Childhood Lymphoblastic Leukemia With Natural Killer Activity: Establishment of the Leukemia Cell Lines Retaining the Activity

By Atsushi Komiyama, Hiroshi Kawai, Yukiaki Miyagawa, and Taro Akabane

Leukemic cells from a child with acute lymphoblastic leukemia (ALL) had high natural killer (NK) activity against K562 as determined by the 51Cr release assay at a 40:1 effector:target ratio; percent lysis was 76.8% (147.4% of normal lymphocyte value) and higher than that of control leukemic cells from 12 childhood ALL (0.1% ± 0.3%). Two leukemic cell lines (SPI-801 and SPI-802) were established from the patient, and they were essentially the same as the freshly harvested leukemic cells in their morphology, cytochemistry, immunologic markers, and functions. The cultured cell lines as well as the fresh leukemic cells had receptors for sheep red blood cells, IgG-Fc, and C3. The cultured cells were OKM1", Ia", and asialo-GM1", and were OKT-3, OKT-4, OKT-6, OKT-8, Leu-7, human mono-
cyte, common ALL", T cell, surface Ig", and cytoplasmic IgM as determined by the immunofluorescence method. The two cell lines shared the same chromosome abnormalities. Their chromosomes were in hypotriplid region (63–73), and 6q-, 11p-, and several marker chromosomes were demonstrated. They had spontaneous cytoxicity against K562 (percent lysis: up to 17.8% and 33.5% of normal lymphocyte value in SPI-801 and SPI-802, respectively) and Molt-3 (38.2% and 27.8% of normal lymphocyte value), but not against Raji and mitogen-induced normal lymphoblasts. Such phenotypic and functional characteristics of the fresh leukemic cells and cultured cells are virtually identical to those of NK cells, demonstrating a new phenotype of childhood ALL of NK cell origin.

IN RECENT YEARS, much attention has been focused on immunologic functions of neoplastic lymphocytes.1–4 For example, Broder et al.5 have demonstrated suppressor cell activity, one of T-cell functions, of neoplastic T cells from a child with acute lymphoblastic leukemia (ALL).

Normal individuals have a lymphocyte subpopulation called a natural killer (NK) cell, which is capable of killing certain tumor-derived cell lines without previous sensitization.5–8 In this article, we report a first case of ALL that had leukemic cells with NK activity against leukemia-derived cell lines such as K562 and Molt-3. In addition, we describe the leukemic cell lines retaining the activity established from the patient, which certainly confirms a new phenotype of childhood ALL of NK cell lineage.

CASE REPORT

A 9-yr-old girl presented on March 2, 1979 with general malaise, pallor, lymphadenopathy, splenomegaly (10 cm), and hepatomegaly (8 cm). The peripheral blood count showed a hemoglobin of 12.3 g/dl, a platelet count of 37,000/cu mm, and a white blood cell (WBC) count of 439,000/cu mm with 4% neutrophils, 4% lymphocytes, 1% monocytes, and 91% lymphoblasts. The bone marrow was hypercellular with 95% lymphoblasts. The lymphoblasts were L2 type cells, 1% monocytes, and 91% lymphoblasts. The bone marrow was hypercellular with 95% lymphoblasts. The lymphoblasts were L2 type cells, 1% monocytes, and 91% lymphoblasts.

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MATERIALS AND METHODS

Sample Preparation

Heparinized peripheral blood was obtained from the patient, as described above, when she was in the terminal stage of ALL and leukemic cells were markedly increased (>99.5%) in the peripheral blood. Leukemic cells were separated by Ficoll-Hypaque density gradient centrifugation, Matsumoto, Japan. In the present studies were obtained from the peripheral blood. The patient died 2 days later. Permission for autopsy was not granted. She had no apparent episodes of pyogenic and viral infections during the observation period.

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(blasts > 98%) were obtained in the same fashion as described above. In addition, heparinized peripheral blood was obtained from 5 healthy volunteers, and mononuclear cells (MNC) were separated by Ficoll-Hypaque centrifugation. MNC were then depleted of monocytes by adherence to plastic surfaces and used as normal lymphocytes.

