Soluble Interleukin 2 Receptors in Sera of Japanese Patients With Adult T Cell Leukemia Mark Activity of Disease

By Norimasa Yasuda, Patrick K. Lai, Stephen H. Ip, Patrick C. Kung, Yorio Hinuma, Masao Matsuoka, Toshio Hattori, Kiyoshi Takatsuki, and David T. Purtillu

Serum concentrations of soluble interleukin 2 receptors (sIL 2R) were measured by an enzyme-linked immunosorbent assay (ELISA) in 30 patients with adult T cell leukemia (ATL), in 9 patients with other hematopoietic malignancies, and in 17 asymptomatic individuals seropositive for human T cell leukemia virus type I (HTLV-I). Sixty HTLV-I seronegative, age-matched controls showed a normal range of form 63.2 to 480.8 U/mL. All asymptomatic carriers of HTLV-I had sIL 2R in their sera within the normal range. sIL 2R in sera was not related to the anti-HTLV-I antibody titer. Eleven patients with acute ATL, a clinical phenotype classified clinically into three subtypes as reported previously, had acute ATL with high numbers of malignant cells in blood and bone marrow, skin involvement, systemic lymphadenopathy, and hepatosplenomegaly. They had elevated serum lactic dehydrogenase (LDH), calcium and bilirubin, and high WBC counts. The malignancy progressed acutely and subacutely, despite chemotherapy, and thus 50% of the patients survived less than 4.4 months. Eleven patients with chronic ATL had an elevated WBC count, cough, and skin disease, but they were not associated with hypercalcemia or hyperbilirubinemia, and a few showed slight lymphadenopathy, hepatomegaly, and high serum LDH levels. These patients usually responded to therapy but had multiple remissions and relapses. Eight patients had smoldering ATL characterized by skin lesions, slight bone marrow infiltration, and a low percentage of ATL cells (0.5% to 3.0%) in their peripheral blood that had persisted for years. Serum LDH and calcium levels were normal, and no lymphadenopathy or hepatosplenomegaly was observed.

sIL 2R levels in sera of Japanese patients with ATL were not significantly correlated with the number of ATL cells, but they were significantly correlated with the number of ATL cells and the disease activity. Longitudinal studies performed in four patients with ATL showed significant correlation between serum concentration of sIL 2R and activity of the malignancy. These findings suggest that the level of sIL 2R in serum indicated tumor load and, possibly, prognosis.

MATERIALS AND METHODS

Patients and controls. Sixty HTLV-I seronegative-age-matched controls, 47 HTLV-I seropositive Japanese, including 30 patients with ATL. 17 asymptomatic individuals, and 9 Japanese patients with other lymphoid malignancies, were studied. Patients with ATL were classified clinically into three subtypes as reported previously. Eleven patients had acute ATL with high numbers of malignant cells in blood and bone marrow, skin involvement, systemic lymphadenopathy, and hepatosplenomegaly. They had elevated serum lactic dehydrogenase (LDH), calcium and bilirubin, and high WBC counts. The malignancy progressed acutely and subacutely, despite chemotherapy, and thus 50% of the patients survived less than 4.4 months. Eleven patients with chronic ATL had an elevated WBC count, cough, and skin disease, but they were not associated with hypercalcemia or hyperbilirubinemia, and a few showed slight lymphadenopathy, hepatomegaly, and high serum LDH levels. These patients usually responded to therapy but had multiple remissions and relapses. Eight patients had smoldering ATL characterized by skin lesions, slight bone marrow infiltration, and a low percentage of ATL cells (0.5% to 3.0%) in their peripheral blood that had persisted for years. Serum LDH and calcium levels were normal, and no lymphadenopathy or hepatosplenomegaly was observed.

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gradient centrifugation. After washing, the cells were stained with Mabs OKT3, 4, 8, 11, and Ia2 (HLA-DR; Ortho Pharmaceutical Co., Dickinson Monoclonal Center, Mountain View, CA) and anti-Tac (kindly provided by Dr. Uchiyama at Kyoto University). The stained cells were analyzed with a laser flow cytometry system (Spectrum III/Ortho Diagnostic System, Westwood, MA).

