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Malignant Melanoma and Renal Cell Carcinoma: Immunological and Haematological Effects of Recombinant Human Interleukin-2

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The immunological and haematological effects of continuous infusion of recombinant human interleukin-2 (rhIL-2) in 6 patients with metastatic melanoma and 6 with disseminated renal cell carcinoma are reported. In patients with malignant melanoma dacarbazine was given before IL-2; in renal cell carcinoma IL-2 alone was given. In malignant melanoma, 1 complete (CR) and 1 partial response (PR) were seen; 2 patients had stable disease (SD) and 2 progressive disease (PD). In renal cell carcinoma 4 patients had SD and 2 PD. Toxicity of IL-2 therapy was minimal. All patients showed increased cytotoxicity, that was not major histocompatibility complex restricted, towards target cells sensitive and insensitive to natural killer cells. These activities varied between individual patients and were less marked in cases of renal cell carcinoma. Cellular proliferative responses increased in all patients, being consistently higher following the first course of therapy, as did HLA-DR, CD16 and CD25 activation marker expression. Hypersegmentation of neutrophils and eosinophilia were commonly observed, and in renal cell carcinoma these changes were accompanied by abnormal lymphocyte morphology.
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INTRODUCTION

OVER THE past 5–10 years, clinical trials with recombinant human interleukin-2 (rhIL-2) have confirmed its antitumour activity in various types of malignancy, particularly malignant melanoma and renal cell carcinoma [1]. The role of IL-2 as an antitumour therapeutic agent was established in preclinical studies where regression of primary tumours and secondary metastases were observed following therapy [2, 3]. The first results from clinical trials of recombinant IL-2 in various types of cancer were published by Rosenberg's group [4] where favourable response rates in malignant melanoma and renal cell cancer were seen (20–25% partial and complete responses [PR and CR]) but at the cost of severe toxicity (e.g. hypotension, fluid retention and life-threatening pulmonary oedema). In these initial clinical trials, the IL-2 was administered in high, 8-hourly, bolus injections. The widely fluctuating serum levels of the cytokine with transient high peak exposures may have contributed to the severe toxicity. In preclinical studies the half-life of rhIL-2 is less than 1 hour [5], suggesting that if IL-2 were given by continuous infusion, antitumour response could be maintained but with less toxicity [6]; this has been confirmed by West *et al.* [6].

Dacarbazine is considered to be the most active cytotoxic agent in patients with metastatic melanoma with response rates varying between 15 and 20% [7]. Thus, it has been suggested that combining this chemotherapeutic agent with IL-2 might provide a significant benefit over single agent administration [8].

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The aim of the present study was to analyse various immunological and haematological responses in patients with metastatic melanoma or renal cell cancer undergoing treatment with continuous infusions of IL-2, and to correlate these with the antitumour clinical response and toxicity of rhIL-2.

MATERIALS AND METHODS

Patients

Patients included in this study had metastatic melanoma or disseminated renal cell carcinoma and had documented tumour progression prior to entry. All gave informed consent. Eligibility requirements were histologically documented metastatic tumour, ECOG performance status of 0-1, age 75 years or less, life expectancy greater than 3 months, no prior chemotherapy, immunotherapy or extensive radiotherapy in the previous 4 weeks and normal haematological parameters. Exclusion criteria were evidence of serious active infection, CNS metastases, significant past or current evidence of cardiovascular disease and previous or concurrent other cancer.

Protocol

The regimens were different for metastatic melanoma and renal cell carcinoma. Intravenous therapy was given through central venous catheter ("Hickman Line"). Patients with metastatic melanoma received dacarbazine intravenously as boluses (250 mg/m^2 per day) over 30 minutes each day for 5 consecutive days. Following 16 days' rest rhIL-2 was given as a continuous intravenous infusion for two 5-day cycles (with 2 days' break between cycles) at 18 $\times$ 10^6 IU/m^2/day (3 $\times$ 10^6 Cetus units/m^2 per day). This completed one course, and after 1 week's rest, patients received a second identical course. Following two treatment courses, patients with progressive disease (PD) were withdrawn from the trial; those with responding or stable disease (SD) were given a further one or two courses. Patients with renal cell carcinoma received rhIL-2 as a continuous infusion at 3 $\times$ 10^6 Cetus units/m^2/day for two cycles of 5 days with 2 days' break between. After 3 weeks' rest a second course was given. After two courses, patients with responding or stable disease received a further two courses; those with progressive disease were withdrawn from the study. The schedules were part of a Eurocetus multicentre phase II trial.

