Chapter I

Side effects of cancer treatment with recombinant human tumor necrosis factor alpha: A new challenge for the intensive care

Submitted as:
Side effects of cancer treatment with recombinant human tumor necrosis factor alpha: A new challenge for the intensive care
Jan H. Zwaveling†, MD; Jan K. Maring†, MD; Armand R.J. Girbes†, MD; Heimen Schraffordt Koops‡, MD

† Division of Intensive Care, Department of Surgery, University Hospital Groningen, Groningen
‡ Division of Surgical Oncology, Department of Surgery, University Hospital Groningen, Groningen
Chapter I

Summary

The purpose of this chapter is to familiarize intensive care staff with the concept of isolated limb perfusion with recombinant human tumor necrosis factor alpha (r-TNF-α), a new modality in cancer treatment, that has attracted considerable attention. The systemic toxicity of this type of treatment, and of cancer therapy with r-TNF-α in general, is reviewed. This review is based on Phase I and Phase II trials published in the English language, along with supportive documentation and data on 64 patients treated with r-TNF-α in our own institution. Guidelines are offered for the successful management of this type of patient.

Treatment with r-TNF-α results in a characteristic clinical syndrome, which resembles the sepsis syndrome. Hypotension and respiratory failure are the main features of this syndrome. Toxicity is largely independent of the route of administration. Very high serum concentrations of TNF-α, if shortlived, can be less toxic than sustained low serum concentrations. Treatment of patients who have undergone isolated limb perfusion with high dose r-TNF-α is feasible and effective in a modern ICU setting, even if high serum concentrations of TNF-α, due to leakage from the perfusion circuit, cannot be avoided. Most patients can be discharged from the ICU within 24 hours.
Side effects of treatment with TNF-α

Introduction

Cancer treatment with bacteria or bacteria-induced serum factors is not new. Late in the previous century, the surgeon William Coley reported considerable success in the treatment of advanced cancers with injections of live bacteria or bacterial extracts, although at the cost of serious morbidity [1-2]. Later, Shear and coworkers could show that endotoxin, a part of the cell wall of Gram-negative bacteria, was indeed capable of inducing hemorrhagic necrosis in laboratory animals with transplanted tumors [3]. Since endotoxin did not kill tumor cells in vitro, the possibility of an intermediate cytotoxic host factor released by the administration of endotoxin was suggested. In a series of elegant experiments Carswell and coworkers were able to demonstrate the presence of such a substance in the serum of BCG-infected mice treated with endotoxin [4]. When this serum was intravenously administered to animals bearing a subcutaneous transplant of a murine sarcoma, visible necrosis of the tumor was observed. Accordingly, the active substance was named tumor necrosis factor (TNF). In the mid-eighties the DNA for human tumor necrosis factor was cloned and, with recombinant techniques, large amounts of human TNF became available for research purposes [5-6]. Since TNF had shown in vitro anti-tumor activity against a number of different cell lines, while showing no effect on human fibroblasts, there was considerable interest to try this new drug in clinical trials in various types of neoplastic disease. Between 1987 and 1991 a series of Phase I and Phase II trials was carried out [7-43]. Unfortunately, none of them showed unequivocal clinical benefit. As toxicity turned out to be pronounced and lethal complications of systemic treatment with TNF were reported, interest in this new drug waned as quickly as it had been aroused. Recently a revival of TNF as antineoplastic drug was introduced by Lejeune and coworkers who combined high-dose human recombinant TNF-α (r-TNF-α) with an alkylating drug in a system of isolated regional perfusion [44]. A response rate of 87% was seen in patients with locally irresectable sarcoma of a limb [45]. Later, encouraging results with this technique were also described in patients with melanoma [46]. Toxicity was considered to be acceptable, although all patients needed intensive care after the procedure [47-48]. This technique of regional perfusion with r-TNF-α in combination with other antineoplastic drugs is rapidly gaining acceptance as a valuable alternative in the treatment of several types of cancer. Post-perfusion patients will find their way to the ICU in increasing numbers and intensivists should be thoroughly familiar with the serious side-effects of treatment with r-TNF-α to be able to provide proper care.