Establishment of Cell Lines

Leukemic cells were suspended in RPMI 1640 medium containing 10% fetal calf serum (FCS; Flow Lab., Rockville, Md.), 10^{-5} M 2-mercaptoethanol, penicillin (50 U/ml), streptomycin (50 μg/ml), and 25% phytohemagglutinin-mitogenic factor (PHA-MF) at a cell concentration of 10^7 cells/ml, and were placed in 20 plastic tissue culture flasks (Falcon Lab., Oxnard, Calif.). PHA-MF was prepared according to the method of Masucci et al. from 48-hr tissue culture medium (RPMI 1640 with 10% FCS) from culture of 1% PHA-P (Difco Lab., Detroit, Mich.) stimulated MNC from healthy volunteers. The cultures were incubated at 37°C in a humidified atmosphere of 5% CO₂ in air. The culture was initiated on November 9, 1980 and established 1 mo later in 2 of 20 flasks. After cell line establishment, subcultures were made usually every 4 days in culture medium without PHA-MF.

Cell Morphology and Cytochemistry

Cell morphology was examined on May-Giemsa-stained smears. For electron microscopy, cells were fixed with 2.5% glutaraldehyde in 0.05 M cacodylate buffer (pH 7.2) and 1% osmium tetroxide in the buffer, dehydrated, embedded, and sectioned as previously described. The ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Hitachi HS-8 electron microscope.

Peroxidase, nonspecific esterase, and PAS cytochemical stains were routinely performed.

Immunologic and Other Cell Markers

Spontaneous rosette formation with 2-aminoethyl isothiouronium bromide hydrobromide (AT-E) treated SRBC (E-rosette) was performed at 4°C and 37°C according to the method of Kaplan and Clark. E-rosette formation by the cultured cells was further studied using neuraminidase-treated SRBC at 4°C, 29°C, and 37°C. Receptors for the Fc portion of IgG (IgG-Fc) and C3 were detected by the rosette method using ox red blood cells (ORBC) coated with purified rabbit anti-ORBC antibody and rabbit IgM-coated ORBC plus fresh human serum complement, respectively. IgG-Fc receptors of the cultured cells were also detected by an immunofluorescent technique using heat-aggregated IgG. For detection of further surface markers, direct or indirect immunofluorescence under non-capping conditions was performed with a Leitz-Dialux 20 EB microscope using several monoclonal and conventional antibodies. Cytoplasmic IgM was detected as described by Volger et al. Monoclonal antibodies were used in antibody excess. Monoclonal OKT-3, OKT-4, OKT-6, OKT-8, and OKM-1 (Ortho Pharmaceutical Co., Raritan, N.J.), monoclonal anti-Leu-7 (HNK-1; Becton Dickinson, Sunnyvale, Calif.), monoclonal anti-human monocyte (Bethesda Research Lab.), and anti-human IgM-F (ab')₂ μ, IgD-F (ab')₂ λ, and IgG-F (ab')₂ λ (Behringwerke, AG, Marburg, F.R.G.) antibodies were commercially obtained. Anti-common-ALL (cALL), anti-T-cell, and anti-IA antisera were kindly supplied by Dr. Shinpei Nakazawa, Department of Pediatrics, Keio University, Tokyo. The anti-cALL was raised in rabbits by immunization with a cell line of KOPN-K, as described by Koshiba et al. The antibody reacted with 75% of non-T, non-B ALL and some of B-cell ALL, but none of T-ALL in children. The anti-T and anti-IA were raised as described by Tsubota et al. with a T-leukemia cell line of KOPT-K1 and a normal B-cell line of SN 1054, respectively. Anti-asialo GM₁, antibody was prepared in rabbits. Terminal deoxypentitol tetransferase (TdT) activity was determined by the Terminal Transferase Immunofluorescence Assay Kit (Bethesda Research Lab.).

Chromosome Analysis

Chromosome analyses were performed by the G-banding method.

