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Correlation of serum cytokine and acute phase reactant levels with alterations in weight and serum albumin in patients receiving immunotherapy with recombinant IL-2

D. J. DEEHAN, S. D. HEYS, W. SIMPSON*, R. HERRIOT†, J. BROOM* & O. EREMIN
*Departments of Surgery, *Biochemistry and †Pathology, University of Aberdeen, Aberdeen, UK*

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SUMMARY

Recombinant IL-2 (rIL-2) has been used alone or in combination with other chemotherapeutic agents to enhance host defences against cancer. Prolonged administration of high doses, required for clinical efficacy, may precipitate serious dose-limiting toxicity. rIL-2-induced 'vascular leak syndrome' leads to hypotension, renal insufficiency, respiratory disturbances and other organ dysfunctions. Serial measurements of serum cytokines and the acute phase protein C-reactive protein (CRP) were performed on nine patients who received high-dose i.v. continuous therapy with rIL-2. The influence of these immunological parameters upon alterations in patients' weight and serum albumin, as indicators of toxicity, was assessed. All patients experienced weight increases during the cycle (3–11% of total body weight). The serum levels of tumour necrosis factor (TNF-α) and CRP were highly predictive of alterations in patients' weight (both $P < 0.001$), while no correlation was found with IL-6 and weight change. Serum albumin fell linearly throughout the infusion cycle, but this showed no correlation with variations in serum levels of IL-6, TNF-α, or CRP. The complement components C3 and C4 were significantly reduced at the end of the infusion, suggesting a possible role for this cascade system in mediating these clinical changes. The strong association between serum TNF-α and weight change, not previously documented, further supports the hypothesis that TNF-α is a key mediator in the pathogenesis of the 'vascular leak syndrome'.

Keywords IL-2 cytokine vascular leak syndrome

INTRODUCTION

IL-2 is a 15·5-kD cytokine produced by T lymphocytes in response to an antigenic or mitogenic stimulus [1]. Increased production of this cytokine amplifies the immunological response to such a challenge. IL-2 stimulates the activation, proliferation and differentiation of various T and B lymphocyte subsets [2], it potentiates the cytotoxicity of natural killer (NK) and lymphokine-activated killer (LAK) cells [3], and induces the release of a number of other cytokines including IL-1, IL-2, IL-4, IL-5, IL-6, tumour necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) [4,5]. Early animal studies documented that IL-2, given by the i.p. route, induced regression of experimentally induced liver tumours, both immunogenic and non-immunogenic [6]. With the development of recombinant (r) DNA technology, rIL-2 has been administered to patients with advanced cancer. However, responses in man to date have been shown to be less beneficial and more selective [7].

The early studies in man used high doses of rIL-2 delivered as bolus or continuous infusions. However, such therapy is associated with a significant morbidity. The development of a 'vascular leak syndrome' is one of the more severe and common side effects identified with this form of anti-cancer therapy [8]. This is related to a systemic decrease in peripheral vascular resistance, and increased vascular endothelial cell permeability leading to the extravasation of intravascular fluid and albumin. Such fluid shifts, if sustained, increase body weight, lead to the development of significant peripheral oedema and prominent effusions into anatomical compartments, and may precipitate organ damage. Pulmonary dysfunction is a recognized adverse event, with a few patients developing hypoxia secondary to adult respiratory distress syndrome (ARDS) [9].

The pathogenesis of this systemic disorder, however, is poorly understood. Recombinant IL-2 is known to induce the production and release of a number of cytokines, IL-1, TNF-α, IL-6 from monocytes/macrophages, and elevated serum concentrations of these cytokines are found in patients receiving rIL-2 [10,11]. TNF-α causes human endothelial cells *in vitro* to become elongated, overlap with each other and rearrange their actin filaments, thereby increasing vascular permeability [12]. Elevated circulating concentrations of IL-5 increase the peripheral circulating eosinophil levels. The latter cells, when exposed

Correspondence: D. J. Deehan, Department of Surgery, Medical School Buildings, Polwarth Building, Aberdeen AB9 2ZD, UK.

to TNF-α, produce superoxide radicals which are directly toxic to endothelial cells. *In vitro* experiments have found that T cells, when stimulated with rIL-2, express surface receptors for complement breakdown products and are capable of cleaving the central C3 component of the complement cascade [13]. Subsequent generation of active C3a and C5a metabolites, by interacting with mast cells and causing the release of histamine and kinins, can also increase vascular permeability. Increased vascular endothelial cell expression of a number of intercellular adhesion molecules (endothelial leucocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1)) is also found with systemic rIL-2 therapy [14]. These surface receptors enhance leucocyte–endothelial interactions, and the released products of these activated cells may disrupt the inter-endothelial junctions.

