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A critical analysis of soluble interleukin-2 receptor levels in kidney allograft recipients

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Abstract Soluble interleukin-2 receptor (sIL-2R) was measured by the Cellfree Kit (T Cell sciences) in 103 pretransplant and 1590 posttransplant samples from 103 patients with cadaveric kidney allografts. The mean values (± SD) detected in pretransplant sera were significantly higher (1932 ± 1389 U/ml) than in 72 healthy adults (267 ± 139 U/ml), but after transplantation they continuously fell towards normal levels within the first 3 postoperative weeks. Recipients with acute rejection episodes showed higher sIL-2R levels (1762 ± 904 U/ml) than those with stable transplants at discharge (937 ± 398 U/ml). Highest values were detected during antirejection therapy with antithymocyte globulin (4996 ± 2166 U/ml) or OKT3 (5905 ± 3910). Increases were also observed during bacterial and viral infections and even, in some cases, without any apparent cause. Because of this lack of specificity, elevated sIL-2R levels should be interpreted cautiously. Nevertheless, sIL-2R level can be useful for monitoring kidney allograft recipients. Increases point to a cellular immune activation process and can predict rejections or infections.

Key words Interleukin-2 receptor · Interleukin-6 · Kidney transplantation · Rejection

Introduction

Allograft rejection is one of the immune responses in which cytokines are considered to play an important role. The stimulation of T cells by foreign antigens results in cellular activation leading to de novo synthesis of interleukin (IL)-2 as well as expression of IL-2 receptor (IL-2R). Helper T cells (Th1 subset) appear to be the major source of IL-2. Membrane-bound IL-2R can be released in a soluble form (sIL-2R) and detected in vivo. This sIL-2R corresponds to a truncated extracellular part of the α chain of IL-2R. It is widely accepted that an elevated serum sIL-2R level reflects the degree of T cell activation. In the field of clinical kidney transplantation, a lot of effort has been made to improve monitoring of graft rejection by the determination of sIL-2R in serum [4, 6, 8, 19, 22, 23, 25–28] and urine [2, 5, 15]. Although most authors described elevated sIL-2R levels in connection with but hardly prior to rejection episodes [4, 6, 8, 19, 22, 23, 25, 27, 28], there were also reports of elevated sIL-2R levels in the immediate postoperative phase [28], during virus infections [4, 8, 19, 22, 27] or treatment with antilymphocytic antibodies [4, 8], and also without any apparent cause [22]. Therefore, in this study we investigated the pattern of IL-2R serum levels in 103 patients submitted for renal transplantation in order to clarify the usefulness of serial sIL-2R determinations and compared these results with the serum level of the proinflammatory cytokine IL-6 as an early indicator of inflammatory tissue injury.

Material and methods

Patients and protocol

One hundred and three patients (mean age 45 ± 11 years; females 32, males 71), who underwent cadaveric renal transplant (first, 93; second/third, 10) between July 1992 and August 1993 at the Kidney Transplant Centre Berlin-Friedrichshain were studied. The immunosuppressive regimen was identical for all recipients. It consisted of oral cyclosporine with the dose adjusted to maintain whole blood levels from 200 to 250 ng/ml, azathioprine (1 mg/kg per day) and methylprednisolone (MP) starting with 40 mg/day. In addition, all but one recipient received the Friedrichshain variant of ATG induction therapy, details are already published [13, 14]. Briefly, this induction consisted of an intraoperative high-dose single ATG or ALG bolus (ATG (Fresenius) 9 mg/kg [$n = 69$], lymphoglobulin (Merieux) 30 mg/kg [$n = 16$], ATGAM (Upjohn) 45 mg/kg [$n = 8$], pressimmun (Behringwerke AG) 60 mg/kg [$n = 10$]). In order to avoid a cytokine-release syndrome, 500 mg MP were given about 1 h pre-ATG/ALG. Rejection episodes were diagnosed by standard clinical and pathological criteria (including a progressive elevation of serum creatinine and urea nitrogen, accompanied by oliguria, albuminuria, IgG-uria, increase of graft size [sonography] and lymphocytic infiltrate [fine needle aspiration biopsy]) and treated with MP for 5 days at 5 mg/kg body weight. Attemps to reverse biopsy-proven MP-resistant rejections by ATG, using a dose-by-T cell-protocol (aspired values: 50–150 T cells/μl), were tried. The number of T cells were determined by flow cytometry (FACScan, Becton Dickinson, Heidelberg, Germany). OKT3 (CILAG, 10 days, 2.5 mg) was given as rescue therapy or primarily in cases of humoral/vascular rejections.