TNF-α toxicity in vivo

TNF-α is generally considered to be the key mediator in the mammalian response to bacterial infection. It is mainly produced by stimulated macrophages and monocytes but many other cells have been reported to be able to synthesize TNF-α. It is a strong pro-inflammatory agent that will affect the function of almost any organ system, either directly or by inducing the formation of other cytokines like IL-1, or prostaglandins. TNF-α, which naturally occurs in a trimeric form, exerts its influence on cells by binding to a specific receptor. Two types have been identified: the smaller 55 kilodalton TNF-R55 receptor, present on virtually all nucleated cells and the larger 75 kilodalton TNF-R75 receptor, mainly present on lymphocytes. The extracellular portions of these receptors can become separated from the cell membrane by proteolysis, and can bind and inactivate TNF-α in the circulation. They are referred to as soluble receptors. Their shedding from cell membranes is upregulated by TNF-α itself. If injected in small amounts, TNF-α is bound rapidly to tissue receptors and degraded in the cells. If administered
in larger amounts it is broken down and partially cleared by the kidneys, and, to a lesser extent, by the liver. The serum half-life of TNF-α is dose-dependent: at doses of 25 µg/m² a serum half-life of 13 min has been described, increasing to 26 min at higher doses [9].

If administered intravenously to laboratory animals it causes a clinical picture very similar to septic shock. Excessive vasodilation will cause hypotension, acidosis and oliguria with renal failure. Increased capillary permeability will result in edema which in the lung will interfere with gas exchange. Activation of the clotting system may lead to a disseminated intravascular coagulopathy. Liver cell necrosis may occur. Even small doses of TNF-α can cause death in susceptible animals. If administered in smaller quantities to human volunteers it causes fever, headache, anorexia, myalgia, hypotension, capillary leak syndrome, increased rates of lipolysis and skeletal muscle protein degradation [49].

**Toxicity of TNF-α in the treatment of cancer: general remarks**

Although the exact mechanism, leading to tumor cell necrosis in vivo, is still largely unknown, TNF-α has been tried for almost any kind of malignant disease. It has been administered subcutaneously, intramuscularly and intravenously in many different dose regimens. It has also been used locally, for instance in tumors of the bladder and in ovarian carcinoma. Intratumoral application of r-TNF-α has been studied extensively and has even been tried in the treatment of brain tumors [23, 24, 40, 41]. Recently, combined treatment of r-TNF-α with alkylating drugs in an isolated perfusion model has received considerable attention [44, 45, 46]. This technique can only be applied with tumors of a limb. After dissection and cannulation of femoral, popliteal or axillary vessels, a perfusion circuit is established using a roller pump and a membrane oxygenator (Fig 1). The system is primed with red blood cells, a colloid solution and heparin and kept at a temperature of 39° C.

![Fig.1 Set-up of isolated limb perfusion](image-url)
After equilibration, up to 4 mg of r-TNF-α is infused into the perfusion circuit. r-TNF-α is usually combined with an alkylating agent such as melphalan to obtain maximal anti-tumor efficacy. Some of these patients are pretreated with recombinant interferon gamma (r-IFN-γ) to enhance the sensitivity of the tumor to r-TNF-α. Human r-IFN-γ increases the number of TNF-receptors on human tumor cells [50, 51]. Additionally, r-TNF-α and r-IFN-γ show synergy in antitumor effects on human tumor cells [52].

Subcutaneous and intramuscular routes of administration have been largely abandoned because of unacceptable local side effects such as soreness and ulceration [12]. Instillation of the drug into body cavities (intravesical, intraperitoneal) does not usually lead to detectable serum levels of TNF-α. Although systemic side effects of this type of treatment are reported to be mild by some [25, 43], others have observed severe systemic toxicity with fever, influenza-like symptoms and prolonged malaise [21]. Intralesional application of r-TNF-α will results in highly variable plasma levels of this cytokine [27], but most reports show plasma concentrations comparable to what is observed after intravenous dosing [23, 24, 27]. Again, systemic side effects may be severe, with intolerable rigors, fatigue and tachycardia [40].

Thus, it appears from the literature that all routes of administration of r-TNF-α can cause serious systemic side effects. