Endogenous Interleukin 6 Levels in Patients with Metastatic Malignant Melanoma: Correlation with Tumor Burden[1]

Roger Mouawad,[2] Ahmed Benhammouda, Olivier Rixe, Eric Charles Antoine, Christian Borel, Marise Weil, David Khayat, and Claude Soubrane

Laboratory of the Medical Oncology Department, Salpetrière Hospital, 47 boulevard de l'Hopital, 75013 Paris, France

ABSTRACT

The involvement of interleukin (IL-) 6 in malignant disease has been investigated in a variety of different malignancies. To evaluate whether serum IL-6 is a useful disease marker in metastatic malignant melanoma (MMM), we studied the time course of endogenous IL-6 secretion in 41 patients treated with cisplatinum, IL-2, and IFN-α. Furthermore, the relationship of endogenous IL-6 concentrations to the tumor burden and/or the clinical response was also evaluated. The baseline serum IL-6 levels were significantly higher in patients with MMM than in the control group (P = 0.002). When tumor burden was taken into consideration, we found that IL-6 levels were higher in patients with high tumor burden than in patients with low tumor burden. During treatment in the whole patient population, a higher serum IL-6 level was observed in nonresponding as compared to responding patients at days 7 (P = 0.0005), 21 (P = 0.002), and 35 (P = 0.009). The follow-up of serum IL-6 in patients with MMM according to the tumor burden and clinical response demonstrated that: (*a*) IL-6 levels were significantly higher at days 7 and 21 in patients with high tumor burden as compared to those with low tumor burden; and (*b*) IL-6 levels remain significantly higher in nonresponding patients as compared to responding patients regardless of the tumor burden. From these results, we can conclude that endogenous IL-6 may play a role in the failure of IL-2 therapy in such patients, since the very early IL-6 increase is correlated with the tumor mass and nonresponse to biochemotherapy. Therefore, it seems that the early detection of endogenous IL-6 may represent valuable information for monitoring the response to biochemotherapy in patients with MMM.

INTRODUCTION

The role of cytokines and growth factors in the development and progression of cancer has recently become an active area of cancer research. Malignant melanoma is a neoplasm that accounts for 1 to 2% of cancer deaths per year in the United States (1). The response rates of patients with advanced metastatic disease remain low to both standard chemotherapy and immunologically based therapies. Treatment with (IL[3]-2) alone or in combination with cytotoxic drugs yields 15–54% partial or complete response with some long-term unmaintained remissions (2–6).

Recombinant proteins such as IL-2 play a pivotal role in inducing immunological responses (5, 7). This is reflected by the production of a cascade of different endogenous cytokines (8) which may play a central role in the efficacy and/or toxicity of IL-2. Among these factors, IL-6 is a pleiotropic immunomodulatory cytokine produced by a variety of cell types and different tumor cells (9). It is a multifunctional cytokine originally identified as a T-cell-derived cytokine that induces terminal maturation of B cells into antibody-producing cells (9–11). It exhibits multiple biological activities that differ widely among various types of tissues and cells (10). IL-6 can promote differentiation and can also induce either growth inhibitory or growth stimulatory activities, depending on the nature of the target cells. Several studies have shown that IL-6 is a growth factor for various tumors such as myeloma, renal cell carcinoma, cervical carcinoma, AIDS-related Kaposi's sarcoma, lymphoma, and prostatic cancer (12–16). In multiple myeloma (9–10, 17), it has been suggested that an elevated serum IL-6 level may predict a poor outcome, possibly due to paracrine and/or autocrine stimulation of myeloma cell proliferation. An elevated serum level of IL-6 may also be associated with a poor response to therapy and with shorter survival in patients with renal cell carcinoma (18), and may have prognostic significance in patients with relapsed Hodgkin's disease (19).

To evaluate whether serum IL-6 is a useful disease marker in MMM, we studied the time course of endogenous IL-6 secretion in 41 patients treated with biochemotherapy. Moreover, the relationship of endogenous IL-6 concentrations with the tumor burden and/or the clinical response was also evaluated.

PATIENTS AND METHODS

Patients. Forty-one patients (19 women and 22 men) with MMM were treated with biochemotherapy in the Medical Oncology Department of the Salpetriere Hospital. The median age was 44 (range, 21–68) years, and the median Eastern Cooperative Oncology Group performance status was 0 (range, 0–2). Sites of metastatic disease included: lymph nodes, 25; skin/soft tissue, 22; lung, 15; liver, 8; bone, 8; and others, 11. Taking into consideration the number of metastatic sites and/or

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[2] To whom requests for reprints should be addressed. Phone: 33 (1) 42 16 04 85; Fax: 33 (1) 43 36 48 41.