Cell-Mediated Cytotoxicity Assay

Cell-mediated cytotoxicity was assayed on ³¹Cr-labeled target cells. Leukemia or lymphoma cell lines such as K562, Mol-3, and Raji and PHA-induced normal T lymphoblasts were used as target cells. Effector cells included the freshly harvested leukemic cells from the present patient and cells from the cultured cell lines in the absence or presence of 3- to 10-day stimulation with 10% PHA-MF, 10% concanavalin-A-MF (Con-A-MF), or mitomycin-C (MMC) treated normal lymphocytes. Con-A-MF was prepared with 100 μg/ml of Con-A (Sigma, St. Louis, Mo.) in the same fashion as described in PHA-MF preparation. As controls, normal lymphocytes in the absence or presence of 24-hr treatment with PHA-MF or Con-A-MF, leukemic cells from the other ALL patients, and several cultured leukemia cell lines including Mol-3, JM, CCRF-CEM, and Jurkat-F 1884 (kindly supplied by Dr. John A. Hansen, Histocompatibility Lab., Puget Sound Blood Center, Fred Hutchinson Cancer Research Center, Seattle, Wash.) were also used as effector cells. Briefly, 2 x 10⁵ target cells were suspended in 2 ml RPMI 1640 containing 10% heat-inactivated FCS, labeled with 100 μCi ³¹Cr (Amersham, Arlington Heights, Ill.) at 37°C for 60 min, and washed 4 times with the culture medium. After the labeling, 3 x 10⁴ of the cells of 0.1 ml of the culture medium were transferred to each plastic experimental tube. Cell mixtures, prepared at a 40:1 effector:target ratio, were brought to a 1 ml final volume, and incubated at 37°C for 4 hr in a 5% CO₂ incubator. In some experiments, the cell mixtures were incubated at 37°C for 4, 8, 12, and 16 hr. The following equation was used to express cytotoxicity: percent lysis = [(cpm experimental release – cpm spontaneous release)/(cpm maximal release – cpm spontaneous release)] x 100. Antibody-dependent cell-mediated cytotoxicity (ADCC) assay was done on ³¹Cr-labeled chicken red blood cells (CRBC). All assays were set up in triplicate.

RESULTS

Establishment of the Cell Lines (SPI-801 and SPI-802)

Two cell lines were established and named SPI-801 and SPI-802, respectively. The cells grew as a single-cell suspension with a doubling time of about 48 hr. Contamination with mycoplasma species was not detected by agar culture.

Cell Morphology and Cytochemistry

Morphological characteristics of both SPI-801 and SPI-802 cells were similar to those of the original leukemic cells. As shown in Fig. 1, most cells had a round nucleus with prominent nucleoli and relatively voluminous basophilic cytoplasm. The cytoplasm contained several to many vesicles and lacked azurophilic granules. Mitotic figures and bi- or trinucleated cells were frequently seen.
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On electron microscopic examination (Fig. 2), cells from the two cell lines were generally 15-18 μ in diameter. They had a round to oval nucleus with diffuse chromatin and 1-3 prominent nucleoli. There were polyribosomes throughout the cytoplasm and no or few profiles of rough endoplasmic reticulum. Mitochondria were numerous in many cells. Golgi apparatus was rather small. In some cells, electron-lucent vesicles and moderately electron-dense pools of amorphous material, probably lipid droplets, were demonstrable.

Cytochemical stains for peroxidase and naphthol-AS-acetate esterase in the fresh leukemic cells were negative. PAS reaction was positive in about one-third of the cells. Both SPI-801 and SPI-802 cells were totally negative for peroxidase, α-naphthyl butyrate esterase, and naphthol-AS-acetate esterase. PAS reaction was granular-positive in 42% of SPI-801 and 50% of SPI-802 cells.

Immunologic and Other Cell Markers

Some of the fresh leukemic cells formed rosettes with AET-treated SRBC at 4°C (positivity: 6.2%) and 37°C (4.5%). IgG-Fc receptors were demonstrated in 10.2% of the cells. Cell markers of the cultured cells were studied several times between 3 and 18 mo after the culture initiation, and the results are shown in Table 1. Both SPI-801 and SPI-802 formed E-rosettes and had receptors for IgG-Fc and C3. Their E-rosette formation was dependent on the SRBC treatment and

