

Constitutive Secretion of Soluble Interleukin-2 Receptor by Human T Cell Lymphoma Xenografted into SCID Mice

Correlation of Tumor Volume with Concentration of Tumor-Derived Soluble Interleukin-2 Receptor in Body Fluids of the Host Mice

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Increased serum concentration of soluble α -chain receptor for interleukin-2 (sIL-2R) has been noted in patients with a variety of inflammatory conditions and lymphoid malignancies including T cell leukemia and lymphoma. Elevated sIL-2R serum levels seen in lymphoid malignancies appear to correlate with the clinical stage of disease. However, because sIL-2R is produced by normal activated lymphocytes, it has been uncertain whether serum sIL-2R in such conditions is derived from tumor cells or normal immune cells responding to the tumor. To address this question, we used a model of human (CD30⁺) anaplastic, large T cell lymphoma transplanted into immunodeficient SCID mice. Reverse transcription polymerase chain reaction of tumor RNA showed that the tumor, designated mJB6, contains mRNA for α -chain of human IL-2R. Furthermore, 15 to 25% of tumor cells stained with anti-human IL-2R α -chain mAb. Solid phase ELISA analysis of serum samples from mice bearing mJB6 lymphoma showed high concentrations of human sIL-2R. None of the control mice without lymphoma or with human nonlymphoid tumors (prostatic carcinoma, ovarian carcinoma, and glioblastoma multiforme) showed detectable human sIL-2R. The sIL-2R serum titers of mJB6-bearing mice cor-

related strongly with tumor volume ($P < 0.0001$). Tumors as small as 0.4 to 0.8 mm³ could be detected by this method. The sensitivity of sIL-2R ELISA exceeded at least 150 times the sensitivity of conventional radioisotopic tumor detection. Total resection of mJB6 tumors resulted in complete clearance of sIL-2R from the murine serum within 48 hours with a half-life of 6 hours. Accordingly, partial resection led to a significant decrease in sIL-2R followed by gradual increase with tumor regrowth. sIL-2R was also detected in the urine of mJB6-transplanted mice. As in serum, urine concentrations of sIL-2R were proportional to tumor mass ($P < 0.02$). Based on these findings we postulate that malignant cells are a major source of serum sIL-2R in patients with lymphoid tumors. In addition, our data further support monitoring sIL-2R concentration in body fluids as a sensitive method to detect change in tumor volume in such patients. (Am J Pathol 1994, 144:1089-1097)

The α -chain of interleukin-2 receptor (IL-2R) is expressed on the surface of T and B lymphocytes in two different forms.¹ When expressed alone, α -chain binds IL-2 protein with low affinity. Combined with β - and, as recently reported,² γ -chain, α -chain forms a high affinity IL-2R capable of transducing a cell-activating signal.^{3,4} No IL-2R α -chain is detected on

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resting T lymphocytes, whereas cell activation results in expression of the α -chain at high concentration. Similarly to other cytokine receptors, the extracellular fragment of IL-2R α -chain is secreted in a soluble form.⁵⁻¹¹ Although the function of sIL-2R remains uncertain,¹² this receptor can be detected at low concentrations in blood and urine of normal individuals.^{12,13} Elevated serum concentrations of sIL-2R have been detected in patients with a broad range of diseases such as leukemias¹⁴⁻¹⁶ and lymphomas,^{17,18} certain autoimmune disorders, viral infections including AIDS, and during rejection of transplanted organs.^{12,13} In lymphoid malignancies sIL-2R serum concentrations appear to correlate with extent or stage of disease.¹⁴⁻¹⁸ It remains uncertain, however, whether elevated sIL-2R levels result from secretion of the receptor by malignant cells or by normal, activated lymphocytes responding to the tumor, because *in vitro* studies show that both the malignant cells and normal activated lymphocytes can secrete sIL-2R.^{5,19} To address this question, we used a model of human anaplastic T cell lymphoma xenografted into SCID mice. Results of our study show that the lymphoma cells constitutively release sIL-2R in this *in vivo* model. Furthermore, there is a direct correlation between tumor volume and sIL-2R levels in blood and urine of the host mice.