Blood samples

Fresh samples comprising 20–30 ml of heparinised, 10 ml of clotted and 5 ml of EDTA blood were collected using Becton Dickinson vacutainers before, during and at the end of each week of treatment. Peripheral blood lymphocytes (PBL) were isolated by centrifugation using the lymphocyte separation method, washed three times with RPMI medium and finally suspended in RPMI-NBCS (RPMI-1640 + 10% newborn calf serum). These cells were then directly tested in all the immunological assays. The serum was separated from clotted blood by centrifugation of the sample at 1500 *g* for 15 minutes and stored at −70°C.

Cytotoxicity assay

A 4 hour ^{51}Cr-release assay was used to measure cytotoxicity that was not major histocompatibility complex (MHC) restricted against K562 (natural killer cell [NK] sensitive; erythroleukaemia cell line) and SW742 (NK resistant; colorectal tumour cell line) targets [9]. The results were expressed as percentage cytotoxicity:

$$\frac{(\text{test release}) - (\text{spontaneous release})}{100 - (\text{spontaneous release})} \times 100.$$

Table 1. Patients' details

Patient	Metastatic disease	Karnofsky score	Toxicity	Courses
Melanoma				
1	Spleen; para-aortic lymph-nodes	100	Recurrent pyrexia	2
2	Chest wall; pleuro pulmonary; hepatic lymph-nodes	100	Axillary vein thrombosis	3
3	Right thigh; external iliac node	100	Recurrent pyrexia	4
4	Right thigh; inguinal lymph-nodes	100	Hypothyroidism	3
5	Pleuropulmonary	100	Peripheral neuropathy	2
6	Left axilla+ neck, small bowel	100	Thrombocytopenia	2
RCC				
7	Pleuropulmonary	100	Hickman line sepsis	4
8	Pleuropulmonary	80	Recurrent pyrexia	4
9	Pulmonary; bone	80	Dyspepsia	3
10	Pulmonary	80	Hickman line sepsis	3
11	Pulmonary	100	Recurrent pyrexia	2
12	Pulmonary; vaginal	100	Recurrent pyrexia	2

RCC = renal cell carcinoma.

Cell proliferation assay

PBL were isolated from the peripheral venous blood and suspended in RPMI-NBCS at 10^6/ml. The cells were added in triplicate to 0.2 ml round-bottomed microtest plate wells in culture medium and autologous plasma. The wells were pulsed with [^{3}H] thymidine (18.5 kBq per well) for 4 h at 37°C in 5% CO_2, and harvested using a Skatron cell harvester. The radioactivity was counted as disintegrations per minute (dpm).

Immunofluorescence staining and flow cytometry

Flow cytometry analyses were performed on fresh PBL using unconjugated monoclonal antibodies against different cellular phenotypic markers: leucocytes (HLE-1), T-cells (anti-CD3), T-helper/inducer cells (anti-CD4), T-suppressor/cytotoxic cells (anti-CD8), NK cells (anti-CD16), B-cells (anti-CD19), IL-2 receptor (Tac) (anti-CD25) and anti-HLA-DR against activated T-cells, monocytes/macrophages and B-cells [9].

Haematological studies

EDTA blood samples were analysed on a Technicon H6000 automatic blood counter to produce a 12 parameter blood profile and six parameter automated white cell differential. Blood films were also stained with May-Grunwald-Giemsa. Any abnormal cells detected by light microscopy were stained by the alkaline phosphatase anti-alkaline phosphatase (APAAP) technique [17].

RESULTS

Clinical response

The clinical details are summarised in Table 1. Of the 12 patients, 1 woman with metastatic melanoma had shown a CR and 1, also with metastatic melanoma, showed a PR. 6 patients had SD and in 4 patients the disease progressed. No severe adverse effects related to IL-2 administration were observed. 1 patient with melanoma developed thrombocytopenia due to dacarbazine and 2 patients had Hickman line sepsis.