Table 1. Cell Markers on SPI-801 and SPI-802

<table>
<thead>
<tr>
<th></th>
<th>SPI-801</th>
<th>SPI-802</th>
<th>Normal Peripheral Lymphocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-rosette*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4°C</td>
<td>56.2-66.5</td>
<td>60.0-68.4</td>
<td>72-85</td>
</tr>
<tr>
<td></td>
<td>(0.8-12.0)</td>
<td>(0.6-13.5)</td>
<td></td>
</tr>
<tr>
<td>29°C</td>
<td>30.5-42.0</td>
<td>36.2-48.5</td>
<td>64-80</td>
</tr>
<tr>
<td></td>
<td>(0.2-9.8)</td>
<td>(0.4-10.5)</td>
<td></td>
</tr>
<tr>
<td>IgG-Fc ORBC</td>
<td>41.5-84.0</td>
<td>45.0-72.5</td>
<td>20-31</td>
</tr>
<tr>
<td>aggregated IgG</td>
<td>92.0-98.0</td>
<td>94.5-98.5</td>
<td>24-36</td>
</tr>
<tr>
<td>C3</td>
<td>19.2-31.6</td>
<td>18.6-34.5</td>
<td>15-26</td>
</tr>
<tr>
<td>OKT-3</td>
<td>0</td>
<td>0</td>
<td>62-82</td>
</tr>
<tr>
<td>OKT-4</td>
<td>0</td>
<td>0</td>
<td>38-56</td>
</tr>
<tr>
<td>OKT-6</td>
<td>0</td>
<td>0</td>
<td>0-1</td>
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<tr>
<td>OKT-8</td>
<td>0</td>
<td>0</td>
<td>20-34</td>
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<td>OKM1</td>
<td>88.5-94.5</td>
<td>90.2-96.8</td>
<td>6-19</td>
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<td>Leu-7</td>
<td>0</td>
<td>0</td>
<td>6-18</td>
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<td>Human monocyte</td>
<td>0</td>
<td>0</td>
<td>2-3</td>
</tr>
<tr>
<td>cALL</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>T cell</td>
<td>0</td>
<td>0</td>
<td>68-86</td>
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<tr>
<td>Surface Ig</td>
<td>0</td>
<td>0</td>
<td>14-21</td>
</tr>
<tr>
<td>Cytoplasmic IgM</td>
<td>0</td>
<td>0</td>
<td>0-0.1</td>
</tr>
<tr>
<td>Ia</td>
<td>99.0-99.5</td>
<td>98.0-99.5</td>
<td>23-50</td>
</tr>
<tr>
<td>Asialo-GM1</td>
<td>63.2-76.8</td>
<td>68.5-80.2</td>
<td>0</td>
</tr>
</tbody>
</table>

*Data obtained using neuraminidase-treated SRBC are shown and those obtained using AET-treated SRBC are in parentheses.

Fig. 1. A light micrograph of SPI-802 cells stained with May-Giemsa.

Fig. 2. An electron micrograph of SPI-802 cells. Note a round to oval nucleus with several nucleoli and abundant polyribosomes distributed throughout the cytoplasm. There is no profile of well developed endoplasmic reticulum. A small Golgi apparatus and many mitochondria are seen in one of the cells (x4800).
temperature. The percentages of cells rosetted with neuraminidase-treated SRBC were apparently higher than those with AET-treated SRBC. The percentages of the former cells at 4°C were 56.2%-66.5% in SPI-801 and 60.0%-68.4% in SPI-802, and they were lower at 29°C and 37°C, indicating their low affinity receptors for SRBC. IgG-Fc receptors were detected by the rosette method and the immunofluorescence method using aggregated IgG. When studied by the latter method between 16 and 18 mo of culture, more than 92% of the cells possessed IgG-Fc receptors. They were not reactive with OKT-3, OKT-4, OKT-6, OKT-8, anti-Leu-7, and anti-human monocyte monoclonal antibodies, and lacked cALL and T-cell antigens. They did not have surface Ig or cytoplasmic IgM. The two cell lines had asialo-GM₁ on the cell surfaces. TdT was not detectable in the cultured cells.

**Chromosome Analysis**

Karyotypic analyses on SPI-801 and SPI-802 were done 3 and 5 mo after the culture initiation, respectively. Fifty metaphases were analyzed on SPI-801 and 30 metaphases on SPI-802. The chromosomes of these two cell lines were essentially the same. Their chromosomes were in hypotriploid region, ranging from 63 to 67. In the vast majority of the cells analyzed, 6q⁻ and 11p⁻ were demonstrated, as shown in Fig. 3. In addition, many cells had several structurally abnormal chromosomes, two of which were probably 8p⁺ and 18p⁺.