Materials and Methods

Origin of the mJB6 Tumor

The JB6 cell line was obtained by *in vitro* culture of peripheral blood leukemic cells from a 12-year-old boy with advanced anaplastic large cell lymphoma.²⁰ The T cell origin of the tumor was established based on the immunohistochemical (positivity for CD2, CD7, and Bf1 antigens) and molecular (clonal rearrangement of β -chain of T cell receptor genes) findings. After several months in culture, 10⁸ JB6 cells were injected intraperitoneally into SCID mice. One mouse developed a widespread tumor. Fragments of mouse abdominal tumor mass (mJB6) were inoculated subcutaneously into several other SCID mice and maintained as described below.

mJB6 Tumor-Bearing Mice

C.B-17 scid/scid mice used in these experiments were kindly provided by Dr. M.J. Bosma, Fox Chase Cancer Center, Philadelphia, PA, and were kept in the animal facility at Joint Center for Radiation Therapy, New England Deaconess Hospital, Boston, MA.

mJB6 Tumor Transplantation

Passage of the mJB6 tumor was performed under anesthesia (ketamine/xylazine mixture) by subcutaneous injection in the right chest wall of 6 to 7 small (0.1 cm in the largest dimension) tumor tissue fragments, unless stated otherwise (see Results). Mice implanted with the mJB6 tumor were maintained up to 6 weeks until tumor growth reached approximately 2 cm in the largest dimension. Even the largest tumors did not have a visible adverse effect on the host mice.

Measurement of the Tumor Volume

Volume of the tumor was determined from the equation, volume = $0.4 \times ab^2$, where a and b designate long and short tumor diameter, respectively.

Collection of Blood and Urine

Blood was collected under anesthesia from the orbital sinus of the right eye or by terminal cardiac puncture. Urine was collected by suprapubic urinary bladder puncture after terminal bleeding. Control serum and urine from athymic nude (NIH-nu/nu) mice bearing human ovarian carcinoma (2008), prostatic carcinoma (PC3), and glioblastoma multiforme (T98G) were kindly provided by Dr. B. Teicher, Joint Center for Radiation Therapy, Dana Farber Cancer Institute, Boston, MA. Serum, heparinized plasma, and urine samples were stored at -80°C for up to 4 months without detectable loss of activity.

Reverse Transcription-Polymerase Chain Reaction

The reaction was performed as described.^{21,22} Briefly, cDNA was obtained from total RNA by incubation with murine Maloney leukemia virus reverse transcriptase (GIBCO-BRL, Gaithersburg, MD), oligo(dT), deoxynucleoside triphosphates, bovine serum albumin, DTT, and RNasin. Aliquots of synthesized cDNA were amplified for 30 cycles in DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT) in the presence of Taq polymerase (Perkin-Elmer Cetus), deoxynucleoside triphosphates, and primer pairs specific for human IL-2R α -chain (CD25), murine IL-2R α -chain, murine β -actin (Clontech, Palo Alto, CA), or human β -actin (synthesized on a Cyclone DNA synthesizer, Bioresearch, San Rafael, CA) and purified using NENSORB PREP columns (DuPont Co., Wilmington, DE) as described.²² Polymerase chain reaction-amplified DNA was visualized in ultraviolet

light after electrophoresis on 2% agarose gel containing ethidium bromide. The product was identified by a predicted molecular weight of the amplified DNA fragment. Its identity was confirmed by restriction mapping. Samples in which no reverse transcriptase was added served as controls.

Immunohistochemical Analysis of the mJB6 Tumor

Immunohistochemical staining of the mJB6 tumor was performed as previously described.^{23,24} In brief, 4- μ sections of frozen, periodate-lysine-paraformaldehyde-prefixed frozen tissue were cut and fixed additionally for 5 minutes in cold acetone. Immunolocalization was performed with anti-human IL-2R α -chain (CD25, Tac, Becton Dickinson, San Jose, CA) and CD30 (BerH2, Dako Corporation, Carpinteria, CA) monoclonal antibodies using a three-step immunoperoxidase method. Slides were developed using 3,3'-diaminobenzidine tetrahydrochloride. Staining was enhanced with osmium tetroxide and slides were counterstained with methyl green.

