A Case of Chronic Lymphocytic Leukemia With Properties Characteristic of Natural Killer Cells

By Kyogo Itoh, Kenya Tsuchikawa, Toshiichi Awataguchi, Kenichi Shiiba, and Katsuo Kumagai

A case of chronic lymphocytic leukemia that consisted of a homogeneous population of cells that had properties similar to those described for natural killer (NK) cells is presented. These leukemic cells had a morphology of large granular lymphocytes (LGL) and receptors for sheep erythrocytes (ER) and the Fc portion of IgG (FcγR). They expressed pan-T antigens OKT3 and Leu-4, but neither helper/inducer T-cell differentiation antigens OKT4 and Leu-3a nor cytotoxic/suppressor T-antigens OKT8 and Leu-2a. HNK 1 antigen, which can be expressed on human NK cells, could be detected on almost all leukemic cells (LGL), whereas a myeloid differentiation antigen, OKM1, which can be expressed on macrophages, granulocytes, and NK cells, was not detected. Thus, it was concluded that the leukemia cells had a characteristic profile of the LGL.10,11 Human LGL reacting to this unique antibody had both NK and K activities and lacked the mitogenic response to phytohemagglutinin (PHA) and concanavalin-A (Con-A).11–13 The HNK-1+ cells also showed variable expression of pan-T-cell antigens like OKT3 and a myeloid antigen OKM1, suggesting the presence of distinct phenotypes of HNK-1+, OKT3−, OKM1+ and HNK-1−, OKT3+, OKM1− in human NK cells.13

We report a case of a leukemia patient with a homogeneous population of LGL that possibly derives from NK cells. The leukemic cells showed homogeneous expression of HNK-1 and pan-T antigens OKT3 and Leu-4, but no expression of T-cell differentiation antigen, OKT4, OKT8, Leu-3a, and Leu-2a, and a myeloid antigen OKM1, and when incubated at 37°C for 24 hr, showed a potent cytotoxic activity accompanied by production of interferon (IFNγ).

CASE REPORT

The patient (T.Y.), a 50-yr-old female, visited the hospital because of hematuria in May 1976. Physical examination revealed an enlarged liver of 5 cm below the right costal margin. The hematocrit (Hct) was 26%; hemoglobin (Hb), 9.4 g/dl; white blood cell (WBC) count, 10,200/cu mm with 97% lymphocytes; platelet count, 97,000/cu mm.

She received no specific treatment and was relatively well until 6 yr later, when anemia was pointed out again at another hospital that she visited because of hemorrhoids, and she was referred to National Sendai Hospital for further evaluation in July 1981. Physical examination revealed a palpable liver and spleen of 5 and 5 cm below the respective costal margins, but there were neither lymphadenopathy nor skin lesions. Laboratory testing revealed Hct 25.8%; Hb 7.8 g/dl; WBC count 8100/cu mm, with 91% lymphocytes, 2% segmented neutrophils, 1% eosinophil, and 6% monocytes; and platelet count 64,000/cu mm. Almost all the lymphocytes were large and possessed numerous large azurophilic granules. The bone marrow...
The CLL patient (T.Y.) described above, was a 3 B-cell type CLL, and Peripheral blood mononuclear cells were obtained by the Ficoll-Hypaque gradient centrifugation from both patients and healthy donors. Patients' bone marrow mononuclear cells were also prepared by Ficoll-Hypaque centrifugation from bone marrow specimens. T cells were defined by their ability to form rosettes with sheep erythrocytes (ER cells) at 4°C. B cells were enumerated by the presence of surface immunoglobulin (sIg) detected by direct immunofluorescence as described previously. Monocytes were identified by phagocytosis of heat-inactivated yeasts. Staining with May-Grünewald-Giemsa and acid phosphatase were carried out on cytotoxicity cell smears.

**Culture of Cells**

The test lymphocytes were suspended with RPMI 1640 medium containing 10% heat-inactivated fetal calf serum, 100 U/ml of penicillin, and 100 μg/ml of streptomycin (RPMI/FCS).

