We examined the expression of cell-surface interleukin-2 (IL-2) receptor (Tac antigen) on peripheral blood leukemic cells and measured soluble IL-2 receptor (sIL-2R) levels in sera from chronic myelogenous leukemia (CML) patients with blastic crisis. Flow cytometric analysis performed by dual immunofluorescence in three cases demonstrated coexpression of Tac antigen with myeloid (CD13, CD14, or CD33) or lymphoid (CD10) antigen on significant proportions of peripheral blood leukemic cells. Radiolabeled IL-2-binding assay demonstrated the specific IL-2 binding sites in each case examined. The exogenous IL-2, however, failed to induce proliferative response. A myeloid cell line, Yut-K3, established from peripheral blood leukemic cells from a CML patient with blastic crisis, also expressed cell-surface Tac antigen and CD13 concurrently. sIL-2R assay showed that Yut-K3 released a detectable amount of sIL-2R in its culture supernatant. The serum sIL-2R levels were significantly elevated (range: 2,580 to 172,000 U/mL) in 12 CML patients with blastic crisis and were slightly elevated in ten patients in chronic phase (range: 250 to 820 U/mL) and in three in accelerated phase (range: 790 to 1,305 U/mL) compared with those in 24 normal controls (range: 70 to 695 U/mL, P < .01). These results indicated that the leukemic cells from CML patients with blastic crisis expressed and released IL-2 receptor (Tac antigen). Longitudinal studies performed in three cases of CML with blastic crisis showed that the change of serum sIL-2R level was closely associated with that of the number of peripheral blood leukocytes and blasts, the percentage of blasts and serum LDH levels, also suggesting that the serum sIL-2R level is a useful clinical indicator of the leukemic cell burden in vivo.

Elevated Serum-Soluble Interleukin-2 Receptor (Tac Antigen) Levels in Chronic Myelogenous Leukemia Patients With Blastic Crisis

By Tomomi Motoi, Takashi Uchiyama, Toshiyuki Hori, Katsuhiko Itoh, Haruto Uchino, and Ryuzo Ueda

INTERLEUKIN-2 (IL-2), which was originally found as a T-cell growth-promoting factor, has been demonstrated to exert its biological effects on a variety of cells, including T cells, B cells, natural killer (NK) cells, monocytes, and oligodendroglial cells. Recent studies have shown that the IL-2 receptor consists of at least two different IL-2–binding proteins, p55 alpha chain (Tac antigen) and p75 beta chain. Furthermore, another soluble form of Tac antigen (sIL-2R) has also been detected in the culture supernatant of activated T cells and sera of patients with various lymphoproliferative diseases such as adult T-cell leukemia, hairy cell leukemia, B-cell chronic lymphocytic leukemia, and Hodgkin’s disease; and its serum level has been considered to be a useful indicator of the number of neoplastic cells expressing IL-2 receptor in vivo. The cell-surface Tac antigen was also demonstrated on the cultured leukemic cells from some patients with acute myelogenous leukemia (AML) and chronic myelogenous leukemia (CML) in chronic phase, although its functional role remains unclear. CML is a clonal disorder of pluripotent hematopoietic stem cells. The stable chronic phase progresses to the stage resistant to chemotherapy (accelerated phase), and most patients finally develop fatal blastic crisis. In spite of the attempts to estimate blastic crisis with cell-surface antigens such as terminal deoxynucleotidyl transferase (Tdt) or common acute lymphocytic leukemia antigen (CALLA), their expressions are restricted to lymphoid crisis and are detectable in only one third of the cases. We examined IL-2 receptor expression in CML patients with blastic crisis to see whether the IL-2 receptor was expressed on leukemic cells and whether the serum sIL-2R level could be an indicator helpful to determine the clinical stage of the disease.

MATERIALS AND METHODS

Patients and normal controls. The study included 25 patients with CML (seven women and 18 men, mean age 44.3 years) and 24 healthy individuals in the same age range (seven women and 17 men, mean age 45.7 years) as normal controls. The patients were diagnosed on the basis of hematologic features and were classified according to the clinical stage as follows: ten in chronic phase (leukocyte count ranges from 19.9 to 123.0 x 10^9/L and the percentage of blasts from 0% to 4.0% in peripheral blood), three in accelerated phase (leukocyte count from 21.4 to 34.7 x 10^9/L and the percentage of blasts from 0% to 1.0% in peripheral blood), and 12 with blastic crisis. Two of 12 patients with blastic crisis were examined at the initial diagnosis of blastic crisis (without any therapy; leukocyte count from 55.0 to 140.2 x 10^9/L and the percentage of blasts from 15.5% to 25.0% in peripheral blood), six after effective therapy (peripheral blood blast less than 10%; leukocyte count from 0.8 to 24.6 x 10^9/L and the percentage of blasts from 0% to 10.0% in peripheral blood), and four in refractory stage in spite of therapy (leukocyte count from 16.6 to 387.0 x 10^9/L and the percentage of blasts from 19.0% to 96.0% in peripheral blood).

