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A phase-II study of low-dose cyclophosphamide and recombinant human interleukin-2 in metastatic renal cell carcinoma and malignant melanoma

Albrecht Lindemann[1], Klaus Höffken[2], Reinhold E. Schmidt[3], Volker Diehl[4], Otto Kloke[2], Heinold Gamm[1], Jörg Hayungs[2], Wolfgang Oster[1], Markus Böhm[1], Jonathan E. Kolitz[5], Chris R. Franks[6], Friedhelm Herrmann[1], and Roland H. Mertelsmann[1]

[1] Department of Hematology, University of Mainz, FRG
[2] Department of Internal Medicine (Cancer Research), University of Essen, FRG
[3] Department of Immunology and Transfusion Medicine, University of Hannover, FRG
[4] Medical Department I, University of Köln, FRG
[5] Hematology/Lymphoma Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY, USA
[6] Cetus Corporation, EuroCetus, Amsterdam, The Netherlands

Summary. Recent preclinical and clinical studies that have demonstrated antitumor activity of high-dose recombinant interleukin-2 (rIL-2), and animal models that demonstrated a synergistic effect of low-dose cyclophosphamide, led us to study rIL-2 (Cetus Corp., Emeryville, Calif) in a phase II clinical trial in combination with low-dose cyclophosphamide in 32 patients, 18 with malignant melanoma and 14 with renal cell carcinoma. rIL-2 was given once daily at 3×10^6 U/m^2, as a 30-min infusion for 14 days in cycle I and for 2×5 days in cycles II and III respectively; if tolerated, the dose was increased to a maximum of 6×10^6 U m^{-2} day^{-1}; the cycles, separated by 1 week treatment-free intervals, were preceded each by a single i.v. bolus of cyclophosphamide at 350 mg/m^2. The most prominent side-effects encountered in this trial consisted of a capillary leak syndrome, myalgia and fever that required dose reduction during the first cycle in one-half of the patients. Given the limit of tolerable toxicities in a standard care unit, the regimen employed achieved minor antitumor activity. No remission was achieved in patients with renal cell carcinoma, and 15% of melanoma patients showed objective responses (partial response + minor response).

Introduction

Interleukin-2 (rIL-2) is a potent inducer of proliferation and functional activation of T cells and natural killer (NK) cells [11], and is believed to be of major relevance in cell-mediated tumor cytotoxicity [5, 12]. Since the physiological role of antigen-specific cytotoxic T cells in antitumor responses has remained controversial [6], recent efforts have focussed on lymphokine-activated non-major-histocompatibility-complex-restricted killing (LAK) [10]. In vitro experiments have demonstrated, that LAK cells are able to lyse a variety of fresh solid tumor targets and tumor cell lines efficiently [17]. In mice significant tumor responses have been induced by the systemic administration of LAK cells in combination with rIL-2 or high-dose rIL-2 as single agent [21].

The experience in man is limited so far. Promising results were recently obtained in selected tumor entities, particularly in renal cell carcinoma and malignant melanoma, when LAK-cell-mediated adoptive immunotherapy was performed in conjunction with high-dose rIL-2 [29, 34]. The general use of this therapeutic concept is precluded by considerable toxicities requiring treatment in an intensive-care unit for a number of patients [29] and intensive laboratory procedures to generate activated LAK cells in vitro. Since the administration of rIL-2 alone can induce LAK activity in vivo and tumor regressions in mice and men [25, 28], the purpose of this study was to investigate the in vivo activity of rIL-2 without adoptive LAK cell transfer, in a schedule that allows treatment in a standard care unit.

