An Ia-like Antigen Positive Null Cell Leukemia Cell Line (THP-5) Lacking in the Stimulating Capacity in Autologous and Allogeneic Mixed Lymphocyte Reaction

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There has been a number of reports about in vitro stimulation of leukemia cells in autologous or HLA-identical (allogeneic) mixed lymphocyte culture (MLR) (Fefer et al. 1974, 1976). The stimulating capacities of established leukemia cell lines on allogeneic MLR have also been demonstrated (Han et al. 1977; Tsubota et al. 1978). Multiple factors may be involved in autologous MLR with leukemia cells; immunocompetence of patients during chemotherapy (Hersh and Oppenheim 1967; Gutterman et al. 1972), and autologous stimulation of normal lymphocyte subpopulations (Opelz et al. 1975). Although HLA-DR plays a critical role in MLR in man (Eijsvoogel et al. 1973), it is not clear whether the stimulation by leukemia cells is due to an increased antigenicity of leukemia-associated antigens or due to the additive effect of stimulation by HLA-D determinations and leukemia-associated antigens (Reinsmoen et al. 1978).
In the present paper we describe the establishment of a null cell leukemia cell line and its stimulation capacities in autologous and allogeneic MLR.

**METHODS AND MATERIALS**

*Patient*

The patient, a 13-year-old girl, was admitted in August, 1982 with the complaints of thigh pain and fever. Her peripheral WBC count on admission was 8600/mm³ with 40% of lymphoblasts, and the bone marrow was occupied with more than 90% of lymphoblasts with characteristics of null cells, positive only for Ia-like antigen. She was treated with induction chemotherapy consisting of predonine, vincristine, cyclophosphamide and L-asparaginase, resulting in complete remission.

*Establishment of cell line*

Lymphoblasts were collected from the peripheral blood of the patient before chemotherapy by Ficoll-Isopaque gradient centrifugation, and were distributed on a tissue culture microplate (Costar, No. 3596, Cambridge, MA) at a density of $6 \times 10^5$ per 0.2 ml per well in RPMI-1640 medium containing 20% of fetal calf serum and 60 µg/ml of kanamycin. They were incubated 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-5, was maintained in 5-ml culture in plastic flasks (Nunclonm, Roskilde, Denmark). No feeder was used.

*Cell markers*

Spontaneous rosette formation with sheep erythrocytes (EN), receptors for complements, C3b (human serum, EAC⁹⁹) and C3d (mouse serum, EAC⁹⁹), and Fe receptors for IgG (EAIgG) and IgM (EAIgM) were examined as described previously (Tsuchiya et al. 1980a). Cell surface immunoglobulins (sIg) and cytoplasmic immunoglobulin (cIg) were detected by direct immunofluorescence. Common ALL antigen (J5), Ia-like antigen, B1 antigen, Mo2 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; Todd et al. 1981). Monoclonal antibodies were purchased from Coulter Corporation (Hialeah, Fla) and Ortho Pharmaceutical Corporation (Raritan, NJ). Terminal deoxynucleotidyl transferase (TdT) was detected by using a terminal transferase assay kit (Bethesda Resarch Lab., Inc., Gaithersburg, MD). Nuclear antigen of Epstein-Barr virus (EBNA) was detected by the method of Reedman and Klein (1973).

*HLA typing*

HLA-A, B, C and DR typing of lymphocytes of the patient in remission and of the established cell line, THP-5, were performed by cytotoxicity technique with anti-HLA sera purchased from Hoechst Japan Corp. (Tokyo), Bosei Science Corp. (Isehara), Biotest (Frankfurt), Behring-werke (Marburg).

*Morphological studies*

Coverslip smears of the cultured cells were stained with Wright-Giemsa solution for light microscopy. The ultrastructures of THP-5 cells were studied by transmission electron microscopy and scanning electron microscopy.

*Mixed lymphocyte culture (MLR)*

MLR tests were performed by means of a modified microculture technique as described by Reinsmoen et al. (1978). As responding cells, peripheral mononuclear cells (PMN) were isolated from the patient in remission and healthy randomly selected donors by Ficoll-Isopaque gradient centrifugation. They were suspended in RPMI-1640 medium with 20%
of fetal calf serum (supplemented with kanamycin) at a concentration of $2 \times 10^5$ per 0.2 ml per well. Stimulating cells were treated with mitomycin C (25 $\mu$g/ml) at 37°C for 30 min. After being washed three times with Hanks' solution, they were suspended in RPMI-1640 medium with 20% of fetal calf serum at a concentration of $2 \times 10^6$ per ml. 0.1 ml of the responding cell suspension was mixed with 0.1 ml of the stimulating cell suspension and cultured in a tissue culture microplate. After 6 days of cultivation, the cultures were labeled for 18 hr with $^3$H-thymidine (0.4 $\mu$Ci/well) and were harvested on glass wool filters by means of a sunction-water-wash apparatus, dried, and counted in a scintillation counter. Proliferative responses of responding cells were expressed as the stimulation index (SI) calculated from the following formula:

$$SI = \frac{cpm \text{ in mixed cell culture} - cpm \text{ in stimulating cell culture}}{cpm \text{ in non-stimulated responding cell culture}}$$

The stimulating capacities of THP-5 cells, EBV-transformed lymphoblastoid cell line (LCL) (Tsuchiya et al. 1980a, b), and PMN of the patient in remission and normal donors were examined in autologous and allogeneic MLR.