Experimental evidence for the participation of cytokines, complement and altered expression of vascular endothelial surface markers is convincing, but changes in immunological variables correlate poorly with clinical findings. In this study, the immunological, biochemical and clinical indices of nine patients who received continuous i.v. infusion of rIL-2 in the treatment of advanced colorectal cancer have been investigated. The data have been examined for correlations between clinical and laboratory findings.

PATIENTS AND METHODS

Patients

Nine patients with metastatic or locally advanced colorectal carcinoma (Dukes C or D) were studied. All patients had an ambulatory performance status (Eastern Cooperative Oncology Group 0–1, Karnofsky ≥80%) with a life expectancy greater than 3 months. Liver and renal function tests were within normal limits, and platelet and leucocyte counts were above $120 \times 10^9/l$ and $3 \times 10^9/l$, respectively. No patient had received systemic chemotherapy, radiotherapy or immunotherapy for the 4 weeks before the rIL-2 infusion. All patients gave written informed consent to participate in the study, which had been approved by the Joint Ethical Committee of the Grampian Health Board and Aberdeen University. Patients were monitored in a surgical high-dependency unit and hourly recordings of pulse, blood pressure, temperature and urine output were documented.

Dosage of rIL-2

A constant i.v. infusion of rIL-2 (Proleukin, Eurocetus Corporation, Amsterdam, The Netherlands) was administered for 5 days. Dosage was calculated according to the schedule, 18×10^6 U/m^2 per 24 h for the total of 120 h. No significant alteration in rate of infusion was required due to severe toxicity.

Timing of sample collection and clinical recordings

Peripheral blood was collected without the use of a tourniquet at times 0, 12, 24, 48, 72, 96 and 120 h from the start of the infusion. Blood was allowed to clot and then spun at 1000 *g* for 10 min. Serum was removed and stored at −80°C until required for analysis.

Weight recordings

The weights of all patients were recorded daily using the same scales, which were regularly calibrated.

Albumin and C-reactive protein

Serum concentrations of albumin were measured by dye binding (bromocresol green) on a DAX-72 autoanalyzer (Bayer Instruments, Basingstoke, UK) using Bayer reagents. C-reactive protein (CRP) was measured by peak rate nephelometry on a Beckman ICS Analyzer II (Beckman Instruments, High Wycombe, UK).

Cytokine assays: IL-6 and TNF-α

Serum concentrations of IL-6 and TNF-α were determined using the 'sandwich' ELISA with a commercially available assay (Quantikine, British Bio-technology Ltd, Abingdon, UK). Samples were analysed in duplicate and intraplate variation was found to be less than 3%. Results were expressed as pg/ml. The minimum detectable level for IL-6 was 3 pg/ml, and that for TNF-α was 10 pg/ml.

Statistical analysis

Regression analyses of the data were performed using the SPSS Windows package. All analyses considered the data to be non-parametric, and analyses of grouped data were carried out with log-transformed data using the paired *t*-test.

RESULTS

IL-6

Baseline IL-6 serum values were below 30 pg/ml in 8/9 patients. In one patient, the basal level was excessively high at 151 pg/ml. With the exception of this patient, serum levels rose to a peak by 12–48 h of commencing the infusion of rIL-2. Median peak level was 42 pg/ml, and this was sustained in all eight patients until cessation of therapy. In the patient with the high resting level, IL-6 fell paradoxically with the infusion. The serum levels did, however, remain above 30 pg/ml throughout therapy. The

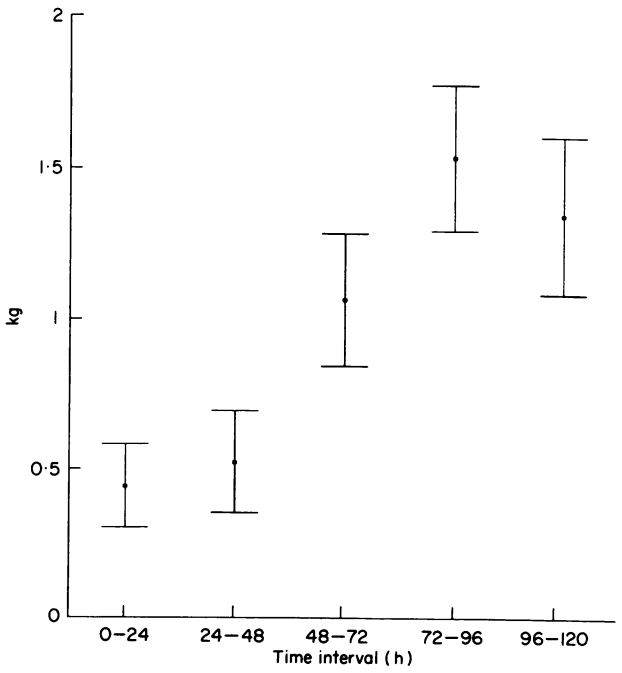

Fig. 1. Weight change with duration of rIL-2 infusion at each 24-h period (mean ± s.e.m.)

Table 1. Correlation of serum cytokine (tumour necrosis factor-alpha (TNF-α), IL-6) and acute phase protein (CRP) levels and weight change over following 24-h period

	B	S.E. B	Beta	T	Significance (*P*)
TNF-α	$1{\cdot}53 \times 10^{-2}$	$4{\cdot}29 \times 10^{-4}$	0·87	11·93	<0·001
CRP	$4{\cdot}18 \times 10^{-3}$	$1{\cdot}42 \times 10^{-3}$	0·41	2·36	<0·001
IL-6	$6{\cdot}3 \times 10^{-5}$	$1{\cdot}6 \times 10^{-3}$	$2{\cdot}87 \times 10^{-3}$	0·04	=0·39

maximum serum level of IL-6 attained by any patient during therapy was 137 pg/ml. The median time to reach peak levels was 48 h from start of therapy.

TNF-α

All patients studied exhibited a detectable serum level of TNF-α at two or more time points. Two patients had a basal detectable circulating concentration of TNF-α (18 and 32 pg/ml). Five patients had no rise in serum concentration until 48 h into the cycle. Median time to reach peak levels was 72 h. Maximum serum concentration of TNF-α attained was 151 pg/ml.

CRP

Four patients had a basal CRP value of less than 15 mg/dl. However, five patients had a resting serum concentration greater than 50 mg/dl (range 51–135 mg/dl, median 93 mg/dl). All but one patient exhibited an acute phase response to the rIL-2 infusion with elevations in serum levels of CRP. In the one exception, serum levels (including basal) did not rise above 10 mg/dl. This patient also had an uncharacteristic IL-6 response with an abnormally high basal level, which fell upon commencement of therapy. Median time to reach peak serum concentrations was 72 h, with maximum value attained being 277 mg/dl.

Weight of patients

There was a wide range in initial weights of the patients, documented before the start of the rIL-2 infusion (57·2–81·4 kg). All patients exhibited a weight gain; the range was 2·1–8·4 kg (3–11%) (Fig. 1). Median increase was 4·6 kg, representing a 7·6% increase in weight over a 120-h infusion of rIL-2. Most weight gain occurred late in the cycle. Median time to reach peak weight increase was 96 h. Least weight change was found in one patient with no significant acute phase protein or cytokine response to the immunotherapy.

Influence of cytokine level upon weight (Table 1)

Serum TNF-α concentration at an individual time point was compared with the change in weight of the patient over the subsequent 24-h period. A plot of serum TNF-α *versus* weight change for all time points in the nine patients ($n=45$) is shown in Fig. 2. Regression analysis of these two variables found a significant correlation ($r^2=0{\cdot}77$, $P<0{\cdot}001$). Dependency of the two variables could be related by the equation:

$$\text{Weight change } (+\text{ve}) = 0{\cdot}213 + (0{\cdot}0152 \times \text{serum [TNF-}\alpha\text{]})$$

The serum level of CRP was also found to be a significant predictor of a weight change, but less so than serum TNF-α ($r^2=0{\cdot}409$, $P<0{\cdot}01$). The interdependency of these variables is shown in Fig. 3. The equation for predicting the relationship of the two is:

$$\text{Weight change } (+\text{ve}) = 0{\cdot}601 + (0{\cdot}0059 \times \text{serum [CRP]})$$

No improvement in the prediction of mass change could be achieved by using the two variables together. In general, greatest weight increase was found in patients with elevated serum concentrations of both TNF-α and CRP.

