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Increased Circulating Concentrations of Interleukin 2 Receptor during Rejection Episodes in Heart- or Kidney-Transplant Recipients

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Concentrations of interleukin 2 receptor (sIL-2R) have been suggested as a marker of rejection episodes after organ transplantation. To evaluate the analytical performance of a "sandwich-type" enzyme immunoassay method for sIL-2R and to verify whether increased concentrations of sIL-2R might be a useful marker of allograft rejection, we quantified sIL-2R in serum samples from heart- or kidney-transplant patients. The mean (±SD) pre-transplant value of sIL-2R (592 ± 209 kilo-units/L) in heart-transplant patients was significantly higher ($P < 0.01$) than that observed in controls (350 ± 101 kilo-units/L). After heart transplantation, the concentrations of sIL-2R slowly decreased to baseline in successfully treated patients but increased significantly (1129 ± 215 kilo-units/L; $P < 0.01$) during acute rejection crisis. However, severe infections were also associated with a significant increase of sIL-2R, so the sIL-2R test is not specific for allograft rejection. The mean pre-transplant concentration of sIL-2R was also increased (1943 ± 878 kilo-units/L) in 26 renal-transplant patients; after transplantation, this value returned to normal, as did that for creatinine, but persisted steadily high in five patients who experienced acute tubular necrosis. In this group of patients, the sIL-2R concentration increased by 1.5- to fourfold, both during acute rejection episodes and in clinically evident infection; thus measurement of creatinine and sIL-2R concentrations can help to distinguish between rejection, infection, and cyclosporine toxicity. In two episodes of mild cyclosporine-induced nephrotoxicity, we observed slight increases in serum creatinine (which returned to baseline when the cyclosporine dose was decreased) not associated with an increase in sIL-2R. We conclude that systematic monitoring of sIL-2R together with other biochemical and clinical markers may be useful in the management of kidney-transplant patients.

Additional Keyphrases: *creatinine · cyclosporine · nephrotoxicity*

Interleukin 2 (IL-2), a T-cell growth factor, is synthesized and secreted after activation with antigen or mitogen in the presence of the monokine interleukin 1 (*1, 2*).[4] The activation of T-lymphocytes is accompanied by expression of specific high-affinity membrane receptors for IL-2 (IL-2R) and release into extracellular fluids of immunoassay-detectable amounts of IL-2R (soluble IL-2R or sIL-2R) (*3, 4*). The sIL-2R glycoprotein has a peptide backbone that is smaller than that of its membrane-associated counterpart (45 000 vs 55 000 Da) and also binds IL-2 (*4, 5*), although with lower affinity (*6*).

Increased circulating concentrations of sIL-2R are detectable in several conditions, e.g., hematological neoplasia (*7, 8*), viral infections (*7*), and granulomatous disorders (*9, 10*). Some have also suggested that the concentrations of serum sIL-2R deriving from activated cell turnover may indicate rejection or infection during organ transplantation (*11–13*). Colvin et al. (*11*) suggested that sIL-2R assay may be useful clinically in the differential diagnosis of renal allograft rejection, especially in distinguishing cyclosporine (CsA) nephrotoxicity; in fact, in kidney-transplant recipients, sIL-2R greatly increased during rejection or viral infections, whereas patients with CsA nephrotoxicity had sIL-2R concentrations significantly below those of patients with rejection (*11*). Moreover, two preliminary reports suggest that an increase of sIL-2R in heart-, heart–lung-, or liver-transplant recipients may be associated with a worse prognosis (*12*) and that the determination of sIL-2R concentrations may prove useful in monitoring cardiac allograft rejection (*13*).

To evaluate the analytical performances of a "sandwich-type" enzyme immunoassay method for measuring sIL-2R and to verify whether increased concentrations of circulating sIL-2R may be considered a useful marker of allograft rejection, we assayed sIL-2R concentrations in serum samples of heart- or kidney-transplant patients.