**Results**

**Establishment of the cell line (THP-5) and its morphology**

The THP-5 cells grew in single cell suspension with a doubling time of 42 hr. The cells appeared uniform and round in shape, and had large, irregular and lobulated nuclei containing a few nucleoli. The basophilic cytoplasm was scarce.

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Fig. 1. Scanning electron micrograph showing a THP-5 cell. Note relatively short microvilli on the cell surface. ×12,000.
and contained a few vacuoles. Scanning electron microscopic observations revealed that the round cells were 5 to 6 μm in diameter and equipped with relatively short microvilli on the cell surface (Fig. 1). Transmission electron microscopic findings showed that the THP-5 cell possessed a large, irregular and lobulated nucleus with one or two prominent nucleoli. The cytoplasm contained a large number of free ribosomes, many mitochondria, a few small vesicles and well-developed Golgi apparatuses (Fig. 2). A few cored vesicles were found in some THP-5 cells. Karyotype analysis of the THP-5 cells revealed a diploid (46, XX) chromosome number.

**Marker profiles**

As detailed in Table 1, THP-5 cells were of a null cell type expressing only Ia-like antigen. They lacked all other cell markers examined including EN, sIg, cIg, EAC^hu, EAC^mo, EA1gG, EA1gM and J5, B1, Mo2, T3, T4, T6, T8 antigens. In HLA typings THP-5 cells were equivocally positive for A9, B12, Cw3 antigens and definitely positive for DRw2 antigen. In remission the patient’s lymphocytes had B40 and DRw9 antigens, in addition to HLA-antigens expressed on the THP-5 cells.
As shown in Table 2, peripheral lymphocytes obtained from the patient in remission and six healthy unrelated donors were cultured with mitomycin C-treated THP-5 cells, EBV-transformed LCL derived from the patient's peripheral blood, and allogeneic lymphocytes. The patient's lymphocytes failed to respond in autologous MLR to mitomycin C-treated THP-5 cells, but responded normally to mitomycin C-treated THP-5 cells, EBV-transformed LCL derived from the patient's peripheral blood, and allogeneic lymphocytes.

**MLR stimulation of THP-5 cells and EBV-transformed LCL**

As shown in Table 2, peripheral lymphocytes obtained from the patient in remission and six healthy unrelated donors were cultured with mitomycin C-treated THP-5 cells, EBV-transformed LCL derived from the patient's peripheral blood, and allogeneic lymphocytes. The patient’s lymphocytes failed to respond in autologous MLR to mitomycin C-treated THP-5 cells, but responded normally to mitomycin C-treated THP-5 cells, EBV-transformed LCL derived from the patient's peripheral blood, and allogeneic lymphocytes.

<table>
<thead>
<tr>
<th>Responding cells</th>
<th>Stimulating cells</th>
<th>Stimulation index (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient (in remission)</td>
<td>THP-5</td>
<td>0.5-1.2 (0.9)</td>
</tr>
<tr>
<td>Normal donors</td>
<td>THP-5</td>
<td>0.2-1.9 (1.1)</td>
</tr>
<tr>
<td>Patient (in remission)</td>
<td>EBV-transformed LCL of patient</td>
<td>4.4-69.4 (36.9)</td>
</tr>
<tr>
<td>Normal donors</td>
<td>EBV-transformed LCL of patient</td>
<td>11.3-24.9 (18.1)</td>
</tr>
<tr>
<td>Patient (in remission)</td>
<td>Allogeneic lymphocytes</td>
<td>1.8-41.0 (10.8)</td>
</tr>
<tr>
<td>Normal donors</td>
<td>Allogeneic lymphocytes</td>
<td>4.5-20.0 (12.2)</td>
</tr>
</tbody>
</table>

*2 x 10⁶ lymphocytes were incubated with 4 x 10⁵ mitomycin C-treated THP-5, EBV-transformed LCL and allogeneic lymphocytes in 0.2 ml RPMI-1640 with 20% fetal calf serum for 6 days. ^H-thymidine incorporation was measured during the last 18 hr of incubation. Stimulation index was calculated by the formula described in the text.

†Lymphocytes of the patient in remission were included.
to autologous EBV-transformed LCL treated with mitomycin C. The stimulating capacity of THP-5 cells in allogeneic MLR was not found, either.