Cell separation and sera preparation. Peripheral blood mononuclear cells were separated from heparinized blood of CML patients by Ficoll-Conray density gradient centrifugation. Sera were stored in small aliquots at −20°C, then thawed at room temperature 30 minutes before assay, and centrifuged at 10,000 g for ten minutes to remove cellular fragments including cell surface IL-2 receptor.

Flow cytometric analysis of cell-surface antigens. Cells were stained with mouse monoclonal antibodies (MoAbs) and fluorescein isothiocyanate (FITC)-conjugated F(ab')2 fragments of goat antimouse IgG (Cappel Laboratories, Cochranville, PA). Dual immunofluorescence study was performed by a three-step procedure: (1) incubation with mouse anti-CD10 MoAb (NU-N1: Nippon Grune & Stratton, Inc.; pp 1052-1057
Table 1. Surface Antigen Analysis of Leukemic Cells From CML Patients With Blastic Crisis

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (Yrs)</th>
<th>Sex</th>
<th>Type of Crisis</th>
<th>Percent Positive Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD2 (T11)</td>
</tr>
<tr>
<td>1.</td>
<td>38</td>
<td>M</td>
<td>Mixed</td>
<td>61.0</td>
</tr>
<tr>
<td>2.</td>
<td>24</td>
<td>M</td>
<td>Lymphoid</td>
<td>32.7</td>
</tr>
<tr>
<td>3.</td>
<td>49</td>
<td>M</td>
<td>Myeloid</td>
<td>40.4</td>
</tr>
<tr>
<td>4.</td>
<td>34</td>
<td>M</td>
<td>Myeloid</td>
<td>0.6</td>
</tr>
<tr>
<td>5.</td>
<td>21</td>
<td>M</td>
<td>Myeloid</td>
<td>13.4</td>
</tr>
<tr>
<td>6.</td>
<td>59</td>
<td>M</td>
<td>Lymphoid</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Abbreviations: CD, cluster designation; M, male; ND, not done.

*Yut-K3: A cell line derived from peripheral blood leukemic cells from case 4.

Reizo, KK, Japan), anti-CD13 (MY7: Coulter Clone, Hialeah, FL), anti-CD14 (MY4: Coulter Clone) or anti-CD33 (MY9: Coulter Clone); (2) incubation with Fab'2 fragments of biotinylated goat antimouse Ig G (Cappel Laboratories); (3) incubation with phycoerythrin (PE)-conjugated streptavidin (Biomeda Corp, Sausalito, CA) and FITC-conjugated anti-Tac.2 Flow cytofluorometric analysis was performed on Spectrum III (Ortho Diagnostic System, Westwood, MA).

Radiolabeled IL-2 binding assay. IL-2 binding assay was performed as previously described18 using 125I-labeled recombinant human IL-2 (Takeda Chemical Industries Inc, Osaka, Japan). The number of IL-2 binding sites and the binding affinity were estimated by Scatchard plot analysis. The cells used in IL-2 binding assay contained more than 90% of blasts as morphologically determined on cytocentrin preparations.

Establishment of a human myeloid cell line, Yut-K3, from a CML patient with blastic crisis. Peripheral blood leukemic cells obtained from a CML patient with blastic crisis (case 4) were cultured in RPMI 1640 medium (Nissui Pharmaceutical Co Ltd, Tokyo) supplemented with 10% fetal calf serum (PCS; GIBCO, Grand Island, NY) and 20 μg/mL tobramycin at 37°C under humid air with 5% CO2. After successful cultivation for more than a year, the analyses of cell-surface antigens and chromosomes were performed. Yut-K3 cells were started to culture at a concentration of 1 x 10^6/mL with medium, and the supernatant was obtained at days 1, 2, 3, 4, 5, 7, 8, and 10. The viability of cells was over 90%. The supernatant was stored in small aliquots at –20°C until the assay of sIL-2R.

Assay of sIL-2R. For sIL-2R measurement, we developed a sensitive sandwich enzyme-linked immunosorbent assay (ELISA) system, as described previously, using two MoAbs, Ta60b23 and anti-Tac,25 which recognize different epitopes of the IL-2 receptor. The sIL-2R reference reagent was a cell-free supernatant of a human T-cell leukemia virus type-I (HTLV-I) infected T-cell line MT-1, cultured at a concentration of 1 x 10^6 cells/mL and obtained 72 hours later. It was defined as containing 1,000 U/mL of sIL-2R.