**Monoclonal Reagents**

Monoclonal HNK-1 antibody was provided by Dr. T. Abo of Alabama University, Birmingham, AL. Anti-Leu-4 (anti-pan-T-cell), anti-Leu-3a (anti-helper/inducer T cell), and anti-Leu-2a (anti-suppressor/cytotoxic T cell) were purchased from Becton Dickinson Laboratory, Sunnyvale, CA. Monoclonal antibodies of OK series, OKT3 (anti-pan-T), OKT4 (anti-helper/inducer T), and OKT8 (anti-suppressor/cytotoxic T), were purchased from Ortho Diagnostics, Raritan, NJ. Anti-asialo GM1 antibody was provided by Dr. K. Okumura of Tokyo University, Tokyo. Cells reactive with these antibodies were enumerated by indirect immunofluorescence.

**Cytokilling by HNK-1 Antibody**

Four million test cells were treated with 0.5 ml of 1:40 diluted HNK-1 antibody (250 μg/ml) or heat–inactivated normal mouse serum (NMS) in RPMI/FCS for 45 min at 4°C. After 2 washings, the cells were treated with 1 ml of 1:4 diluted rabbit complement (Behring Inst., Marburg, Germany) in RPMI/FCS for 45 min at 37°C. The viability of cells after the treatment was judged by dye exclusion. The viable cells were adjusted to 2 × 10⁶ cells/ml, incubated for 1 day at 37°C, and then assayed for their NK activity and interferon production.

**Natural Killer and ADCC Assays**

Target cells used for NK assay were erythroleukemic cells K562, three T-cell lines (Molt 4F, CEM and P12), two B-cell lines (Daudi and Raji), a monocytic line (U937), and concanavalin-A (Con-A) blast T cells, which were obtained from the peripheral lymphocytes of a healthy donor after incubation with 2.5 μg/ml of Con-A for 4 days. For antibody-dependent cell-mediated cytotoxicity (ADCC) assay, mouse mastocytoma cell line, P815, and chicken red blood cells (CRBC) were used as target cells in the presence of rabbit antiserum as described previously. These two target cells were resistant to natural cytotoxicity.

NK and ADCC assays were carried out as described previously. Briefly, 10⁷ target cells were incubated with 100 μCi of Na₂¹⁴C₁₄O₄ (Daiichi Laboratory, Tokyo, 0.5 mCi/0.5 ml) for 1 hr at 37°C. The labeled cells were then washed 3 times, resuspended in RPMI/FCS, and further incubated for 2 hr at 37°C. After incubation, the cells were washed twice and adjusted to 5 × 10⁶ cells/ml in the medium. Then, 200 μl lymphocyte suspension was mixed with 2 μl of 5 × 10⁶ target cells in triplicate in Nuncron round-bottomed micro-test plates. For ADCC assays, 2 μl of antibody to target cells (10⁻⁵) were added into the mixtures. After incubation in 5% CO₂/95% air at 37°C for 4 hr, 100 μl of supernatant was removed from each well for counting released ¹⁴C₁₄O₄ by an autogamma counter. The specific NK and ADCC activities were calculated by the formula described previously. The NK activity was also expressed as lytic units (LU)/10⁶ cells with 1 LU being the number of effector cells required to cause 30% lysis of target cells.

**Separation of the Patient's Serum**

The serum of the NK-leukemia was fractionated by 33% ammonium sulfate [(NH₄)₂SO₄] precipitation at 4°C with gentle stirring. The precipitates, which were obtained by 33% (NH₄)₂SO₄ saturation, were isolated by centrifugation at 10,000 g for 10 min, dissolved in 4 ml of phosphate-buffered saline (PBS) at pH 7.2, and finally dialyzed extensively against PBS. This solution was applied to a calibrated 2.5 × 85 cm Sephacryl S-200 (Pharmacia, Uppsala, Sweden) column equilibrated in PBS (4°C). The column was eluted at 4°C at a rate of 15 ml/hr. The major protein peak of the 7S obtained after gel-filtration was applied to immunoelectrophoresis and identified as IgG. Fractions were collected, concentrated in Amicon ultrafiltration system with UM-10 Diaflow membrane (Amicon Corp. Lexington, MA), and assayed for inhibitory activity to NK lysis by lymphocytes of healthy donors and the patient. The supernatant of ammonium sulfate precipitation was concentrated, dialyzed, and then assayed for suppressive activity for NK lysis. Human IgG (polyclonal IgG; purity, 99%, Behring Inst., Marburg, Germany) and bovine serum albumin (Wako Chemical Co., Japan) were used as inhibition controls.