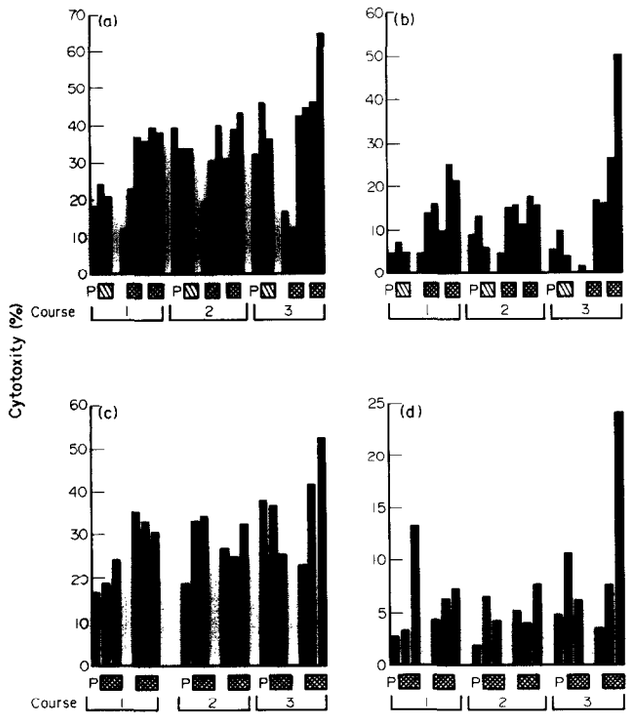

Fig. 1. Mean cytotoxic activity of PBLs of 6 melanoma and 6 renal cell carcinoma patients: (a) against K562 tumour cell targets, (NK sensitive erythroleukaemic cell line) and (b) against SW742 tumour cell targets (NK resistant-LAK sensitive colorectal tumour cell line), in melanoma patients; (c) against K562 tumour cell targets (NK sensitive erythroleukaemic cell line) and (d) against SW742 tumour cell targets (NK resistant-LAK sensitive colorectal tumour cell line) in renal cell carcinoma patients. (a–d) E:T = 20:1. P = pretreatment, ▨ = dacarbazine, ▩ = rhIL-2.

Non-MHC restricted cytotoxicity

Cytotoxic activity was monitored using the NK-sensitive K562 tumour cell line and an NK-resistant SW742 tumour cell line in a 4 hour ^{51}Cr-release assay. Figure 1 summarises the results in all patients monitored during three courses of treatment. In patients with melanoma during the week of dacarbazine therapy, there was no change in the cytotoxic potential of PBLs but the percentage cytotoxicity gradually increased towards the end of the second week of rhIL-2 administration (Figs 1a and b). This response increased in subsequent courses and was maximum at the end of the third course of treatment (65% and 50% cytotoxicity at effector to target [E:T] ratio of 20:1 for K562 and SW742 targets, respectively). In patients with renal cell carcinoma, cytotoxic activity increased after rhIL-2 therapy was started (Figs 1c and d). During the first two courses of rhIL-2 the cytotoxic activity against SW742 cells (lymphokine activated killer [LAK] target cells) was only slightly augmented (usually less than 10% killing at a 20:1 E:T), and it was only during the third course of treatment that a pronounced increase in cytotoxicity was observed against K562 and SW742 targets (50% and 25%, respectively, at 20:1 E:T). The cytotoxic potential against K562 and SW742 targets was greater in patients with metastatic melanoma than in those with renal cell carcinoma. Although no definite relationship was found between clinical response and cytotoxicity, NK and LAK cell activity were greater in patients showing good clinical responses, both in terms of tumour regression (Table 2) and improvement in general well being. The patient with melanoma who showed CR

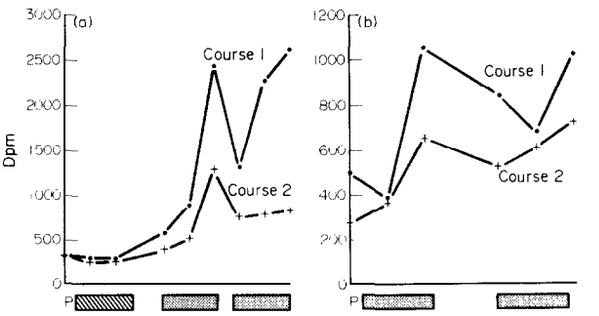

Fig. 2. Mean cellular proliferative activity in 6 melanoma (a) and 6 renal cell carcinoma patients (b). Note different scales for y-axis.

was re-assessed 3 months after completion of four courses of treatment; an increase in NK and LAK activity, compared with pretreatment levels, was still evident (greater than 50% cytotoxicity at E:T 20:1).