In an attempt to block induction of counteracting suppressor cells by rIL-2, low-dose cyclophosphamide was added as an additional immunomodulating agent. A single cyclophosphamide dose of 50–350 mg/m^2 does not have antitumor activity on its own, but is capable of selectively inhibiting suppressor T cell functions [2, 22, 24], facilitating antitumor responses in IL-2-treated tumor-bearing mice [6, 30]. In a previous study [14] we have shown that pretreatment with a single dose of cyclophosphamide at 50 mg/m^2 or 350 mg/m^2 was safe when followed by rIL-2 at 1×10^6 U/m^2 given by 6-h i.v. infusion. The present study was designed to evaluate (a) whether repeated administration of cyclophosphamide would show a stronger synergizing effect than a single dose and (b) whether a 30-min infusion of a higher dose of rIL-2 (3×10^6 U/m^2) would result in a more effective and better-tolerated schedule in a multicenter trial.

Materials and methods

Patient population. Thirty-two patients (age 28–67 years) were entered in the study, 14 with metastatic renal cell carcinoma and 18 with metastatic malignant melanoma. The clinical characteristics of all patients are shown in Table 1. All patients were required to have a Karnofsky score of 70% or more, bidimensionally measurable sites of disease manifestation and a minimum life expectancy of 3 months. Serious infections, a history of cardiac disease or central nervous system metastases were regarded as exclusion criteria. Any form of chemo-, immuno-, or hormonal therapy had to be discontinued 6 weeks before entering the study. Patients with platelet counts of $< 100000/\mu$l, white blood cell counts of $< 4000/\mu$l, serum creatinine of > 2 mg/dl, and serum bilirubin of > 1.5 mg/dl were excluded. The

Offprint requests to: R. H. Mertelsmann, Department of Hematology, University of Mainz, Langenbeckstr. 1, 6500 Mainz, FRG

Table 1. Patient characteristics

Characteristic	Malignant melanoma	Renal cell carcinoma	Total
No. of patients	18	14	32
Male/female	12/6	13/1	25/7
Age			
Range	28–67	41–64	28–67
Median	54	52.5	53
Site od disease			
Lung	12	9	21
Liver	10	7	17
Skin	7	1	8
Bone	2	3	5
Lymph nodes	5	5	10
Others	6	3	9
Previous treatment			
None	1	0	1
Surgery	17	13	30
Chemotherapy	7	2	9
Radiotherapy	2	5	7
Hormonal therapy	1	4	5
Immunotherapy	4	3	7
Any two or more	10	8	18
Any three or more	5	4	9

study was approved by the institutional review board and written informed consent was obtained.

Study design. Patients under study were clinically staged before treatment and after each cycle, tests including electrocardiography, chest X-ray, and appropriate measurements of marker lesions. Bolus administration of 350 mg/m^2 cyclophosphamide i.v. was followed 3 days later by rIL-2, given in a conventional care unit as a single 30-min infusion once daily for 2 weeks (Fig. 1). The *E. coli*-expressed rIL-2 was supplied by Cetus Corp. (Emeryville, Calif) and provided in lyophilised form, formulated with 171 μg sodium dodecyl sulphate/mg rIL-2 (specific activity 3×10^6 Cetus units/mg protein; 2.3 Biological Response Modifier Program units are equivalent to 1 Cetus unit, as refered to in the text). Compared to the native IL-2 molecule, the recombinant material in our study is slightly altered by the replacement of cysteine at position 125 by serine. – After a 1-week rest, two further cycles were administered with rIL-2 given, from Monday to Friday for 2 consecutive weeks. If tolerated, the dose was increased to 4.5×10^6 U/m^2 and 6×10^6 U/m^2 in cycles II and III respectively. Observation of grade 3 toxicity (WHO criteria) resulted in reduction of the rIL-2 dose by 50%; in the case of grade 3 hematological toxicity, the cyclophosphamide dose was reduced by 50% as well. Patients suffering from grade 4 toxicity were excluded from further therapy. Disease progression after the first treatment cycle led to patient removal from study.