**Interferon Assay**

Mononuclear cell suspensions were suspended to contain 2 × 10⁶ cells/ml in RPMI/FCS and incubated in RPMI/FCS at 37°C in 5% CO₂/95% air. After incubation for 1 or 2 days, the cell suspensions were centrifuged and the supernatant fluids were assayed for antiviral activity using human amnion (WISH) cells as previously described.

The type of interferon produced by the patient's lymphocytes was determined by antiserum neutralization and acid stability tests. Rabbit antiserum neutralizing human IFNα and IFNβ was obtained from Toray Industries, Inst., Japan. For neutralization, 0.2 ml of test interferon preparations at 100-200 U/ml was mixed with an equal volume of antiserum or culture medium as control. After incubation
RESULTS

Morphology of Leukemic Cells

May-Grünwald-Giemsa staining (Fig. 1) showed that the patient’s mononuclear cells at any time tested contained 80%-90% leukemic cells with a morphology quite similar to human large granular lymphocytes (LGL), which were identified as a major effector of the human NK system:10-14 large-sized lymphoid cells with pale and characteristically granular cytoplasm, a reniform nucleus, and a high cytoplasmic:nuclear ratio compared with other conventional lymphocytes. They did not adhere to plastic, did not phagocytize yeast or other particles, did not carry peroxidase, but they did contain acid-phosphatase enzyme in the cytoplasm.

Marker Analysis of Leukemic Cells

As shown in Table 1, marker analysis by rosette formation for surface receptors and T and NK differentiation antigens using monoclonal antibodies of HNK-1, OKT, and Leu series revealed that nearly all of the patient’s mononuclear cells (90%-98%) were ER+ and Fcy-R+. A few Fcy-R+ cells, slg+ cells, and monocytes were found. Of the cells, 80%-90% were LGL and positive for HNK-1 antigen, which was expressed on human NK cells (LGL). The percentages to the similar levels of pan-T antigen OKT3+ and Leu-4+ cells were also found, whereas a few cells were helper/inducer T antigens OKT4+ and Leu-3a+ and killer/suppressor T antigens OKT8+ and Leu-2a+. These results indicate that the leukemic cells (LGL) were HNK-1+, OKT3+, Leu-4+, OKT4+, OKT8+.

Table 1. Analysis of Surface Receptors, Antigens, Morphology (LGL), and Function (Phagocytosis) of the Patient’s Peripheral Blood Mononuclear Cells (PBMC) and Bone Marrow Cells (BMC)

<table>
<thead>
<tr>
<th>Marker</th>
<th>PBMC*</th>
<th>BMC†</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER (4°C)</td>
<td>90-98</td>
<td>75</td>
</tr>
<tr>
<td>Fcy-R</td>
<td>90-98</td>
<td>85</td>
</tr>
<tr>
<td>Fcy-R</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Surface Ig</td>
<td>&lt;1</td>
<td>&lt;5</td>
</tr>
<tr>
<td>Phagocytosis</td>
<td>&lt;5</td>
<td>24</td>
</tr>
<tr>
<td>OKT3</td>
<td>80-90</td>
<td>ND</td>
</tr>
<tr>
<td>OKT4</td>
<td>&lt;5</td>
<td>ND</td>
</tr>
<tr>
<td>OKT8</td>
<td>&lt;5</td>
<td>ND</td>
</tr>
<tr>
<td>Leu-4</td>
<td>80-90</td>
<td>ND</td>
</tr>
<tr>
<td>Leu-3a</td>
<td>&lt;5</td>
<td>ND</td>
</tr>
<tr>
<td>Leu-2a</td>
<td>&lt;5</td>
<td>ND</td>
</tr>
<tr>
<td>HNK-1</td>
<td>80-90</td>
<td>52</td>
</tr>
<tr>
<td>OKM1</td>
<td>&lt;5</td>
<td>ND</td>
</tr>
<tr>
<td>ASGM1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>Large granular lymphocytes (May-Giemsa)</td>
<td>80-90</td>
<td>40</td>
</tr>
</tbody>
</table>

*Results represent the values obtained with 7-8 PBMC specimens sampled at different times during a 6-mo period.
†Values with BMC sampled in February 1982.
ND, not done.