[3] The abbreviations used are: IL, interleukin; NR, nonresponding; MMM, metastatic malignant melanoma.

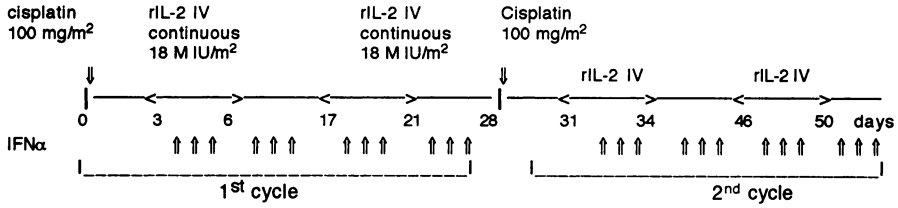

Fig. 1 Therapeutic protocol. IFN-α was administered s.c., 9 million IU/day, three times/week. *rIL-2*, recombinant interleukin 2.

tumor size, patients were divided into two groups: a low tumor burden group (n = 21), defined as <10 metastatic sites and/or tumor size <6 cm, and a high tumor burden group (n = 20), defined as ≥10 metastatic sites and/or tumor size ≥6 cm.

Controls. Serum samples from 40 healthy donors with comparable sex and age distribution characteristics were also evaluated simultaneously with the patient serum samples in a blinded fashion.

Therapeutic Protocol. The therapeutic protocol is illustrated in Fig. 1. Patients received 100 mg/m² cisplatin over 4 h on day 1. From days 3 to 6 and from days 17 to 21, patients received recombinant IL-2 (Proleukin; Chiron, Amsterdam, the Netherlands) at a dose of 18 million IU/m²/day by continuous i.v. infusion. IFN-α (Roferon A; Roche, Neuilly, France; 9 million IU/day) was given s.c. three times weekly. The same cycle was repeated on day 28 (6).

Cytokine Evaluation. Sera were obtained from whole-blood samples collected on a serum separator tube; they were aliquoted and stored at −80°C until assayed. Serum IL-6 levels were determined in duplicate just before starting IL-2 therapy (day 0), then at days 7, 21, 35, and 49 during the treatment. A commercially available ELISA kit was used which was able to detect concentrations as low as 4 pg/ml (Immunotech, Marseille, France). All results were expressed in pg/ml as the mean of the two measurements ± SD.

Statistical Analysis. The statistical significance of the difference between responding and NR patients was performed using the nonparametric Mann-Whitney U test; Fisher's exact test (two tailed) was used for the coefficient of correlation: all P values given are two-sided, and $P \leq 0.05$ was considered statistically significant.

RESULTS

Clinical Response

All patients received the two induction cycles and could be fully evaluated. Overall response rate was 54% (95% confidence interval; 38–70) with 5 of 41 patients (12%) achieving a complete response (for 44+, 38+, 24, 5, and 4 months, respectively) and 17 of 41 (42%) patients achieving a partial response with a median duration of 7 (3–12+) months. Responder patients have a median survival of 14 months, and NR patients have a median survival of 7 months (P = 0.0001; Fig. 2). Complete and partial responses were determined according to WHO criteria. Responding sites included: lymph nodes, 17; soft tissue, 11; lung, 6; liver, 3; bone, 2; and others, 2. The response rate was the same in patients with visceral or nonvisceral metastases. No difference in age and sex was observed between responding and NR patients.

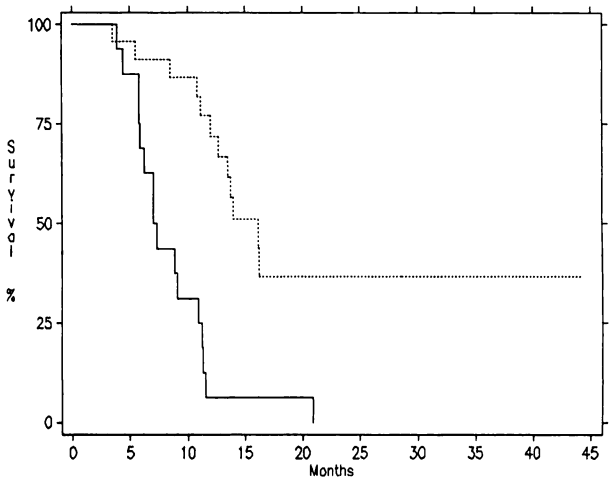

Fig. 2 Survival curve according to response. —, nonresponders; · · ·, responders.

