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Serum Soluble Interleukin-2 Receptor Levels in Patients with Adult T-cell Leukemia and Human T-cell Leukemia/Lymphoma Virus Type-I Seropositive Healthy Carriers

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Using an enzyme-linked immunosorbent assay (ELISA) technique, we measured the soluble interleukin 2 receptor (s-IL-2R) levels in the sera of patients with adult T-cell leukemia (ATL) in Japan. The s-IL-2R levels in the sera of the ATL patients were markedly higher (range 540–310, 400 U/ml, mean ± SD = 62,800 ± 81,000 U/ml, n = 42) than those in normal individuals (range 42–950 U/ml, mean ± SD = 322 ± 198 U/ml, n = 35, $P < 0.01$). The patients with acute-type or lymphoma-type ATL had high s-IL-2R levels (range 11,900–310,400 U/ml, mean ± SD = 110,340 ± 370 U/ml, n = 15; range 26,400–214,400 U/ml, mean ± SD = 90,170 ± 59,040 U/ml, n = 7, respectively). All of the patients with hypercalcemia (Ca > 10 mg/dl) or elevated serum LDH levels (LDH > 500 IU/liter) also had s-IL-2R levels above 10,000 U/ml. The high s-IL-2R levels in the sera of ATL patients indicate abnormal IL-2 receptor production and its release from the leukemic cells *in vivo*. Thus, the serum s-IL-2R level may be a sensitive and useful marker to monitor the total amount of tumor cells in ATL, especially in the lymphoma type. We next examined the serum s-IL-2R levels in human T-cell leukemia/lymphoma virus type-I (HTLV-I) seropositive healthy carriers to investigate whether there might be abnormal IL-2 receptor expression in such individuals. However, there was no statistically significant difference between the s-IL-2R level of 71 HTLV-I seropositive healthy carriers (range 65–880 U/ml, mean ± SD = 394 ± 212 U/ml) and that of 71 age- and sex-matched normal individuals (range 33–950 U/ml, mean ± SD = 357 ± 224 U/ml) who lived in Okinawa Prefecture.

Key words: Soluble interleukin-2 receptor — Adult T-cell leukemia — Human T-cell leukemia/lymphoma virus type-I

Human T-cell leukemia/lymphoma virus type-I (HTLV-I)[1, 2] has been considered to be an etiological agent[2–5] of adult T-cell leukemia (ATL),[6] although its precise role in the development of ATL still remains unclear. Interleukin-2 (IL-2) receptor (Tac antigen) is detectable on peripheral blood leukemic cells from most ATL patients[7–9] and HTLV-I-infected cell lines.[10] The close association of IL-2 receptor expression and HTLV-I infection has been reported.[8, 10–12] One of the attractive hypotheses concerning the leukemogenesis of ATL is that the abnormally (constitutively) expressed IL-2 receptor plays an important role in the transformation of HTLV-I-infected cells and the development of ATL. It is, therefore, important to study the IL-2 receptor expression in HTLV-I-infected cells from HTLV-I seropositive healthy carriers as well as leukemic cells from ATL patients as one of the approaches to examine the validity of this hypothesis.

It has recently been demonstrated that the soluble form of IL-2 receptor (s-IL-2R) is released from cells expressing the surface IL-2 receptor, including HTLV-I-infected cell lines.[13–16] In addition, the serum s-IL-2R level was reported to be a useful indicator of the IL-2 receptor expression *"in vivo"* in many lymphoproliferative disorders.[17–24] It is especially useful in cases where it is difficult to obtain enough cells to examine the cell surface IL-2 receptor expression. In the present study, we measured serum s-IL-2R level in 42 patients with various types of ATL and 71 HTLV-I seropositive healthy carriers.

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Materials and Methods

Patients and HTLV-I Seropositive Healthy Carriers The patients were 42 ATL patients (25 females and 17 males, age range 26–83 years, mean =56.1 years). The diagnosis was made on the basis of clinical and hematological features and positive serum anti HTLV-I antibody. The clinical types classified according to the clinical features and course,[25] sex of the patient and the time when the serum or plasma was obtained were as follows; 15 acute type (7 females and 8 males; 12 before therapy and 3 at the progressive stage in spite of therapy), 19 chronic type (13 females and 6 males; all of them before therapy), 7 lymphoma type (4 females and 3 males; one before therapy, one at relapse and 5 at the progressive stage) and one smoldering type (female; before therapy). The sera of 35 normal volunteers (22 females and 13 males) were examined as controls. Seventy-one HTLV-I seropsitive healthy carriers (42 females and 29 males, age range 27–87 years, mean=65.9 years) and 71 healthy individuals with comparable age range (41 females and 30 males, age range 23–90 years, mean=65.7 years) living in the same area of Okinawa Prefecture were also studied.