Detection of sIL-2R and IL-6

Soluble IL-2R levels were determined by means of the Cellfree IL-2R Kit (T cell Sciences, Cambridge, Mass., USA) and IL-6 by the Quantikine IL-6 Immunoassay (R & D Systems, Minneapolis, Mass., USA) according to the instructions of the manufacturers. In Germany, both immunoassays are distributed by DPC Biermann GmbH, Bad Nauheim. Serum for IL-2R and IL-6 assays were taken before transplantation and three times a week thereafter (always between 7.00 and 8.00 a.m.) up to discharge and stored at − 20 °C. Altogether 103 pretransplant sera and 1590 posttransplant sera were examined and the results compared with those obtained from 72 healthy adults.

Table 1 Serum IL-2R levels in kidney transplant recipients (*CMV* cytomegalovirus, *sIL-2R* soluble interleukin-2 receptor)

Sample	Number	sIL-2R [U/ml]
Healthy adults	72	267 ± 139
Pretransplant (dialysis)	103	1932 ± 1389
Posttransplant (day 1–3)	93	2109 ± 1212
Stable transplant at discharge (event-free courses)	34	937 ± 398
Rejection		
Methylprednisolone (MP)-sensitive		
Pre-MP	16	1469 ± 911
During MP	16	1762 ± 904
MP-resistant		
Pre-ATG	6	3644 ± 2033
During ATG	6	4996 ± 2166
Humoral/vascular or MP-resistant		
Pre-OKT3	8	2111 ± 1867
During OKT3	8	5905 ± 3910
Infections		
CMV disease (mild) at the time of leukocytopenia	6	1081 ± 600
Bacterial infections (serious)	8	2593 ± 1764

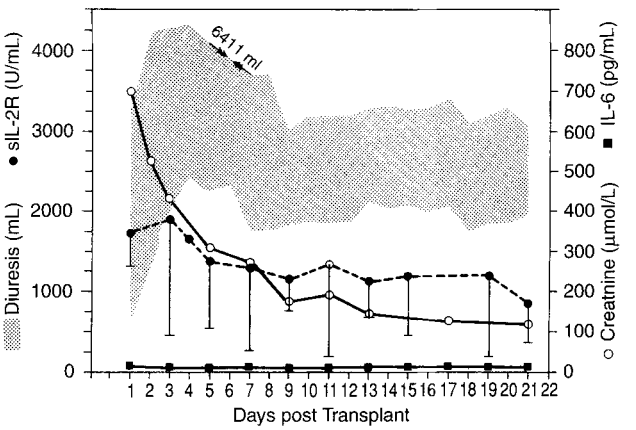

Fig. 1 Course of sIL-2R (mean ± SD) and IL-6 serum levels (mean ± SD) compared with serum creatinine (mean values) and diuresis (± SD, *hatched area*) in 26 renal graft recipients with immediate early graft function, no rejection episodes and no infections in the early posttransplant period up to discharge from transplant centre

Results

The range of sIL-2R levels in the serum of 72 normal individuals is between 17–737 U/ml, with a mean value of 267 U/ml. The upper normal limit ⩾ + 3 SD) is 683 U/ml. IL-6 is not detectable in healthy subjects. The mean values (± SD) of sIL-2R detected in pretransplant sera (i.e. sera from patients on chronic dialysis) were 1932 ± 1389 U/ml (Table 1). These values were significantly higher than in healthy adults (267 ± 139 U/ml, $P < 0.001$). The immediately posttransplant elevated sIL-2R serum levels (2109 ± 1212 U/ml) continuously fell towards normal levels within the first 3 weeks after transplantation. Samples from patients with stable renal function (Fig. 1) after event-free courses showed values hardly above normal at discharge (937 ± 398 U/min), but significantly decreased as compared to pretransplant levels ($P < 0.01$). Recipients with acute cellular rejections episodes (Fig. 2) had significantly ($P < 0.01$) higher sIL-2R levels than stable patients (1762 ± 904 U/ml). In comparison to MP-sensitive rejections, in both MP-resistant and humoral/vascular rejections the sIL-