Cellular proliferation

In patients with melanoma, no change in cellular proliferative response was noted during dacarbazine administration, but rhIL-2 infusion significantly enhanced cell proliferation by the end of the first week of the treatment cycle. Assessments before the beginning of the second week of rhIL-2 therapy (2 days' rest was given between each week) showed a minor fall in the proliferative response but with recommencement of rhIL-2 infusion it increased, reaching maximum at the end of the first treatment course (Fig. 2a). With subsequent courses of treatment the pattern of cellular proliferative activity remained the same but the degree of proliferation decreased with each subsequent course. This was seen in each patient, irrespective of clinical outcome. Table 2 summarises the results obtained for LAK activity and proliferation relative to the clinical response.

In patients with renal cell carcinoma, the proliferative responses were similar to those found in malignant melanoma (Fig. 2b). Again there was no correlation between cell proliferation and clinical response and treatment toxicity; cellular proliferation for both patient groups corresponded with serum levels of soluble IL-2 receptor (data not shown).

Table 2. Response and lymphocyte function and proliferation

	Response	LAK activity			Proliferation		
No. of courses		1	2	3	1	2	3
Melanoma*							
1	PD	+	+	NT	++	−	NT
2	SD	++	++	+++	+++	++	+
3	CR	+	+	+++	+++	++	++
4	PR	−	+	++	+++	++	++
5	SD	+	+	NT	++	+	NT
6	PD	+	−	NT	++	+	NT
RCC†							
7	SD	−	−	+	+	+	+
8	PD	−	−	−	+++	++	+
9	PD	−	−	NT	++	+	+
10	SD	−	−	NT	+	++	NT
11	SD	−	−	NT	++	+	NT
12	SD	−	−	NT	++	+	NT

NT: not tested; *rhIL-2 + dacarbazine; † IL-2.

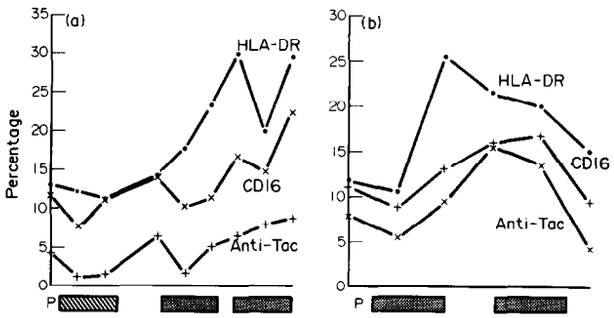

Fig. 3. Phenotypic and activation markers on lymphocytes in 6 melanoma (a) and 6 renal cell carcinoma patients (b).

Lymphocyte phenotypic analyses

No significant change was noticed in HLE-1, CD3, CD4, CD8 or CD19 cell markers during treatment (data not shown). The percentage of cells expressing HLA-DR antigen after starting rhIL-2 infusion gradually increased towards the end of the first course of treatment (Fig. 3), increasing with each subsequent course of therapy. The percentage of cells expressing CD16 increased in the same manner. The expression of the p55 low-affinity receptor for IL-2 (Tac) also increased during rhIL-2 infusion; the mean intensity of Tac antigen expression was relatively greater than the actual increase in the percentage of cells. These changes in phenotypic/activation marker status were seen in all patients during each treatment course irrespective of clinical response.

Haematological studies

In all patients the haemoglobin, red cell count and other red cell parameters showed no significant change during rhIL-2 therapy (data not shown). Patients with melanoma showed a decrease in white cell count and platelet count during dacarbazine treatment which then increased above pretreatment levels