Samples Serum or plasma was stored in small aliquots at $-20°$, then thawed at room temperature 30 min before assay, and centrifuged at 10,000g for 10 min to remove cellular fragments including cellular surface IL-2 receptors.

Monoclonal Antibodies Two murine monoclonal antibodies directed against different epitopes of human IL-2 receptor, anti Tac[26,27] of the IgG_{2a} subclass and Ta60b[28] of the IgG_1 subclass, were purified from hybridoma ascites by gel filtration and DEAE cellulose chromatography. Biotinylated anti Tac was prepared using *n*-hydroxysuccinimidobiotin (Pierce Chemical Co., Rockford, IL).

Assay of S-IL-2R Wells of a flat-bottomed 96-well microtiter plate were coated with 100 μl of a 5 μg/ml solution of Ta60b in coating buffer (0.05M Tris-HCl buffer, pH 7.55) and incubated for 72 hr at 4°. The plate was washed 6 times with washing buffer (coating buffer supplemented with 0.05% polyoxyethylene(20)sorbitan monolaurate), then 350 μl of blocking buffer (coating buffer plus 0.1% bovine serum albumin) was added to the wells to cover the nonspecific protein binding sites, and the plate was incubated overnight at 4°. The plate was washed, 50 μl of sample was added to each well in triplicate, and incubation was carried out overnight at 4°. After washing of the wells, 100 μl of a 5 μg/ml solution of biotinylated anti Tac was added per well and incubation was continued for 2 hr at 4°. Washing was repeated more than 6 times, then 100 μl of a 0.2 U/ml solution of alkaline phosphatase conjugated Avidin D (Vector Laboratories Inc., CA) was added per well and the plate was incubated for an hour at 4°. The wells were washed 6 times and 50 μl of 0.1mM beta-NADP (nicotinamide adenine dinucleotide phosphate) in substrate buffer (50mM diethanolamine-HCl buffer, pH 9.5, with 1mM $MgCl_2$ and 0.1mM $ZnCl_2$) was added. After incubation for 20 min at 37°, 100 μl of a 20 mM phosphate buffer (pH 7.2) containing 4% (v/v) ethanol, 0.55mM *p*-iodonitrotetrazolium violet, 0.5% bovine serum albumin, 0.02% alcohol dehydrogenase (EC 1.1.1.1) and 0.015% diaphorase (EC 1.6.4.3) was added to each well without washing. After a 10-min incubation at 25°, 50 μl of a 2N H_2SO_4 solution was added to stop the reaction and the absorbance at 492 nm was determined by using a Titertek Uniskan II (Flow Laboratories Inc., McLean, VA).

Standards The dilution buffer alone was defined as 0 U/ml of s-IL-2R. The culture supernatant of the HTLV-I-infected cell line MT-1 cultured at a concentration of 1×10^5/ml for 72 hr was centrifuged at 10,000g for 30 min, passed through a 0.22-μm filter (STERIVEX-GS; Millipore Corporation, MA), and defined as the standard sample containing 1,000 U/ml of s-IL-2R. The absorbance of the standard diluted to eight different concentrations was measured in triplicate. The average absorbance was plotted against the s-IL-2R concentration in U/ml to obtain the standard curve.

Results

Specificity of ELISA for S-IL-2R Biotinylated anti Tac was preblocked by anti-IL-2 receptor antibodies to evaluate the specificity of the assay system. When the wells coated with Ta60b and reacted with the supernatant of the MT-1 were preblocked for two hours by "non"-biotinylated anti Tac (0.5 μg/ml, 100 μl/well), biotinylated anti Tac could not bind to the receptor (absorbance; 0.080). In contrast, buffer or other monoclonal antibodies recognizing different epitopes of the IL-2 receptor such as 7G7/B6 or HIEI, at 100-fold higher concentration, did not block the binding of the biotinylated anti Tac to its receptor (absorbance; 0.680–0.712). These results indicate that this assay system is specific for the molecule bound by Ta60b and biotinylated anti Tac.

Serum or Plasma Soluble IL-2 Receptor Levels in the Patients with ATL The mean value of s-IL-2R levels in the serum or plasma of ATL patients was much higher than that of age- and sex-matched normal controls (62,800$\pm$81,000 U/ml, n=42 versus 322$\pm$198 U/ml,

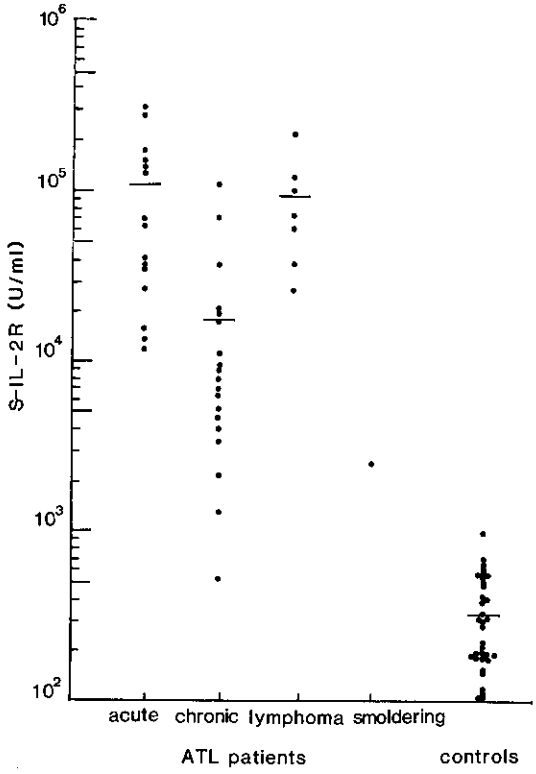


Fig. 1. Soluble IL-2 receptor levels in the patients with ATL. Patients with acute- or lymphoma-type ATL showed a higher soluble IL-2 receptor level (>10,000 U/ml). The bars indicate the geometric mean values.

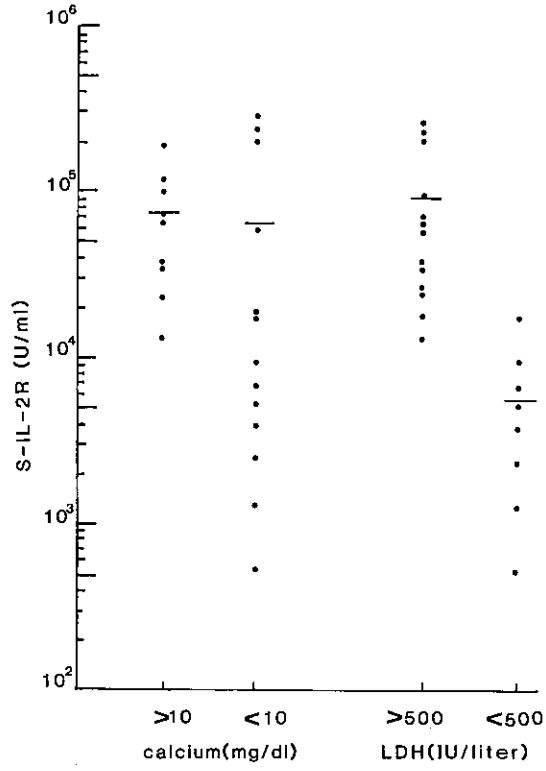
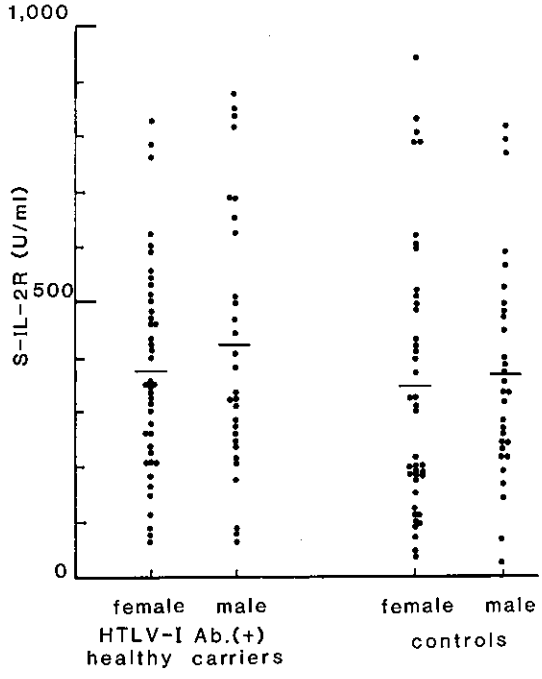


Fig. 2. Serum soluble IL-2 receptor levels in the patients with or without hypercalcemia and in those with or without elevated serum LDH. All patients with hypercalcemia (Ca > 10 mg/dl) showed elevated serum soluble IL-2 receptor level (>10,000 U/ml). Patients with elevated serum LDH level (LDH>500 IU/liter) also showed higher soluble IL-2 receptor level (>10,000 U/ml). The bars indicate the geometric mean values.

n=35, $P<0.01$; Fig. 1). The serum s-IL-2R level of the patients with acute-type ATL ranged from 11,900 to 310,400 U/ml (mean ±SD=110,340±102,370 U/ml, n=15), and that of those with lymphoma-type ATL ranged from 26,400 to 214,400 U/ml (mean ±SD=90,170±59,040 U/ml, n=7). The serum s-IL-2R level of the patients with chronic-type ATL ranged from 540 to 110,400 U/ml (mean±SD=18,370±26,870 U/ml, n=19) and that of the patient with smoldering-type ATL was 2,530 U/ml (n=1).

Relationship between S-IL-2R Level and Serum Calcium or LDH Level in the ATL Patients In some cases, ATL is associated with hypercalcemia[29] of unknown etiology. Although the serum s-IL-2R level of the patients with a normal serum calcium level varied greatly (range 540–299,200 U/ml, mean±SD=68,170±103,600 U/ml, n=13), it was more than 10,000 U/ml (range 13,500–193,640 U/ml, mean±SD=74,430±53,370 U/ml) in all patients with hypercalcemia (Ca >10 mg/dl, n=9; Fig. 2). The serum s-IL-2R level of the patients with elevated serum LDH level (LDH>500 IU/liter; normal range 228–475 IU/liter; all of those with elevated serum LDH level were classified as acute or lymphoma type of ATL) was more than 10,000 U/ml (range 13,500–299,200 U/ml, mean±SD=93,930±91,590 U/ml, n=13), whereas that of the patients with a normal serum LDH level also varied greatly (range 540–18,200 U/ml, mean±SD=6,050±5,380 U/ml, n=8; Fig. 2).



Serum S-IL-2R Level in the HTLV-I Seropositive Healthy Carriers The serum s-IL-2R level of the 71 HTLV-I seropositive healthy carriers ranged from 65 to 880 U/ml (mean $\pm$SD=394$\pm$212 U/ml) and that of the age- and sex-matched 71 normal individuals ranged from 33 to 950 U/ml (mean$\pm$SD =357$\pm$224 U/ml; Fig. 3). There was no significant difference between these two values.

The Change of Serum S-IL-2R Level during the Clinical Course of Lymphoma-type ATL A 44-year-old man, born in Nagasaki Prefecture, was admitted to our hospital because of general peripheral lymphadenopathy in De-

Fig. 3. Serum soluble IL-2 receptor level in the HTLV-I seropositive healthy carriers. There was no significant difference between the serum soluble IL-2 receptor level of the HTLV-I seropositive healthy carriers and that of age- and sex-matched normal individuals. The bars indicate the geometric mean values.