**Determination of sIL-2R.** Serum concentration of sIL-2R was determined by using a commercially available test kit specific for IL-2R (Cellfree™, T Cell Science Inc., Cambridge, MA). This enzyme-linked immunoassay employs two anti-CD25 Mabs directed against two distinct epitopes on the 55-kd α chain of IL-2R. The first Mab (ie, 2R1.2, an alias of the clone AT-1) that reacted with the IL-2 binding site was coated onto 96-well microtiter plate; the other (ie, 7G7/B6) was linked to horseradish peroxidase for detection. The assay is specific for IL-2R with a coefficient correlation of 3.2% and 10% for intra-assay and interassay precisions (n = 30), respectively. Sera from patients were coded and tested without knowledge of clinical disease. Serial dilutions of each serum were tested in duplicate according to the manufacturer's instruction and the absorbance was read at 490 nm in a Dynatech MR600 automatic reader (Dynatech Laboratories Inc., Alexandria, VA). sIL-2R values in sera were expressed in U/mL relative to a set of standard IL-2R preparations supplied with the test kit.

**Specificity of the anti-IL-2R Mabs.** Cell iodination was performed by suspending 1 × 10⁷ three-day-PHA-activated peripheral blood mononuclear cells in 1 mL phosphate-buffered saline (PBS) containing 0.5 mCi Na¹²⁵I (Amersham, Arlington Heights, IL) and 200 μg lactoperoxidase (Sigma Chemicals, St. Louis). Labeled cells were solubilized with 0.5% Nonidet P-40 solution at a concentration of 1 × 10⁶ cells/ml. Detergent extracts (500 μg) of the cells were immunoprecipitated with 1 μg of anti-Tac, 2R1.2, 7G7/B6 Mabs, or a control antisera for two hours at 4°C, followed by a one-hour incubation with 10% *Staphylococcus aureus* (Cowan strain 1, ATCC 12598, ATCC, Rockville, MD). The immunoprecipitates were extensively washed, were resuspended in buffer, and were analyzed on a 12.5% sodium dodecyl sulfate (SDS) polyacrylamide gel followed by autoradiography.

Sequential immunoprecipitations were performed on the labeled polypeptides. Aliquots were prepared as described above and immunoprecipitated first with 2R1.2, 7G7/B6, or anti-Tac Mab. The supernatants from these immunoprecipitates were removed and then immunoprecipitated with either the same or alternate Mabs.

**Statistical analysis.** For statistical analysis, correlation coefficient and one-way test for analysis of variance were employed.

## RESULTS

**Specificity of the anti-IL-2R Mabs.** The two Mabs employed in this study were specific to the 55-kd α chain of the IL-2R. Both Mabs immunoprecipitated a 55- to 60-kd band from cell surface iodinated phytohemagglutinin (PHA)-activated T cells (Fig 1). After the cell extracts were cleared with 2R1.2 Mab, either 7G7/B6 or 2R1.2 Mab did not precipitate any molecule from the extracts of activated T cells (data not shown). The reciprocal experiment showed similar results. Thus 2R1.2 and 7G7/B6 Mabs bound to an identical protein on activated T cells.

The enzyme-linked immunoassay using these two Mabs detected IL-2R in serum (Fig 2). Addition of recombinant IL-2 at high concentration to serum prior to assay interfered with the binding of serum IL-2R to the Mab linked to solid phase with 50% inhibition of binding at 5 × 10² U/mL. IL-2 (data not shown).

**Relation of serum sIL-2R to HTLV-I infection.** All symptomatic carriers of HTLV-I have sIL-2R in their serum within the normal range. The normal range in 60 healthy individuals seronegative to HTLV-I was defined as the mean ± 2 SD was 6.32 to 480.8 U/mL. No correlation was found between serum concentrations of sIL-2R and antibody titers to HTLV-I (r = .2173207, P > .05; Fig 2).