Leu-3a+, and Leu-2a-. These were negative for OKM1, a human myeloid antigen, and ASGM1, a marker for murine NK cells.16,17

In the mononuclear cells obtained from the bone marrow, 75% of ER+, 85% of Fcy-R+, and 52% of HNK-1+ cells were found. Almost all of HNK-1+ cells in the bone marrow were also identified morphologically as LGL under the microscope.

NK Activity of Patient’s Lymphocytes

NK activity of the patient’s lymphocytes was assayed by 51Cr release from K562 targets (Table 2). No definite activity was detected in the freshly prepared patient’s peripheral lymphocytes. When incu-

Table 2. Appearance of NK Activity in Leukemic Cells After Incubation at 37°C

<table>
<thead>
<tr>
<th>Incubation at*</th>
<th>Percent NK Lysis at E/T of 106 cells</th>
<th>LU‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>— (before incubation)</td>
<td>7  3  2.5</td>
<td>0.1</td>
</tr>
<tr>
<td>37°C</td>
<td>86  71  60</td>
<td>160</td>
</tr>
<tr>
<td>4°C</td>
<td>20  17  13</td>
<td>2</td>
</tr>
<tr>
<td>37°C in the presence of 10³ U/ml IFNβ</td>
<td>94  89  71</td>
<td>1,000</td>
</tr>
</tbody>
</table>

*Incubation in RPMI/FCS for 24 hr.
†NK cytotoxicity against K562 cells. Values represent the means of 5 different experiments.
‡Lytic units/10⁶ cells, with 1 LU being the number of effector cells required to cause 30% lysis of target cells.
Fig. 2. NK activity of the patient's lymphocytes as a function of time. Peripheral blood mononuclear cells of the present patient (---●---), a healthy donor (-----○-----), and a patient with B-cell CLL (-----△-----) were individually incubated with RPMI/FCS at 37°C in 5% CO₂/95% air for 2 days. Before and after incubation for 1 or 2 days, NK activity against K562 was assayed at E/T ratio of 5.

bated at 37°C for 24 hr, however, the lymphocytes showed a potent NK activity against the same target. When incubated at 37°C in the presence of human interferon (IFNβ), the increase of NK activity was much more demonstrable. When the lymphocytes were incubated at 4°C for 24 hr, they showed a much lesser activity as compared with those incubated at 37°C, indicating that the appearance of NK activity in the patient's lymphocytes during culture was dependent on temperature.

Figure 2 shows a demonstrable increase in NK activity of the patient's lymphocytes in culture in contrast with no changes in NK activities of the lymphocytes from a normal donor and a patient with B-cell leukemia in culture at least for 2 days. Neither increase nor decrease in number of cells in culture was seen throughout the observation periods.

Profile of NK Cytotoxicity by the Patient's Lymphocytes

The cytotoxic profile of the patient's lymphocytes, which appeared after cultivation for 24 hr, was characterized by 51Cr assay using various lymphoid cell lines and Con-A-activated normal human lymphocytes (Con-A-blast) (Table 3). The lymphocytes were highly cytotoxic to an erythroleukemic K562 cell line and three T-cell lines: Molt 4F, CEM, and P12. They also showed a low NK activity against a monocytic cell line, U937. No activity was found against the B-cell lines, Daudi and Raji, and Con-A-blast. Bone marrow mononuclear cells also showed a high NK activity against K562 and T-cell lines, but no or a low cytotoxicity against other targets.

As shown in Table 3, the patient's lymphocytes had an NK activity more preferentially effective with T-cell lines, as compared with the NK cytotoxicity by peripheral blood lymphocytes from normal donors assayed at the same time using the same targets.