Follow-Up of Serum IL-6 Concentrations

Before Treatment. Seventeen of 41 patients (41%) had detectable serum IL-6 concentrations, whereas levels were detectable only in 6 of 40 (15%) healthy donors (P = 0.038). Moreover, a significant difference (P = 0.002) was found in the mean IL-6 level in patients (8.07 ± 4.9 pg/ml) as compared to healthy donors (2.65 ± 2.9 pg/ml; Fig. 3).

When tumor burden was taken into consideration, we found that IL-6 levels were higher in patients with high tumor burden (11.3 ± 6.7 pg/ml) as compared to those with low tumor burden (5.7 ± 1.4 pg/ml); no significant difference in IL-6 levels was observed between patients with liver metastasis and those with other metastatic sites.

During Treatment. The IL-6 level was always higher in patients with high tumor burden as compared to patients with low-tumor burden; this difference was significant at days 7 and 21 (Fig. 4). Moreover, we found that mean IL-6 levels were significantly higher in NR patients at days 7 and 21 as compared with responding patients with low tumor burden as well as in those with high tumor burden at days 7, 21, and 35 (Table 1).

When serum IL-6 concentrations were evaluated in the whole group of patients, the largest and the most rapid increase occurred among NR patients (Fig. 5). In fact, a significant difference was found between NR and responding patients at days 7 (P = 0.0005), 21 (P = 0.002), and 35 (P = 0.0089). However, at the end of the treatment, no difference in IL-6 levels was observed.

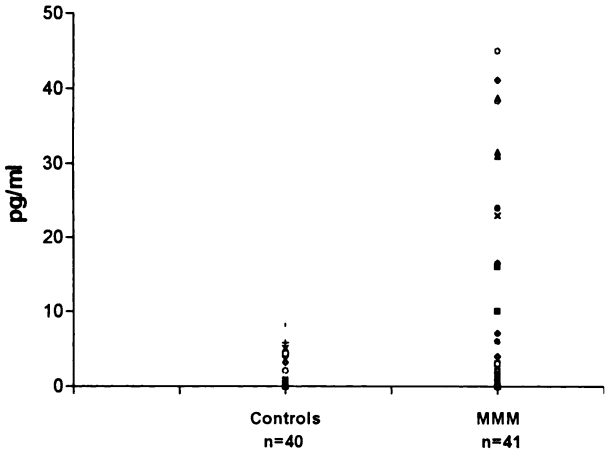

Fig. 3 Serum levels of endogenous IL-6 in healthy subjects (n = 40) and patients with MMM (n = 41).

Table 1 Follow-up of serum IL-6 in patients with MMM according to the tumor burden and clinical response[a]

	Days	NR (n = 8)	Responding (n = 13)	P
Low tumor burden	0	10.5 ± 6.2	5.33 ± 3.2	0.88
	7	52.1 ± 12.3	15.4 ± 6.2	0.025
	21	131.7 ± 28.9	31.7 ± 20.6	0.022
	35	96.6 ± 30.2	77.5 ± 20.5	0.55
	49	68.9 ± 17.4	44.3 ± 22.3	0.79
		NR (n = 11)	Responding (n = 9)	
High tumor burden	0	9.8 ± 4.5	5.8 ± 2.4	0.9
	7	87.4 ± 12.6	23.1 ± 15.3	0.023
	21	154.8 ± 33.7	58.9 ± 27.3	0.037
	35	147.5 ± 34.3	43.2 ± 22.3	0.001
	49	116.1 ± 25.6	67.5 ± 18.9	0.22

[a] Results are expressed in pg/ml as mean ± SD. The Mann-Whitney U test was used for the statistical analysis.

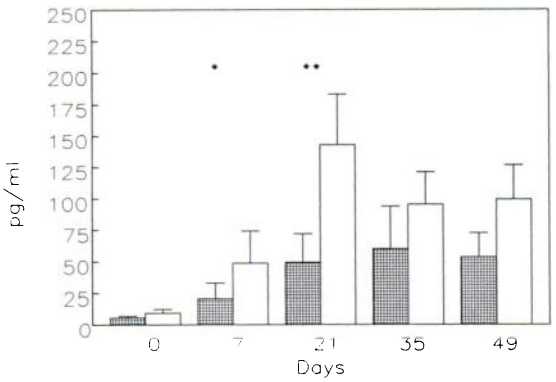

Fig. 4 Follow-up of IL-6 levels in patients with low (▦, n = 21) and high (□, n = 20) tumor burden. Results are expressed in pg/ml as mean ± SD. The Mann-Whitney U test was used for statistical analysis. *, P = 0.02; **, P = 0.009.