**Cell-Mediated Cytotoxicity**

Representative results of the cytotoxicity assay are shown in Table 2. The freshly harvested leukemic cells had NK activity against K562: percent lysis was 76.8%, being 147.4% of the value of normal lymphocytes (52.1% ± 6.5%, mean ± SD) and higher than that of control leukemic cells from 12 childhood ALL patients (0.1% ± 0.3%). The first cytotoxicity assay of the cultured cells (exp. 2 in Table 2) was done on May 28, 1981. SPI-801 cells demonstrated cytotoxicity in the absence of any stimulation against K562 and Molt-3, but they did not show the activity against Raji. Similarly SPI-802 had spontaneous cytotoxicity against K562 but not against Raji. The stimulation of effector cells with PHA-MF or Con-A-MF had no significant augmentation effects on their cytotoxicity (p > 0.05), although in the separate experiments the
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Table 2. Results of Cytotoxicity Assay

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Effector Cells Were Stimulated With</th>
<th>Effector Cells</th>
<th>Cytotoxicity</th>
<th>Percent Lysis</th>
<th>Cytotoxicity</th>
<th>Percent Lysis</th>
<th>Cytotoxicity</th>
<th>Percent Lysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>K562</td>
<td></td>
<td>Molt-3</td>
<td></td>
<td>Raji</td>
<td></td>
</tr>
<tr>
<td>Exp. 1*</td>
<td>Fresh leukemic cells</td>
<td>None</td>
<td>1,699 ± 12</td>
<td>76.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
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<tr>
<td></td>
<td>Normal lymphocytes</td>
<td>None</td>
<td>1,268 ± 113</td>
<td>52.1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
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<tr>
<td>Exp. 2†</td>
<td>SPI-801</td>
<td>None</td>
<td>596 ± 12</td>
<td>8.5</td>
<td>1,630 ± 120</td>
<td>15.3</td>
<td>390 ± 10</td>
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<tr>
<td></td>
<td>SPI-802</td>
<td>None</td>
<td>464 ± 17</td>
<td>4.2</td>
<td>1,706 ± 100</td>
<td>16.9</td>
<td>383 ± 11</td>
<td>0</td>
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<td></td>
<td>SPI-802</td>
<td>Con-A-MF</td>
<td>652 ± 14</td>
<td>10.3</td>
<td>1,309 ± 170</td>
<td>8.6</td>
<td>371 ± 9</td>
<td>0</td>
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<tr>
<td></td>
<td>SPI-802</td>
<td>PHA-MF</td>
<td>826 ± 103</td>
<td>16.0</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
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<tr>
<td></td>
<td>SPI-802</td>
<td>PHA-MF</td>
<td>834 ± 127</td>
<td>16.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td></td>
<td>SPI-802</td>
<td>Con-A-MF</td>
<td>574 ± 22</td>
<td>7.8</td>
<td>ND</td>
<td>ND</td>
<td>406 ± 16</td>
<td>0.5</td>
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<td></td>
<td>Normal lymphocytes</td>
<td>None</td>
<td>1,807 ± 184</td>
<td>47.8</td>
<td>2,815 ± 286</td>
<td>40.1</td>
<td>433 ± 10</td>
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<td></td>
<td>Molt-3</td>
<td>None</td>
<td>340 ± 30</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
<td>390 ± 16</td>
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<tr>
<td></td>
<td>JM</td>
<td>None</td>
<td>338 ± 41</td>
<td>0</td>
<td>860 ± 50</td>
<td>0</td>
<td>386 ± 25</td>
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<td></td>
<td>CCRF-CEM</td>
<td>None</td>
<td>322 ± 18</td>
<td>0</td>
<td>892 ± 36</td>
<td>0</td>
<td>401 ± 8</td>
<td>0</td>
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<td></td>
<td>Jurkat-F 1884</td>
<td>None</td>
<td>345 ± 16</td>
<td>0</td>
<td>910 ± 68</td>
<td>0</td>
<td>392 ± 30</td>
<td>0</td>
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</table>

*Exp. 1 was done on November 9, 1980. Effector and target cells were mixed at 40:1 and incubated for 4 hr; cpm spontaneous release: 359; cpm maximal release: 2,104. Fresh leukemic cells were obtained from the present patient.
†Exp. 2 was done on May 28, 1981. Effector and target cells were mixed at 40:1 and incubated for 4 hr; cpm spontaneous release: 333 (K562), 900 (Molt-3), and 389 (Raji); cpm maximal release: 3416 (K562), 5675 (Molt-3), and 4019 (Raji).
‡Cpm: expressed as mean ± SD.
ND, not done.

treatments of normal lymphocytes with the PHA-MF and Con-A-MF increased the values of their percent lysis against K562 from 52.0% ± 4.7% to 85.8% ± 6.0% and 73.6% ± 2.3%, respectively. Leukemia cell lines of Molt-3, JM, CCRF-CEM, and Jurkat-F 1884 did not have cytotoxic activity against K562, Molt-3, and Raji.

