Acta Oncologica Vol. 34, No. 5, pp. 599–603, 1995

SERUM LEVELS OF CYTOKINES AND SOLUBLE CYTOKINE RECEPTORS IN PATIENTS WITH METASTATIC RENAL CELL CARCINOMA OR MALIGNANT MELANOMA RECEIVING IL-2/INTERFERON-α COMBINATION THERAPY

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Elevated serum levels of soluble tumour necrosis factor receptor (sTNFR-55) and (at a lesser degree)of sTNFR-75 were found in most of the patients with metastatic renal cell carcinoma (RCC) or malignant melanoma (MM) before immunotherapy and with further increase during treatment (intravenous infusions of interleukin-2 [IL-2] and subcutaneous interferon-α [IFN-α]). In the majority of the patients with MM the pretreatment serum levels of IL-2 and soluble IL-2 receptor (sIL-2R) were increased, whereas fewer patients with RCC presented with increased serum levels of IL-2 and sIL-2R. Twelve days' treatment with IL-2/IFN-α, with a rest on days 6 and 7, resulted in a consistent further increase in the serum levels of sIL-2R and sTNFRs. In most patients the increase of sIL-2R and sTNFRs lasted for at least 3 weeks after treatment discontinuation. The clinical significance of the increase remains unknown.

During the last 10 years immunotherapy has increasingly been used in patients with metastic renal cell carcinoma (RCC) and advanced malignant melanoma (MM) (1–5). Most commonly interleukin-2 (IL-2) and interferon-α (IFN-α) have been applied given either as single drug treatment or as combination therapy. Objective responses of 10–30% and of 8–33% have been obtained in ECC and MM respectively.

IL-2 stimulates cytotoxic lymphocyte activity and release of endogenous cytokines involved in the activation of cytotoxic cell functions (e.g. IL-1, tumour necrosis factor alfa (TNF-α), IL-2) (6–11). IFN-α has similar but weaker lymphocyte stimulating effect and enhances the MHC-1 expression on the tumour cell surface (for review, see (12)). In vitro studies have demonstrated a synergestic cytotoxic activity of IFN and IL-2 on cells from solid tumours (13, 14). In addition, IFN-α has direct anti-proliferative activity in cell cultures of RCC and inhibits angiogenesis (15).

In order to become active within cells, cytokines have to bind to cytokine receptors on the cell surface. IL-2 receptors (IL-2R) have been demonstrated on all mononuclear cell subsets and also shed into the circulation. Soluble IL-2Rs (sIL-2R) can usually serve as markers of immunological activation. Two types of TNF receptors (TNFRs) have been isolated, p55 TNFR and p75 TNFR (16, 17), derived from various leukocyte subsets, endothelial cells and tumour cells. Soluble TNFRs (sTNFR) can be measured in the serum of patients (18, 19). From in vitro studies sTNFRs are known either to facilitate or inhibit TNF functions in vivo (20).

Only limited knowledge is available about the serum levels of cytokines and cytokine receptors in patients with advanced RCC and MM before, during and after combination treatment with IL-2 and IFN. In the present study our group has therefore analysed the serum levels of sIL-2R and of sTNFRs in patients with metastatic RCC and MM receiving induction treatment with IL-2 and IFN

Received 3 October 1994.
Accepted 14 March 1995.
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 ISSN 0284-186X

in the setting of a phase II study (21). These serum levels were measured before, during and after the immunotherapy.

Material and Methods

Patients. From 1989 to 1991, 16 patients with RCC and 15 patients with MM respectively were included into two separate phase II studies, evaluating the efficacy and toxicity of continuous IL-2 infusions (Proleukin, EuroCetus BV, Amsterdam, The Netherlands) and subcutaneously applied IFN (Roferon-A, Hoffman La Roche, Basel, Switzerland). The most important eligibility criteria of the patients were 1) progressing and bidimensionally measurable locally advanced non-resectable lesions or metastatic disease, 2) performance status (WHO ≤ 1), 3) no previous immunotherapy or chemotherapy, and 4) no brain metastases. All patients signed an informed consent document. All patients were followed until death or until December 1993.

