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# Treatment of Malignant Melanoma and Renal Cell Carcinoma with Recombinant Human Interleukin-2: Analysis of Cytokine Levels in Sera and Culture Supernatants

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**In this study we evaluated the clinical response of 12 patients with malignant melanoma and renal cell carcinoma (RCC) following administration of recombinant human interleukin-2 (rhIL-2) by continuous infusion. Serum samples taken before, during and following sequential courses of IL-2 were assayed for the presence of tumour necrosis factor alpha (TNF-α) IL-1α, IL-6 and interferon gamma (IFN-γ) and the presence or changes in these cytokines were examined with respect to clinical response data: our results did not show any direct correlation between the parameters measured and clinical outcome. In addition, peripheral blood mononuclear cells (PBMC) derived from 3 RCC patients were cultured in a serum-free environment and the resulting supernatants assayed for the production of these cytokines and compared to the corresponding serum levels. During one or more courses of treatment only 1 patient, who had metastatic bone disease, demonstrated detectable serum TNF-α; serum IL-6 levels were elevated in a proportion of all patients studied and a sustained IL-6 response occurred in a patient who had complete disease remission; IL-1α was detected in the serum of 3 RCC patients; IFN-γ could not be detected in any serum sample tested. Cytokine levels in sera and supernatants derived from 3 RCC patients were compared but no correlation was found: TNF-α and IL-6 were shown to be present at much higher concentrations in supernatants when compared to sera whereas the levels of IL-1α were almost undetectable. This lack of correlation is probably due to the presence of "interfering" proteins in sera which either depress or enhance the ability to detect cytokines in sera using enzyme immunoassays.**
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## INTRODUCTION

INTERLEUKIN-2 (IL-2) promotes the cytotoxic potential of large granular lymphocytes (LGL) and monocytes [1,2], induces the production and release of cytokines such as interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α), and interleukin-6 (IL-6) [3,4], and is the principle factor required for the induction and growth of lymphokine-activated killer (LAK) cell activity [5]. It is on the basis of these activities and results from preclinical therapy experiments [6] that clinical trials are now being conducted to evaluate the potential of IL-2 as a therapeutic agent in the treatment of human malignant disease: the results of initial studies indicate that a 20–30% clinical response rate can be achieved with some human tumours [7–11].

The mechanism by which some patients with renal cell carcinoma or malignant melanoma respond to recombinant human IL-2 (rhIL-2) therapy is not understood and is the subject of debate and controversy. IL-2 is known not to exert a direct antitumour effect on solid malignancies, and tumour regression, in part, may be a consequence of direct or indirect activation of major histocompatibility complex (MHC)-restric-

ted or non-restricted cellular immune responses. Experimental studies support the contention that IL-2-activated effector cells play a direct role in tumour cell destruction, and some reports have suggested a correlation between peripheral blood LAK activity and tumour regression in melanoma patients [12]. However, this is not a consistent finding [13], and successful adoptive immunotherapy may be dependent on the recruitment of MHC-restricted activated T lymphocytes, as suggested by data demonstrating the greater efficacy of tumour-infiltrating lymphocytes (TIL) in mediating tumour regression [14]. To date, no consistent *in vitro* or *in vivo* correlation for clinical outcome has been documented. IL-2 is known to activate lymphocytes to release additional (secondary) cytokines, which are likely to play a significant role in immunoregulation [15,16], although few IL-2 based clinical studies have investigated the production of cytokines during successive courses of IL-2 therapy [17,18].

In the present study, we evaluated the clinical response of 12 patients with renal cell carcinoma (RCC) and malignant melanoma following administration of rhIL-2 by continuous infusion. This regimen was well tolerated with minimal *in vivo* toxicity. In previous studies we were unable to relate immunological and haematological responses to clinical outcome [19]: here we have analysed serum taken before, during and following sequential courses of rhIL-2, for the presence of TNF-α, interleukin-1 alpha (IL-1α) and IL-6, and compared the results with the clinical response. In addition, the production of TNF-α, IL-1α, IL-6 and IFN-γ by cultured peripheral blood mononuclear cells (PBMC) derived from 3 RCC patients was determined and compared with the corresponding serum levels.

## PATIENTS AND METHODS

*Patients*

Patients' inclusion criteria and treatment protocols are given in Hayat *et al.* [19]. Briefly, renal cell carcinoma patients received $3\times10^6$ cetus units/m$^2$/day, by continous infusion, for two sets of 5 days separated by a 2-day break: the cycle was then repeated at least once after a 3-week rest period. Malignant melanoma patients received dacarbazine as a bolus injection (250 mg/m$^2$ per day) for 5 days followed by rhIL-2, after a 16-day rest, in the same way as above: the cycle was repeated at least once after a 1 week rest. These schedules were part of a Eurocetus multicentre phase II trial.

*Collection of sera*

10 ml venous blood were taken from patients or normal individuals (undergoing routine occupational health check-ups) and allowed to clot overnight at 4°C. Serum was collected and stored at −20°C until required.

*Collection of supernatants*

10 ml of heparinised blood were taken from patients or volunteer blood donors and PBMC isolated using lymphoprep. PBMC were cultured at a density of $10^6$ cells/ml in AIM V (chemically defined, serum-free) medium (Gibco) for 24 h at 37°C in 5% $CO_2$ in air. Supernatants were then harvested and stored at −20°C until required.

*Table 1. A summary of reagents and suppliers for cytokine EIAs*

| Cytokine | Capture antibody | Standard cytokine | Second antibody | Third antibody |
|---|---|---|---|---|
| TNF-α | Polycolonal goat anti-TNF-α (H34)† | rhTNF-α (yeast)§ ($6\times10^7$ U/mg)* | Monoclonal (101/4)† | Biotinylated sheep anti-mouse Ig¶ |
| IFN-γ | Polyclonal sheep anti-IFN-γ† | rhIFN-γ (*E. coli*)§ ($2\times10^7$ U/mg) | Monoclonal (4S3B)† | Biotinylated sheep anti-mouse Ig¶ |
| IL-6 | Monoclonal (CLB-IL6-8)‡ | rhIL-6 (*E. coli*)‖ ($4\times10^7$ U/mg) | Biotinylated polyclonal sheep anti-IL-6‡ | Not applicable |

*Specific activity.
Supplied by †A. Meager, NIBSC; ‡L. Aarden, CLB (Amsterdam); §Boehringer Ingelheim; ‖ICI; and ¶Amersham.

*Cytokine measurement*

A specific enzyme immunoassay (EIA) was used to detect TNF-α, IL-6 and IFN-γ in patients' sera and supernatants based on the methods of Meager [20] (TNF-α and IFN-γ) and L. Aarden (CLB, Amsterdam) (IL-6). The reagents used are summarised in Table 1. The dilutions used were optimised prior to the routine use of these assays and checked routinely for optimum sensitivity.

Flexible 96-well assay plates (Dynatech) were coated with 50 μl capture antibody diluted in 0.05 mol/l carbonate buffer (pH 9.6) and incubated for 1 h at 37°C (TNF-α and IFN-γ) or for 2 h at room temperature (IL-6). Non-specific sites were blocked overnight at 4°C with 150 μl 2.5% BSA (TNF-α and IFN-γ) or 5% BSA (IL-6) diluted in Tris buffered saline (50 mmol/l Tris HCl; 150 mmol/l NaCl; pH 7.5) (TBS). Plates were washed three times with TBS + 0.02% Tween 20 (BDH) at this step and in between each of the following steps using a Wellwash 4 automatic plate washer (Denley Instruments, Billinghurst, UK). Standards were diluted in prescreened human AB sera (a generous gift of Sheffield Blood Transfusion Service) or in AIM V medium as appropriate, and 50 μl added to each well, in parallel with the samples, followed by either a 1 h incubation at 37°C (TNF-α and IFN-γ) or a 2 h incubation at room temperature (IL-6). Plates were washed followed by the addition of the second antibody diluted in TBS + 1% heat inactivated fetal calf serum (HIFCS) and incubated for 1 h at 37°C (TNF-α and IFN-γ) or room temperature (IL-6) followed by a further wash. In the TNF-α and IFN-γ assays the third antibody was diluted in TBS + 1% HIFCS, 50 μl were added to each well and the plate incubated for 1 h at 37°C prior to a further wash. Streptavidin-conjugated alkaline phosphatase (Amersham) was diluted in TBS + 1% HIFCS and 50 μl added to each well and incubated for 30 min (TNF-α and IFN-γ) or 1 h (IL-6) at 37°C.

Phosphatase substrate (Sigma) was prepared at 10 mg/ml in distilled water and stored in the dark until ready to use. After a final wash, 50 μl alkaline buffer solution (1.5 mol/l 2-amino-2-methyl-1-propanol, pH 10.3) (Sigma) were added to each well,

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followed by 50 μl substrate. Plates were incubated at 37°C until the colour was fully developed (usually 30 min) when 50 μl 0.1 mol/l NaOH were added to stop the reaction. The optical density at 414 nm was determined using an Anthos 2001 plate reader (Denley Instruments). The reaction was decolourised with 50 μl of 4 mol/l HCl and the optical density determined as above: this reading was then subtracted from the first to give the optical density due to the specific enzyme reaction. The amount of cytokine present in each sample was determined with reference to the standard curve included on each plate.

Assay sensitivities are given in Table 2. Studies undertaken in our laboratory have shown these assays to be specific and not to crossreact with IL-1α, IL-1β, IL-2, IL-6, TNF-α or IFN-γ.

*Measurement of IL-1α*

This was performed using an immunoradiometric assay (IRMA), [21]: the method was modified in that all samples were diluted 1:1 in the PBS-Hb (3%) buffer and the standards were prepared by diluting rhIL-1α in prescreened AB serum or AIM V medium as above. The sensitivity of this assay is given in Table 2. rhIL-1α used for the standards (a generous gift of Glaxo Institute for Molecular Biology S.A., Geneva) had a specific activity of $2.22 \times 10^7$ U/mg.

## RESULTS

*Clinical response*

Full details of the observed clinical responses are given in the paper by Hayat *et al.* [19] and summarised in Table 3 along with the IL-6 results.

*Detection of cytokines in sera*

Serum from 27 healthy individuals was collected, stored at −20°C and subsequently assayed for cytokines. IL-6, TNF-α, IL-1α and IFN-γ were normally undetectable in the sera of healthy individuals, as shown in Fig. 1. It is interesting to note that when one cytokine was detected in a normal serum sample it was often accompanied by elevated levels of at least one other cytokine: for example, 1 individual had TNF-α present at a concentration in excess of 100 U/ml accompanied by IL-6 and IFN-γ in excess of 1000 U/ml and IL-1α at 5.4 U/ml. This was probably due to an underlying infection.

During one or more courses of treatment only 1 patient (suffering from renal cell carcinoma: patient 9) demonstrated detectable serum TNF-α. This was detected in his sera during three courses of treatment, and was accompanied by elevated levels of IL-6 but not IL-1α (this patient's sera was not assayed for the presence of IFN-γ). It is interesting that this patient alone had metastatic bone disease and was hypercalcaemic, supporting the view that increased bone turnover may be responsible for the high levels of TNF-α produced.

As shown in Table 3, IL-6 was detected in 3 out of the 6 RCC patients' sera both before and after therapy; thus, if IL-6 was present prior to the administration of rhIL-2 it remained present throughout the course of therapy. This was shown not to occur in patients with melanoma although IL-6 was detected in some patients both prior to and during therapy.