ELISA for Human sIL-2R

The sIL-2R ELISA plates were obtained from T cell Diagnostics, Cambridge, MA, and the assay was performed as recommended by the manufacturer as previously described.²⁵ In brief, 50 μ l of standards, serum, or urine diluted 1:5 to 1:40 were applied in duplicate into wells precoated with anti-human IL-2R monoclonal antibody. Next, 50 μ l of a monoclonal antibody against a different IL-2R epitope conjugated with horseradish peroxidase were added and the plates were incubated for 3 hours at room temperature on a rotator set at 150 rpm. After washing of the wells, 100 μ l of the O-phenylenediamine solution was applied into each well. Plates were incubated at room temperature for 30 minutes and then 50 μ l of 2 N H₂SO₄ were added to inhibit the enzymatic reaction. The optical density value was determined using an automated ELISA reader (Molecular Devices Corp., Menlo Park, CA) and data were processed and plotted using Softmax (Molecular Devices Corp.) and Microsoft Excel (Microsoft Corp., Redmont, WA) software. The data are shown as a mean of the duplicate samples (SD was <10%).

Surgical Removal of the Tumor

The implanted mJB6 tumors were either partially or totally resected in a laminar flow hood under sterile

conditions. The dermal wounds were closed with sterile surgical staples that, in case of partial resection, were removed after 7 days.

Radioisotopic Detection of the Tumor

mJB6 tumors were implanted under the skin over the right scapula and allowed to grow to approximately 1 to 2 cm in diameter. Tumor-bearing mice were then injected into the tail vein with 250 μ Ci of Ga-67 as the citrate. Nine-minute posterior whole body images (approximately 8×10^4 counts each) were obtained at 72 hours after injection using a pinhole collimator (4-mm aperture) fitted to a small field of view gamma camera (Siemens LEM, Hoffman Estates, IL). Scintigraphic data were collected on a PCS 512 computer (Picker International Inc., Highland Heights, OH) in a 128 \times 128 matrix for further evaluation.

Statistical Analysis of the Results

The data were analyzed using the Prophet system, a national computer resource sponsored by the Division of Research Resources, NIH. The correlation between tumor volume and sIL-2R concentration in serum and urine was evaluated using the Pearson's product moment (simple) correlation coefficient. Differences between serum and urine sIL-2R concentration were analyzed using slope and intercept covariance analysis.

Results

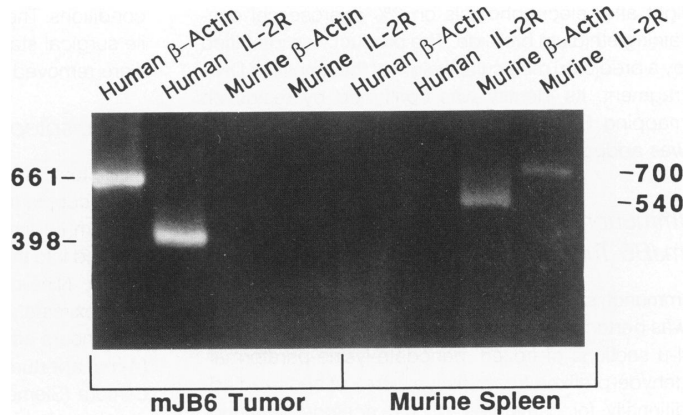
Expression of mRNA for IL-2R by mJB6 Tumor

To examine whether the mJB6 tumor cells produced a message for IL-2R α -chain, we performed a reverse transcription-polymerase chain reaction test with primers specific for the human α -chain. Three additional sets of primers directed against human β -actin and murine IL-2R α -chain and β -actin served as controls. As shown in Figure 1, total RNA isolated from the mJB6 cells contained transcripts for human IL-2R α -chain and β -actin but no detectable message for their murine counterparts. In contrast, murine spleen contained no transcripts recognizable by both human oligoprobes and contained mRNA for murine IL-2R α -chain and β -actin.

