Establishment of Five Human Malignant Non-T Lymphoid Cell Lines and Mixed Lymphocyte-Tumor Reaction

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Establishment of truly leukemia and lymphoma cell lines has provided useful tools for the study of normal hematopoietic cell differentiation (Koeffler 1983), the function of hematopoietic cells at various stages of differentiation (Steinberg et al. 1985), cytokines secreted by unique types of hematopoietic cells (Robb 1982; Uittenbogaart and Fahey 1982), and the mechanisms of leukemogenesis (Greaves 1979). A number of investigators have routinely tried to establish permanent cell lines from leukemia or lymphoma patients (Minowada et al. 1977; Miyoshi et al. 1977; Hurwitz et al. 1979). We have already reported the establishment of several leukemia/lymphoma cell lines; the acute monocytic leukemia cell line (THP-1) (Tsuchiya et al. 1980), two common ALL cell lines (THP-3-1 and THP-3-2) (Tsuchiya et al. 1983a), the Ia-positive null ALL cell line (THP-5) (Imaizumi et al. 1984), and the B cell lymphoma cell line (THP-2) (Tsuchiya et al. 1983b).

This communication describes the newly established five human malignant non-T lymphoid cell lines, two of which are CALLA positive ALL cell lines.

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(THP-4 and THP-7), two null ALL cell lines (THP-6 and THP-8), with one a B cell lymphoma cell line (THP-9).

**MATERIALS AND METHODS**

**Patients**

The patients were admitted between December 1981 and October 1984. Diagnoses on admission and the present state of the patients are listed in Table 1. Four patients were diagnosed as having acute lymphoblastic leukemia (ALL), two of the common type (patients Y.S. and A.A.) and the two of the null type (patients M.S. and Y.K.). One patient (patient Y.A.) was diagnosed as having B cell malignant lymphoma. Patient Y.S., a 2-year-old boy, was suffering from central nervous system involvement on admission and relapsed soon after the first bone marrow remission. Patients M.S., a 6-year-old girl and A.A., a 13-year-old girl, are at present maintaining the first remission. Patient Y.K., a 7-month-old girl, had a relapse soon after the first bone marrow remission. Patient Y.A., a 7-year-old boy, had a huge abdominal mass and ascites on admission and during the remission induction therapy developed a leukemic transformation of malignant lymphoma.

**Cell cultures**

Lymphoblasts from peripheral blood, bone marrow blood, or ascites were isolated by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) density gradient centrifugation. Six to $10^6$ cells/0.2 ml of RPMI-1640 medium containing 20% fetal calf serum and 60 μg/ml of kanamycin were delivered to individual wells of flat-bottom 96-well microtiter plates (Falcon, No. 3040, Oxnard, CA, USA). They were cultured at 37°C in an atmosphere of 5% CO$_2$ in air. The medium was changed twice a week. After being established, the cell lines were maintained in 5- or 8-ml culture in plastic flasks (Nuncion, Roskilde, Denmark). No feeder cells or special supplements for cell culture were used. Mycoplasma contamination of continuous cell lines was examined for using bioassay systems with mycoplasma detection kit (Bioassay Systems Co., Woburn, MA, USA). All cell lines were proved to be free of mycoplasma contamination.

**Characterization of cell surface markers**

Spontaneous rosette formation with sheep erythrocytes (EN), receptors for complement, C3b (human serum, EAC$^{3b}$) and C3d (mouse serum, EAC$^{3d}$), and Fc receptors for IgG (EAG) and IgM (EAM) were examined as described previously (Tsuchiya et al. 1980). Cell surface immunoglobulins and cytoplasmic immunoglobulins were detected by direct