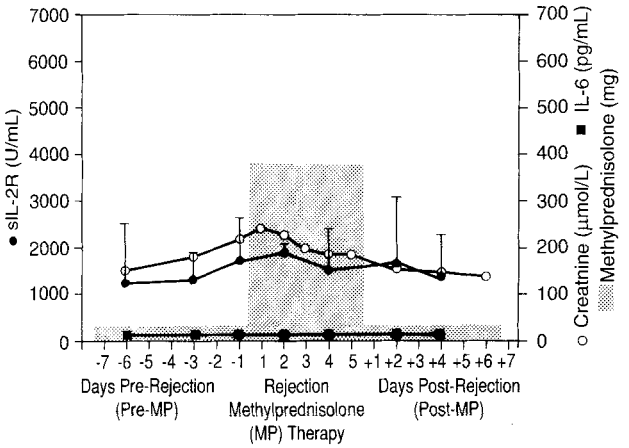

Fig. 2 Course of sIL-2R (mean ± SD) and IL-6 (mean ± SD) serum levels compared with serum creatinine (mean values) in 16 renal graft recipients with methylprednisolone(MP)-sensitive rejections. The treatment consisted of 5 mg MP/kg body weight for 5 consecutive days (MP dose = mean value)

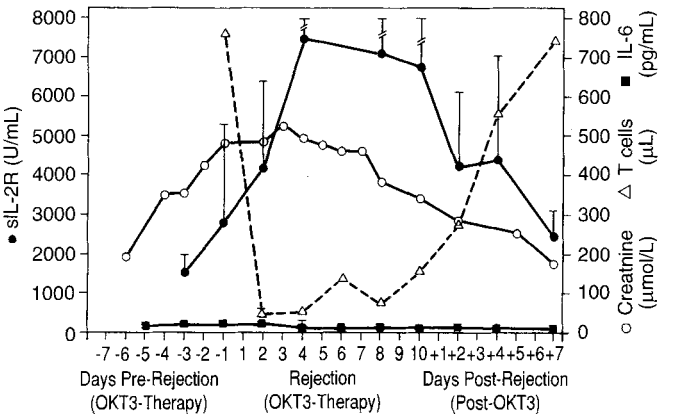

Fig. 3 Course of sIL-2R (mean ± SD) and IL-6 (mean ± SD) serum levels compared with serum creatinine (mean values) and T cell count (mean values of absolute numbers of CD3 positive cells) in eight renal graft recipients with methyl-prednisolone-resistant or humoral/vascular rejections during successful OKT3 therapy (2.5 mg/day for 10 consecutive days)

2R levels were significantly higher before antirejection therapy had begun (Table 1). Shortly after treatment with poly- or monoclonal anti-T cell antibodies (ATG or OKT3) sIL-2R levels further increased. The highest values were seen during OKT3 therapy (Fig. 3). In patients with mild cytomegalovirus (CMV) disease the sIL-2R levels were only slightly elevated at the time of leukocytopenia (1081 ± 600 U/ml). In contrast, serious bacterial infections (pneumonia, sepsis) were associated with marked but very different elevations of sIL-2R levels (2593 ± 1764).

With regard to the interpretation of elevated sIL-2R levels, the 103 sIL-2R serum peaks were grouped according to the clinical events and compared with the distribution of IL-6 peaks (Table 2). The data show that after transplantation two events are especially associated with sIL-2R serum level elevations, the immediate postoperative period (43/103 peaks, 41.7 %) and rejection episodes (37/103 peaks, 35.9 %). Because infectious diseases and even event-free phases could also be connected with increased sIL-2R levels, these data do not support the hypothesis that the sIL-2R level can be used to predict rejection. In comparison to sIL-2R, the IL-6 peaks showed a different distribution. Beside rejections and surgical trauma, 27 % of IL-6 peaks are associated with infections, but also, in apparently uncomplicated courses, IL-6 can be detected at very low levels.

Discussion

In healthy individuals the sIL-2R mean value was determined to be 267 U/ml and, using the same kit, Pizzola [21] described mean values of approximately 250 U/ml. These values are likely to reflect the lymphocyte activation normally occurring upon physiological stimuli. The upper normal sIL-2R limit is 683 U/ml (≥ + 3 SD). Only 2 out of 72 sIL-2R concentrations of the healthy control population were above this limit (734 and 729 U/ml). In contrast, only 2 out of 103 patients on chronic dialysis showed sIL-2R concentrations below this limit. This is in agreement with the findings of other investigators [4, 22, 27]. The reason for the sIL-2R elevation in chronic uraemia is not known. Köhler et al. [16] discussed a monocytic defect with an insufficient co-stimulatory signal transduction to T cells leading to a reduced IL-2 secretion with compensatory increase of IL-2R expression. This deficient production of IL-2 may partly explain the reduced response of uraemic lymphocytes in vitro [18]. In spite of the elevated sIL-