*Table 2. Cytokine assay sensitivity*

| Cytokine | Sensitivity (U/ml) | Assay |
|---|---|---|
| TNF-α | 1–10 | EIA |
| IFN-γ | 10–20 | EIA |
| IL-6 | 10–30 | EIA |
| IL-1α | 5–10 | IRMA |

EIA = enzyme immunoassay, IRMA = immunoradiometric assay.

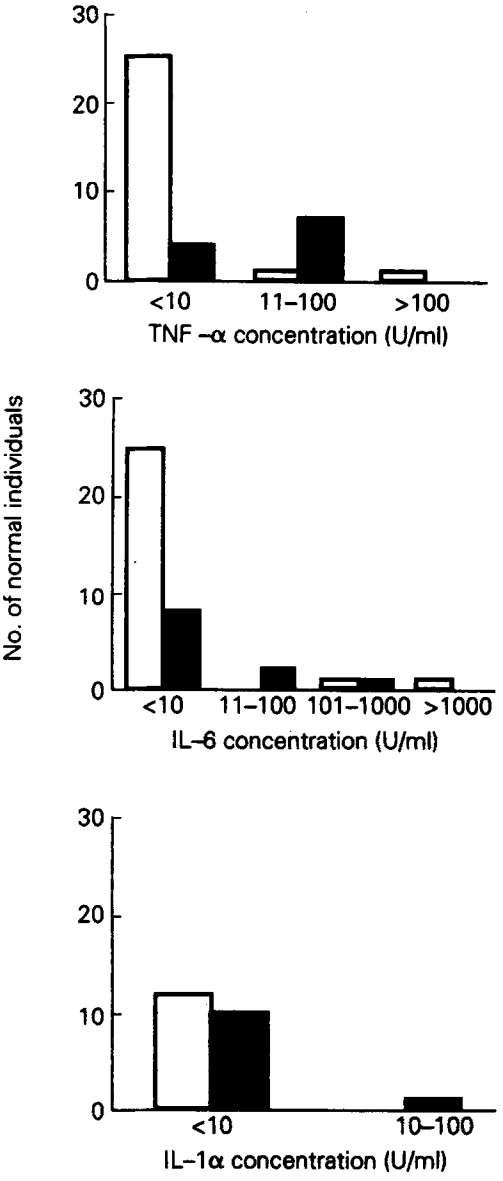


**Fig. 1. Normal cytokine levels found in sera and supernatants.**

The most consistent and interesting results came from a female patient (patient 3) who went on to attain a complete clinical response. IL-6 was undetectable in sera both prior to therapy and during the first course of treatment. However, upon commencing the second cycle of rhIL-2, IL-6 was detected and continued to remain at high levels during the subsequent two courses she received (Table 3). A post-therapy sample taken 14 weeks after cessation of IL-2 therapy still showed elevated levels of IL-6 (230 U/ml), indicating a sustained production of IL-6 which became independant of exogenous rhIL-2 administration. The sera of this patient was also screened for a variety of acute phase reactants (APR) including C-reactive protein (CRP), α1-antichymotrypsin, α1-chymotrypsin, haptoglobin and α1-acidglycoprotein, to determine whether the IL-6 levels reflected an acute phase reaction. However, CRP was detected during

*Table 3. IL-6 levels (maximum) detected in sera of malignant melanoma and renal cell carcinoma patients*