Treatment. The treatment consisted of 2 induction cycles: IL-2 (18×10^6 U/m^2/day) was given as intravenous (i.v.) continuous infusions on day 1–5 and 8–12 together with subcutaneous (s.c.) IFN (3×10^6 U/m^2/day). A 3-week rest was scheduled between the two induction cycles. Three weeks after discontinuation of the two induction cycles maintenance treatment was started in non-progressing patients who did not experience unacceptable toxicity. Maintenance treatment consisted of 4 monthly 5-day continuous infusions of IL-2 and s.c. IFN (doses and schedule identical with those mentioned above). IL-2 was infused by an indwelling catheter inserted in the subclavicular vein.

Serum sampling. According to the protocol serum from 10 ml blood was supposed to be obtained from the patients on day 0 or in the morning of day 1 before starting the IL-2 infusion (pretreatment sample), and on days 5, 8 and 12 of cycles 1 and 2. All serum samples were stored at −20°C until analysis in December 1992. However, due to several administrative difficulties (critically ill patients transferred to the intensive care unit, holidays, erroneous

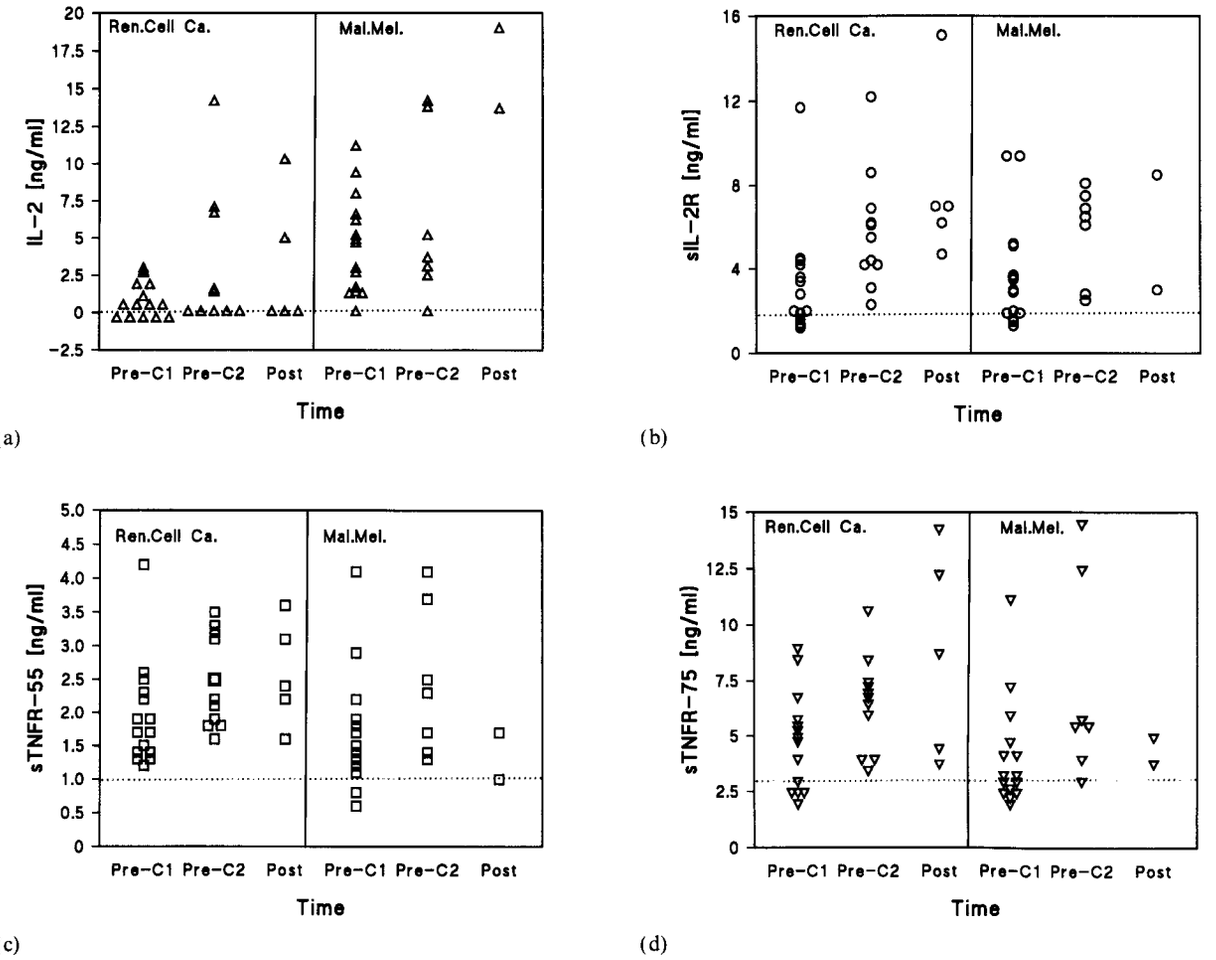


Fig. 1. Serum levels of IL-2 (a), sIL-2R (b), sTNFR-55 (c) and sTNFR-75 (d) in patients with metastatic renal cell carcinoma (RCC) or malignant melanoma (MM) analysed before the first induction cycle of immunotherapy with IL-2 and IFN-α, (Pre-C1), before the second induction cycle (Pre-C2) and 3–6 weeks after induction immunotherapy (Post). – – – Upper limit of the normal range

storing) and due to medical reasons (premature discontinuation of treatment) the sampling schedule was not strictly maintained, and only 70% of the expected serum samples were finally available for analysis.

Analysis of cytokines and cytokine receptors. The following cytokine receptors were analysed in the thawed serum samples by previously described techniques (22): sIL-2R (normal range: $\leqslant 2$ ng/ml); sTNFR-55 (normal range: $\leqslant 2$ ng/ml); sTNFR-75 (normal range: $\leqslant 3$ ng/ml). Serum levels of IL-2 were determined by sandwich enzyme immuno-assays (3).