Laboratory analysis. Complete blood counts and serum chemistries were obtained every third day. Surface marker profiles were determined in most patients before therapy, during treatment cycles and in the rebound phase after discontinuation of infusion. The surface phenotype was determined by two-color fluorescence analysis on an EPICS V cell sorter (Coulter, Hialeah, Fla), using the following panel of monoclonal antibodies (mAb): directly fluoresceinated OKT3 (CD3), OKT26a (CD25) and a phycoerythrin-conjugated NKH-1 were purchased from Coulter Immunology, anti-(HLA-DR) and phycoerythrin-conjugated mAbs directed against CD25 from Becton Dickinson (Mountain View, Calif); non-specific isotype-identical mAbs were used as controls throughout the study. Serum samples were collected before therapy and 24 h after the infusion of rIL-2, and frozen (−20° C) immediately until assayed. Interferon-γ levels were measured by radioimmunoassay using a kit developed by Centocor Inc. (Malvern, Pa). Sensitivity is 0.1 U/ml as indicated by the manufacturer. Serum IL-2 receptor (sIL-2R) levels were determined by an ELISA, obtained from T Cell Sciences Inc. (Cambridge, Mass). The detection limit of this test kit is 50 U/ml sIL-2R. Normal sIL-2R values in healthy adult donors range from 50 U/ml to 500 U/ml (mean, 273 U/ml).

Response criteria. A minimum of 10 treatment days or of 30×10^6 U/m^2 total rIL-2 dose was required for assessment of response. Response was evaluated using standard criteria, with complete response defined as total disappearance of all measurable and evaluable lesions for at least 4 weeks, and partial response as more than 50% decrease in the sum of the products of the perpendicular diameters of all lesions for 1 months without simultaneous increase at any site. A minor response was defined as 25%–50% objective regression of the tumor; a state of unchanged tumor size for at least 4 weeks was called stable disease. Increase of any lesion to more than 25% or the appearance of new tumor sites was defined as progressive disease. Response duration was measured from the time the criteria of remission were fulfilled until progression of disease.

Results

Antitumor activity

Of the 32 patients who had received a minimum cumulative dose of 30×10^6 U/m^2 rIL-2, 29 were evaluable for response (Table 2). None of these patients achieved a complete response. Two patients with malignant melanoma responded with a partial remission with a median duration of response of 3 months and 4 months respectively. One additional patient had a minor response lasting for 3 months. The disease remained unchanged in one patient with renal cell carcinoma (2 months) and in three patients with malignant melanoma (2, 2, 3 months respectively).

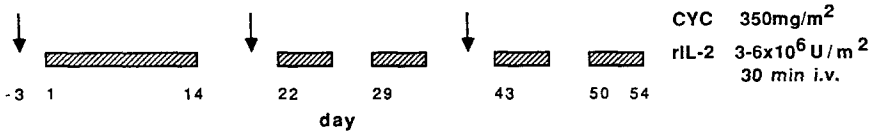

Fig. 1. Protocol for administration of high-dose recombinant interleukin-2 (rIL-2) and low-dose cyclophosphamide (CYC)

Table 2. Clinical response to combination therapy of recombinant interleukin-2 and low-dose cyclophosphamide

Response[a]	Malignant melanoma (%)	Renal cell carcinoma (%)	Total (%)
Patients evaluable	18	11	29
CR	0	0	0
PR	2	0	2
MR	1	0	1
SD	3	1	4
PD	12	10	22
CR + PR + MR	3 (17)	0 (0)	3 (10)

[a] CR, complete response; PR, partial response; MR, minor response; SD, stable disease; PD, progressive disease

Table 3. Toxicity of the high-dose recombinant interleukin-2 and low-dose cyclophosphamide regimen

Side-effect	Overall (%)	> Grade 3[a] (%)
Fever	32 (100)	9 (28)
Chills	32 (100)	– –
Skin rash	19 (59)	– –
Myalgia[b]	19 (59)	4 (13)
Neurotoxicity	7 (22)	– –
Nausea/vomiting	31 (97)	– –
Mucositis	10 (31)	– –
Diarrhea	9 (28)	– –
Weight gain[c] (edema)	32 (100)	3 (10)
Dyspnoea	28 (87)	10 (31)
Hypotension[d]	32 (100)	10 (31)
Cardiac (rhythm)	2 (6)	– –
Bilirubin↑	6 (19)	– –
Alkaline phosphatase↑	19 (59)	– –
Creatinine↑	6 (19)	– –
Anemia	18 (56)	– –
Thrombocytopenia	7 (22)	– –