**Influence of mitomycin C-treated THP-5 cells on allogeneic MLR**

Allogeneic MLR in healthy nonrelated donors were examined with or without the addition of THP-5 cells treated with mitomycin C at the same cellular concentration as responding cells (Table 3). Two healthy donors examined showed strong responses to each other in MLR (SI 38.8 and 70.5) but no response to mitomycin C-treated THP-5 cells (SI 0.6 and 1.9). The addition of THP-5 cells treated with mitomycin C to the above allogeneic MLR systems showed no effect on their reactions (SI 38.8 vs. 38.0 and 70.5 vs. 69.8).

**DISCUSSION**

A null-cell leukemia cell line, THP-5, was characterized by the presence of Ia-like antigen but lacking all other markers. Although there has been reported an acute myeloblastic leukemia cell line with similar surface characters (Minowada et al. 1981), the morphological and cytochemical features and reactivity with Mo2 antigen of the THP-5 indicate it to be of non-myeloid cells. This cell line is unique in expressing only Ia-like antigen among established null-cell leukemia cell lines. Null-cell leukemia cell lines including NALL-1 (Miyoshi et al. 1977), KM-3 (Schneider et al. 1977), Reh (Rosenfeld et al. 1977), NALM-1 (Minowada et al. 1977), NALM-6 (Chechik and Minowada 1979), were positive for

<table>
<thead>
<tr>
<th>Responding cells</th>
<th>Stimulating cells</th>
<th>THP-5 cells (MMC treated)</th>
<th>Mean cpm</th>
<th>Stimulation index</th>
</tr>
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<tbody>
<tr>
<td>C1</td>
<td>-</td>
<td>-</td>
<td>812</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>-</td>
<td>-</td>
<td>334</td>
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</tr>
<tr>
<td>C1</td>
<td>C2</td>
<td>-</td>
<td>31,869</td>
<td>38.8</td>
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<tr>
<td>C2</td>
<td>C1</td>
<td>-</td>
<td>24,204</td>
<td>70.5</td>
</tr>
<tr>
<td>C1</td>
<td>C2</td>
<td>+</td>
<td>32,010</td>
<td>38.0</td>
</tr>
<tr>
<td>C2</td>
<td>C1</td>
<td>+</td>
<td>24,116</td>
<td>69.6</td>
</tr>
<tr>
<td>C1</td>
<td>-</td>
<td>+</td>
<td>758</td>
<td>0.6</td>
</tr>
<tr>
<td>C2</td>
<td>-</td>
<td>+</td>
<td>854</td>
<td>1.9</td>
</tr>
<tr>
<td>-</td>
<td>C1</td>
<td>-</td>
<td>667</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>C2</td>
<td>-</td>
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<td></td>
</tr>
<tr>
<td>-</td>
<td>-</td>
<td>+</td>
<td>210</td>
<td></td>
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</tbody>
</table>

*2×10⁶ lymphocytes of healthy unrelated donors (C1 and C2) were incubated with 4×10⁶-mitomycin C-treated allogeneic lymphocytes with or without the addition of mitomycin C-treated THP-5 cells in 0.2 ml RPMI-1640 with 20% fetal calf serum for 6 days. "H-thymidine incorporation was measured during the last 18 hr of incubation. Counts per minute (cpm) given are the mean of triplicate cultures.
J5 antigen as well as Ia-like antigen (Minowada et al. 1981). Among these cell lines, two (NALL-1, NALM-1) were demonstrated to have stimulating capacities in MLR to the same degree as normal lymphocytes (Han et al. 1977). Although there is much evidence for heterogeneity of null-cell ALL in a variety of antigen phenotypes demonstrated (Ueda et al. 1982), Ia-like antigen positive leukemia cells commonly stimulate allogeneic lymphocytes in MLR. THP-5 cells had no stimulating capacity in allogeneic MLR as well as autologous MLR in spite of the expression of Ia-like antigen and HLA-DR antigen. It is unlikely that mitomycin C-treated THP-5 cells suppress directly the proliferation of responding cells. The culture supernatant of THP-5 cells suppressed neither the proliferation of lymphocytes in MLR nor proliferative responses to mitogens (data not shown).

Kourilsky et al. (1968) demonstrated no difference in the leukocyte antigen expression between peripheral lymphocytes of leukemia patients and their own leukemia cells, and Han et al. (1972) reported that new HLA antigens could be expressed on the cultured tumor cell lines, without inducing any significant increase of stimulation in MLR. The THP-5 cells have an HLA-phenotype different in its expression from that of the patient’s lymphocytes in remission but no giving rise to new HLA-antigen. The significance of such altered HLA expression in THP-5 cells is unknown.

Further studies on the escape mechanism of THP-5 cells from the recognition of allogeneic lymphocytes may provide important information about the role and function of HLA-DR antigen in non-self recognition.

References


8) Hersh, E.M. & Oppenheim, J. (1967) Inhibition of in vitro lymphocyte transforma-