Materials and Methods

sIL-2R assay. The sIL-2R concentration was assayed by a sandwich enzyme immunoassay method (T Cell Science, Inc., Cambridge, MA 02139) that involves two different anti-IL-2R monoclonal antibodies; the first one is adsorbed onto polystyrene microtiter wells and the other is conjugated with horseradish peroxidase (EC 1.11.1.7). Soluble IL-2R present in the serum sample binds to antibody on the well and, after washing of the unreacted materials, the enzyme conjugated to the anti-IL-2R antibody is added to complete the sandwich. The unbound enzyme-conjugated antibody is then removed and a substrate solution (hydrogen peroxide/*o*-phenylenediamine) is added to form a colored product (measured at 490 nm), the quantity of which is proportional to the amount of sIL-2R present in the sample. The concentration of unknown samples is estimated by interpolation on a calibration curve prepared with five sIL-2R standards. sIL-2R concentration is expressed in kilo-units per liter, 1 kilo-unit being defined as the amount of sIL-2R released or present in 1 mL of a

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[4] Nonstandard abbreviations: IL-2, interleukin 2; sIL-2R, soluble receptor for IL-2; and CsA, cyclosporine.
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standard preparation of cell-culture supernatant fluid from phytohemagglutinin-stimulated peripheral blood cells (T Cell Science, Inc.).

The mean precision profile was cumulatively computed from 10 assays, by use of a previously described computer program (*14, 15*); the sensitivity (lowest detection limit) was read on the precision profile as the lowest concentration distinguishable from zero (confidence limit ±2 SD) or the concentration corresponding to a CV of 50%; the working range, also derived from the precision profile, was the concentration range measurable with a within-assay precision (CV) <10%. The concentration of CsA in blood samples was measured by fluorescence polarization immunoassay (TDx; Abbott Labs., N. Chicago, IL), as previously reported (*16*).

Healthy subjects. We studied 38 adult blood donors (26 men and 12 women, ages 18–60 years).

Heart failure and transplantation. We studied 13 heart-transplant patients: six who developed a biopsy-proven episode of acute rejection that was successfully treated with supplementary immunosuppression, five with a severe nonviral infection, and two others with acute viral infections. All these patients were receiving standard therapy with CsA and steroids. A baseline value for sIL-2R was established for each patient in a clinically and instrumentally determined rejection-free and infection-free interval. These heart rejection episodes were graded histologically as moderate, according to Billingham (*17*). The patients underwent routine endomyocardial biopsy weekly in the first month, every two weeks for the second and third month, once every month thereafter until the sixth month, and whenever clinically indicated. Supplementary treatment with pulsatile steroids (1 g of methylprednisolone intravenously or 100 mg of oral prednisone for three days) was administered for moderate rejection or mild rejection plus clinical finding of heart failure. Persistent rejection after this therapy with steroids was treated with horse anti-lymphocyte globulin (20 mg/kg of body wt, intravenously, for 10 days).

Kidney end-stage disease and transplantation. We studied 39 patients (27 men and 12 women, ages 32–76 years) with end-stage renal disease who had been treated by hemodialysis for from six months to 22 years; 35% of them were undergoing hemodialysis with cuprophan dialyzers, 65% with polyacrylonitrile.

Finally, we monitored for 15 months a group of 26 patients (ages 18–59 years) who underwent renal transplantation. All these patients were on standard triple immunosuppressive therapy: azathioprine, low-dose CsA (8 mg/kg of body wt daily), and steroids. The dose of CsA administered was determined after monitoring the circulating concentrations of the drug (target range 300–600 μg/L). Biopsy-proven rejection episodes were treated with steroids (methylprednisolone, 500 mg/day, for three to five days) and, if the patient did not respond, with OKT3 monoclonal antibody (5 mg/day for 14 days). The clinical diagnosis of acute CsA nephrotoxicity was made when the renal function, as reflected by the concentration of serum creatinine, improved with a decrease of CsA dose.

Results

Analytical performances of the sIL-2R assay. The dose–response curve of the sIL-2R assay was approximately linear in the range 100–1600 kilo-units/L; the working range extended from 250 to 1600 kilo-units/L; the mean sensitivity (lowest detection limit) was 53 ± 20 kilo-units/L. The between-assay variability (CV), as assessed by measuring (in eight runs) three serum pools containing different amounts of sIL-2R (349 ± 37, 628 ± 66, or 989 ± 102 kilo-units/L) was 10.7%, 10.5%, and 10.3%, respectively. Serial sample dilution (parallelism test) showed a linear relationship (r = 0.999) with dilution.