[a] WHO toxicity score, unless indicated otherwise. The following side-effects were classified as grade 3 toxicity: [b] myalgia requiring dose reduction of rIL-2, [c] weight gain of more than 10% of body weight, and [d] decrease of systolic blood pressure > 530 kPa (40 mm Hg) or requiring fluid replacement

Progression was noted in the remaining 21 patients. All responders belonged to the group of patients who showed best tolerance to rIL-2; all of them were able to receive more than 120×10^6 U/m^2 total dose. The responses were consistently observed after the first treatment cycle, with no further responses seen following the second or third cycles. This would suggest that toxicity and antitumor activity of rIL-2 were not correlated, and that the mechanisms underlying side-effects may be different from those mediating tumor regression.

Another observation of interest was made in an additional three patients, who were classified as showing progressive disease, but did show "mixed responses". An example of these patients is presented in Fig. 2, demonstrating a chest X-ray of patient no. 4 before and after treatment with a cumulative dose of 30×10^6 U/m^2 rIL-2. The marker lesions in the right lung resolved completely, while an enlarged lymph node tumor on the other side of the thoracic chest and two liver metastases developed at the same time. The reasons for this variability of response at different tumor sites remain to be elucidated. Possible explanations include the emergence of different tumor cell subclones with varying sensitivity to therapy or different influences of the tumor microenvironment (e.g. vascularization or presence of inhibitors) to tumor accessibility of rIL-2-activated effector cells.

Clinical toxicity

In most patients rIL-2 toxicity prevented dose increase to 6×10^6 U m^{-2} day^{-1}. Three patients were able to tolerate the maximum dose in cycle III, in eight additional cases 4.5×10^6 U m^{-2} day^{-1} was tolerated in cycle II. The starting level of 3×10^6 U m^{-2} day^{-1} in cycle I could be administered to 10 patients for 10 days and to 15 patients for 2 weeks as decribed in the protocol. In seven cases the treatment had to be discontinued before day 10 of rIL-2 treatment because of grade 3–4 toxicity. Within the large range of side-effects listed in Table 3, the "capillary leakage" syndrome, associated with hypotension, weight gain, edema, and lung congestion, was the major dose-limiting toxicity. The leading symptoms were dyspnoea in six, and hypotension in five cases. However, no patient re-

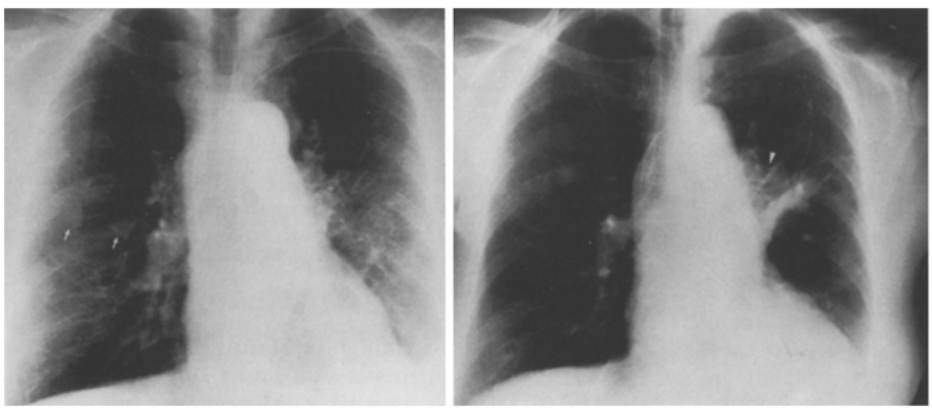

Fig. 2. The chest X-rays of patient no. 4 before treatment (31. 03. 87) and after (21. 04. 87) application of a cumulative dose of 30×10^6 U/m^2 rIL-2 (31. 03. 87–13. 04. 87) reveal a complete disappearance of metastases (*arrows*) of the right lung, while an enlarged lymph node tumor (*arrow*), confirmed by computed tomography scan, developed concomitantly on the left side of the thoracic chest