Results

IL-2. Before start of immunotherapy, elevated IL-2 levels were found in 6 of 15 patients with RCC and 14 of 15 patients with MM, resulting in a statistically significant difference of the median values (RCC: 0; MM: 4.6, $p < 0.001$) (Fig. 1a). After one cycle (immediately before start of cycle 2) 6 of 7 patients with MM and all patients with RCC had detectable serum IL-2 levels. Three to 6 weeks after cycle 2, 5 RCC patients and both evaluable MM patients had raised IL-2 levels.

sIL-2R. In spite of the differences between RCC and MM patients regarding the pretreatment IL-2 levels, no statistically significant difference was found between the two malignancies with respect to sIL-2R before start of immunotherapy. After the first cycle sIL-2R was elevated in the serum in all studied 18 evaluable patients and remained elevated in each patient 3–6 weeks after cycle 2 (Fig. 1b).

sTNFRs (Fig. 1c, d). sTNFR-55 serum levels and—to a lesser degree—those of sTNFR-75 were elevated in the majority of patients before start of immunotherapy. Both types of TNFRs tended to increase further after the first cycle without any difference between RCC and MM patients.

Changes of development of cytokine receptors in individual patients during treatment (Fig. 2). Analysis of sequential serum samples was possible in only 8 patients with MM and 5 patients with RCC. sIL-2R and sTNFRs increased from day 1 to day 12 of each induction cycle, in general, reaching a maximum on day 12. No principal differences between RCC and MM patients was detected. The pattern of cytokine changes in the few responding patients did not differ from those observed in non-responders and was similar in long-term survivors and in patients with short survival, the median survival time serving as cut-off point.

