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Increase in Soluble Interleukin-2 Receptor and Neopterin Serum Levels during Immunotherapy of Cancer with Interleukin-2

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Both immunostimulatory and immunosuppressive events would occur during the immunotherapies of cancer, including interleukin 2 (IL-2) therapy. The marked increase in soluble IL-2 receptor (SIL-2R) levels during IL-2 therapy could represent a potentially negative biological effect, because of the receptor's capacity to bind IL-2 and compete for it with IL-2 cell surface receptor. Since it has been observed that macrophages stimulate *in vitro* the release of SIL-2R, a study was started to evaluate *in vivo* the role of macrophages in IL-2-induced SIL-2R rise by measuring neopterin, which is a marker of macrophage activity. The study included 9 advanced renal cancer patients, treated subcutaneously with IL-2 at 1.8 × 10^6 IU/m^2 twice daily for 5 days/week for 6 weeks. Both SIL-2R and neopterin serum mean levels significantly increased during IL-2 treatment, and the highest concentrations were reached on the second week of therapy. SIL-2R rise was significantly correlated to that of neopterin. This study, by showing a positive correlation between SIL-2R and neopterin rise, would suggest a macrophage involvement in the stimulation of SIL-2R release during IL-2 immunotherapy of cancer.
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INTRODUCTION

RECENT DISCOVERIES have demonstrated that most antitumour immune responses are interleukin 2 (IL-2) dependent biological functions [1, 2]. From this point of view, IL-2 would represent the most promising immunotherapeutic strategy of human neoplasms. In fact, objective tumour regressions have been described with IL-2 in several tumour histotypes [3–5].

Before the clinical use of IL-2, the previous cancer immunotherapies, such as BCG, had made relatively little impact in the treatment of human tumours. This failure was attributed to the capacity of immunotherapeutic agents to activate suppressor cells, as well as host defenses against tumour [6, 7]. Macrophages have been shown to play an important role in cancer immunosuppression, either in the clinical course of the neoplastic disease or in response to the immunotherapies of cancer investigated in the past years [6, 8]. IL-2 immunotherapy itself, in addition to the activation of antitumour immune reactions [3–5], has been proven to determine concomitantly immunosuppressive events, such as a decreased delayed type hypersensitivity response [9]. The mechanisms responsible for these immunosuppressive events have still to be better characterised, but they would include an increased production of transforming growth factor-β (TGF-β), which strongly inhibits the IL-2 induced antitumour immune response [10], as well as of soluble IL-2 receptors (SIL-2R) [11, 12], which could reduce IL-2 availability by binding IL-2 and competing for it with IL-2 cell surface receptors [13]. However, the biological and prognostic significance of SIL-2R rise during IL-2 therapy is still obscure, and in particular it remains to be established whether the enhanced secretion of SIL-2R simply reflects lymphocyte activation, or whether it is due to alterations of IL-2 cell surface receptor expression. *In vitro* results [14] have demonstrated that macrophages are involved in the stimulation of SIL-2R release from activated lymphocytes. Because of the documented role of macrophages in the *in vitro* release of SIL-2R [14], a study was started to

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Table 1. Clinical data of 9 patients with advanced renal carcinoma treated subcutaneously with IL-2

Patient (sex, age)	Sites of disease	Previous treatment*
1 (F, 55)	Lung, bone	N, VLB + IFN
2 (F, 68)	Bone	N
3 (M, 59)	Lung	N, VLB + IFN
4 (M, 24)	Retroperitoneal nodes	N, VLB + IFN
5 (M, 60)	Lung, liver	N, VLB + IFN
6 (M, 67)	Retroperitoneal nodes	N, IFN
7 (M, 59)	Lung	N
8 (F, 52)	Bone	N, VLB + IFN
9 (M, 49)	Bone	N, IFN

* N = nephrectomy, VLB = vinblastine, IFN = interferon-α.

establish the relation between SIL-2R release and macrophage activity during IL-2 immunotherapy of cancer. The macrophage activity was indirectly evaluated by determining neopterin, which is a specific marker of macrophagic functions [15].