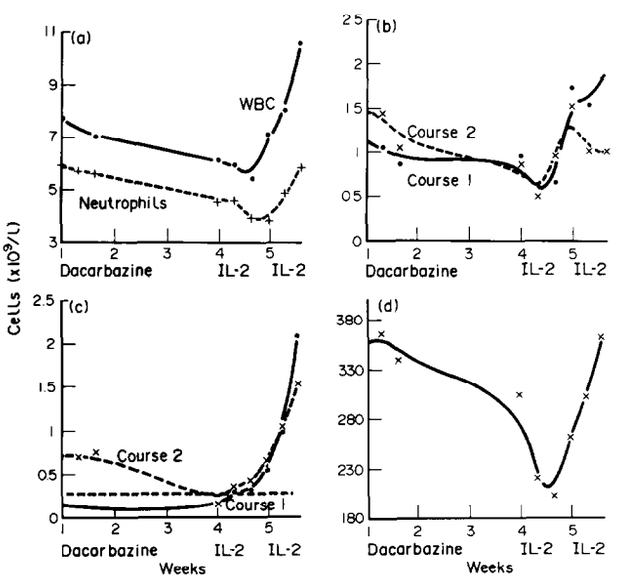

Fig. 4. Haematological changes in melanoma patients undergoing rhIL-2 treatment. (a) White cells and neutrophils (course 1); (b) lymphocytes (courses 1 and 2); (c) eosinophils (courses 1 and 2); (d) platelets (course 1).

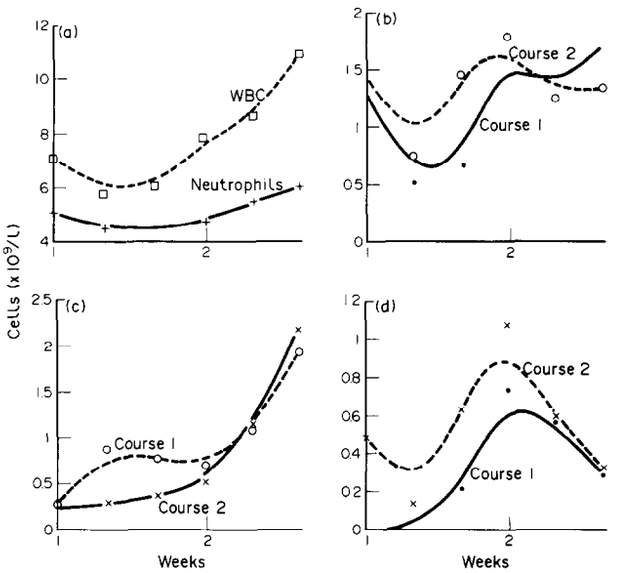

Fig. 5. Haematological changes in renal cell carcinoma patients undergoing rhIL-2 treatment. (a) White cells and neutrophils (course 1); (b) lymphocytes (courses 1 and 2); (c) eosinophils (courses 1 and 2); (d) Pinocchio cells (courses 1 and 2).

during rhIL-2 therapy (Fig. 4). Renal cell carcinoma patients receiving rhIL-2 alone showed a pattern of response similar to melanoma patients during their rhIL-2 therapy (Fig. 5). With both melanoma and renal cell carcinoma patients, neutrophil and monocyte counts were not significantly affected, although neutrophils became more hypersegmented with successive courses of treatment, as exemplified by measurement of neutrophil lobe index (Fig. 6). Nearly all patients showed some degree of eosinophilia (maximum count observed 6.3 × 10^9/l) (Figs 4c, 5c), together with increasing eosinophil hypersegmentation in each subsequent course of rhIL-2 therapy. Quantitative and qualitative changes were seen in lymphocytes. Lymphopenia occurred after dacarbazine therapy but normalised when rhIL-2 therapy was started; however, at no time was there rebound lymphocytosis (Fig. 4b). Morphological changes included the appearance of increasing numbers of large granular lymphocytes, prolymphocytes, occasional blast cells and cells showing signs of activation with deeply staining cytoplasm (so-called plasmacytoid cells). Cells with nuclear protuberances, described as Pinocchio cells by Paciucci *et al.* [19], were seen in patients with renal cell carcinoma who had received rhIL-2 therapy alone and reached maximum numbers midway through each cycle.

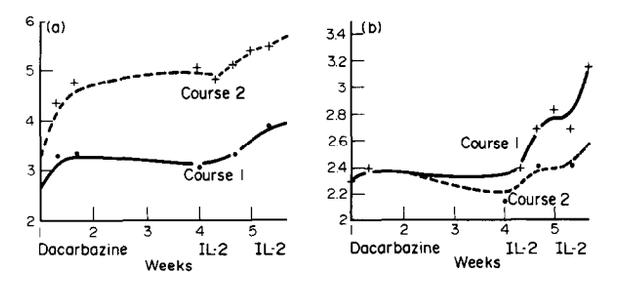

Fig. 6. Lobe index of neutrophils (a) and eosinophils (b) in melanoma patients (total number of nuclear lobes/ number of cells counted).