Control subjects. The mean (±SD) sIL-2R concentration in 38 healthy subjects was 350 ± 101 kilo-units/L (range 165–630 kilo-units/L), without any significant difference between men and women.

Heart-transplant patients. The mean pre-operative value of sIL-2R (592 ± 209 kilo-units/L) found in 13 heart-transplant patients was significantly (P <0.01) higher than that observed in controls. In six transplant patients, sIL-2R increased significantly over the baseline values (1129 ± 215 kilo-units/L, P <0.01 by paired t-test) during acute rejection crisis documented by clinical findings and myocardial biopsy; these increases were significantly reduced to baseline after successful treatment (544 ± 395 kilo-units/L, P <0.01). Moreover, we observed that severe bacterial (n = 5) or viral (n = 2) infections were also accompanied by a significant increase of sIL-2R concentrations in serum (1076 ± 263 vs 486 ± 146 kilo-units/L, P <0.01, in bacterial infections; 1290 ± 368 vs 370 ± 85 kilo-units/L in viral infections).

Patients with end-stage renal disease or renal transplantation. sIL-2R concentrations were increased both before (1829 ± 797 kilo-units/L) and after hemodialysis (1846 ± 805 kilo-units/L) in 39 patients with end-stage renal disease; there were no significant differences between membranes, length of dialysis, and type of primary renal disease. The mean (±SD) pre-operation concentration of sIL-2R was also increased (1943 ± 878 kilo-units/L) in the patients who received a renal transplant; after transplantation, the sIL-2R decreased to normal values, following the pattern of creatinine concentrations. The rate of decrease varied in all uneventful transplants but values stayed high in five patients who experienced acute tubular necrosis requiring dialysis for the first week or so after transplantation. Figure 1 shows a typical time-course for the concentrations of sIL-2R and serum creatinine in one

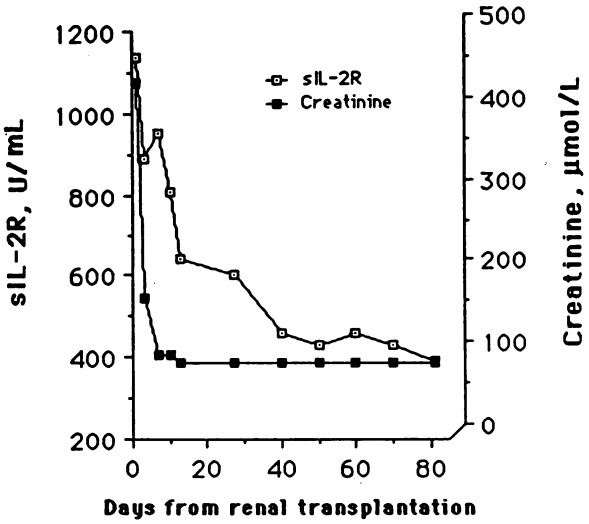

Fig. 1. Typical time-course of the concentration of sIL-2R and serum creatinine in a patient with uneventful kidney transplant

patient with uneventful kidney transplant. The difference in decay rate of sIL-2R after renal transplant was independent of the type of allograft. In addition, high pre-operation concentrations of sIL-2R did not correlate with immunological post-transplant events and did not predict subsequent allograft loss.

Eight kidney-transplant patients had 11 rejection episodes (documented by biopsy) that were reversed by OKT3 treatment. The concentration of IL-2R at the beginning of acute rejection showed a 1.5- to fourfold increase. Moreover, we observed that in five infection episodes (including two cases of interstitial pneumonias) sIL-2R was increased up to sixfold. Figure 2 shows the time-course of sIL-2R in a patient who experienced an episode of acute rejection (reversed by treatment) and two episodes of viral infection. During the rejection episode, the concentrations of sIL-2R and creatinine simultaneously increased, whereas the infections produced an increase of sIL-2R only, with no change of serum creatinine.