**TABLE 1. Clinical findings of the patients**

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Diagnosis</th>
<th>Present state</th>
<th>Cell line</th>
<th>Source of malignant cells for in vitro culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Y.S.</td>
<td>M</td>
<td>2Y</td>
<td>Common ALL</td>
<td>Dead</td>
<td>THP-4</td>
</tr>
<tr>
<td>2</td>
<td>M.S.</td>
<td>F</td>
<td>6Y</td>
<td>Null ALL</td>
<td>CCR</td>
<td>THP-6</td>
</tr>
<tr>
<td>3</td>
<td>A.A.</td>
<td>F</td>
<td>13Y</td>
<td>Common ALL</td>
<td>CCR</td>
<td>THP-7</td>
</tr>
<tr>
<td>4</td>
<td>Y.K.</td>
<td>F</td>
<td>7M</td>
<td>Null ALL</td>
<td>Dead</td>
<td>THP-8</td>
</tr>
<tr>
<td>5</td>
<td>Y.A.</td>
<td>M</td>
<td>7Y</td>
<td>Malignant lymphoma</td>
<td>Dead</td>
<td>THP-9</td>
</tr>
</tbody>
</table>

M, male; F, female; ALL, acute lymphoblastic leukemia; CCR, continuous complete remission; PB, peripheral blood; BM, bone marrow.
immunofluorescence. CALLA, Ia, B1, B2, B4 antigens, T3, T4, T6, T8, T11 antigens, My9, OKM1, and Leu 11a antigens were detected by indirect immunofluorescence with respective monoclonal antibodies (Kung et al. 1979; Breard et al. 1980; Reinherz et al. 1980; Ritz et al. 1980; Stashenko et al. 1980; Nadler et al. 1981a, b, 1983; Phillips and Babcock 1983; Griffin and Schlossman 1984). These monoclonal antibodies were purchased from Coulter Clone TM Corporation (Hialeah, Fla), Ortho Pharmaceutical Corporation (Raritan, NJ, USA), and Becton Dickinson Immunocytometry Systems (Mountain View, CA, USA).

Nuclear antigen of Epstein-Barr virus (EBNA) was detected by the method of Reedman and Klein (1973).

**Morphological and cytochemical studies**

Coverslip smears of the cultured cells were stained with Wright-Giemsa, peroxidase, naphthol AS-D chloroacetate esterase, α-naphthyl butyrate esterase, and periodic acid-Schiff (PAS) solution by the standard methods.

**Mixed lymphocyte-tumor reaction (MLTR)**

MLTR tests were performed by means of a modified microculture technique as described by Reinsmoen et al. (1978). As responding cells, peripheral mononuclear cells were isolated from healthy randomly selected donors and patients in remission by Ficoll-Paque density gradient centrifugation. They were suspended in RPMI-1640 medium with 10% AB-human serum at a concentration of 1×10⁶ cells per ml. Stimulating cells were inactivated with mitomycin C (25 to 300 μg/ml) at 37°C for 30 min. After being washed three times with RPMI-1640 medium, they were suspended in RPMI-1640 medium with 10% of AB-human serum at a concentration of 1×10⁶ cells per ml. One tenth ml of the responding cell suspension was mixed with 0.1 ml of the stimulating cell suspension in triplicate in a 96-well tissue culture microplate (Falcon, No. 3072, Oxnard, CA, USA). After 6 days of cultivation in a humid 5% CO₂ incubator at 37°C, the cultures were labelled for 18 hr with ³H-thymidine (0.4μCi/well) and were harvested on glass wool filters by means of a suction-water-apparatus, dried, and counted in a liquid scintillation counter. Proliferative responses of the responding cells were expressed as the stimulation index (SI) of the MLTR according to 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 5 THP cell lines were examined in allogeneic MLTR. Peripheral blood of two patients with ALL in continuous complete remission (CCR) was used in autologous MLTR. A stimulation index of greater than 2.3 indicated significant stimulation (Sengar and Terasaki 1971).

**Results**

**Establishment of the cell lines and their morphological and cytochemical findings**

All of the cell lines have been cultured for over one year and grown in single cell suspension. The doubling times of the cell lines, as listed in Table 2, varied from 24 to 68 hr. The maximal cell density was also variable from 0.8 to 4.2×10⁶ cells per ml.

