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Correlation between Clinical Response to Interleukin 2 Therapy and Sustained Production of Tumor Necrosis Factor[1]

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ABSTRACT

Twenty-five previously untreated patients with metastatic renal cell carcinoma were treated with 5-day cycles of continuous infusion of interleukin 2 (IL2) and lymphokine-activated killer cell reinfusion. Five achieved a partial response. Three patients were found to have detectable tumor necrosis factor (TNF) in serum before initiation of therapy. On the fifth day of therapy, 24 patients had circulating TNF with immunoradiometric assay whereas 13 had detectable biological activity. Two days after the end of IL2 therapy, TNF concentration (immunoradiometric assay) decreased in most cases but was still detectable in 17 patients. Thirteen patients had still circulating TNF bioactivity. Although there was no significant difference between TNF levels observed on the fifth day of therapy in the responder and nonresponder groups, 48 h after the end of IL2 infusion, both the TNF concentration and the biological activity were significantly higher in the group of responder patients. This result suggests that the clinical response to IL2 therapy in patients with metastatic renal cell carcinoma is correlated to a sustained production of TNF after the end of IL2 infusion.

INTRODUCTION

IL2,[3] with or without LAK cell infusion, induces the regression of evaluable tumor sites in 20 to 30% of patients with metastatic renal cell carcinoma (1–3). *In vitro*, it has been shown that IL2-activated lymphocytes kill LAK-susceptible cells, in a non-MHC-restricted manner, after a contact between target and effector cells (4). However, the exact mechanism of the *in vivo* antitumoral effect of IL2 in humans remains unclear. Although cytolytic activity of LAK cells has been found in some reports to correlate with the *in vivo* antitumoral effect (2, 5), several recent studies failed to demonstrate any correlation between the lytic capacity of peripheral blood lymphocytes and the clinical response *in vivo* (6, 7). Moreover, observations that human LAK cells apparently do not move to sites of metastatic tumors suggest the possible involvement of soluble factors in the antitumoral effect of IL2 *in vivo* (7, 8).

It has been reported that IL2-activated lymphocytes release detectable concentrations of cytokines, such as TNFα, a monokine with cytotoxic properties on tumor cells and immunoregulatory activities (10, 11). Recent reports have shown the presence of TNF in the serum of patients shortly after IL2 bolus infusion (12, 13). In 25 patients treated with continuous infusion of interleukin 2 for metastatic renal cell carcinoma, TNF serum level was measured by an immunoradiometric assay and a biological assay. Results suggest that the antitumoral activity of IL2 therapy is correlated with the persistence of detectable TNF in the serum after cessation of IL2 therapy.

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[3] The abbreviations used are: IL2, interleukin 2; TNF, tumor necrosis factor α; IRMA, immunoradiometric assay; LAK, lymphokine-activated killer.

PATIENTS AND METHODS

Patients

Twenty-two patients received IL2 and LAK cells and 3 received IL2 without LAK cells because it was not possible to culture LAK cells for two patients in the same week. IL2 was given as continuous infusion of 3×10^6 units/m^2/day in 2 cycles. A first cycle of 120 h (days 1 to 5) was followed by 36 h of rest and 4 days of leukophereses (days 8 to 11); the second cycle of IL2 lasted for 108 h (days 12 to 16), with the reinjection of the 4 leukophereses on days 12, 13, and 15. Patients who did not undergo leukophereses and LAK cell reinjection received 2 cycles of IL2 with the same dose and schedule. Patients who later experienced partial response and progressive disease did not differ in their clinical status before therapy and received comparable doses of Il2 and reinjected LAK cells (Table 1). Serum samples were obtained for all the patients during the first course of IL2 at day 0 (before the onset of therapy), day 5 (at the end of the first IL2 continuous infusion), and at day 8, between the two cycles of IL2. All samples were frozen and stored before the assays.

Activation of Mononuclear Cells *in Vitro*. After processing of the peripheral blood, the platelet-rich plasma was separated from the mononuclear pellet by slow centrifugation and mononuclear cells were enriched by a Ficoll-Hypaque sedimentation (IBM Cobbe 2997 processor). Cells were then resuspended at 3×10^6 nuclear cells/ml in RPMI 1640 containing antibiotics, glutamine, 2% heat-inactivated human AB serum, or autologous plasma and IL2 (1500 units/ml) and incubated in 3-litre polyolefin bags (Dupont de Nemours or Baxter) at 37°C with 5% CO_2 during 4 days. After incubation, cells were washed in 0.9% sodium saline with the Stericell processor (Dupont de Nemours) before reinjection.

TNFα Immunoradiometric Assay. TNFα was measured retrospectively using an IRMA (Medgenix, Fleurus, Belgium) according to the kit procedure. Briefly, standards or samples were added to anti-TNFα-coated tubes in the presence of ^{125}I-labeled anti-TNFα antibody directed against a different TNF epitope. After 18 h incubation at room temperature, tubes were washed with 20% Tween 20 and the remaining radioactivity, reflecting TNFα concentration, was measured on a Gamma counter (Kontron).