These cells stained positive for CD4, CD8, CD19 and CD25 using the APAAP technique (data not shown).

DISCUSSION

In clinical studies rhIL-2 has been used alone, or in combination with other antitumour agents such as cytotoxic drugs, other cytokines or *in vitro* IL-2 activated LAK cells [9, 18, 20]; several regimens for rhIL-2 administration have been reported varying from high dosage bolus injections [1] to slow continuous intravenous infusions [6]. Although the clinical responses observed have been encouraging (with 20–25% CR or PR being reported in metastatic melanoma and renal cell carcinoma), the biological basis of this response is ill-defined.

In the present study, 12 patients were monitored for immunological and haematological changes during successive courses of therapy. 6 patients with metastatic melanoma were treated with dacarbazine and rhIL-2, and 6 with metastatic renal cell carcinoma were treated with rhIL-2 alone. It has been reported that in melanoma the administration of chemotherapy with rhIL-2 appears not only to increase therapeutic efficacy but also enables the use of lower doses of rhIL-2 with less toxicity [8, 10]. This synergism could in part be due to the antitumour effect of chemotherapy reducing tumour burden, suppressor cell activity and/or alteration of tumour immunogenicity, thus increasing tumour susceptibility to cellular immune responses [8]. The observed clinical responses in the present study (1 CR and 1 PR, and 2 patients with SD) occurred in patients with melanoma without major toxic effects. In renal cell carcinoma 3 out of 6 patients had SD, and again major toxicity did not occur. It is of interest that all patients admitted to these trials showed improvement in their general medical condition as reflected by general medical condition.

Melanoma patients showed no change in NK cell activity during dacarbazine therapy, but this was significantly increased by the end of the second week of rhIL-2 infusion; activity reached a maximum by the end of the third course of rhIL-2 treatment. It has been reported in various studies that LAK activity of PBLs isolated from peripheral blood is difficult to demonstrate in most patients receiving various rhIL-2 regimens [6, 8, 11,12]; however, it has been suggested that prolonged treatments and continuous infusions of IL-2 induce LAK cells [15, 16]. To demonstrate reproducibly LAK cell activity in fresh PBLs, it is necessary to include rhIL-2 in the culture medium during the cytotoxicity assay [13, 15]. In our study, we were able to demonstrate significant levels of LAK activity without the addition of rhIL-2 to the culture medium. Cytotoxicity against the NK-resistant LAK sensitive target SW742 was higher in patients with melanoma than in those with renal cell cancer.

In malignant melanoma dacarbazine therapy did not alter PBL proliferation, but rhIL-2 infusion resulted in significant cellular proliferation, which reached a maximum by the end of the first course. With each subsequent course of treatment the proliferative response was comparatively less; this was commensurate with the levels of soluble serum IL-2 receptors in the same patients. Similar patterns of cellular proliferative responses and serum IL-2 receptor levels were seen in patients with renal cell carcinoma. It has previously been suggested that a correlation exists between the cellular proliferative response and the expression of the alpha chain (Tac) of the IL-2 receptor on lymphocytes [16], but in our studies such a correlation was not observed. One possible explanation for these findings is that Tac antigen is only transiently expressed on activated lymphocytes, and very quickly shed into serum as a soluble component. Among the various phenotypic markers studied, we observed increased expression of MHC class II HLA-DR antigen on PBL following rhIL-2 infusion in each course; possibly as a result of other cytokines, such as interferon-γ, from activated T-cells [20]. The expression of IL-2 receptor (Tac) was also increased, which is consistent with previous studies [16]. The results of the present study suggest that continuously infused "low-dose" rhIL-2 has clinical antitumour activity with minimal toxicity in some patients. In some patients there appears to be a correlation for NK and LAK activity and clinical response; however, we were unable to correlate changes in other immunological and haematological markers.

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

P. Lissoni, E. Tisi, F. Brivio, S. Barni, F. Rovelli, M. Perego and G. Tancini

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.
Eur J Cancer, Vol. 27, No. 8, pp. 1014–1016, 1991.

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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