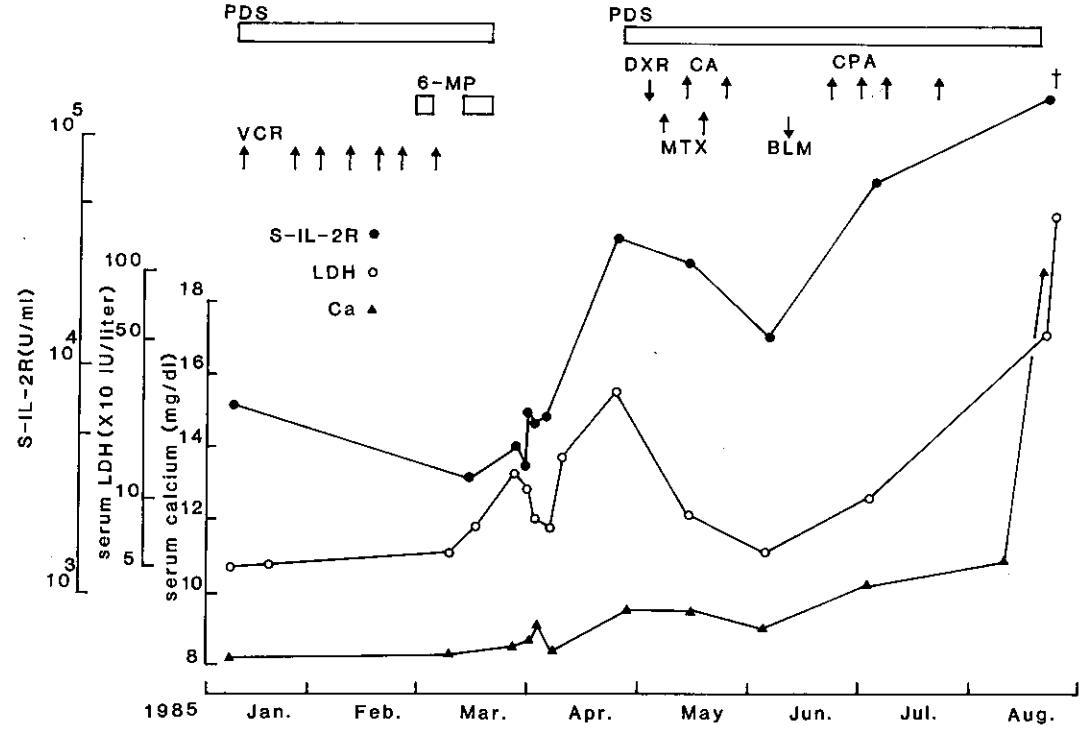


Fig. 4. Soluble IL-2 receptor level and other clinical parameters in a case of lymphoma-type ATL. Soluble IL-2 receptor level (●) changed in association with the serum LDH level (○) and serum calcium level (▲). Abbreviations: PDS, prednisolone; 6-MP, 6-mercaptopurine; VCR, vincristine; DXR, doxorubicin; CA, cytarabine; MTX, methotrexate; BLM, bleomycin; CPA, cyclophosphamide.

cember, 1984. His peripheral WBC counts ranged from 9,000 to 10,000/mm^3 and only 4 to 5% of pathological cells were observed in peripheral blood. He had been treated with prednisolone, vincristine and 6-mercaptopurine. S-IL-2R was below 10,000 U/ml, until April, 1985, when infiltration of the leukemic cells into the bilateral kidneys was noted by whole-body CT scanning. The s-IL-2R, serum LDH and calcium level gradually increased on April 24, and decreased after intensive therapy (Fig. 4). At the terminal stage, the s-IL-2R level was remarkably elevated (maximum: 149,800 U/ml on August 22), together with a marked elevation of the serum LDH level (maximum: 11,000 IU/liter on August 23) and the appearance of severe hypercalcemia (maximum: 18.6 mg/dl on August 19). Thus, serum s-IL-2R level is a useful indicator that changes in association with the serum LDH level, and may be a more sensitive clinical marker in ATL.

Discussion

The serum or plasma s-IL-2R level in the patients with ATL was much higher than that in normal individuals as examined by a sensitive ELISA using two different anti-IL-2 receptor antibodies. A markedly higher level of serum s-IL-2R was detected especially in the patients with an acute clinical course (acute type), hypercalcemia or elevated serum LDH level. We have been studying IL-2 receptor expression in ATL and reported that the IL-2 receptor was constitutively expressed on peripheral blood leukemic cells from most of the ATL patients examined[7, 8]; we also demonstrated a close association between IL-2 receptor mRNA expression and HTLV-I viral RNA expression in short-term-cultured leukemic cells.[30] Although the fresh peripheral blood leukemic cells from most patients with ATL express the IL-2 receptor, the expression is usually weak to moderate and it is difficult to examine this expression in a lymphoma-type ATL because of the paucity of leukemic cells in the peripheral blood. It appears, therefore, important to determine whether leukemic cells strongly express the receptors *in vivo* in association with HTLV-I expression. Our results showing very high s-IL-2R levels in the sera of the patients with ATL strongly suggest that the IL-2 receptor is actively produced by, and its soluble form is released from, leukemic cells *in vivo*. Leukemic cells in the organs or tissues other than peripheral blood may express more IL-2 receptors than peripheral blood leukemic cells. In addition, it is noteworthy that the serum s-IL-2R level was very high in patients with lymphoma-type ATL in whom it is often difficult to study cellular surface IL-2 receptor expression on neoplastic cells.

It is an attractive hypothesis that the abnormally expressed IL-2 receptor plays a key role in the leukemogenesis of ATL. In this context, we also studied IL-2 receptor expression in HTLV-I seropositive healthy carriers, but the results were similar to those in age- and sex-matched normal individuals. This suggests that the proportion of HTLV-I-infected T-cells expressing the IL-2 receptor and releasing its soluble form is too small to be detected by this assay, or those T-cells do not express IL-2 receptor in healthy carriers.

The biological role and significance of serum s-IL-2R *in vivo* is still obscure although its capability of binding IL-2 with low affinity has been recently demonstrated.[31, 32] A large amount (one thousand-fold more than normal in some ATL cases) of serum s-IL-2R may affect the immune system by binding IL-2 and consequently be associated with the immunodeficiency state in ATL. Thus, s-IL-2R appears to be a useful parameter to monitor the IL-2 receptor expression *in vivo* and to study the mechanism underlying the abnormal expression of IL-2 receptor in ATL.

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