There was little correlation between the serum level of IL-6 and corresponding change in weight over the following 24-h period.

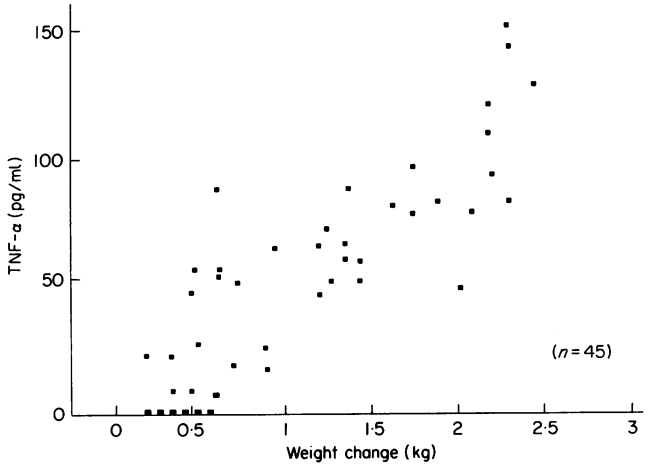

Fig. 2. Serum tumour necrosis factor-alpha (TNF-α) concentration *versus* 24-h weight change.

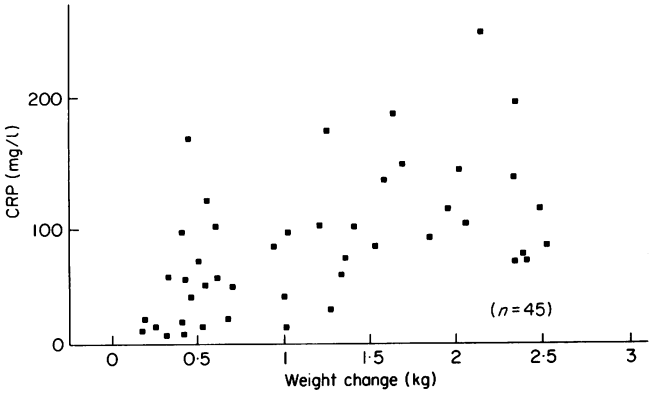

Fig. 3. Serum C-reactive protein (CRP) concentration *versus* 24-h weight change.

Table 2. Correlation of serum cytokine (tumour necrosis factor-alpha (TNF-α), IL-6) and acute phase protein (CRP) levels and albumin change over following 24-h period

	B	S.E. B	Beta	T	Significance (*P*)
TNF-α	$-1{\cdot}5\times10^{-3}$	$2{\cdot}03\times10^{-3}$	$-4{\cdot}09\times10^{-2}$	−0·21	=0·708
CRP	$2{\cdot}5\times10^{-3}$	$3{\cdot}6\times10^{-3}$	0·14	0·7	=0·416
IL-6	$3{\cdot}34\times10^{-3}$	$6{\cdot}1\times10^{-3}$	$8{\cdot}6\times10^{-2}$	0·55	=0·518

Influence of cytokine level upon albumin change (Table 2)
All patients experienced a fall in the serum level of albumin during the rIL-2 infusion. The median net fall over the 120-h period was 6 g/*l*, with a range of 3–13 g/*l*. The least change was identified in the patient with a poor cytokine and acute phase response, who also had a low albumin level (26 g/*l*). Serum albumin levels fell linearly during the rIL-2 infusions, as seen in Fig. 4. However, albumin levels returned to pretreatment values within 5 days of cessation of therapy.

No correlation was identified between variations in serum albumin concentration and the circulating levels of IL-6, CRP and TNF-α, either individually or in combination.