Discussion

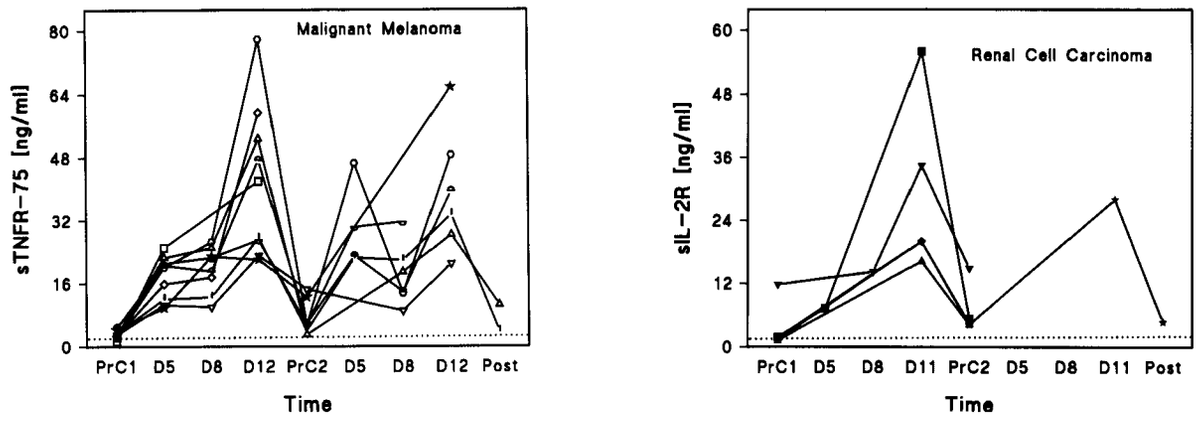
The anti-tumour activity of the IL-2/IFN-α combination was disappointing: Only 3 of 12 evaluable patients with metastic RCC and 2 of the 13 evaluable MM patients responded (21). Most of the responses were partial and lasted for only a few months. All patients suffered from considerable and sometimes life-threatening toxicity.

The present study indicates significant immunological response in the majority of patients receiving the IL-2/IFN-α combination treatment. Serum levels of IL-2, sIL-2R and sTFNR generally increased during treatment and remained high in the majority of patients during the treatment-free interval between cycle 1 and 2 and for 3–6 weeks after induction treatment. Our results are thus in agreement with the observations of Belldegrun et al. (23) and Lotze et al. (24).

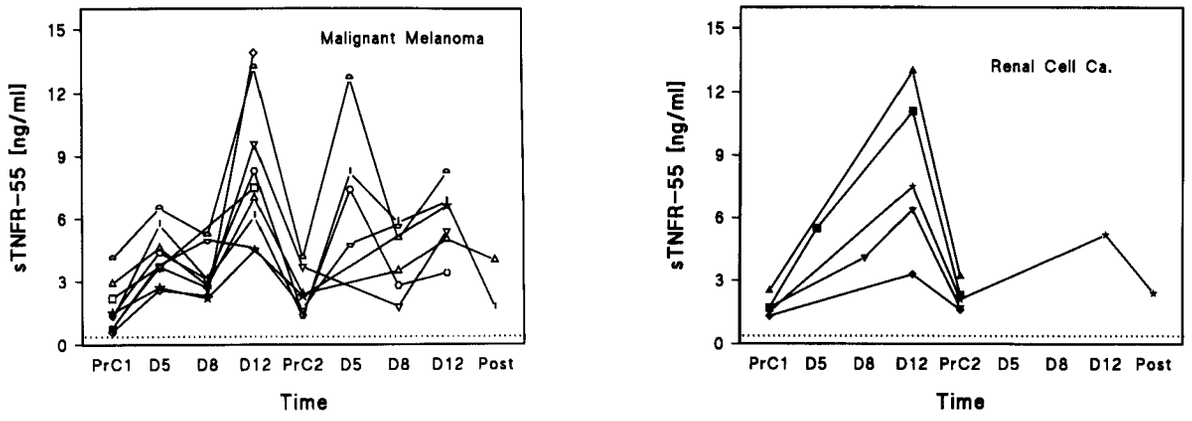
An interesting observation was that the majority of MM patients, but not the RCC patients, had increased IL-2 serum levels before IL-2/IFN-α treatment was started. In most patients with MM the pretreatment levels of sIL-2R were above the normal range. In patients with RCC, sIL-2R could be elevated in the serum, but less often than in patients with MM. In all the patients the serum levels of sTNFR-75 and, in particular, sTNFR-55 were elevated before the onset of immunotherapy. These observations suggest that some spontaneous immunoreaction takes place in patients with advanced MM and metastatic RCC, and that it probably is more pronounced in MM than in RCC patients.