Statistical analysis. The geometric mean and the SD of the mean were determined for each group of patients and controls. The Student’s t test was used to compare individual groups.

RESULTS

Flow cytofluorometric analysis of cell-surface antigens. Flow cytofluorometric analysis of cell-surface antigens, performed in six cases (case 1 at the initial diagnosis of blastic crisis, case 2 after effective therapy, and cases 3 to 6 in refractory stage) out of 12 with CML blastic crisis, demonstrated that significant proportion (19.0% to 95.3%) of peripheral blood mononuclear cells from patients expressed cell-surface IL-2 receptor (Tac antigen; Table 1). In four cases of CML blastic crisis in refractory stage, dual immunofluorescence study using FITC and PE revealed the
coexpression of Tac antigen with CD13 (MY7) in case 4 (data not shown) and case 5 (Fig 1A), with CD14 (MY4) in case 3 and case 4 (data not shown), with CD33 (MY9) in case 3 and case 4 (data not shown), and with CD10 (CALLA) in case 6 (Fig 1B), which confirmed that the leukemic cells (blasts) expressed surface Tac antigen.

**Radiolabeled IL-2 binding assay.** In three cases radiolabeled IL-2 binding assay exhibited that peripheral blood leukemic cells expressed specific IL-2 binding sites (Table 2). The number of binding sites varied case by case. In case 4, 2,870/cell low affinity \( (k_d = 4.8 \text{ nmol/L}) \) and 80/cell high affinity \( (k_d = 33 \text{ pmol/L}) \) receptors were expressed. In cases 1 and 6, 4,680/cell low affinity \( (k_d = 4.6 \text{ nmol/L}) \) and 17,400/cell low affinity \( (k_d = 12.7 \text{ nmol/L}) \) receptors (Fig 2) were demonstrated, respectively.

**Analysis of a human myeloid cell line Yut-K3.** A myeloid cell line, Yut-K3, established from a CML patient with blastic crisis (case 4) and considered to be derived from the leukemic cell clone based on the same chromosome abnormalities, also expressed cell-surface Tac antigen, CD13 and CD33 antigen. The cell line spontaneously released sIL-2R in its culture supernatant (maximum: 2,940 U/mL at day 10). In case 4 and the cell line Yut-K3, the exogenous recombinant IL-2 failed to induce proliferative response of leukemic cells estimated by \(^{3}H\)-thymidine deoxyribose uptake (data not shown).

**Assay of sIL-2R.** As shown in Fig 3, 25 patients with CML had higher serum sIL-2R levels (range: 250 to 172,000 U/mL, mean \( \pm \text{SD} = 13,878 \pm 38,856 \text{ U/mL}) \) than those of the age- and sex-matched 24 normal controls (range: 70 to

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**Table 2. Radiolabeled IL-2 Binding Assay in CML Patients With Blastic Crisis**

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Percent Tac Positive Cells</th>
<th>Binding Sites (molecules/cell)</th>
<th>( k_d ) (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. NH</td>
<td>38</td>
<td>M</td>
<td>43.2</td>
<td>4,680</td>
<td>4.6</td>
</tr>
<tr>
<td>4. NT</td>
<td>34</td>
<td>M</td>
<td>19.0</td>
<td>2,870</td>
<td>4.8</td>
</tr>
<tr>
<td>6. MK</td>
<td>59</td>
<td>M</td>
<td>95.3</td>
<td>17,400</td>
<td>12.7</td>
</tr>
</tbody>
</table>

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695 U/mL, mean \( \pm \text{SD} = 280 \pm 161 \text{ U/mL}) \). Ten CML patients in chronic phase and three in accelerated phase had slightly higher serum sIL-2R levels (range: 250 to 820 U/mL and 790 to 1,305 U/mL, mean \( \pm \text{SD} = 557 \pm 217 \text{ U/mL} \) and 1,035 \( \pm \text{SD} = 211 \text{ U/mL} \), respectively) than controls.
ELEVATED SERUM-SOLUBLE IL-2R IN CML

On the contrary, sera from 12 CML patients with blastic crisis showed much higher sIL-2R levels (range: 2,580 to 172,000 U/mL, mean ± SD = 28,191 ± 52,453 U/mL, P < .01). Furthermore, sera from patients with CML blastic crisis after effective therapy (peripheral blood blast percentage: 0% to 10.0%, the absolute number of blasts: 0 to 1.476 x 10^9/L, n = 6) showed less elevated sIL-2R levels (range: 2,580 to 7,200 U/mL, mean ± SD = 4,020 ± 1,630 U/mL) than those (range: 3,200 to 9,680 U/mL mean ± SD = 6,440 ± 3,240 U/mL) at initial diagnosis of blastic crisis (prior to the treatment; peripheral blood blast percentage: 15.5% to 25.0%, the absolute number of blasts: 13.75 to 22.01 x 10^9/L, n = 2) and those (range: 4,600 to 172,000 U/mL, mean ± SD = 75,323 ± 70,075 U/mL) in