Complement levels
Circulating levels of the complement components C3 and C4 fell progressively during the 120-h rIL-2 infusion. The C3 component fell from a mean pretreatment level of 211 ± 12·3 mg/dl to a level of 165 ± 11·07 mg/dl at 120 h ($P<0{\cdot}05$) (Fig. 5). The complement component C4 fell from a baseline value of 39·1 ± 3·7 mg/dl to post-treatment level of 27·2 ± 3·4 mg/dl ($P<0{\cdot}05$) (Fig. 6). Both complement components (C3, C4) returned to baseline levels within 10 days of cessation of therapy.

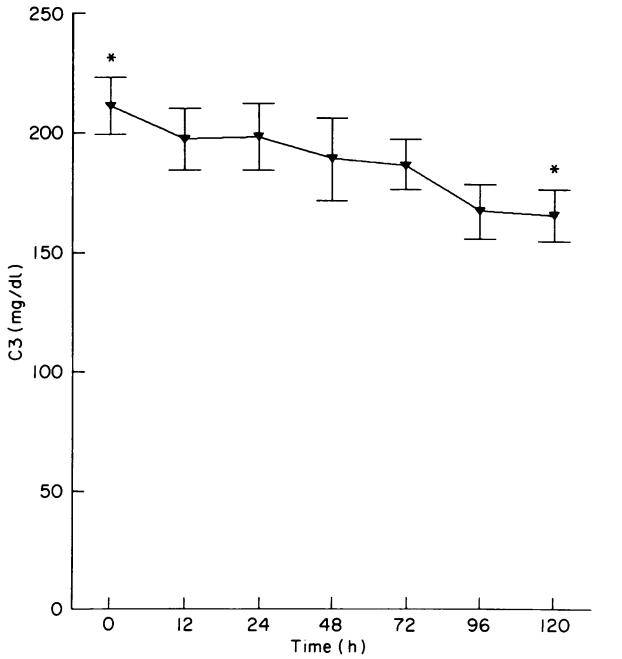

Fig. 5. Serum C3 component during rIL-2 infusion (mean ± s.e.m.) (*$P<0{\cdot}05$, comparing values at time 0 h and 120 h).

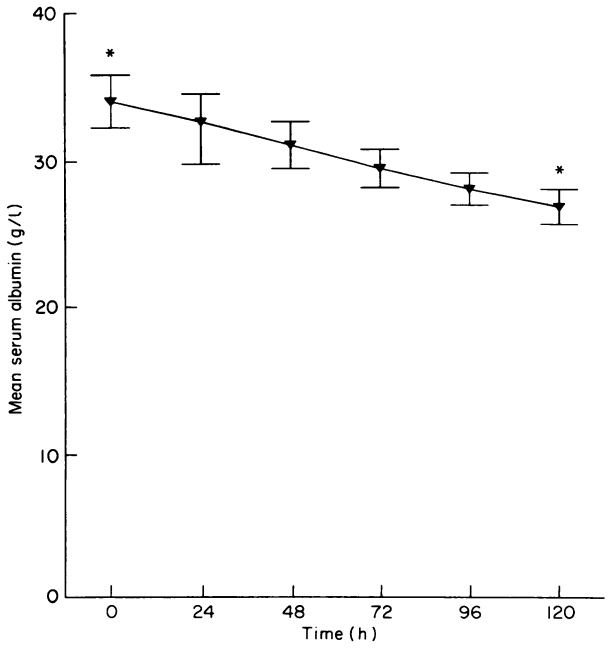

Fig. 4. Serum albumin level during rIL-2 infusion (mean ± s.e.m.) (*$P<0{\cdot}05$, comparing values at time 0 h and 120 h).