No or a slight ADCC activity was found in the

Table 3. A Profile of NK and K Cytotoxicities by Leukemic Cells

<table>
<thead>
<tr>
<th>Targets</th>
<th>Cell Origin</th>
<th>Patient's Peripheral Lymphocytes</th>
<th>Patient's Bone Marrow Cells</th>
<th>Peripheral Lymphocytes From Healthy Donors</th>
</tr>
</thead>
<tbody>
<tr>
<td>NK cytotoxicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K562</td>
<td>Erythro-leukemia</td>
<td>77*</td>
<td>36*</td>
<td>46 ± 7†</td>
</tr>
<tr>
<td>Molt 4F</td>
<td>T</td>
<td>68</td>
<td>23</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>CEM</td>
<td>T</td>
<td>72</td>
<td>32</td>
<td>31 ± 10</td>
</tr>
<tr>
<td>P12</td>
<td>T</td>
<td>90</td>
<td>37</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Con-A-blast‡</td>
<td>T</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Daudi</td>
<td>B</td>
<td>&lt;1.0</td>
<td>5</td>
<td>19 ± 4</td>
</tr>
<tr>
<td>Raji</td>
<td>B</td>
<td>&lt;1.0</td>
<td>2</td>
<td>18 ± 8</td>
</tr>
<tr>
<td>U937</td>
<td>Monocyte</td>
<td>12</td>
<td>&lt;1.0</td>
<td>12 ± 6</td>
</tr>
<tr>
<td>ADCC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P815</td>
<td>Mastocytoma (mouse)</td>
<td>&lt;1.0</td>
<td>ND</td>
<td>12 ± 4</td>
</tr>
<tr>
<td>CRBC</td>
<td></td>
<td>4.0</td>
<td>ND</td>
<td>21 ± 6</td>
</tr>
</tbody>
</table>

*Values represent the means of one representative experiment in which all NK assays for each target were carried out at the same time.
†Values represent the means ± SD of five healthy donors.
‡Test cells were the peripheral blood lymphocytes of a healthy donor after incubation with 2.5 μg/ml of Con-A for 4 days.
human fibroblast IFNa and IFNβ at 300 U/ml was used.

The antiserum neutralizing IFNa and IFNβ, but not IFNγ, markedly inactivated the interferon.

Antibody neutralization of the interferon produced by the patient's lymphocytes tested using either P815 cells or CRBC as a target (Table 3).

**Interferon Production Induced Concomitantly With the Expression of NK Activity in Culture**

We have also found that the patient's lymphocytes in culture produced an antiviral activity in the culture fluids. An antiviral activity produced was shown to be interferon by the criteria based on the antiviral spectrum, species specificities, enzymatic degradation, and others (data not shown). As shown in the representative results of Table 4, the interferon at 180–240 U/ml was produced by the cells in the culture fluids concomitantly with the appearance of cytotoxic activity in the cells after cultivation at 37°C. Neither interferon production nor NK activity was induced in the culture at 4°C. A low level of interferon (10 U) was produced in the lymphocyte culture from normal donors. The interferon titers produced in these cell cultures appeared to correlate well with the NK activity induced in culture.

The type of interferon produced by the patient's lymphocytes in culture was determined by antiserum neutralization and acid lability tests (Table 5). Treatment with pH 2 markedly inactivated the interferon. The antiserum neutralizing IFNa and IFNβ, but not IFNγ, failed to neutralize the interferon produced by

<table>
<thead>
<tr>
<th>Lymphocytes</th>
<th>Interferon Production (U/ml)</th>
<th>NK Lysis (LU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient's peripheral lymphocytes</td>
<td>37°C, 180</td>
<td>115 (0.1)‡</td>
</tr>
<tr>
<td>Patient's peripheral lymphocytes</td>
<td>4°C, &lt;10</td>
<td>1</td>
</tr>
<tr>
<td>Patient's bone marrow cells</td>
<td>37°C, 60</td>
<td>18 (0.1)</td>
</tr>
<tr>
<td>Peripheral lymphocytes of healthy donors</td>
<td>37°C, 10§</td>
<td>17§</td>
</tr>
</tbody>
</table>

*Two million cells per ml were incubated with RPMI/FCS for 24 hr.
†Lytic units/10⁶ cells.
‡The numerals in parentheses represent LU before incubation.
§Values represent the means of three different healthy donors.

**Characterization of NK Effector Cells and Interferon-Producing Cells**

When the patient's lymphocytes were treated with HNK-1 antibody and complement at 37°C, 70% or more of the patient's cells (LGL) were destroyed, as revealed by dye exclusion. Therefore, in order to characterize the effector cells of NK cytotoxicity and the cells involved in interferon production, we have examined both activities of cells treated with HNK-1 antibody and complement after culture at 37°C for 24 hr (Table 6). The results clearly show that both NK activity and ability to produce interferon were completely lost from the patient's lymphocytes after treatment. Antibody or complement alone had no effect on either activity. These results indicate that HNK-1-positive leukemic cells may be effector cells in NK cytotoxicity, and cells involved in interferon induction are probably producers of interferon.