Table 2 Distribution of sIL-2R and IL-6 serum peaks in 103 kidney allograft recipients

[a] Peak means the highest concentration in the posttransplant course independent of the height of the absolute value

	sIL-2R peaks[a]			IL-6 peaks		
	n	%	≥ ± s (U/ml)	*n*	%	≥ ± s (pg/ml)
Posttransplant (day 1–3)	43	41.7	2649 ± 1967	27	26.2	49 ± 70
Rejection	37	35.9	4914 ± 3372	26	25.2	32 ± 20
Infection	7	6.8	3397 ± 1800	28	27.1	37 ± 45
Event-free phase	16	15.5	2193 ± 1623	22	21.4	10 ± 6

2R level, only 14.3 % of our patients showed pretransplant more than 8 % IL-2R$^+$ CD3$^+$ cells (mean ± 1 SD = 5.3 ± 3.7 %). Raskova et al. [24] described even a reduced expression of IL-2R in the plasma membrane of PHA-stimulated lymphocytes. Another explanation for the immune defect in chronic renal failure could be the binding of IL-2 by sIL-2R competing in this way with the membrane-bound IL-2R on T cells. In vitro experiments performed by Chopra et al. [3] support this hypothesis.

Soon after transplant there was a further, but not significant increase, of sIL-2R as compared to the pretransplant level. This fact seems to be related, to some extent, to the intraoperative high-dose ATG bolus; comparable results were found after heart transplantation [11]. Postoperatively, elevated sIL-2R serum levels were also described in patients undergoing major operations and discussed as non-specific response to the trauma of surgery [1, 17]. The elevation of sIL-2R which then interferes with IL-2-dependent immunity could play a role in postoperative immune deficiency [17]. The pre- and postoperatively elevated sIL-2R serum levels continuously fell towards normal levels within the first 3 weeks after transplantation. Samples from patients with stable renal function after event-free courses showed values hardly above normal at discharge (937 ± 398 U/ml). Patients with normal sIL-2R levels at discharge ($\leq$ 683 U/ml, $n = 18$) had significantly less (3/18 vs 34/75, $P < 0.05$) need for rehospitalisation within the first year after grafting than patients ($n = 75$) with sIL-2R levels > 683 U/ml. There were no instances of rehospitalisation for rejection treatment in patients discharged with normal sIL-2R levels. In heart transplant recipients, a mean level < 1000 U/ml predicted long-term survival with a 76 % sensitivity, 79 % specificity and 88 % negative predictive value [29].

Patients with acute rejections had significantly higher sIL-2R levels than healthy individuals and stable patients. Rejections occurring early after transplantation will be difficult to detect because the background level is so high. In comparison to MP-sensitive rejections, the sIL-2R levels in MP-resistant or humoral/vascular rejections were clearly higher before antirejection therapy had begun. Shortly after treatment with poly- or monoclonal anti-T cell antibodies sIL-2R levels further increased. The highest values were seen after OKT3 therapy, therefore, reflecting the response to antirejection therapy. In agreement with Colvin et al. [4], our data also make no claim that the sIL-2R levels can be used to predict rejection. Comparable results were reported after heart [11, 12] and lung [9] transplantation. Nevertheless at the time of rejection most investigators observed elevated sIL-2R levels. Because infectious diseases in transplant recipients are also associated with elevated sIL-2R levels [4, 7, 8, 10, 19, 20, 22, 27], distinguishing between rejection and infection on the basis of sIL-2R serum level is not possible without knowledge of other clinical or laboratory parameters. Perkins et al. [20] recommend the exclusion of CMV and the confirmation of rejections by biopsy when sIL-2R levels increase. In our experience the simultaneous determination of IL-6 can be useful for distinguishing bacterial and viral infection. With regard to the interpretation of elevated sIL-2R levels, we should keep in mind that 16 out of 103 sIL-2R peaks (2193 ± 1623 U/ml) were not associated with clinical or otherwise detectable events. In these patients, the simultaneously determined IL-6 levels were not or only slightly elevated (10 ± 6 pg/ml) indicating no systemic inflammatory process. We conclude from our results that elevations of sIL-2R are not specific for allograft rejection, since increase of sIL-2R serum levels were also observed during bacterial and viral infections, after treatment with mono- and polyclonal antilymphocytic antibodies and even without any apparent cause. Because of this lack of specificity, elevated sIL-2R levels must be assessed carefully. Nevertheless sIL-2R level can be useful for monitoring kidney allograft recipients. Increases point to a cellular immune activation process and can predict rejections or infections.

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