| Patient | Course | IL-6 concentration (U/ml) Pre-therapy | Dacarbazine | IL-2 Week 1 | IL-2 Week 2 | Response |
|---|---|---|---|---|---|---|
| Melanoma | | | | | | |
| 1 | 1 | 55 | 175 | 180 | 170 | PD |
| | 2 | | 210 | 100 | 170 | |
| 2 | 1 | <30 | <30 | <30 | <30 | SD |
| | 2 | | 100 | <30 | <30 | |
| | 3 | | <30 | <30 | <30 | |
| 3 | 1 | <30 | <30 | <30 | <30 | CR |
| | 2 | | 280 | 125 | 230 | |
| | 3 | | 210 | 100 | 190 | |
| | 4 | | 110 | 100 | 210 | |
| 4 | 1 | [4]NA | 78 | 84 | 86 | PR |
| | 2 | | 110 | 78 | 180 | |
| | 3 | | 74 | 110 | 200 | |
| 5 | 1 | 440 | <30 | <30 | 340 | SD |
| | 2 | | 240 | <30 | <30 | |
| 6 | 1 | <30 | <30 | <30 | <30 | PD |
| | 2 | | <30 | <30 | | |
| Renal cell carcinoma | | | | | | |
| 7 | 1 | <30 | | <30 | <30 | SD |
| | 2 | | | <30 | <30 | |
| | 3 | | | <30 | <30 | |
| | 4 | | | 43 | 39 | |
| 8 | 1 | <30 | | <30 | <30 | PD |
| | 2 | | | <30 | <30 | |
| | 3 | | | <30 | <30 | |
| | 4 | | | <30 | <30 | |
| [5]9 | 1 | 270 | | 225 | 220 | PD |
| | 2 | | | 390 | 470 | |
| | 3 | | | 520 | 520 | |
| 10 | 1 | 52 | | 86 | 48 | SD |
| | 2 | | | 47 | 48 | |
| | 3 | | | 30 | 40 | |
| | 4 | | | 38 | 30 | |
| 11 | 1 | <30 | | <30 | 225 | SD |
| | 2 | | | <30 | <30 | |
| 12 | 1 | 460 | | 580 | 510 | SD |
| | 2 | | | 510 | 520 | |
| | 3 | | | 460 | 660 | |

PD = progressive disease, SD = stable disease, CR = complete response, PR = partial response, NA = not assayed.
Patient 9 had metastatic bone disease and was hypercalcaemic.

the first cycle when no IL-6 was present, and although CRP increased during IL-2 administration the levels attained did not parallel that of IL-6 (results not shown). Furthermore, dacarbazine did not stimulate CRP, whereas high levels of IL-6 were detectable during this stage of the protocol in the second and subsequent cycles. Other APR gave similar results showing a lack of correlation with IL-6 levels.

Representative samples of sera from 5 RCC and 4 melanoma patients were assayed for IL-1α during treatment (up to four cycles): serum IL-1α was detected in 3 RCC patients as shown in Table 4. These 3 patients' serum samples were also assayed for IFN-γ but none could be detected.

*Detection of cytokines in culture supernatants*

Supernatants derived from PBMC, cultured for 24 h in serum-free AIM V medium, from 11 healthy individuals were stored at −20°C and subsequently assayed for the presence of cytokines. IL-6, TNF-α IL-1α and IFN-γ were usually undetectable as shown in Fig. 1. A detectable level of one cytokine was usually associated with the presence of at least one other. For example, 1 individual was shown to have in excess of 100 U/ml of TNF-α, 370 U/ml IL-6, 16 U/ml IL-1α and 70 U/ml IFN-γ: as proposed earlier, this could possibly be due to a common infection.

Cytokine levels detected in culture supernatants and accompanying sera derived from 3 RCC patients (patients 10, 11 and 12) are shown in Table 4. IL-6 was found to be present at high levels and often at concentrations greater than 1000 U/ml in supernatants but without any correlation to the corresponding serum levels. TNF-α was shown to be present in 19 out of the 27 supernatants tested at concentrations up to 100 U/ml even though no TNF-α had been detected in the accompanying sera. In contrast, IL-1α was detected in sera whereas it could be