TNF Biological Assay. TNF cytotoxic activity was analyzed using D-actinomycin-treated L929 cells as described (14, 15). Serial dilutions of serum were incubated with L929 cells for 18 to 20 h at 37°C with duplicates for each dilution. After incubation, the plates were washed and cell lysis was determined by staining cells with 0.5% crystal violet in methanol/water (1/4, v/v). Dye uptake was calculated using an automated micro-enzyme-linked immunosorbent assay autoreader (Titertek; Multiscan, MC). One unit is defined as the amount of TNF required to kill 50% of target cells. Standards using recombinant TNF (Eurocetus; 10^7 units/mg) were routinely tested during each assay. When repeated assays were performed on the same sera, only minor interassay variations were observed (<15%).

Statistical Analysis. Data were compared using the nonparametric Mann-Whitney test and Yates corrected χ^2 test.

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Table 1 *Clincal data during treatment with interleukin 2*

	Dose of IL2 ($\times 10^6$ units/m^2/course)	No. of LAK reinjected ($\times 10^{10}$)	No. of metastatic sites	Sites	Response[a]	Capillary leak syndrome[b]
Responders						
PAH	28.3	5.2	3	Lung	PR	+
				Residual kidney	PD	
				Liver	PD	
GUE	22.9	5.0	2	Lymph nodes	PR	+
TER	24.1	0	3	Lung	PR	+
				Residual kidney	PR	
				Bone	PD	
BOU	20.2	7.56	2	Lung	PR	+
				Lymph nodes	PR	
DEL	23.7	4.85	3	Bone	PD	+
				st. lesions	CR	
				Lung	CR	
Nonresponders (median)	25.7	5.74	2[c]			4/22[d]

[a] According to WHO criteria: CR, complete response; PR, partial response; PD, progressive disease.
[b] Increase of creatinine >200 μmol/liter and hypotension (systolic blood pressure ≤80 mm Hg) and oliguria (<400 ml/24 h) and weight gain >5%.
[c] Range, 1–4.
[d] Three of them had high TNF levels by IRMA and/or bioassay.

RESULTS

TNF Concentration and Biological Activity in the Sera of Patients Treated with IL2 (Table 2). Among the 25 patients, 3 had detectable TNFα before the onset of IL2 infusion. Two of them had low TNFα concentrations (IRMA < 25 pg/ml) without bioactivity. Only the third patient had 108 pg/ml TNFα with detectable activity in the L929 bioassay. On the fifth day of IL2 continuous infusion, 24 patients had detectable concentrations of circulating TNFα by IRMA (mean, 59 pg/ml; range, 13 to 158 pg/ml); detectable biological activity was observed in 13 patients.

Forty-eight h after interruption of IL2 infusion (day 8), 17 patients still had measurable TNF by IRMA in serum with a lower mean level (mean, 24.7 pg/ml; range, 5 to 130 pg/ml); TNF biological activity was detected only in 13 patients. Twenty patients had detectable TNF by either of the 2 methods. No significant difference of TNF levels was observed in patients who did not receive reinjected LAK cells. During the IL2 course, 9 patients showed a capillary leak syndrome, as defined in Table 1. Capillary leak syndrome was found to be significantly associated with response to therapy ($P = 0.005$).

Correlation between TNF Levels and Clinical Response to Therapy. TNFα concentrations in responder and nonresponder patients were compared using the nonparametrical Mann-Whitney *U* test. Analysis of the data obtained at day 8 indicated that TNFα concentrations detected by IRMA were significantly higher in the responder subgroup ($P < 0.05$). Four of the 5 responder patients had more than 20 pg/ml of TNFα whereas such level in the sera was observed in only 4 of the 20 nonresponder patients. These differences were found to be statistically significant (Table 3). In contrast, at day 5, no significant

Table 2 *TNF concentrations in the serum of patients during the first course of IL2*

TNF was measured at day 0, before the onset of therapy, at day 5 at the end of the first continuous IL2 infusion, and at day 8, 48 h after the end of the first continuous IL2 infusion.

		TNF concentration (IRMA) (pg/ml)			TNF activity (bioassay) (units/ml)		
Patients	Clincal response[a]	Day 0	Day 5	Day 8	Day 0	Day 5	Day 8
1. PAH	PR	0	35	64	0	0.7	0.8
2. GUE	PR	25	80	118	0	0.2	0.8
3. TER[b]	PR	0	43	22	0	1.1	1.0
4. BOU	PR	0	37	30	0	0	0
5. DEL	PR	0	52	6	0	0.4	0.4
6. PIT	MR	0	158	38	0	0.4	0.3
7. FOI	MR	0	77	28	0	0.4	0.2
8. BOU	PD	0	55	14	0	0	0
9. CAC	PD	19	124	19	0	0.5	0
10. BOI	PD	0	95	60	0	0	0
11. JAR	PD	0	19	130	0	0	0
12. DID	PD	0	71	5	0		0
13. CON	PD	0	70	0	0	0	0
14. VAC	PD	0	52	0	0	0	0
15. CHA	PD	0	96	15	0	0.3	0
16. LAC	PD	0	29	0	0	0.7	0.3
17. PER	PD	0	24	0	0	0	0.7
18. CAT[b]	PD	0	92	0	0	0	0
19. BER	PD	0	52	14	0	0.4	0.2
20. BES	PD	0	61	15	0	0.5	1.2
21. GAI	PD	0	13	0	0	0	0
22. MAR	PD	0	21	10	0	0	0
23. COU	PD	0	0	0	0	0	0
24. PAT[b]	PD	108	65	15	0.7	0.6	0.1
25. DEL	PD	0	53	0	0	0.3	0.5
Mean results		6 (0–108)	59 (0–158)	24.7 (0–130)	0	0.27 (0–1.1)	0.27 (0–1.2)