**Suppressive Effect of Patient's Serum on NK Activity**

To clarify the mechanisms by which the NK activity of the patient's leukemic cells in the blood was almost completely suppressed, we examined the possible effect of the patient's sera suppressing NK activity of patient's and normal lymphocytes. Figure 3 shows the results of experiments in which the NK activity of lymphocytes from two healthy donors were assayed in the presence of sera from the patient and healthy donors. The results clearly show that NK activities of both specimens were greatly suppressed by the addition of the patient's serum at 5%. The serum of a normal donor, even at 20%, had no effect on the NK activity.

The patient's serum contained immunoglobulins (Ig) at 30–40 mg/ml, which were concentrations 2–3-fold higher than those in normal serum. Therefore, we isolated the crude Ig from the patient's serum by 33% ammonium sulfate precipitation and tested for their suppressive effect on the NK activity of patient's
lymphocytes in which a high level of NK activity was expressed after culture. The results depicted in Fig. 4 show that the crude Ig from the patient’s serum at 1–2 mg/ml greatly suppressed the NK activity of patient’s lymphocytes, whereas the serum protein depleted of Ig had no effect on the activity. The IgG preparations purified from healthy humans and bovine serum albumin, even at 2 mg/ml, had no effect on the NK activity. The IgG fraction of the patient’s crude Ig at 0.5 mg/ml, but not other fractions, separated by chromatography on Sephadex G-200 also suppressed the NK activity (data not shown).

Comparison of the Surface Marker Profile and NK Activity of our Leukemic Cells and Those of Other Leukemic Cells

Preliminary experiments with other leukemia patients showed that at least 1 T-ALL, 1 null-ALL, 3 B-CLL, and 1 CML had no higher percentages of HNK-1 + cells (<1% to 11%) and LGL (<1% to 5%) as compared with those of HNK-1 + cells (11% ± 7%) and LGL (7% ± 4%) in normal donors. All the patients, with the exception of 1 B-CLL, showed no NK activity even after incubation of cells at 37°C. No interferon production was detected in all leukemia cases (data not shown).

The leukemia disease that should be discriminated from our case may be T-CLL cases in which ER + and pan-T-antigen-positive leukemia cells were found. In the present study, we could not examine whether these T-CLL also show LGL-like leukemic cells with HNK-1 antigenicity and NK activity, because T-CLL is uncommon in Japan. Table 7 shows a comparison of surface marker profile and NK activity of our leukemic cells with those of T-CLL cells reported by previous investigators for which adequate marker analysis was provided.1–9 As shown in Table 7, all cases of T-CLL, without exception, were positive for ER and OKT3. Fcγ-R also appears to be a common property of these and our leukemia cases, with the exception of...
cases 2 and 5 in which high percentages of OKT4+ cells were found.

It appears that all these T-CLL cases reported are included in the T populations with OKT4 and/or OKT8 as follows: case 1, OKT8+; 2, OKT4+; 3, OKT8-; 4, OKT4+ and OKT8-; and 5, OKT4+. Our case has no definite T-cell differentiation antigens of either OKT or Leu series. No NK activity has been detected in any case of T-CLL reported, although ADCC activity could be found in 4 of 5 cases. Our leukemic cells showed no definite ADCC. Thus, it is concluded that our case has a unique profile with respect to T-cell differentiation antigens and NK (K) activity as compared with those of T-CLL previously reported.

**DISCUSSION**

In this article we present a patient with a homogeneous population of leukemic cells that can possibly be identified as of NK cell origin. Identification of the cell origin as NK was based on the following characteristics: (1) a morphological feature of large granular lymphocytes (LGL), (2) a unique profile of surface markers including HNK-1 antigenicity, (3) a potent NK activity, (4) a clear response to interferon with augmentation of NK activity, and (5) production of interferon in culture.