Fig 4. Longitudinal studies of the changes of serum-soluble IL-2 receptor levels and other clinical parameters in two CML patients with blastic crisis (cases 3 and 4). The serum sIL-2R levels changed in association with the count of leukocytes and the absolute number and percentage of blasts in peripheral blood.
refractory stage (peripheral blood blast percentage: 19.0% to 96.0%, the absolute number of blasts: 16.6 to 387.0 x 10^9/L, n = 4). In cases 3, 4, and 5, the serum sIL-2R level was determined at various times of the clinical stage and was compared with the changes of other clinical parameters such as serum LDH level (normal range: 228 to 475 IU/L), the absolute number of leukocytes and blasts, and the percentage of blasts in peripheral blood (Fig 4A and B). The serum sIL-2R level changed closely in parallel with these parameters in cases 3 and 4 (Fig 4A and B). In case 5, serum sIL-2R level at blastic crisis was 153,600 U/mL, which was remarkably high, and declined to 73,728 U/mL after combination chemotherapy 1 month later. Then it increased again to 112,128/mL 2 months later when chemotherapy was no longer effective.

**DISCUSSION**

The present study showed that peripheral blood leukemic cells from CML patients with blastic crisis spontaneously expressed cell surface IL-2 receptors and that serum sIL-2R levels were significantly elevated in these patients. Although flow cytometric analysis demonstrated that the significant proportion of the cells examined expressed IL-2 receptor (Tac antigen), we could not completely exclude the possibility that the cells expressing IL-2 receptor (Tac antigen) could be residual normal T cells but not leukemic cells, especially in cases 1, 2, and 3. However, dual immuno-fluorescence studies in cases 4, 5, and 6 clearly demonstrated the coexpression of Tac antigen with CD13 (My7), CD33 (MY9), or CD10 antigen (CALLA) on peripheral blood leukemic cells. In other words, leukemic cells (blasts) of CML patients with blastic crisis expressed IL-2 receptor (Tac antigen).

A considerable number of IL-2 binding sites (2,950 to 17,400/cell) were detected in leukemic cells from cases 1, 4, and 6. A small number of high-affinity receptors were detected in case 4. However, only low-affinity receptors were detectable in the remaining two cases. Most of the IL-2 receptors expressed on myeloid cells may be low-affinity receptors. Results obtained from IL-2 binding assay also confirmed IL-2 receptor expression on leukemic cells from CML patients with blastic crises.

In addition, the demonstration of IL-2 receptor (Tac antigen) and IL-2 binding sites on myeloid cell line (Yut-K3) cells strongly supported the IL-2 receptor (Tac antigen) expression on blast cells from CML patients. Although IL-2 was shown to enhance O_2^- production and a microbicidal activity of activated monocytes, the functional role of IL-2 receptors on myeloid cells, including blasts from CML patients, remains unclear.

sIL-2R assay revealed markedly elevated sIL-2R levels in blastic crisis and slightly elevated levels in chronic phase of CML. Serum sIL-2R levels in patients in accelerated phase of CML were intermediate. In blastic crisis, serum sIL-2R levels were higher in patients prior to the treatment or in refractory stage than in those to whom an effective treatment was given.

Taken together, IL-2 receptor (Tac antigen) is apparently expressed spontaneously on fresh leukemic cells (blasts) in the peripheral blood from CML patients with blastic crisis, and its soluble form appears to be released from leukemic cells in vivo. Slight elevation of the sIL-2R levels in chronic phase or accelerated phase of CML in contrast to the marked elevation in blastic crisis and the lack of the expression of IL-2 receptor (Tac antigen) on differentiated myeloid cells may indicate that the IL-2 receptor expression is restricted to myeloid blasts in CML.

Finally, the serum sIL-2R level may be a useful indicator in monitoring the clinical course of CML patients with blastic crisis and the effectiveness of the treatment as shown by the good correlation between serum sIL-2R levels and other clinical parameters obtained from the longitudinal studies on cases 3, 4, and 5. The marked elevation of serum sIL-2R levels in the blastic crisis of CML, in contrast to its slight elevation in chronic and accelerated phase, implies its usefulness in following the case and detecting the transition to the crisis phase.

**REFERENCES**

ELEVATED SERUM-SOLUBLE IL-2R IN CML


Elevated serum-soluble interleukin-2 receptor (Tac antigen) levels in chronic myelogenous leukemia patients with blastic crisis

T Motoi, T Uchiyama, T Hori, K Itoh, H Uchino and R Ueda