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

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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 with median survival rate of 4.4 months, had markedly elevated sIL 2R (11,100 to 99,000 U/mL), but eight patients with smoldering ATL had low sIL 2R values (<480.8 U/mL) comparable to controls. Eleven patients with chronic ATL had intermediate elevated levels of sIL 2R (480.8 to 37,300.0 U/mL). Serum levels of sIL 2R correlated with the number of ATL cells (r = 0.812) and CD25-positive cells (r = 0.725) circulating in the peripheral blood. 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.

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gradient centrifugation. After washing, the cells were stained with Mabs OKT3, 4, 8, 11, and 1a2 (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 immunosassay 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 × 10⁶ 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 immunoprecipitations 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 immunosassay 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 × 10⁴ U/mL IL 2 (data not shown).

**Relation of serum sIL 2R to HTLV-I infection.** All asymptomatic 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 < .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 1) 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,
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 in the peripheral blood (Figs 5 and 6). The serum level of sIL 2R correlated with the number of ATL cells that were 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<, 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% μ 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
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 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 correlation 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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