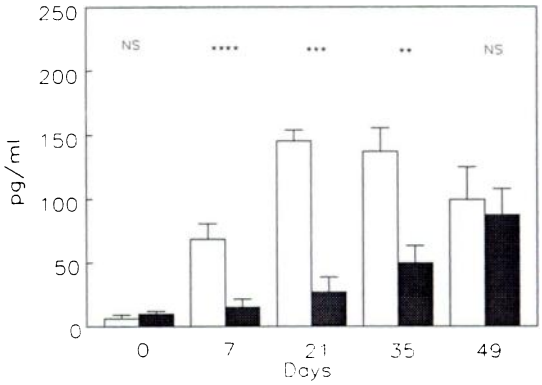

Fig. 5 Follow-up of endogenous IL-6 concentrations in responding (■, n = 22) and NR (□, n = 19) patients. Results are expressed in pg/ml as mean ± SD. The Mann-Whitney U test was used for statistical analysis. **, P = 0.0089; ***, P = 0.0021; ****, P = 0.0005; *NS*, not significant.

DISCUSSION

The achievement of remission is the primary aim of any treatment, including biochemotherapy. Early detection of biological parameters might allow the identification of patients who will respond to different therapeutic strategies. In addition, the kinetics of these biological parameters might also be used to indicate treatment failure and the need for alternative therapy. We previously demonstrated the involvement of soluble (20) and cellular IL-2 receptors (21) in the clinical response of patients with MMM treated with biochemotherapy. In the present study, our primary objective was to define the role of endogenous IL-6 in 41 patients with MMM before and following biochemotherapy. In addition, the relationship of endogenous IL-6 concentrations with the tumor burden and/or the clinical response was also evaluated.

When pretreatment samples were compared with those of healthy donors, we found that IL-6 levels were significantly increased in the serum of patients with MMM (P = 0.002): 41% of the patients had detectable serum IL-6 concentrations. In contrast, only 15% of healthy donors had detectable IL-6. These results are in agreement with those obtained in patients with aggressive multiple myeloma (17, 21), plasma cell leukemia (22), ovarian cancer (23), and metastatic renal cell carcinoma (18). Interestingly, when tumor burden has been taken into consideration, IL-6 levels were higher in patients with high tumor burden as compared to those with low tumor burden. This suggests that IL-6 might indeed be produced by tumor cells and released in the circulation as observed *in vitro* by different authors with human malignant melanoma cells (24, 25).

It has been shown that following IL-2 administration, the serum IL-6 level transiently increases in 40–50% of the patients (8, 26–29). In our study, during treatment, we recorded an increase of endogenous IL-6 levels in the sera of 70% of the patients with MMM; this increase lasted until the end of the treatment. But it is noteworthy that the largest and the most rapid increase occurred in NR patients as soon as biochemotherapy began (25-fold higher at day 21 as compared to day 1). In fact, a significant difference was observed between NR and

responding patients at days 7 ($P = 0.0005$) and 21 ($P = 0.002$). Moreover, when patients were classified according to the tumor burden and the clinical response, we found that IL-6 levels remained significantly higher in NR patients as compared to responding patients, regardless of the tumor burden.

These results prompted us to ask whether this endogenous IL-6 had any function on melanoma cell growth or resistance to biochemotherapy. It has been previously demonstrated that IL-6 acts as a bifunctional cytokine for human melanoma cells during malignant tumor progression (30–33). Lu *et al.* (31) showed that IL-6 inhibited the growth of early stage melanoma cells, which were unable to metastasize, by a paracrine mechanism. This growth inhibitory effect was lost in the advanced stage of these melanoma cells which were able to metastasize and to synthesize and use IL-6 as an intracellular autocrine growth factor. Furthermore, these cells also exhibited resistance to other inhibitory factors such as IL-1 β, tumor necrosis factor-α, and tumor growth factor-β. These resistance phenomena were often associated with spontaneous IL-6 secretion by the advanced stage cell lines (30). Moreover, endogenous IL-6 production and its possible contribution to autocrine cell growth have been implicated as a key step in the progression of some types of hematological (17, 21) and other malignancies (34–36). Thus, it is conceivable that endogenous IL-6 may play a role in the failure of IL-2 therapy in such patients, since the very early IL-6 increase is associated with tumor mass and nonresponse to biochemotherapy.

From our results, we can conclude that the detection of endogenous IL-6 in patients with MMM may represent valuable information for monitoring response to biochemotherapy. An appropriately designed prospective study will be required to address this issue.

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