As shown in Fig. 1a and 1c, the original cells of THP-4 and THP-7 were classified as L1 according to the criteria of FAB (French-American-British co-operative group 1976). Cells of THP-6 and THP-8 were classified as L2 (Fig. 1b and 1d) and those of THP-9 as in L3 (Fig. 1e). All cell lines were negative for peroxidase staining, naphthol AS-D chloroacetate esterase staining and α-
Fig. 1. Comparative cytological appearance of THP cell line cells. a, THP-4; b, THP-6; c, THP-7; d, THP-8; e, THP-9. Wright-Giemsa × 600.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Time (hr)</th>
<th>Maximal cell density ($\times 10^9$/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>THP-4</td>
<td>52</td>
<td>4.2</td>
</tr>
<tr>
<td>THP-6</td>
<td>24</td>
<td>1.3</td>
</tr>
<tr>
<td>THP-7</td>
<td>50</td>
<td>2.8</td>
</tr>
<tr>
<td>THP-8</td>
<td>68</td>
<td>0.8</td>
</tr>
<tr>
<td>THP-9</td>
<td>30</td>
<td>1.4</td>
</tr>
</tbody>
</table>
Human Malignant Lymphoid Cell Lines

naphthyl butyrate esterase staining. THP-9 cells were strongly positive for PAS staining.

**Characterization of cell surface markers**

THP-4, THP-6 and THP-7 did not have receptors for EN, EAC\textsubscript{nu}, EAC\textsubscript{mo}, EAG and EAM on their surface (Table 3). THP-8 contained a small number of C3b receptor positive cells as detected by EAC\textsubscript{nu} rosette formation. Less than 11% of THP-9 cells possessed C3b and C3d receptors.

The surface markers defined by monoclonal antibodies are shown in Table 4. THP-4 cells were pre-B cell type expressing cytoplasmic \( \mu \) immunoglobulins, B4, CALLA and Ia. THP-7 cells were common type, and expressed B4, CALLA and Ia. THP-8 cells were the other null cell type lacking CALLA, but expressing B4 and Ia. THP-9 cells were B-cell type; they possessed surface \( \mu \)-\( \lambda \) immunoglobulins, B1, B4, CALLA and Ia. The cell antigens such as T3, T4, T6, T8, T11, My9, OKM1 and Leu-11a were negative in all cell lines. EBNA was also negative in all cell lines.

**MLTR stimulation of THP cell lines**

The results of mixed lymphocyte-tumor reactions between allogeneic peripheral blood lymphocytes (responding cells) and THP cell lines (stimulating cells) are shown in Table 5. Four cell lines, THP-4, THP-7, THP-8 and THP-9, which were positive for Ia, showed a strongly stimulating capacity (S.I., 35.1, 45.5, 78.1 and 35.1, respectively). In contrast, an Ia negative cell line, THP-6, had a low but distinct stimulating capacity (S.I., 7.0).

In autologous MLTR (Table 6), lymphocytes obtained from the patient M.S. in a CCR state responded to mitomycin C-treated THP-6 cells exhibiting a S.I. of

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**Table 3. Rosette forming assay**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>% of rosette forming cells with</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EN</td>
</tr>
<tr>
<td>THP-4</td>
<td>0</td>
</tr>
<tr>
<td>THP-6</td>
<td>0</td>
</tr>
<tr>
<td>THP-7</td>
<td>0</td>
</tr>
<tr>
<td>THP-8</td>
<td>0</td>
</tr>
<tr>
<td>THP-9</td>
<td>2.9</td>
</tr>
</tbody>
</table>

EN, neuraminidase-treated sheep red blood cells (SRBC); EAC\textsubscript{nu}, IgM antibody (human complement)-coated SRBC; EAC\textsubscript{mo}, IgM antibody (C5-deficient mouse complement)-coated SRBC; EAG, IgG antibody-coated SRBC; EAM, IgM antibody-coated SRBC. The mean \( \pm \) S.D. of EN, EAC\textsubscript{nu}, EAC\textsubscript{mo}, EAG and EAM rosette-forming cells from control subjects is 66.2 \( \pm \) 6.5, 33.5 \( \pm \) 5.4, 18.6 \( \pm \) 8.2, 15.8 \( \pm \) 6.7 and 55.3 \( \pm \) 3.3, respectively.
but lymphocytes obtained from the patient A.A. in a CCR state had little responding ability to the autologous THP-7 cells (S.I., 1.7).