The source of the increased cytokines and cytokine receptors before and during IL-2/IFN-α therapy remains unclear (23). There is no doubt that IL-2 induces the production of various cytokines in the peripheral mononuclear blood cells, often combined with leukocytosis (24). Normal endothelial cells represent another source of cytokines. On the other hand, it has also been demonstrated that cancer cell lines produce cytokines in vitro (25–27).

Due to the observed inter-patient variations of the measured levels of IL-2 and the cytokine receptors, the low response rate and the limited number of patients, it was not possible to assess a statistical correlation between the observed immunological changes with the patients' clinical course. In the individual responding patient there was, however, no obvious association between survival, response or toxicity, on the one hand, and immunological serum parameters on the other. Our results so far do not support the suggestion by Belldegrun et al. (23) of a correlation between clinical response and immunological changes, at least in some patients receiving immunotherapy. One explanation for a probable lack of correlation may be that the serological immuno-parameters do not sufficiently mirror the immunological processes taking place at the tumour site.



(a)



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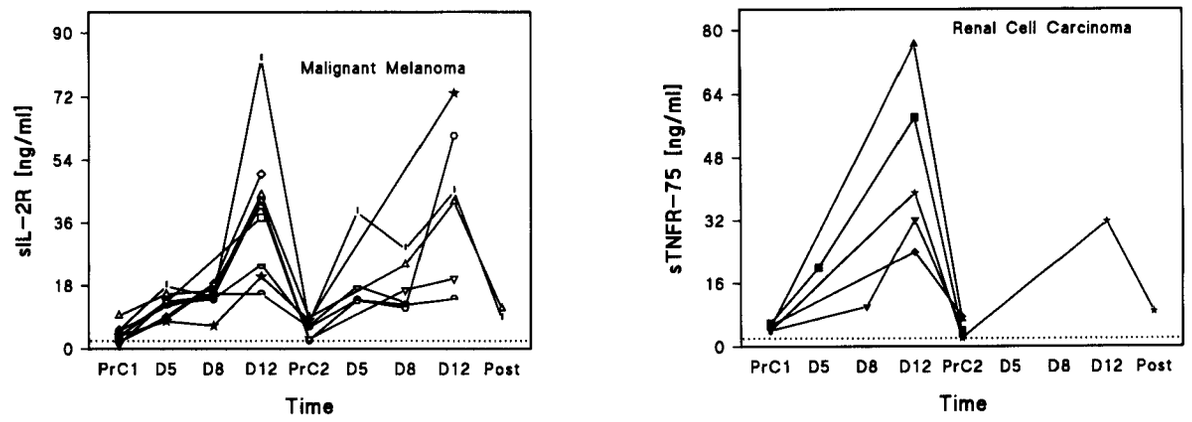

(c)

Fig. 2. Individual serum levels of sIL-2R (a), sTNFR-55 (b) and sTNFR-75 (c) before (PrC1/PrC2), during (days 5, 8 and 12 of cycle 1 and cycle 2) and after (Post) induction immunotherapy with IL-2 and IFN-α of patients with metastatic renal cell carcinoma (RCC) or malignant melanoma (MM). --- Upper limit of the normal range

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