*Table 4. Cytokine levels detected in the sera and supernatants of 3 renal cell carcinoma patients*

| Patient | Day of treatment | IL-6 Sera | IL-6 Sup. | TNF-α Sera | TNF-α Sup. | IFN-γ Sera | IFN-γ Sup. | IL-1α Sera | IL-1α Sup. |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 0 | 52* | >1000 | <5 | 1.5 | <1 | <10 | 70 | <1 |
| | 1 | 86 | >1000 | <5 | 1.4 | <1 | <10 | 30 | NA |
| | 6 | 48 | 740 | <5 | <1 | <1 | <10 | 36 | <1 |
| | 35 | 47 | 650 | <1 | 16 | <1 | <10 | 28 | <1 |
| | 45 | 48 | 320 | <1 | <1 | <1 | <10 | 25 | NA |
| | 92 | 30 | >1000 | <1 | 19 | <1 | <10 | 10 | 5 |
| | 101 | 40 | >1000 | <1 | 10 | <1 | <10 | 12 | 7.8 |
| | 125 | 11 | >1000 | <1 | 11 | <1 | <10 | 14 | 8 |
| | 129 | 38 | 980 | <1 | 100 | <1 | <10 | 64 | 13.6 |
| 11 | 1 | <30 | >1000 | <5 | 2.2 | <1 | <10 | 28 | <1 |
| | 6 | 225 | >1000 | <5 | <1 | <1 | <10 | 24 | <1 |
| | 8 | <30 | >1000 | <5 | <1 | <1 | <10 | 26 | <1 |
| | 25 | <1 | >1000 | <1 | >100 | <1 | <10 | 16 | <1 |
| | 28 | <1 | >1000 | <1 | 32 | <1 | <10 | 6 | 7.5 |
| | 32 | <1 | >1000 | <1 | 5 | <1 | <10 | 16 | <1 |
| | 33 | <1 | 470 | <1 | <1 | <1 | <10 | 48 | 8 |
| | 35 | <1 | >1000 | <1 | 9.4 | <1 | <10 | 42 | 13.6 |
| 12 | 6 | >1000 | >1000 | <1 | <1 | <1 | <10 | 25 | <1 |
| | 8 | NA | >1000 | <1 | 1.5 | <1 | <10 | 84 | 8 |
| | 10 | 510 | >1000 | <1 | 3.8 | <1 | <10 | 28 | 16 |
| | 34 | 520 | >1000 | <1 | 1 | NA | <10 | NA | <1 |
| | 36 | NA | >1000 | <1 | <3 | <1 | <10 | 12 | <1 |
| | 38 | 510 | >1000 | <1 | 6.4 | <1 | <10 | 6 | <1 |
| | 41 | NA | >1000 | <1 | <3 | <1 | <10 | 8 | 13.9 |
| | 43 | 520 | >1000 | <1 | 17 | NA | <10 | NA | 70 |
| | 85 | 460 | >1000 | <1 | 74 | <1 | <10 | 25 | 480 |
| | 92 | 660 | >1000 | <1 | 35 | <1 | <10 | 14 | 14 |

IL-2 was administered on days 1–5 and 8–12 inclusive: the treatment cycle was then repeated commencing on days 36, 71 and 106 as appropriate.
*U/ml.
Sup. = supernatant, NA = not assayed.

detected in only 13 out of the 25 supernatants. IFN-γ was not detected in any patient's serum or supernatant sample assayed.

## DISCUSSION

Response rates in malignant melanoma to therapy with either rhIL-2 or dacarbazine have been generally reported to be between 20 and 30% [7–11]. Stoter and Mitchell [9,10] both suggested that a combination of chemotherapy and IL-2 might be more efficacious than immunotherapy alone, and two small studies have reported promising results for dacarbazine followed by continuous infusion of IL-2 with 5/14 (36%) patients responding. The study reported here does not show an improvement in response rates of melanoma patients using this combination therapy (11%), although the number of patients admitted into this trial was too small to allow statistical evaluation. This investigation was undertaken to establish whether serum cytokine levels were influenced by the treatment protocol and whether the values obtained reflected the clinical response in individual patients. The results show that although no direct correlate could be found between any of the parameters measured and clinical outcome, there were several interesting findings which would benefit from further study.