Detection of IL-2R α -chain Protein in mJB6 Tumor Tissue

To determine whether mJB6 tumor cells synthesize IL-2R α -chain protein, we performed an immunocy-

Figure 1. Expression of mRNA for α -chain of human IL-2R by mJB6 tumor. Total RNA derived from mJB6 tumor or spleen of the tumor host SCID mouse was reversibly transcribed and the cDNA was PCR amplified in the presence of primer pairs specific for fragment of human β -actin (predicted molecular weight 661 bp), human IL-2R α -chain (398 bp), murine β -actin (540 bp), or murine IL-2R α -chain (700 bp). The amplified DNA was visualized in ultraviolet light after electrophoresis on 2% agarose gel containing ethidium bromide. Identity of the product was confirmed by restriction mapping of the amplified fragment (data not shown). Tumor-derived cDNA is in lanes 1 to 4, spleen-derived DNA in lanes 5 to 8.



tochemical analysis of tumor tissue sections using murine monoclonal antibody specific for human IL-2R α -chain (CD25). Figure 2, in addition to showing the histological appearance of the mJB6 tumor (panel A), revealed strong staining of the tumor cells in a characteristic membranous pattern (panel B). It is noteworthy that only a subpopulation of cells, approximately 15 to 25% depending on the microscopic field examined, expressed IL-2R α -chain. In contrast, vir-

tually all tumor cells were positive for the CD30 (Ki-1) antigen, which is characteristic of anaplastic large cell lymphoma (panel C).

Production of sIL-2R by mJB6 Tumor

To address the question of whether mJB6 tumor produces a soluble form of IL-2R α -chain, we tested serum samples from mice with two different mJB6 tumor

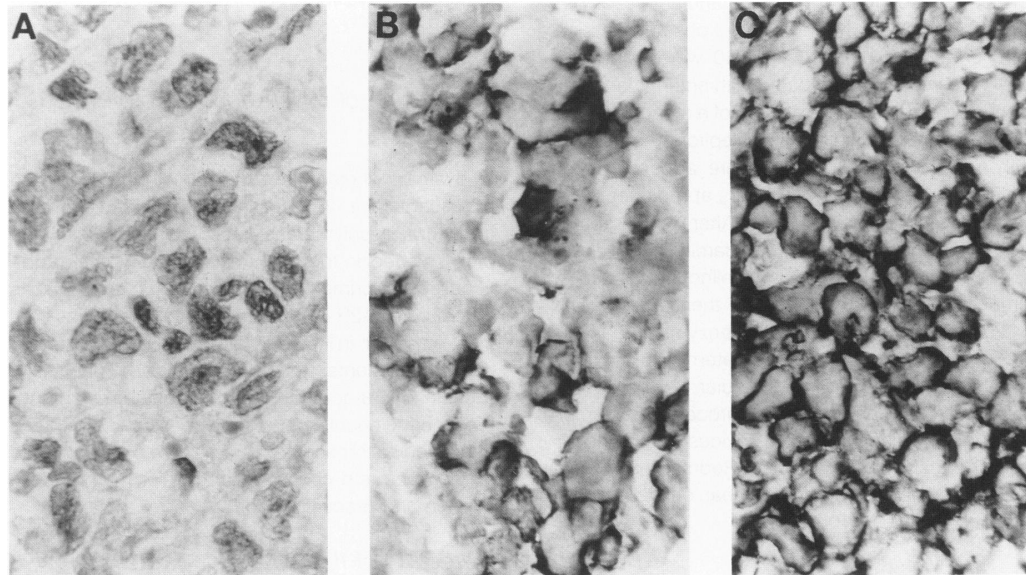


Figure 2. Immunohistochemical detection of IL-2R α -chain protein in mJB6 tumor tissue. Sections of periodate-lysine-paraformaldehyde-prefixed frozen mJB6 tumor tissue were fixed in cold acetone. Antigen immunolocalization was performed by three-step immunoperoxidase method. The staining was enhanced with osmium tetroxide and methyl green was used as a counterstain. A: Morphologic appearance of mJB6 tumor (hematoxylin and eosin stain). B: Immunohistochemical staining with anti-human IL-2R α -chain (CD25) monoclonal antibody. C: staining with anti-human CD30 (BeeH2) monoclonal antibody.

volumes in a solid phase ELISA specific for human sIL-2R. As controls, we used serum samples from mice with no tumor or with three different types of human nonlymphoid tumors. As shown in Figure 3, only sera from mJB6-bearing mice contained human sIL-2R at high concentrations. In contrast, sera from mice with no tumor or human prostatic carcinoma, ovarian carcinoma, or glioblastoma, all of which had a volume comparable to mJB6 tumors, contained no detectable human sIL-2R. Importantly, the sIL-2R concentration in mJB6-bearing mice appeared to correlate directly with tumor volume because the larger tumor (3.36 cm³) yielded higher sIL-2R serum titer (58,683 U/ml) than the smaller one (0.38 cm³ and 27,548 U/ml, respectively).