**Relations of serum sIL-2R to ATL.** Serum sIL-2R concentrations in 30 patients with ATL varied from normal to remarkably elevated values (Fig 3). All patients with acute ATL had sIL-2R values beyond 10,000 U/mL, whereas patients with smoldering ATL had normal concentrations of sIL-2R (< 480.8 U/mL). The 11 patients with chronic ATL had intermediate elevated levels of sIL-2R.
ranging from 480.8 to 37,300 U/mL. Three had values over 10,000 U/mL, and eight had intermediate values between 480.8 and 10,000.0 U/mL. Hence serum concentrations of sIL 2R differentiated from the three clinical subtypes of ATL.

Using the one-way test for analysis of variance, we found that neither the number of ATL cells ($F = 3.01, P > .05$) nor the number of CD25-positive cells ($F = 3.17, P > .05$) in peripheral blood differed significantly among the three clinical subtypes of ATL. In contrast, the number of leukocytes (Fig 4; $F = 4.11, 0.01 < P < 0.05$), the number of CD4-positive cells ($F = 5.11, 0.01 < P < .05$) in peripheral blood, and serum LDH ($F = 3.39, 0.01 < P < 0.05$) in ATL patients differed significantly according to clinical subtypes. However, concentrations of sIL 2R in sera varied much according to whether cases were acute, chronic, or smoldering ATL ($F = 20.04, P < .00001$). This finding suggests that the serum concentration of sIL 2R might be a more superior marker for staging HTLV-I-associated ATL than the other parameters.

**Serum sIL 2R and the evolution of ATL.** Longitudinal study of four patients was performed. Three patients with acute ATL had high serum concentrations of sIL 2R that fell dramatically at remission (patients 1 and 2, Table I) or partial remission (patient 4). The third patient had chronic ATL that progressed to the acute stage six months after the diagnosis. Concomitant to the progression of the malignancy,

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**Table 1. Activity in Four Patients With ATL and Concentration of sIL 2R**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Date of Sample Collection</th>
<th>Clinical Stage</th>
<th>Soluble IL 2R* (U/mL)</th>
<th>WBC × 10³</th>
<th>CD25* cells (%)</th>
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<tbody>
<tr>
<td>1</td>
<td>3-29-85</td>
<td>Active acute ATL</td>
<td>57,800</td>
<td>17.6</td>
<td>48.5</td>
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<tr>
<td></td>
<td>5-30-85</td>
<td>Remission</td>
<td>126</td>
<td>4.6</td>
<td>ND†</td>
</tr>
<tr>
<td></td>
<td>6-5-85</td>
<td>Remission</td>
<td>127</td>
<td>4.4</td>
<td>13.2</td>
</tr>
<tr>
<td></td>
<td>11-14-85</td>
<td>Remission</td>
<td>137</td>
<td>4.7</td>
<td>11.9</td>
</tr>
<tr>
<td>2</td>
<td>8-6-85</td>
<td>Active acute ATL</td>
<td>11,100</td>
<td>20.5</td>
<td>22.7</td>
</tr>
<tr>
<td></td>
<td>11-11-85</td>
<td>Remission</td>
<td>868</td>
<td>8.6</td>
<td>42.4</td>
</tr>
<tr>
<td></td>
<td>6-24-86</td>
<td>Relapse</td>
<td>18,200</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>6-25-85</td>
<td>Active chronic ATL</td>
<td>4,765</td>
<td>20.6</td>
<td>58.5</td>
</tr>
<tr>
<td></td>
<td>12-13-85</td>
<td>Active acute ATL</td>
<td>57,400</td>
<td>207.0</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>1-28-15</td>
<td>Active acute ATL</td>
<td>42,700</td>
<td>80.2</td>
<td>56.7</td>
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<tr>
<td></td>
<td>4-17-85</td>
<td>Partial remission‡</td>
<td>1,340</td>
<td>2.8</td>
<td>5.4</td>
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<tr>
<td></td>
<td>11-14-85</td>
<td>Partial remission</td>
<td>505</td>
<td>2.5</td>
<td>ND</td>
</tr>
</tbody>
</table>

*The normal range, defined by mean ± 2 SD was 63.2 to 480.8 U/mL.
†ND, not done.
‡Although no abnormal cell was found in peripheral blood, slight lymphadenopathy persisted.
the serum level of sIL 2R in this patient rose from 4,765 to 57,400 U/mL (Table 1). The second patient also showed high sIL 2R level in relapse, suggesting that the concentration of serum sIL 2R was proportional to activity of the tumor.