The next experiment was performed to examine whether the cultured cells had the same cytotoxic activity as cytotoxic T cells and whether they had the activity against a target of normal cells. SPI-801 and SPI-802 were stimulated with MMC-treated lymphocyte mixtures from 5 volunteers, although we failed to study their HLA-DR, and then the cytotoxicity of the cells was assayed on PHA-induced normal T lymphoblasts derived from the counterpart of the stimulator lymphocytes. They did not demonstrate, however, apparent cytotoxicity in the experiment: values of percent lysis were less than 2.6% (4.6% of normal lymphocyte value). To further investigate characteristics of the cytotoxic activity of SPI-801 and SPI-802, a kinetic study was performed with Molt-3 as a target in which percent lysis was evaluated at 4 time points over a period of 16 hr. As shown in Fig. 4, the highest percent lysis was observed at 12 hr: percent lysis by SPI-801 and SPI-802 was 33.4% (83.5% of normal lymphocyte value) and 29.2% (73.0%), respectively. In the latest 4-hr assay for cytotoxicity on a K562 target 14 mo after the culture initiation, percent lysis by SPI-802 and SPI-802 was 4.6% (9.2% of normal lymphocyte value) and 5.2% (10.4%), respectively.

The cultured cells did not show significant levels of ADCC against antibody-coated CRBC in a single assay: percent lysis by SPI-801 was 0.8% ± 0.4% and that by SPI-802 was 1.0% ± 0.2% as compared with the value of normal lymphocytes of 31.2% ± 5.4% (mean ± SD).

DISCUSSION

The most striking finding in our patient is that the freshly harvested leukemic cells had NK activity against a leukemia-derived cell line of K562. In the other 12 ALL patients, the leukemic cells did not have any such NK activity, and this was not related to the stage of the illness or its chemotherapy. Fortunately, we were able to establish the leukemia cell lines retaining the activity and to confirm the results of studies on the fresh cells.

Cellular and functional similarities between the fresh leukemic cells and cultured cells indicate that the cell lines were derived from the leukemic cells. A decrease of the cytotoxic activity of the cultured cells as compared with that of the fresh leukemic cells may be explained by the usual decrease of the cytolytic activity during culture as demonstrated in T killer cell lines.23,24 Abnormal karyotypes in our cultured cell
lines, although we failed to perform chromosome analyses on the fresh cells, support their leukemic nature. The finding that the two cell lines, SPI-801 and SPI-802, shared the same chromosome abnormalities is indicative of an outgrowth of the leukemic cells of a neoplastic clone derived from a single cell rather than that of independent two normal NK cells. In addition, a 6q anomaly in our cell lines certainly favors their leukemic origin, as it is an anomaly closely associated with lymphoid malignancies and is not infrequent in ALL. The spontaneous growth of non-B lymphoblasts in the absence of T-cell growth factor also supports their leukemic nature. Another proof of the leukemic origin is the presence of asialo-GM1, a high density on the cultured cells, because the glycosphingolipid has been demonstrated to be a cell surface marker of ALL lymphoblasts. NK cells are considered to be non-T, non-B lymphocytes. Most NK cells have low affinity receptors for SRBC and avid receptors for IgG-Fc, and some of them have receptors for C3. Recent studies with monoclonal antibodies have demonstrated that human NK cells do not express T-cell-associated antigens such as OKT-3, OKT-4, and OKT-6 despite the presence of E-receptors, and most of them are positive for OKM1 or Leu-7 (HNK-1). Some NK cells express an Ia antigen. NK cells have ability to kill certain cultured cell lines and, unlike cytotoxic T cells, are not under any major histocompatibility complex restriction. NK activity is many times stronger against tumor (leukemia) derived cell lines, such as K562 and Molt-3, than cell lines derived from normal lymphocytes. Of tumor-derived cell lines, Raji is an exception, as it is virtually resistant to NK activity. The freshly harvested leukemic cells as well as the cultured cells had receptors for SRBC, IgG-Fc, and C3. The cultured cells were cALL-negative and lacked surface Ig and cytoplasmic IgM, excluding the possibility that they were B cells at any stage of maturation. They did not express T-cell-associated antigens as defined by anti-T heteroantiserum and monoclonal antibodies such as OKT-3, OKT-4, OKT-6, and OKT-8. They did not react with anti-Leu-7 or anti-human monocyte antibodies, but possessed OKM1 and Ia antigens. The fresh and cultured cells had spontaneous cytotoxicity against K562. The cultured cells had the cytotoxicity against Molt-3 as well but not against Raji and normal lymphoblasts. Such phenotypic characteristics and target-cell dependent spontaneous cytotoxicity of the freshly harvested and cultured cells described here are, therefore, identical to those of NK cells.