More than 80% of the peripheral blood lymphocytes and 40%–50% of bone marrow mononuclear cells of our patient were identified as a homogeneous population of cells that was morphologically undistinguishable from human LGL: large-sized lymphoid cells with a pale cytoplasm containing azurophilic granules, a reniform nucleus, and a high cytoplasmic/nuclear ratio. A high content of acid phosphatase was also found in the cell. The patient's leukemic cells were found to be ER+, Fcγ-R-, Fcμ-R-, and sIg-. The same profile of these surface markers has been shown in human NK cells.10-14 The leukemic cells also had pan-T antigens OKT3 and Leu-4, whereas OKT4 and Leu-3a (helper/inducer T antigens) and OKT8 and Leu-2a (killer/suppressor T antigens) were not detected on the cell. This marker profile is similar to that of murine NK cells, in which a T-cell antigen (Thy-1) is variably present, but no differentiation antigens (Lyt-1 and Lyt-2) can be expressed24 (Suzuki et al., J Immunol 130:981, 1982).

Analysis of surface markers also demonstrated that almost all LGL in this patient's lymphocytes were positive for HNK-1, which was recently shown by monoclonal antibodies to be expressed on human NK cells in a high concentration.10,11 The leukemic cells showed no antigenicity of OKM-1, which is expressed on NK cells as well as on monocytes and granulocytes. Abo and Balch10 and Abo et al.11,12 reported that HNK-1+ cells showed variable expression of OKT3 and OKM-1, suggesting the presence of distinct phenotypes of HNK-1+, OKT3+, OKM-1+ and HNK-1+, OKT3-, OKM-1+ in human NK cells. The phenotype of our leukemia cells is coincident to the latter.

Repeated experiments at different times during the course of her disease always demonstrated no NK activity in the freshly isolated leukemic cells. However, when the cells were incubated at 37°C in conventional culture medium, they showed a potent NK activity. The specific NK activity of the patient's leukemic cells assayed by K562 was tenfold higher than that of the healthy donor. This high level of specific NK activities in the patient's leukemic cells coincided with the high frequencies of LGL. Bone marrow cells, in which HNK-1+ LGL were found, also showed a potent NK activity after culture. The demonstration of leukemic cells (LGL) being responsible for NK activity in the patient's lymphocytes was also verified by a concomitant destruction of the cells with inactivation of the NK activity by anti-HNK-1 and complement. The NK activity of leukemic cells was also markedly reduced after treatment with OKT3 antibody and complement, although the reduction rate varied depending on the experiments (data not shown).

The patient's leukemic cells of supposedly monoclonal origin showed cytotoxicity with a wide spectrum of target cells of erythroleukemic, T-cell, and monocyte origin. However, these were not cytotoxic to B-cell

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**Table 7. Surface Receptors, Antigens Defined by Monoclonal Antibodies, and NK and ADCC Activities in the Peripheral Blood Lymphoid Cells of our Patient and Other Cases Reported as T-CLL**

<table>
<thead>
<tr>
<th>Case Report</th>
<th>Surface Receptors</th>
<th>Antigens</th>
<th>ADCC Activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ours</td>
<td>ER</td>
<td>Fcγ-R</td>
<td>Fcμ-R</td>
<td>OKT3</td>
</tr>
<tr>
<td>1</td>
<td>95-98</td>
<td>90-98</td>
<td>&lt;5</td>
<td>80-90</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>NR†</td>
<td>NR</td>
<td>96</td>
</tr>
<tr>
<td>3</td>
<td>&gt;90</td>
<td>55-70</td>
<td>&lt;10</td>
<td>98</td>
</tr>
<tr>
<td>4</td>
<td>+</td>
<td>50</td>
<td>30</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>+</td>
<td>0</td>
<td>6</td>
<td>91</td>
</tr>
</tbody>
</table>

*Signs in parentheses represent the NK or ADCC activity of the cell after overnight incubation at 37°C.
†Not reported.
lines tested that were susceptible to lysis by normal human lymphocytes. Although many investigators initially considered the cytotoxic activity of NK cells to be nonspecific, increased evidence has accumulated for a selective pattern of reactivity and for multiple antigenic specificities on susceptible target cells.23,25

Recently, a major question has been whether each NK cell can recognize the full range of susceptible targets or whether there are subpopulations of NK cells, each reactive with a more limited number of targets.23,25

This case may be useful in resolving such a question about the specificity of NK cells. No ADCC activity was found in the leukemic cells, even after cultivation at 37°C. We do not at present have a satisfactory explanation for this failure of detection of ADCC activity in the patient’s lymphocytes.