quired treatment in the intensive-care unit or intubation. Episodes of hypotension were usually manageable by fluid replacement. Twice dopamine had to be given transiently. Cardiac toxicity was seen in two patients, who developed transient atrial fibrillation and supraventricular tachycardia, which did not require further intervention after discontinuation of rIL-2. Three patients experienced a rise of creatinine of up to 4.3 mg/dl. Two of these had been previously nephrectomized and the pretreatment creatinine level was minimally elevated to 1.7 mg/dl in one of them. Fever, a common side-effect as previously reported [1], was usually well controlled by paracetamol. Several patients experienced grade-2 chills, that were controlled by i.v. pethidin-HCl. Skin reactions (scaling dermatitis) and gastrointestinal side-effects (nausea, diarrhea) did not require specific therapeutic interventions. Hyperbilirubinemia up to 5 mg/dl occurred in two patients with extensive liver metastases. Neurological symptoms, such as paresthesia or headaches, were of minor degree. Onset of severe myalgias required dose reduction in four cases.

Treatment-associated changes in peripheral blood cell counts and phenotypes

The administration of high-dose rIL-2 has been shown to be associated with a decline of white blood cell counts, mainly as a result of lymphocytopenia [19]. In our study, after an initial decrease, probably caused by cell sequestration in lung, liver, renal capsule, and spleen [8, 16], the number of lymphocytes increased again, despite continuation of daily rIL-2 therapy, from 300/μl (median) to 3400/μl (median) in the last third of cycle I (Table 4 and Fig. 3). An additional rebound lymphocytosis (4200/μl median) occurred after termination of treatment with maximum lymphocyte counts 48 h later. Peripheral blood mononuclear cell counts and distribution did not differ significantly in patients with or without pretreatment of low-dose cyclophosphamide in our previous study [16]. The extent of rebound lymphocytosis in patients treated with 3×10^6 U/m^2 rIL-2 in cycle I (14 days) and II (2×5 days) respectively did not exhibit a marked difference. The response to dose increase was heterogeneous, with some patients generating higher lymphocyte counts, while lymphocyte counts were unaffected by dose increase in others (e.g. Fig. 3).

The overall non-lymphoid cell counts decreased in the early phase of treatment mainly as a result of a drop of polymorphonuclear neutrophils, often accompanied by an initial increase of band forms. While slowly recovering during the first 14-day course of rIL-2 administration, neutrophils attained pretreatment values within a week after termination of rIL-2 infusion. An increase of eosinophils became apparent after a few days of rIL-2 application. While falling to near normal values in the 7-day treatment-free interval, an increase of eosinophils of up to 100-fold was encountered in the following cycles (Fig. 3). Platelet counts exhibited a continuous decrease during treatment, followed by a moderate rebound. The decrease in hemoglobin levels was usually less than 2 g/dl. In two cases with anemic pretreatment values, supportive treatment was required with 2 units and 4 units of packed red cells respectively.

Surface marker studies were performed at the time of lymphocytosis in the last third of cycle I and in the rebound phase after termination of treatment. Representative data of two patients are shown in Table 5. A marked increase of NKH-1-positive, CD3-negative cells became detectable in the last phase of cycle I. Induction of an enhanced surface HLA class-II surface expression, indicating in vivo activation of NK cells, was found to correlate