**DISCUSSION**

Four ALL cell lines from the peripheral blood or bone marrow of four patients, and one B cell lymphoma cell line from the ascites of a patient with malignant lymphoma were established. Two of the four ALL cell lines, THP-4 and THP-7, were positive for Ia, B4 and CALLA, but only THP-4 cells for...
cytoplasmic $\mu$-chains. From these results, THP-4 was represented as a pre-B cell ALL cell line and THP-7 as a common ALL cell line. THP-8 cells were positive for Ia and B4 but negative for CALLA. THP-6 cells had no surface markers which could be detected by the lymphoid cell specific monoclonal antibodies employed. These two cell lines could be designated as null cell ALL cell lines, which were derived from different stages of differentiation. THP-9, which was established from a patient with malignant lymphoma, was a mature B cell line with a surface $\mu$- chain immunoglobulin.

A series of monoclonal antibodies that defines B cell-restricted and-associated antigens has already been utilized in an attempt to characterize tumors of B lineage (Anderson et al. 1984). Five immunologically characterized THP cell lines, in the present study, showed five different patterns in antigen expression that probably represented special stages of B cell differentiation. THP-6, designated as a null cell line, might represent the most immature stage of B cell differentiation, followed by THP-8 with Ia and B4, THP-7 with Ia, B4 and CALL, THP-4 with Ia, B4, CALLA and cytoplasmic $\mu$ chain and THP-9 with surface immunoglobulins, B1, CALLA, B4 and Ia in a sequential order of differentiation. These cell lines seem to form a very important set of cell lines which clearly represented major differentiation stages of B cell lineage. As for tumor cells isolated from patients with non-T cell ALL, they could be assigned consecutively to four subgroups: Ia alone, Ia+B4+, Ia+B4+CALLA+, and Ia+B4+CALLA+B1+ (Nadler et al. 1984). However, Ia+B4+CALLA+B1+ cells, which can be identified in most non-T cell ALLs seen in children over 2 years and under 16 years old, were not found in THP-series ALL cell lines.

MLTR has been used to analyse the cellular antigens on tumor cells, showing the in vitro proliferation of responding lymphocytes by the stimulation of tumor cells (Tsubota et al. 1978; Grimm et al. 1984). Known candidates of epitopes which evoke MLTR are histoincompatible Ia and tumor specific antigens (Reinsmoen et al. 1978; Han et al. 1979).

Allogeneic MLTR generally reflects differences between the histocompatibility antigens of responding and stimulating cell, but some tumor cells have ability to stimulate responding cells without Ia on the tumor cells (Han et
al. 1977). In the latter case the presence of tumor specific antigens could be hypothesized. In the present study, Ia-positive cells, such as THP-4, THP-7, THP-8 and THP-9, showed strongly positive MLTR responses, suggesting that responding cells were stimulated by the presence of Ia. On the other hand, Ia-negative cells of THP-6 also evoked a distinct stimulation to allogeneic responding lymphocytes. This non-Ia mediated proliferative response might have probably been caused by some kind of leukemia-associated antigens on THP-6 cells. The autologous lymphocytes from patient M.S. who was in CCR responded well to the mitomycin C-treated THP-6 cells, strongly suggesting the presence of leukemia-associated antigens on THP-6 cells. These findings seem to be in agreement with the data reported by other investigators that showed the presence of cell mediated immunity mediated by leukemia-associated antigens in the patients with ALL (Viza et al. 1969; Halterman and Leventhal 1971; Powles et al. 1971).

Autologous MLTR using lymphocytes from patient A.A. who was also in CCR showed other interesting findings. Autologous lymphocytes did not respond to THP-7 cells, in spite of a strong response of allogeneic lymphocytes to THP-7 cells. This fact may imply the absence of leukemia-associated antigens on THP-7 cells. The clinical importance, however, of these two modes of MLTR remains to be resolved. Further analysis of a surface molecular structure of THP-6 cells recognized by both allogeneic and autologous responding cells might give an insight into immunological responses to leukemic cells.

Acknowledgments

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References