The failure to detect NK activity in the patient’s freshly isolated lymphocytes has been shown to be due to the suppression by a serum factor(s), one of which may be IgG. Recently, Sulica et al.26 reported that binding of IgG to human peripheral blood mononuclear cells reversibly inhibited their NK activity. They also showed that the inhibitory IgG was monomeric IgG that had properties with those described for cytophilic IgG and was mediated through the Fc region of IgG, proposing a novel mechanism for negative regulation of NK activity. The present patient showed 2–3-fold increase of serum IgG concentrations as compared with those of healthy persons. Therefore, it might be possible that the increased IgG in the patient's serum contained such cytophilic IgG, which could effectively suppress the NK activity. This possibility is under investigation using further purified patient’s serum IgG.

We also found that the patient’s lymphocytes, when incubated at 37°C, spontaneously produced IFNγ in the culture fluids. Treatment of cells with anti-HNK-1 and complement resulted in a complete reduction of IFNγ production, as was the case with NK activity, indicating that the leukemic cells were involved in the induction of IFNγ production. We have shown that murine NK cells and NK clones when incubated at 37°C in the presence of interleukin-2 (IL-2) produce IFNγ in the culture fluids (Handa et al., submitted for publication). Human NK cells from normal donors isolated by Percoll density gradient centrifugation were also found to produce IFNγ when grown in IL-2 (unpublished observation), although when incubated in the absence of IL-2, these produced no definite levels of IFN spontaneously. These results indicate that NK cells may have the potential to produce IFNγ, which can be expressed in the presence of IL-2. However, it remains uncertain whether this IFN production in leukemic cell cultures involves IL-2 or other unknown factor(s) that might be carried over from the patient’s serum. Hooks et al. also reported that CLL patients’ lymphocytes that consisted of 95% Tγ cells (OKT3+, OKT8+, OKT4+) spontaneously produced IFNγ in vitro, unlike lymphoid cells from normal individuals and patients with other lymphoid malignancies.

Neither HNK-1 antigenicity nor NK activity was found in the leukemic cells of at least 1 T-ALL, 1 null-ALL, 3 B-CLL, and 1 CML patients. Leukemic cells of all these cases showed no morphology like LGL, indicating that these can easily be discriminated from our case with respect to both surface markers and morphology. However, earlier studies27,28 showed the presence of CLL cases with clinical appearance and cell morphology similar to that of our case. Toben and Smith27 characterized a group of T-CLL as “clinically atypical T-CLL” because either the white blood count was low (4000–18,000/cu mm), marrow infiltration was equivocal, or the patient was young (20–50 yr old). None of these patients had lymphadenopathy and skin lesions, and at least one patient lived more than 5 yr with his disease. Brouet et al.28 also described a group of CLL patients in which the blood and bone marrow was moderate. These T-CLL had leukemic cells morphologically similar to ours: large-sized lymphoid cells of ER+ and Fcγ-R+ with reniform nucleus and pale cytoplasm containing a high content of lysozomal enzymes and azurophilic granules. In both cases, the presence or absence of T-differentiation antigens or NK activity has not been described.

Recent investigations using monoclonal antibodies have also characterized T-CLL in which leukemic cells with pan-T antigens OKT3 or Leu-4 as well as ER and Fcγ-R are found.49 However, all these leukemias, as far as we could infer, had either helper/inducer T antigens (OKT4) or killer/suppressor T antigens (OKT8) or both on the surface, which were not detected on the leukemic cells of our patient (Table 7). With all cases of these leukemias reported, no NK activity has been detected or described. Therefore, our case appears to have unique characteristics differing from T-CLL, although we have at present no data on the NK activity and HNK antigenicity with such T-CLL cases, as the disease is uncommon in Japan. Further investigations on their antigenic profiles using anti-T-cell differentiation antigens and HNK-1 and on their functional profiles such as NK activity and interferon production may enable us to classify non-B chronic lymphocytic leukemia in more detail.

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A case of chronic lymphocytic leukemia with properties characteristic of natural killer cells

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