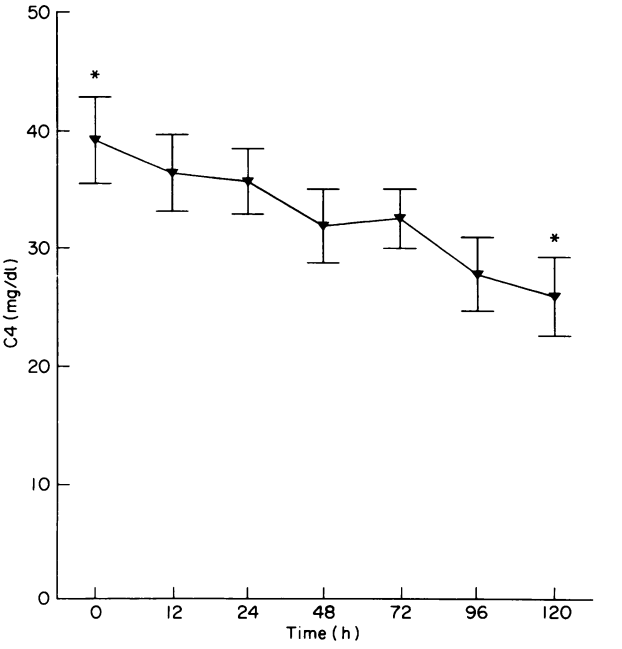

Fig. 6. Serum C4 component during rIL-2 infusion (mean ± s.e.m.) (*$P<0{\cdot}05$, comparing values at time 0 h and 120 h).

DISCUSSION

Immunotherapy with rIL-2, either alone or in combination with a number of chemotherapeutic agents, has been used to induce tumour regression in patients with advanced cancers [15]. The best clinical and pathological response rates have been achieved with renal carcinoma and malignant melanoma, and are of the order of 35% [16]. However, the administration of high-dose therapy for prolonged periods, required to achieve such responses, has been associated with a significant level of morbidity [7,17].

Alterations in haemodynamic homeostasis represent one of the most serious and often dose-limiting toxicities [18]. Therapy may cause increased systemic vascular permeability, with 'third-space' fluid retention resulting in weight gain, peripheral oedema and, if severe, can precipitate circulatory failure and organ dysfunction. Invasive monitoring has identified minimal changes in pulmonary capillary wedge pressure, but an increase in left ventricular end-diastolic volume consequent upon lowered ejection fractions [19,20]. While rIL-2 is known to be a direct myocardial depressant [21], this is not believed to be the principal mechanism behind such altered haemodynamics, but rather rIL-2-mediated decrease in peripheral vascular resistance. The exact pathogenesis of this fluid shift into the extravascular space is incompletely understood, but TNF-α is believed to be an important mediator of this extravasation. TNF-α is detected in high concentrations in the serum of patients receiving rIL-2, when given either as a bolus or continuous infusion [22]. In our study, persistent elevations of serum TNF-α were documented. Also, we found a strong correlation between serum levels of TNF-α at a given time point and increase in weight over the ensuing 24 h. *In vitro* culture of vascular endothelial cells with TNF-α leads to rearrangement of intracellular cytoskeletal structure, with narrowing of cell shape [12], and increased expression of a series of intercellular adhesion molecules [23]. Altered shape may expose a greater pore space for fluid transfer, and changes in the level of expression of some adhesion molecules results in high local accumulations of activated leucocytes. Furthermore, serum from patients with adult ARDS, a condition not dissimilar to that seen in patients with more severe toxicity from rIL-2, is found to contain very high levels of TNF-α [24–26]. IL-2 stimulation of monocytes induces production of IL-1β, both *in vitro* and *in vivo* [27,28], and this cytokine is believed to augment the vascular reactivity of TNF-α.

Both IL-1β and TNF-α are capable of inducing the vascular expression of ELAM-1, selectins present on endothelial cells at sites of inflammation [29]. Also, IL-2 induces an increased expression of vascular cell adhesion molecules (vascular cell adhesion molecule-1 (VCAM-1)) and ICAM-1 on endothelial cells and dermal tissues [30]. These surface molecules are believed to contribute to the vascular 'leak' by increasing the adherence of a number of activated leucocytes to the endothelium [31]. These trapped cells then release locally high concentrations of various proinflammatory mediators, such as C3a, C5a and superoxide radicals, which may disrupt the endothelial intercellular junctions, leading to increased vascular permeability. IL-2, therefore, probably mediates its vascular effects through the combined actions of TNF-α and IL-1β. However, IL-1β is detected infrequently in the serum of patients receiving rIL-2, possibly because most of it is retained intracellularly and the serum half-life of IL-1β is less than 12 h.

Immunotherapy with rIL-2 is found to induce a peripheral blood eosinophilia [32]. The increase in this cell type occurs towards the end of the infusion, with peak levels being attained approximately 2–3 weeks after cessation of therapy. With the associated lymphopenia, eosinophils may account for up to 50% of the peripheral blood leucocyte count. This effect is mediated through IL-2 induction of IL-5 release from monocytes. Incubation of eosinophils with TNF-α *in vitro* generates large quantities of superoxide radicals which are capable of inducing endothelial cell damage [33]. There is, however, a lack of temporal correlation with the clinical effects, as weight gain occurs during the infusion and resolves after cessation [34].