[a] Clinical response according to WHO criteria: PR, partial response; MR, mixed response; PD, progressive disease.
[b] Treated with IL2 without LAK cells.

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Table 3 *Clinical response in patients with low or high TNF levels and/or activity in the sera at day 8 post-IL2 therapy*

	Bioassay (units/ml)		IRMA (pg/ml)		Bioassy or IRMA		Bioassay and IRMA	
	<0.4	≥0.4	<20	≥20	≥0.4	≥20	≥0.4	≥20
Responders (*n* = 5)	1	4	1	4	5		3	
Nonresponders (*n* = 20)	17	3	16	4	7		0	
Statistical analysis	$\chi^2 = 5.47$	$P < 0.02$	$\chi^2 = 4.15$	$P < 0.05$	$P < 0.03$		$P < 0.01$	

difference in TNFα concentrations by IRMA was observed between the responders and the nonresponders (data not shown). We then compared TNF bioactivity in responders and nonresponders. Four of 5 patients responding to therapy were found to have TNF activity over 0.4 unit/ml at day 8, whereas such levels were observed in only 4 of 20 nonresponders; TNF activity was then found to be significantly associated with clinical response (Table 3). In contrast, no significant difference in TNF activity was observed at day 5 between the 2 subgroups (data not shown). Moreover, the 2 patients who achieved mixed response with clear disappearance of all metastatic but appearance of a new metastatic site had both high TNF concentration by IRMA at days 5 and 8.

DISCUSSION

Contrasting with previous report on cancer patients (16), detection of circulating TNF prior to any therapy was uncommon in our series of 25 patients with metastatic renal cell carcinoma since only one had both high concentration of TNF and biological activity. Continuous infusion of IL2 induced an increase of TNF serum level in 24 patients thus confirming previous reports (12, 13). Interestingly, whereas TNF is known to have a short half-life in serum (17), we observed sustained TNF concentration 48 h after the end of IL2 infusion in several patients. This observation is likely to be due to a prolonged TNF production by IL2-stimulated mononuclear cells and suggests a persistent lymphocyte activation 48 h after the end of IL2 infusion.

Of note this high TNF serum level was not due to severe infectious diseases as described previously (18).

We observed several discrepancies between TNF concentration measured by bioassay and by IRMA. Some patients had high TNFα concentration by IRMA but low or undetectable biological activity. These observations could be related to biological inactive forms of TNFα or to the presence of endogenous or exogenous substance affecting TNF cytotoxic activity. It is noteworthy that patient 11, in whom the major difference was observed, had received, during the first IL2 course, high doses of methylprednisolone known to inhibit TNF cytotoxic activity (19).

Few patients had low concentration of TNF measured by IRMA and high TNF bioactivity in the serum. Since lymphotoxin, produced by IL2-activated lymphocytes (20), shares a common receptor with TNF and also has cytotoxic activity against the L929 cell line used in the bioassay, this observation is likely to be due to lymphotoxin production. Interestingly, the mean level of TNFα measured by IRMA decreased 48 h after the end of IL2 infusion whereas the biological activity remained stable. *In vitro,* lymphotoxin is detectable after 4 days of stimulation with IL2 whereas TNFα is measurable 24 h after lymphocyte stimulation with IL2 (20). Consistent with these observations, the sustained TNF activity observed *in vivo* could be due to a delayed lymphotoxin production, following TNFα, during IL2 infusion. This persistence of TNF after the end of IL2 infusion could reflect a prolonged activation of the immune system involved in the *in vivo* antitumor activity and the clinical response.

It has been reported that TNF may be partially responsible for the toxicity of IL2 in animal models (12). Our results support this observation since capillary leak syndrome occurred in all patients who experienced partial response to IL2 therapy, suggesting common biological mechanisms for response and toxicity.

IL2 and LAK cell infusion induce a tumor regression in only 25 to 30% of patients with metastatic renal cancer (1–3). It would then be a considerable interest for the physicians to determine biological parameters correlated with clinical response and then to delineate the subgroups of patients who could benefit of IL2 therapy. In this study, we report that sustained TNF serum concentration 48 h after the end of IL2 infusion is correlated with clinical response to therapy. This criterion might prove useful in avoiding some toxic effects of retreatment in patients who are unlikely to respond and in the design of new therapeutic approaches such as combination of cytokines. Ongoing studies are designed to explore the value of TNF measurement in a larger series.

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