IL-2 has been shown to stimulate TNF-α production both *in vivo* [17] and *in vitro* [22], and is also known to play a role in the necrosis of some tumours [23]. In the present study, TNF-α was not detected routinely in the sera of either melanoma or renal cell carcinoma patients on rhIL-2 therapy. The only patient with measurable serum TNF-α (patient 9) was shown to be hypercalcaemic, and had metastasis to the bone; this serum also had the highest levels of IL-6 detected. Overall this cytokine release was most likely to be directly attributable to activated cells in bone rather than IL-2 mediated activation of PBMC [24]. This patient also had high pretreatment levels and our data showed that there was no correlation between treatment and clinical status.

IL-6 has been shown to be an important immunoregulatory agent, particularly as an acute phase reactant. Tabibzadeh *et al.* [25] have shown that a variety of tumours stain positively for IL-6, and there is evidence for an increase in circulating IL-6 in cancer patients [26]. This cytokine has been shown to inhibit the proliferation of tumour cells derived from certain carcinomas [27] and to enhance the proliferation of myeloma cells [28]. In mice bearing transplantable solid tumours, the levels of IL-6 correlates directly with the tumour burden, and more recently IL-6 has been shown to synergise with IL-2 in the induction of LAK activity *in vitro* [29].

The results given here suggest that the only patient who had complete remission of disease had an rhIL-2-dependent rise in IL-6 levels which was sustained post-rhIL-2 therapy and may represent an important correlate of response. This however remains to be confirmed in other responding cases.

IL-1α was detected in the sera of only 3 RCC patients and was below the level of detectability in the remaining 9 patients. Moldawer *et al.* [30] have shown that biologically active IL-1 is not generally found in the plasma of weight-losing cancer patients and that a in number of individuals a marked downregulation in their monocytic IL-1 production occured. Evidence exists to suggest that such a downregulation in IL-1 and/or apparent TNF-α production may be responsible for some of the defects in both host MHC-restricted or non-restricted cellular immunity seen in patients with advanced cancer [31,32].

Subsequent culture of 3 RCC patients' PBMC revealed an alternative picture of cytokine production which did not correlate with the corresponding serum levels. IL-6 was detected at extremely high levels (often > 1000 U/ml) in patients' supernatants compared to the much lower concentrations found in sera. TNF-α was found in some supernatants whereas it had been conspicuously absent in sera. In contrast, IL-1α was present at much higher levels in sera compared to supernatants. The most likely explanations for these differences is the presence of "interfering" proteins present in sera which serve to either mask the true cytokine levels or cross react so as to apparently enhance cytokine levels when assayed in a EIA. Several lines of evidence support this view.

Preliminary data from our own laboratory has revealed that patients sera can inhibit the detection of TNF-α in an EIA, and suggests that "quenching" of TNF-α by the putative inhibitors occurs. These results are similar to those documented by Bellotti *et al.* [33] in which a putative IL-2 inhibitor was detected in the sera of both normal controls and in patients with Hodgkins disease [34] and a TNF-α inhibitor of 40–60 kD has also been reported in urine. A prime candidate for these inhibitors is a component of the cytokine receptors [35] which are shed upon cell activation [36]. Autoantibodies against cytokines are also thought to be important inhibitors [37]. Such cytokine inhibitors have been reported both under normal physiological conditions [38] and in a variety of disease states [39, 40] and there is also evidence that alpha-2 macroglobulin can act as a cytokine carrier in sera [41]. All of the above explanations would serve to inhibit cytokine detection, especially in an EIA where only cytokine protein is detected irrespective of its biological activity.

Recent reports have demonstrated a soluble IL-1 antagonist to be present naturally in sera, and is structurally similar to IL-1α and IL-1β but which does not have the capacity to initiate signal transduction when bound to the IL-1 receptor [42,43]. It is possible that the antibodies used in an EIA could detect this protein and could be mistakenly interpreted to signify the presence of IL-1α or IL-1β: only a bioassay would reveal if this were indeed true.

We propose that the removal of these interfering proteins by *in vitro* culture of PBMC, in the absence of autologous sera, reveals a more accurate reflection of cytokine production *in vivo* and should be considered at least in parallel with serum cytokine levels when monitoring patient responses to therapy.

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