MATERIALS AND METHODS

The study included 9 consecutive patients affected by advanced renal adenocarcinoma (M/F:6/3; median age 59 years, range 24–68). 2 patients were untreated for their advanced disease, while the other 7 patients had been previously treated with vinblastine and/or interferon-alpha [2]. Clinical data of cancer patients are reported in Table 1.

Human recombinant IL-2 (Euro-Cetus, Amsterdam) was given subcutaneously into different parts of the abdominal wall, each cycle consisting of a 2-day IL-2 pulse of 9×10^6 IU/m^2 every 12 h as an induction phase, followed by 6 weeks of IL-2 at 1.8×10^6 IU/m^2, twice daily for 5 days per week. This study was a single-agent study not part of the multicentre combination trials. Clinical response and toxicity were evaluated according to WHO criteria.

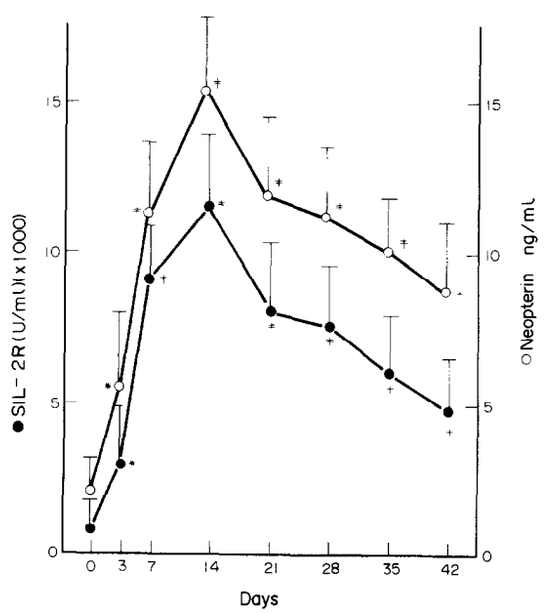

Fig. 1. Mean (S.D.) serum levels of SIL-2R and of neopterin during one subcutaneous cycle of IL-2 in 9 patients with advanced renal adenocarcinoma. *$P < 0.05$, †$P < 0.005$, ‡$P < 0.001$ vs. before treatment.

Table 2. Individual maximum increase in SIL-2R and neopterin serum levels during IL-2 subcutaneous therapy in 9 advanced renal cancer patients

	Prior to study		Maximum on study	
Patient	SIL-2R U/ml	Neopterin (ng/ml)	SIL-2R U/ml	Neopterin (ng/ml)
1	1294	2.9	10 860	12.1
2	627	1.9	19 520	19.3
3	1293	3.3	11 792	21.2
4	624	2.8	6 163	14.9
5	2265	4.1	16 735	20.4
6	478	0.9	7 450	10.3
7	735	2.9	16 784	22.1
8	432	0.8	8 347	12.4
9	793	3.1	9 430	13.7

For immune detections, venous blood samples were collected at 0800 before the start of IL-2 therapy, 3 and 7 days after, then weekly until the end of the IL-2 cycle. No patient was under therapy with steroids and/or other drugs influencing the immune functions during the study. In each sample, serum levels of SIL-2R and neopterin were simultaneously determined. SIL-2R concentrations were measured with an enzyme immunoassay, using commercially available kits (T Cell Sciences, Cambridge, MA). Serum levels of neopterin were detected with a double-antibody radioimmunoassay with commercial kits (Henning, Berlin). Normal values obtained in our laboratory were: SIL-2R, < 480 U/ml; neopterin, < 2.5 ng/ml. Data are reported as mean (S.D.) and statistically analysed by the Student's t test, analysis of variance and determination of coefficient of correlation, as appropriate.