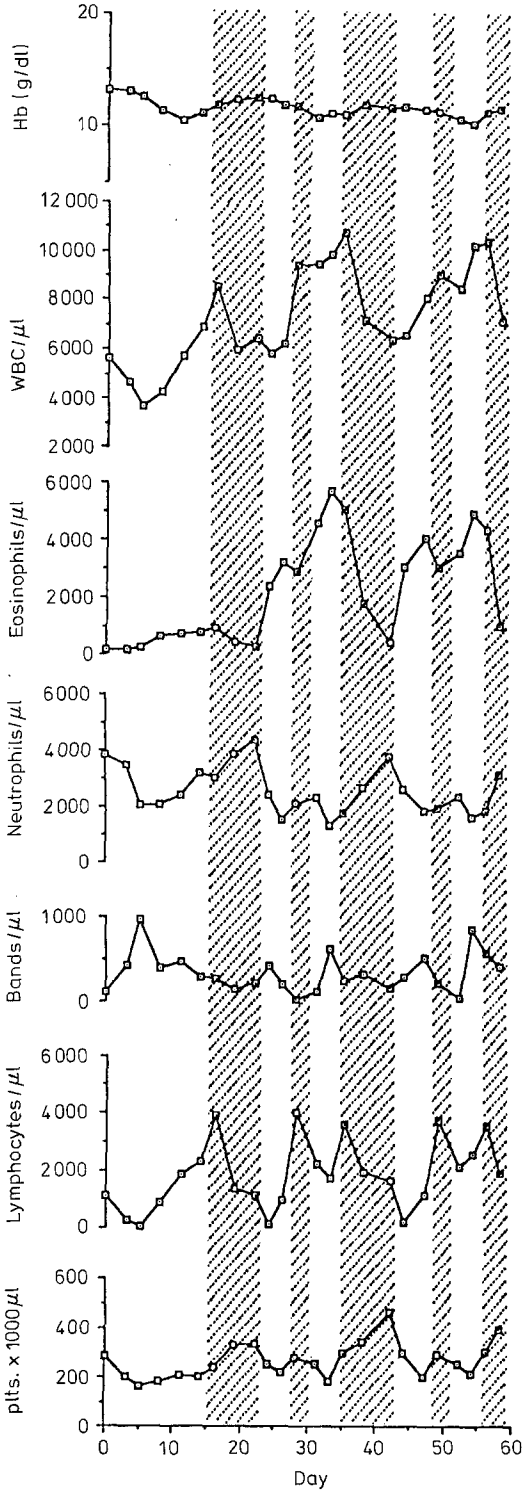

Fig. 3. A representative course of peripheral blood cell counts on therapy with rIL-2, exemplified in one patient, who was treated according to protocol including dose increase escalation. *Hatched areas* represent the treatment-free intervals

Table 4. Hematological effects of cycle I and II of therapy in 15 patients

Cells	10^{-3} × Cell count/ µl	No. of patients (%)	Pre-treatment	10^{-3} × Cell counts/µl; median (range)					Post-treatment[d]
				Cycle I			Cycle II		
				Nadir	Peak[a]	Peak[b]	Nadir	Peak[c]	
Lymphocytes	>5.0	5 (33)	1.5	0.3	3.4	4.2	1.2	3.8	2.0
	>4.0	8 (53)	(0.6–2.4)	(0.07–0.6)	(0.8–5.5)	(2.7–8.4)	(0.8–1.6)	(1.0–8.8)	(1.1–4.0)
Eosinophils	>7.0	2 (13)	0.1	–	1.2	1.5	–	5.4	0.6
	>5.0	8 (53)	(0.0–0.4)	–	(0.4–2.7)	(0.0–3.8)	–	(2.9–8.8)	(0.1–5.5)
Neutrophils	<2.0	14 (93)	5.0	2.6	3.7	4.7	1.8	3.3	3.6
	<1.0	2 (13)	(2.3–7.4)	(1.4–3.7)	(2.5–5.2)	(1.6–10.0)	(0.8–3.1)	(2.0–6.7)	(1.7–8.8)
Platelets	<100	5 (33)	315	154	223	278	180	267	359
	< 50	0 (–)	(138–507)	(83–353)	(160–451)	(183–544)	(64–546)	(161–548)	(260–811)

[a] Peak values in the last third of cycle I; [b] within 3 days post-treatment; [c] within cycle II including 72 h post-treatment; [d] cell counts 1 week after discontinuation of therapy