Correlation Between mJB6 Tumor Volume and sIL-2R Serum Concentration

To confirm the apparent relationship between the tumor volume and serum level of sIL-2R, we tested a number of blood samples from five mice with mJB6 tumors at various stages of tumor growth. The results (Figure 4) show a strong direct correlation between the tumor volume and sIL-2R concentration ($P < 0.0001$).

Effect of Surgical Resection of mJB6 Tumor on sIL-2R Serum Concentration

To further investigate the relationship between the tumor growth and sIL-2R in serum, we performed partial and total resection of the several mJB6 tumors (Figure 5). Total resection of three small tumors (volume 0.18 to 0.23 cm³) resulted in a rapid decrease in circulating sIL-2R. A 50% decrease occurred within 6 hours after

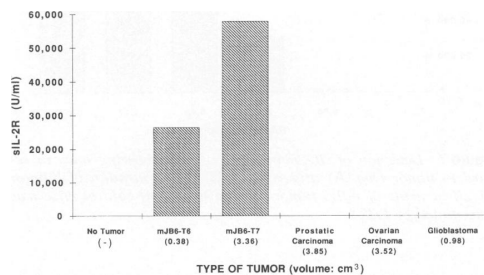


Figure 3. Production of sIL-2R α -chain by mJB6 tumor. Concentration of tumor-derived sIL-2R in serum of the host mice was determined by solid phase ELISA using plates precoated with anti-human sIL-2R monoclonal antibody and horseradish peroxidase conjugated to the second monoclonal antibody against a different epitope of human sIL-2R. Mice with no tumor or xenografted with human prostatic carcinoma, ovarian carcinoma, and glioblastoma multiforme served as controls.

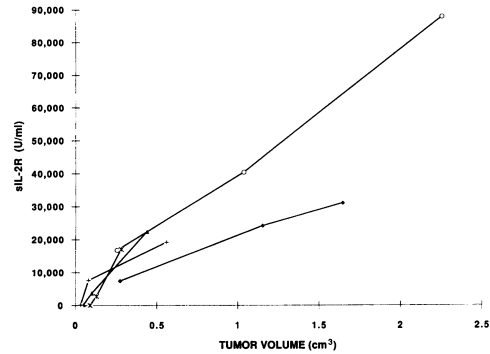


Figure 4. Correlation between mJB6 tumor volume and sIL-2R concentration in serum. Serum samples from five mice bearing mJB6 tumor of different volumes were collected from each mouse on three separate occasions at 7- to 10-day intervals. Three mice (labeled +, *, and \blacktriangle) had small tumors at the time of first bleeding, the remaining two (\circ and \blacklozenge) had larger tumors. Concentration of tumor-derived sIL-2R in serum of the mice was determined by ELISA specific for human sIL-2R.

resection with complete serum clearance by 48 hours. Similarly, a marked decrease in circulating sIL-2R was seen 24 hours after partial resection of two relatively large tumors (0.63 and 1.04 cm³). Regrowth of these partially resected tumors resulted in a continuous increase in sIL-2R serum concentration.

Sensitivity of sIL-2R ELISA in Detection of mJB6 Tumor

Next, we tested the limit of sensitivity of tumor detection by measurement of serum sIL-2R. For this purpose mice were injected subcutaneously with a single fragment of tumor rather than with 6 to 7 fragments as in the previous experiments (see Materials and Methods). We reasoned that such an approach would permit better vascularization of the small tumors. Inadequate angiogenesis is not only a factor limiting the tumor growth^{26,27} but in this case it might also decrease serum sIL-2R concentration in the smallest palpable tumors. As shown in Figure 6, tumors as small as 0.80 mm³ were easily detectable by sIL-2R ELISA method (386 U/ml). Even a smaller tumor (0.40 mm³) could be detected yielding 193 U/ml of sIL-2R compared with 0 U/ml for the serum from a mouse with no palpable tumor. This, however, appears to be the limit of sensitivity for this system because in some experiments a nonspecific background of up to 100 U/ml for serum from mice with no injected tumor was noted (data not shown). The sensitivity of sIL-2R ELISA for detection of mJB6 compared very favorably with a radioisotopic method that is routinely used for

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