein (1973).

Plating efficiency

Colony formation in semi-solid agar (SeaPlaque agarose, FMC Corpor., Rockland, MD) without feeder cells was performed as described previously (Tsuchiya et al. 1980).

RESULTS

Establishment of the cell line (THP-2) and morphological features

Forty-two days after the start of in vitro culture, the proliferating cells were transferred to 8-ml cultures in stopped plaque bottles. The cells grew in single cell suspension, occasionally forming loose clumps of less than 10 cells, with a doubling time of 24 hr. The cells appeared uniform and round in shape, with diameter of 8 to 10 μ m and had large, irregular and indented nuclei containing prominent nucleoli, and basophilic cytoplasm with a few vacuoles. SEM revealed many microvilli on the cell surface (Fig. 2). TEM findings showed many small vesicles and free ribosomes in the cytoplasm (Fig. 3). Karyotype analysis of the THP-2 cells after one-year culture revealed that almost all the cells had a diploid (46, XY) chromosome number.

Marker profile

Multiple markers of THP-2 cells were analyzed six times from two months to

cells at various times after cultivation

Rosette formation with (%)				Reactivity with monoclonal antibody (%)			TdT positive cells (%)
EAox	EAC ^{hu}	EAC ^{mo}	EN	B1	Ia	J5	
53	8	7	18	ND	ND	ND	ND
81.3	0.1	0.1	0	ND	ND	ND	ND
60.6	0.2	1.0	0	ND	ND	ND	ND
24.8	0	0	0	ND	ND	ND	ND
0	0	0	0	96.7	98.2	98.8	0
0	0	0	0	97.5	97.2	98.1	0
0	0	0	0	0	0	23.2	0

rocyte-IgG complex (EAox), with ox erythrocyte-IgM-complement (EAC^{hu}, terminated.

Fig. 2. Scanning electron microscopic appearance of a THP-2 cell. Note a number of microvilli on the cell surface. $\times 8,400$.

Fig. 3. Ultrastructural appearance of a THP-2 cell. Note an indented nucleus and a number of small vesicle and free ribosomes. $\times 7,500$.

five years after cultivation (Table 1). At the first examination 81% of the cells had Fc receptors, while only 0.1% were positive for complement receptors. However, the number of Fc receptor⁺ cells decreased with the time of cultivation and were not detected after two years of cultivation. There were no EN⁺ cells. Immunofluorescent studies showed that THP-2 cells bore μ - λ immunoglobulin on their surface and reacted with the monoclonal antibodies against B1, Ia-like and common ALL (J5) antigens. However, such surface markers of cells as Fc receptor, IgM, B1 antigen and Ia-like antigen were not detected when examined after 5 years of cultivation, while common ALL (J5) antigen was still present in 23% of the cells. Cytoplasmic immunoglobulins, TdT and EBNA were not detected by immunofluorescence at any time during culture. Monoclonal antibodies against T cells and their subsets (OKT3, OKT4, OKT6, OT8) did not react with THP-2 cells.

Plating efficiency

Cultured THP-2 cells formed colonies in 0.3% semi-solid agar with a plating efficiency of 2.3% 28 days after the seeding of the cells in the plate. The cells of each colony were transformed into a suspension culture in order for cloned cell lines of THP-2 to be obtained. Ten clones of THP-2 were obtained growing in a suspension culture and bearing the same surface antigen profiles as shown in the parent THP-2 cells. There were several unsuccessful attempts to transplant the THP-2 cells into the subcutaneous tissue of nude mice.

DISCUSSION

We have established an EBNA negative Burkitt's lymphoma cell line from a Japanese patient with Burkitt's lymphoma. Two other EBNA negative cell lines derived from Burkitt's lymphoma taken from Japanese patients have been established (Miyoshi et al. 1977; Hayashi et al. 1980). These three lines established in Japan have all been grown in single cell suspensions and bore surface immunoglobulins. In addition, THP-2 cells were positive for Ia-like antigen, common ALL antigen (J5) and human B cell specific antigen (B1) on their surface. B1 antigen, known to be a unique B cell surface differentiation antigen, was positive in all B cell lymphoma cells reported (Nadler et al. 1981). On the other hand, the expression of common ALL antigen, which permits classification of B cell lines, has not always occurred on the Burkitt's lymphoma cells; these were common ALL positive "B-blast I" and common ALL negative "B-blast II", which are considered to be in different differentiation stages (Minowada et al. 1981). In the sense of the stage of differentiation, the marker profile of THP-2 cells coincided with that of B-blast I. In year-5 culture surface markers of THP-2 cells, present for 3 years such as IgM, B1 antigen and Ia-like antigen were not detected, while the cells had common ALL antigen on their surface. This phenotypic alteration with time may suggest that THP-2 cells became less differentiated along the B cell lineage during long term culture, though the significance of this is unknown at present.

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