Correlation between serum sIL 2R and the number of CD25-positive cells. We determined the relationship of sIL 2R to tumor cells and membrane-bound IL 2R by comparing the sIL 2R concentration in sera with the number of CD25-positive cells identified morphologically in peripheral blood smears (Fig 5; r = -.812). Likewise, the serum concentration of sIL 2R in patients with ATL corresponded with the number of CD25-positive cells identified by fluorescence-activated cell sorter (FACS) analysis in 31 patients with ATL or other hematopoietic malignancies (Fig 6; regression line A, r = -.725). Figure 6 also shows that the results of studies from patients with acute ATL are located to the upper right of the regression line A. In contrast, results from most patients with chronic and smoldering ATL are located below the regression line. Segregation of patients with chronic and smoldering ATL from those with acute ATL permitted further analysis of results. A more significant correlation was found between sIL 2R and CD25-positive cells (Fig 6; regression line B, \( r = .803971 \)).

As an indicator of sIL 2R production per one CD25-positive cell, the ratios of sIL 2R values to CD25-positive cell numbers among patients with acute, chronic, and smoldering ATL were compared. The ratio (sIL 2R concentration: CD25-positive cell number) in acute ATL was significantly different than the other subtypes (\( P < .05 \)) but no difference was observed between chronic and smoldering cases ATL (\( P > .05 \)).

Relation of sIL 2R to other hematopoietic malignancies. An elevated serum concentration of sIL 2R was observed in some but not all patients with other hematopoietic malignancies (Table 2). These patients were HTLV-I seronegative except for patient 7. Patient 5, with CD4 chronic lymphocytic leukemia, and patient 10, with non-T-non-B acute lymphoblastic leukemia (78.4% J5, 8.8% T11`, 12.3% B1`, 92.4% DR`), had high serum levels of sIL 2R and percentage of CD25-positive cells in peripheral blood. Patient 12 had acute myeloblastic leukemia with 54.7% DR`, 70.8% Mo-1`, 38.2% platelet glycoprotein (GP) IIb/IIIa`, and 7.4% CD25` cells in the peripheral blood and an intermediate level of sIL 2R. In contrast, patient 11 had relatively low sIL 2R, yet 56.1% of the peripheral blood cells had membrane-bound IL 2R. This patient had a leukemic conversion from a non-Hodgkin’s B cell lymphoma with 96.5% DR`, 85.8% \( \mu \) chain (of immunoglobulin) and 29.6% Leu 12` cells in the peripheral blood. Similarly, patients 8 and 9 had normal level of sIL 2R in serum, although 3.8% or 4.0% of the peripheral blood cells were positive for CD25. Other patients had a low percentage of CD25` cells in their peripheral blood, and their serum levels of sIL 2R were within the normal range.

**DISCUSSION**

Diagnosis and staging of ATL depends on a triad of clinical, hematologic, and laboratory findings. Serum levels of LDH, total WBC counts, and the number of CD4-positive cells in the peripheral blood stage the malignancy. We

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**Table 2. Values of Soluble sIL 2R in 12 Patients With Non-ATL Hematopoietic Malignancies**