Table 5. Antigenic phenotype of circulating lymphocytes

Cell type	Percentage of total lymphocytes (%)			
	Pre	I (11)	I (+2)	II (+2)[a]
Patient no. 7				
T CD3+	59	54	50	57
NKH1+	3	4	10	7
Tac+	3	24	32	31
NK CD3-NKH1+	9	28	32	35
Ia+	3	18	24	25
Tac+	<2	4	7	5
Patient no. 11				
T CD3+	73	39	41	52
NKH1+	3	2	7	2
Tac+	4	11	18	22
NK CD3-NKH1+	12	45	41	28
Ia+	<2	31	32	19
Tac+	<2	3	<2	4

[a] Blood samples were collected before treatment (Pre), on day 11 of cycle I [I(11)] and 2 days after completion of cycles I [I(+2)] and II [II(+2)] respectively. Two-color immunofluorescence analysis was done as indicated in Materials and Methods

with LAK activity as has recently been reported [25]. Induction of Tac expression, however, was restricted to CD3-positive T lymphocytes.

Cytokine assays in serum samples

Serum samples were drawn 24 h after the last dose of rIL-2 at different times during the first cycle of therapy. Serum IL-2 receptor (sIL-2R) levels rose to maximum of 12000 U/ml on day 9 and fell to pretreatment values within 1 week after discontinuation of therapy (Fig. 4A). There was no correlation of sIL-2R levels with lymphocyte counts, lactate dehydrogenase levels [26] or specific side-effects. However, in several patients pretreatment sIL-2R levels were found to be elevated in comparison to normal controls. Interferon-γ levels exhibited a minor increase during therapy to a maximum of 1.5 U/ml after the last dose of rIL-2 (Fig. 4B).

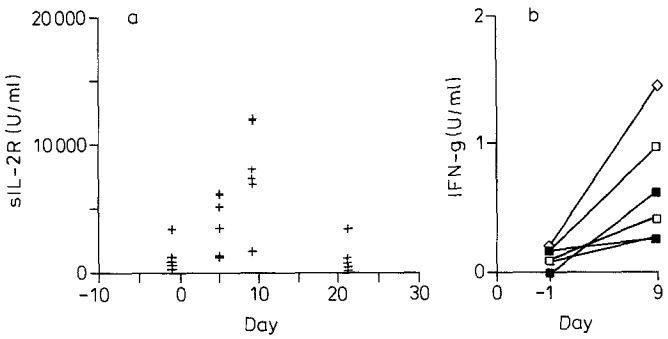

Fig. 4. Effect of administration of recombinant interleukin-2 (rIL-2) on serum IL-2 receptor (sIL-2R) levels (**A**) and serum interferon-γ (IFN-g) levels (**B**) in six patients

Discussion

Recently a number of studies exploring the use of autologous lymphokine-activated killer (LAK) cells and systemic administration of high-dose rIL-2 were reported in patients with advanced cancer [29, 34]. Objective evidence of tumor regression was noted in 25%–50% of patients with renal cell carcinoma and malignant melanoma. Compared to these results the overall antitumor activity of rIL-2 preceded by low-dose cyclophosphamide, as used in our trial, demonstrated no major responses in renal cell carcinoma and a 15% objective response rate in malignant melanoma. Although the differential sensitivity of malignant melanoma and renal cell carcinoma to single-agent rIL-2 is in line with the first of these reports [29], the difference in antitumor activity remains unclear. In fact, the protocol described by Rosenberg et al. involves higher daily doses of rIL-2 for a 5-day period without demonstrating, however, a significant advantage of additional LAK cell infusions in the therapy of malignant melanoma [29]. West et al. used the same daily dose as we did, given as a continuous infusion in combination with adoptive LAK cell transfer, and achieved a remission rate of 50%; higher doses of rIL-2 exhibited unfavorable effects [34]. Thus, the role of LAK cells activated in vivo and the importance of administrating rIL-2 at the maximum tolerated dose as a crucial prerequisite of response remains questionable [21]. It should

be noted that all previous reports were from single institutions while our study is a multicenter trial, which tends to generate lower response rates possibly because of unbiased recruitement of consecutive patients.