RESULTS

A partial response was observed in 3/9 (33%) patients. No cardiovascular toxicity was seen. The only relevant side-effect was temperature higher than 38°C in all patients during the induction phase.

Before the start of IL-2 therapy, abnormally high serum levels of SIL-2R and of neopterin were seen in 7/9 and in 6/9 patients, respectively. In all patients, IL-2 subcutaneous injection induced an evident increase in both SIL-2R and neopterin; mean concentrations of both SIL-2R and neopterin became significantly higher than those seen before from the third day of IL-2 injection ($P < 0.05$). The highest mean levels of both SIL-2R and neopterin were reached on the second week of therapy ($P < 0.001$ vs. before IL-2). They then slowly decreased, though still remaining significantly higher than those seen before IL-2 therapy until the last week of therapy ($P < 0.05$ vs. before). Serum mean levels of SIL-2R and of neopterin seen before and during IL-2 cycle are illustrated in Fig. 1. The individual maximum values of SIL-2R and neopterin observed during IL-2 therapy are reported in Table 2. A significant correlation was seen between SIL-2R and neopterin increases ($r = 0.83$).

DISCUSSION

In accord with the results previously reported by Lotze *et al.* [11] with IL-2 intravenous administration and as previously reported by Urba *et al.* [12] on a lower number of cancer

patients, this study shows that IL-2 given subcutaneously also markedly enhances SIL-2R serum levels. The SIL-2R serum concentrations rapidly increase in response to IL-2 injection, and remain high until the end of IL-2 cycle, even though the peak is reached on the second week of therapy. The mechanisms responsible for the increase in SIL-2R in response to IL-2 exogenous administration need to be further investigated. The results of this study, by showing a concomitant and positively correlated increase in neopterin, which main source is represented by macrophages [15], would suggest that SIL-2R release during IL-2 therapy may be at least in part modulated by an enhanced macrophage function. Therefore, this study would confirm *in vivo* the importance of macrophages in influencing SIL-2R release from lymphocytes into the blood, as previously demonstrated *in vitro* by Nelson *et al.* [14]. However, these results are not exhaustive to demonstrate a causative link between SIL-2R and neopterin increases. The progressive fall in SIL-2R and neopterin levels after the first 2 weeks of therapy might depend on a down-regulation of IL-2 receptors on lymphocyte and macrophage cell surfaces, induced by the prolonged exogenous administration of IL-2.

At present, it is still unknown whether the increase in neopterin levels may be related to a macrophage activation in a suppressive or in an antitumour way. Since suppressor activity in cancer patients seems to be mainly due to macrophages [5, 8], either in the clinical course of the neoplastic disease or as an unfavourable event during tumour immunotherapy, an enhanced neopterin release could be the expression of an enhanced macrophage suppressor function. In fact, a concomitant increase in both SIL-2R and neopterin blood levels, with a negative prognostic significance, has been also described in immunodeficiencies, such as AIDS [16]. If this hypothesis is true, an inhibition of macrophage suppressor activity could enhance the IL-2 efficacy. The stimulation of SIL-2R release might represent one of the possible mechanisms through which macrophages determine immunosuppression of IL-2-dependent functions. However, even though SIL-2R have been proven to link IL-2, it remains to be demonstrated that the increase in SIL-2R levels may determine a reduced IL-2 availability to stimulate IL-2 cell surface receptors on lymphocytes [13].

Further studies will be needed to establish a relation between clinical efficacy of IL-2, and SIL-2R and neopterin increases during IL-2 immunotherapy of cancer. A more detailed knowledge of IL-2 stimulatory and suppressive immune effects, mainly those concerning the macrophage activity, could allow the elaboration of new immunotherapeutic strategies; in particular, immunotherapeutic combinations with IL-2 and other cytokines capable of selectively modulating the different functions of macrophages and of other cells provided by a suppressive function, might make it possible to treat cancer patients by selectively activating host cells involved in the defence against cancer, while neutralising suppressor cells that impede the generation of an effective antitumour immune response.

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