Activation of the biologically active molecules of the complement system results in the generation and release of a number of reactive metabolites capable of decreasing vascular resistance [35]. Activated components of the alternate (Ba), and classical (C4d) pathways increase 8- and 4·8-fold at the end of a 5-day infusion of rIL-2, respectively, compared with pretreatment values [13]. $CD3^+$ cells stimulated with IL-2 show increased expression of receptors for breakdown products of complement, and can cleave native C3 *in vitro*. A correlation has also been found between plasma levels of C3a and weight gain during rIL-2 infusion [36]. In our study, both C3 and C4 fell significantly by the end of the infusion cycle. We did not attempt to correlate changes in the levels of these components with the clinical parameters, as they represent the net effect of consumption and generation of the two mediators. It would appear that in our patients activation of the complement pathway occurred at such a rate that hepatic production was unable to match consumption during the rIL-2 infusion. Confirmation of this theory would require measurement of breakdown products and/or formation of multimolecular complexes or detection of neoantigens in classical and alternate pathway components. We were unable to perform these assays due to the lack of available plasma, but hope to do so in a future study.

In our study, serum albumin was found to fall linearly throughout the 120-h period of infusion of rIL-2. This is unlikely to be due to the semi-starved state of the patients, as previous workers have not found complete starvation over a similar period of time to influence serum albumin levels significantly [37]. Neither was this fall secondary to a dilution effect, as no significant variation in the mean peripheral blood haematocrit was identified during the cycle (pre-cycle $0{\cdot}35 \pm 0{\cdot}02$; post-cycle $0{\cdot}36 \pm 0{\cdot}02$). No correlation was identified, however, between serum levels of IL-6, TNF-α or CRP at a certain time point and change in the circulating levels of albumin over the following 24-h period.

Serum levels of IL-6 are elevated in patients with severe burns [38] or sepsis [39], and correlate with the magnitude of the trauma. Elevations in the serum concentrations of IL-6 in these patients, corresponding to high TNF-α levels, reflect activation of the monocyte. In our patients there was a variable elevation of IL-6 with rIL-2 infusion, but no significant correlation with weight gain. The role of other monocyte-derived mediators, induced by the administration of rIL-2, needs to be investigated. Serum concentrations of prostaglandin E_2 (PGE_2) have been found to rise from baseline (< 30 pg/ml) to excessively high levels (200–500 pg/ml) during rIL-2 infusions (authors' unpub-

lished work), and this eicosanoid may, by inhibiting activation of the monocyte, limit or ameliorate such toxicity.

A weaker (but significant) correlation was found between weight increase and the serum concentration of CRP. CRP is an acute phase reactant produced by the liver in response to elevated monocyte production of IL-1, TNF-α and IL-6. IL-6 is considered the final mediator in the induction of hepatic production. CRP is a co-factor with IL-2 in the generation of LAK cells. It is also an opsonin for pathogenic bacteria, and high circulating concentrations can activate the classical arm of the complement system [40]. This correlation, however, may be an epiphenomenon, as the effect of CRP on the vascular endothelium is unknown. T lymphocytes isolated from patients who received infusions of rIL-2 had increased surface expression of receptors for both breakdown products of complement and CRP [41]. CRP may synergize with rIL-2 in stimulating the activity of T ($CD3^+$) cells which cleave the mature C3 component of complement. In this way, CRP is capable of activating complement via two related pathways. The activation of complement generates high concentrations of C3a and C5a which are able to increase vascular permeability. CRP may further activate leucocytes, locally chemoattracted and adherent to endothelial cells, to generate and release large quantities of locally vasoreactive intermediates.

This study has shown a significant correlation between the serum levels of TNF-α and CRP and increases in body weight, in patients undergoing immunotherapy with rIL-2 infusion. To the best of our knowledge, this is the first documentation of such correlations. This lends indirect support to the postulate that TNF-α may be the key mediator in the etiology of the vascular leak syndrome. However, it has yet to be seen whether reductions in patient serum levels of TNF-α could ameliorate this toxicity from rIL-2 administration and lead to safer therapy.

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