A major impact of low-dose cyclophosphamide on the therapeutic efficacy of our schedule appears unlikely, although a synergistic antitumor effect of this treatment in comparable settings has been reported [3, 30]. A somewhat higher response rate as compared to our study was achieved by Mitchell et al. using a similar regimen [20]. However, in that study shorter treatment cycles were administered, which may be more effective and, by generating less toxicity, allow more frequent recycling. The extent of rIL-2-induced lymphocytosis (Table 4) and expansion of $NKH1^+DR^+$ cells (Table 5), which has been shown to correlate with LAK activity [25], was somewhat lower in patients treated according to our regimen as compared to other studies [9, 34]. It remains to be determined, however, if LAK cells are the therapeutic principle of rIL-2-induced antitumor activity, as suggested by in vitro LAK cell assays. The contrast of high sensitivity of sarcomas and colon carcinomas in vitro [17, 27] as compared to modest clinical responses [29, 34] as well as the lack of significant tumor infiltration by LAK cells in vivo [23], may indicate that other effector cells are contributing to tumor regressions in patients receiving rIL-2 therapy.

The side-effects of our treatment schedule were acceptable in comparison to what has been reported elsewhere [29]. However, owing to the limits of toxicity tolerable in a standard care unit, dose reduction or early termination of therapy was required in a considerable number of patients. As objective and subjective toxicity were positively correlated to dose and duration of rIL-2 therapy, repeated short-term cycles of continuously infused rIL-2, separated by 3–5-day rest periods, may be better tolerated, while generating large numbers of activated lymphocytes in vivo [31].

Since this is the first report of a human study of high-dose rIL-2 given as a short-term infusion for more than 10 consecutive days, some new observations were made in this study, not previously reported in trials using treatment schedules with shorter cycles [9, 25]. Lymphocytosis of mostly NKH-1-positive cells was not restricted to the post-treatment rebound phase, but had already evolved, although to a lesser extent, in the second half of a 2-week treatment period. Eosinophil counts increased early during therapy and showed a dramatic rise during subsequent cycles, suggesting involvement of an rIL-2-induced eosinophil growth and differentiation factor, e.g. IL-5, IL-3 or GM-CSF [23, 36]. The changes within the neutrophil lineage might reflect an increased consumption of polymorphonuclear neutrophils paralleled initially by an increased demargination of band forms, that may be linked to therapy-induced elevation of cortisol levels [18].

The clinical relevance of the treatment-associated rise of serum IL-2 receptor (sIL-2R) values is probably less than initially expected. This parameter seems to be of minor specificity [33] and lacks a strict correlation to the cell type of interest in this setting, i.e. NK, LAK or cytotoxic T lymphocyte cells. Elevated interferon-γ serum levels, however, may be important in view of its macrophage-activating properties. In vitro, interferon-γ and rIL-2 act synergistically to induce macrophage secretion of interleukin-1 and tumor necrosis factor α [13], molecules possibly involved in the generation of therapy-related side-effects, like fever and capillary leakage [4, 7, 32] as well as of antitumor effects [35].

Summarizing the results of the present study, rIL-2 in combination with low-dose cyclophosphamide can lead to objective tumor regressions in malignant melanoma while no significant activity was seen in renal cell carcinoma. The frequency of responses appears somewhat lower than that reported by other investigators [20, 29, 34], possibly because of the unbiased inclusion of patients in this multicenter trial. A clear benefit of the additional use of low-dose cyclophosphamide appears unlikely, although controlled studies are required for a definitive statement. In all patients therapy was manageable in a conventional care unit. Frequent dose reductions because of toxicity argue in favor of shorter treatment cycles and a mode of application more easily tolerable. Further empirical clinical studies may evaluate whether the generation of maximum lymphocyte counts [31] or the combination with synergizing agents like interferons or tumor necrosis factor [15, 35] is capable of improving efficacy without increasing toxicity.

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