Two episodes of mild CsA nephrotoxicity were observed; in these patients only a slight increment of serum creatinine (which returned to baseline when the CsA dose was decreased) was found, whereas the sIL-2R was unaffected. Table 1 reports concentrations of CsA, sIL-2R, and creatinine in one of these two patients.

Discussion

We found the enzyme immunoassay technique used in the present study to be suitable and reliable for the measurement of sIL-2R concentration in transplant patients. Our results indicate that sIL-2R concentrations increase to the same extent both during allograft rejection and infection episodes in heart- or kidney-transplant patients; therefore, the sIL-2R test is nonspecific and cannot distinguish rejection from infection. However, in renal transplant, rejection produces a characteristic pattern, i.e., a parallel increase in both creatinine and sIL-2R. Moreover, we were able to observe in these patients two episodes of mild CsA nephrotoxicity in which creatinine (and CsA) increased while sIL-2R remained steady. These data confirm the findings of Colvin et al. (*11*), who suggested that sIL-2R measurement may be useful in distinguishing between CsA nephrotoxicity, rejection, and viral infection. In the rejection episodes, however, the concomitant increase of sIL-2R and creatinine suggest that the sIL-2R test is not more sensitive than the creatinine assay.

We conclude that systematic monitoring of sIL-2R as well as other biochemical and clinical markers can be a useful tool in managing kidney-transplant patients.

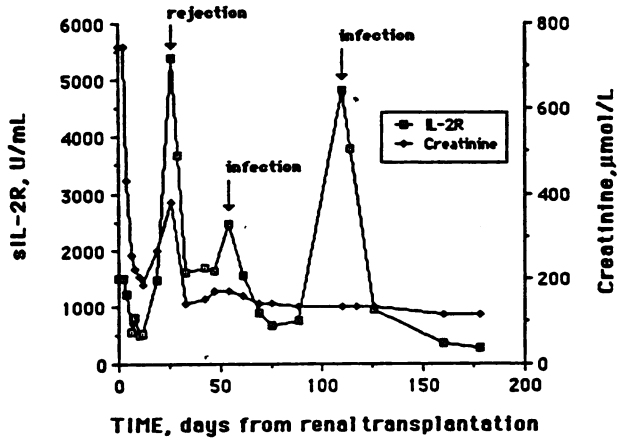

Fig. 2. Time-course of sIL-2R and creatinine concentrations in a kidney-transplant patient with one episode of allograft rejection and two episodes of viral infections

Note the simultaneous increase of sIL-2R and creatinine during the rejection episode, whereas during infections only sIL-2R increased with no change in serum creatinine concentrations

Table 1. Serum Concentrations of Creatinine and sIL-2R and Blood CsA Concentrations in a Renal-Transplant Recipient with Mild CsA Nephrotoxicity

Day after transplantation	Serum creatinine, µmol/L	Blood CsA, µg/L	sIL-2R, kilo-units/L
0	433	—	1130
5	106	537	940
8	97	550	800
21[a]	115	1137	740
40	97	535	450
80	88	382	380

[a] CsA nephrotoxicity episode documented by the slight increase in serum creatinine concomitantly with a doubling of the CsA blood concentration. Two to three weeks after, when the CsA dose was decreased, the concentrations of CsA and creatinine returned to their normal ranges. The concentrations of sIL-2R declined progressively and continuously throughout this period, reaching the normal range one month after transplantation.

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Insufficient Accuracy and Specificity of Polyanion Precipitation Methods for Quantifying Low-Density Lipoproteins

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Recently, polyanion precipitation assays for low-density lipoprotein (LDL)-cholesterol have been found to underestimate their analyte in normolipidemic samples (Siekmeier et al., Clin Chim Acta 1988;177:221–30). Therefore, accuracy, specificity, and interference by nonesterified fatty acids have been studied for three precipitants (obtained by heparin, dextran sulfate, or polyvinyl sulfate precipitation). At normal concentrations of LDL, precipitation is incomplete, whereas it is nearly quantitative at high concentrations of LDL. The polyvinyl sulfate reagent markedly responds to variations in the amount of non-LDL protein present in the precipitation mixture. In the dextran sulfate and the polyvinyl sulfate method, but not in the heparin method, the percentages of LDL precipitated notably increase as the concentration of the polyanion compound is decreased. In either assay, very-low-density lipoproteins, but not high-density lipoproteins, are significantly coprecipitated (dextran sulfate 28%, polyvinyl sulfate and heparin 66%) in a concentration-independent fashion. Increased concentrations of nonesterified fatty acids markedly interfere with the dextran sulfate and polyvinyl sulfate assay, but do not much affect results with the heparin reagent.