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Diagnosis</th>
<th>Soluble sIL 2R* (U/mL)</th>
<th>WBC x 10⁹</th>
<th>CD25` cells (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>T6-CLL</td>
<td>22,000</td>
<td>29.2</td>
<td>44.7</td>
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<tr>
<td>4</td>
<td>T4-CLL</td>
<td>121</td>
<td>14.0</td>
<td>0.3</td>
</tr>
<tr>
<td>7</td>
<td>T8-CLL</td>
<td>57</td>
<td>10.3</td>
<td>0.6</td>
</tr>
<tr>
<td>8</td>
<td>T4-ML</td>
<td>212</td>
<td>41.5</td>
<td>3.8</td>
</tr>
<tr>
<td>9</td>
<td>T-ALL</td>
<td>206</td>
<td>NA</td>
<td>4.0</td>
</tr>
<tr>
<td>10</td>
<td>ALL</td>
<td>51,500</td>
<td>20.6</td>
<td>57.1</td>
</tr>
<tr>
<td>11</td>
<td>B-NHL</td>
<td>561</td>
<td>14.5</td>
<td>56.1</td>
</tr>
<tr>
<td>12</td>
<td>AML(M7)</td>
<td>1,180</td>
<td>52.4</td>
<td>7.4</td>
</tr>
<tr>
<td>13</td>
<td>AML(M1)</td>
<td>111</td>
<td>78.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

Abbreviations: CLL, chronic lymphocytic leukemia; ML, malignant lymphoma; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin’s lymphoma; AML, acute myeloblastic leukemia.

*The normal range, defined by mean ± 2 SD, was 63.2 to 480.8 U/mL.*
examined the relationship of serum sIL-2R to the subtype and stage of ATL. sIL-2R in sera from patients with acute ATL were markedly elevated. In contrast, asymptomatic carriers of HTLV-I had normal values. The sIL-2R level in patients with chronic ATL ranged from 480.8 to 37,300 U/mL. The concentration of sIL-2R in patients with ATL correlated with the clinical subtypes of the malignancy and the number of ATL cells in the peripheral blood. Noteworthy is the strong correlation between the sIL-2R concentration and evolution of ATL from active to remission phase or from chronic to acute phenotypes. These findings suggest that the concentration of sIL-2R in sera might reflect the tumor load, and assessing sIL-2R in patients with ATL might have diagnostic and prognostic values.

The source of sIL-2R in serum is unknown, but the number of CD25-positive cells in peripheral blood determines the level in sera, suggesting that soluble form of IL-2R might be released by these cells in vivo. Whether these CD25-positive cells were malignant cells or normal, activated T cells is unclear, as one-color FACS analysis was used. Potentially both cell types have the capacity to release sIL-2R in the serum. In vivo sIL-2R might be produced by normal lymphoid cells responding to stimulation by the malignant cells. We think this scenario is unlikely because the correlation we observed was high between sIL-2R concentration and the tumor load. Malignant cells from patients with ATL were identified morphologically as ATL cells, and their numbers in peripheral blood correlated with the concentration of sIL-2R in serum. Although morphological identification of ATL cells is sometimes difficult and subjective, this finding suggests that CD25-positive malignant cells in vivo may be the source of serum sIL-2R. This interpretation is consistent with observations that HTLV-I-transformed cells and cell lines established from ATL patients released sIL-2R in vitro. However, our interpretation remains a conjecture, and verification of the source of sIL-2R in patients with ATL requires further investigation.

The kinetics of release of sIL-2R in vivo varied in acute, chronic, and smoldering ATL. The ratio of serum sIL-2R to CD25-positive cells was higher in patients with acute ATL than in patients with chronic or smoldering ATL, suggesting that more sIL-2R might have been released by each CD25-positive cell in patients with acute ATL. Alternatively, acute ATL cells might have invaded other organs apart from the peripheral blood, and sIL-2R may be released from these invading cells.

Asymptomatic carriers of HTLV-I and patients with smoldering ATL had normal concentrations of sIL-2R in their sera. This finding was unexpected. Southern blot analysis has revealed a significant amount of virus or HTLV-I-infected cells in peripheral blood from patients with smoldering ATL but not from asymptomatic individuals. Moreover, HTLV-I infection of T cell lines induced the production of sIL-2R. Our findings suggest that the presence of virus or virus-infected cell is in itself not sufficient to enhance the production of sIL-2R in vivo. Alternatively, certain pathologic actions of HTLV-I may be suppressed in smoldering ATL.

In summary, the results of this study suggest that measuring the concentration of sIL-2R in sera can provide diagnostic and prognostic information in patients with ATL by predicting disease activity, progression, and tumor burden.

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