It is of interest that our cultured cells had avid receptors for neuraminidase-SRBC but modest receptors for AET-SRBC. As NK cells have affinity for neuraminidase-SRBC and the AET E-rosette formation is rather specific for T cells, this may be further evidence to support that the cultured cells are of NK cell origin. One of our unexpected results was the absence of a Leu-7 antigen on our cell lines. It is well known that lymphocyte surface antigens change with maturation. It is likely, therefore, that the Leu-7 expression on NK cells is restricted to mature cells in the circulation, and our patient’s leukemic cells were at earlier stages of maturation not to express the antigen. Alternatively, since NK cells are heterogeneous in the surface markers, there is a possibility that the leukemic cells were derived from an OKM1+ and Leu-7- population of NK cells.

Interleukin 2 (IL-2) has been shown to augment NK cell activity. Our MF containing IL-2, indeed, augmented normal NK cell activity. The NK activity of our cell lines, however, was not augmented by the MF treatment. Further studies are needed to determine whether the absence or marked decrease of their response to IL-2 resulted from the cell abberation during the leukemic transformation or long-term culture in vitro.

Evidence has been presented on the morphological association of human NK cells with large granular lymphocytes (LGL). LGL have relatively abun-
dant cytoplasm with many mitochondria, sparse endoplasmic reticulum, and electron dense granules.\textsuperscript{44} Our patient's leukemic cells had abundant cytoplasm with many mitochondria and no or few profiles of endoplasmic reticulum as do LGL, but lacked azurophilic granules. The absence of cytoplasmic granules in the leukemic cells with NK activity suggests that they lost the granules during the leukemic transformation or that NK cells comprise several lymphocyte populations different in the morphology. The morphological resemblance in several structures between LGL and the leukemic cells and our recent results that anti-SPI-802 rabbit antisera was reactive with LGL (data not shown) are in favor of the former possibility.

One may think it is surprising that our cell lines had NK activity but not ADCC, since NK cells have been found in the same lymphocyte population with IgG-Fc receptors as ADCC effector cells (K cells).\textsuperscript{32} In recent years, however, Neville\textsuperscript{33} has clearly demonstrated that NK cells and K cells are distinct subsets of IgG-Fc receptor-bearing lymphocytes. With respect to the relationship between effector cells for NK and ADCC activity, of interest is the demonstration of ALL with such functions, since the leukemia results from the proliferation of a malignant clone derived from a single cell of lymphocyte lineage. Pandolfi et al.\textsuperscript{4} reported an interesting patient with chronic lymphocytic leukemia whose leukemic cells had ADCC but not NK activity. The patient is, therefore, in contrast with ours in the functions of the leukemic cells. The clonal expansion of a lymphocyte population with either NK or ADCC activity as demonstrated in these patients certainly supports the view that NK and K cells are distinct subsets of lymphocytes.

As our patient's leukemic cells had a function of NK activity, it is of interest to determine clinical characteristics of the disease. Our patient had massive leukemic infiltration, high blast cell counts, and a poor prognosis. On the basis of the hematologic findings and E-rosette formation by the leukemic cells, the diagnosis of T-ALL was initially made. It was an unexpected finding that a chest x-ray film failed to demonstrate mediastinal enlargement in our patient, because most T-ALL patients with such clinical features as those of the patient have thymic enlargement.\textsuperscript{45,46} Since NK cells are thymus-independent cells,\textsuperscript{5,22} however, the absence of thymic enlargement despite massive leukemic infiltration in our patient appears conceivable.

NK cells play important roles in host defense against virus infections\textsuperscript{48,49} as well as tumor development and metastasis\textsuperscript{47,50} in vivo. In our patient, apparent episodes of infections were not found during the observation period. It is unclear, however, whether the absence of infections was due to the NK activity of the leukemic cells. Further studies on such patients as ours will disclose the clinical characteristics of ALL of NK cell origin.

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Childhood lymphoblastic leukemia with natural killer activity: establishment of the leukemia cell lines retaining the activity

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