Additional Keyphrases: *intermethod comparison · cholesterol · analytical error*

Hypercholesterolemia represents one of the primary risk factors for the premature development of atherosclerosis. Whereas increased concentrations of low-density lipoproteins (LDL) confer a high cardiovascular risk, high-density lipoproteins (HDL) are thought to exert protective effects (*1–3*).[2] Hence, individual risk profiles should include assays for HDL and LDL. A common approach to the quantification of lipoprotein classes has been the measurement of HDL-C after precipitation of apolipoprotein (apo) B-containing lipoproteins (*4–7*) and subsequent calculation of LDL-C according to Friedewald et al. (*4*). In the meantime, methods aimed at the selective precipitation and direct quantification of LDL-C have been designed and are commercially available (*8–10*).

Starting from our recent observation that concentrations of LDL-C after precipitation with polyanion compounds are lower than those obtained with a combined ultracentrifugation–precipitation procedure (*11*), we have evaluated the accuracies and specificities for LDL-C of three assays (*8–10*) based on precipitation of LDL with either heparin, dextran sulfate (DS), or polyvinyl sulfate (PVS).

Materials and Methods

Apparatus. For preparative ultracentrifugation we used a Model L 8-70 (Beckman Instruments, Fullerton, CA) or a Model TGA 75 (Kontron AG, Analytical Division, Zürich, Switzerland) ultracentrifuge with fixed-angle rotors (Kontron types TFT 50.38 and TFT 45.6). Densities were monitored with a DMA 55 digital precision density meter (A. Paar KG, Graz, Austria).

Reagents and other materials. Reagent kits for cholesterol (Monotest CHOD-PAP) and triglycerides (GPO-PAP) were purchased from Boehringer Mannheim, Mannheim, F.R.G. The "NEFAC" test for the enzymatic determination of nonesterified (free) fatty acids was obtained from Wako Chemicals, Neuss, F.R.G. Celite 545 (analytical grade), palmitic acid, stearic acid, and Tris were from Serva Feinbiochemica, Heidelberg, F.R.G.; other chemicals were from E. Merck, Darmstadt, F.R.G.

Blood collection. Blood samples from ostensibly healthy donors, mostly women, ages 20 to 30 years, were obtained by venipuncture after an overnight fast and drawn into tubes containing K_2EDTA at a final concentration of 1.5–2 g/L (Sarstedt, Nümbrecht, F.R.G.). Plasma was recovered by centrifugation (1500 × *g*, 30 min) and stored at 4 °C. Lipid analyses were completed within no more than five days after blood collection.

Quantification of LDL-C. LDL-C was determined either after precipitation with heparin (LDL-C_{HEP}), dextran sulfate (LDL-C_{DS}), polyvinyl sulfate (LDL-C_{PVS}), or by a combined ultracentrifugation and phosphotungstic acid/$MgCl_2$ method (LDL-$C_{UC/PhT}$).

For LDL-C_{HEP} (*8*), we added 100 μL of sample to 1.0 mL of precipitant (sodium citrate, 64 mmol/L, pH 5.04, and heparin, 50 000 U/L), swirled, and incubated the mixture

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[2] Nonstandard abbreviations: VLDL, LDL, HDL, very-low-, low-, and high-density lipoproteins; VLDL-C, LDL-C, HDL-C, cholesterol in VLDL, LDL, and HDL; DS, dextran sulfate; PVS, polyvinyl sulfate; LDL-C_{HEP}, LDL-C_{DS}, LDL-C_{PVS}, LDL-C as measured after heparin, DS, or PVS precipitation; $LDL_{UC/PhT}$, LDL-C measured after a combination of ultracentrifugation and phosphotungstic acid/$MgCl_2$ precipitation; and FFA, free fatty acids.
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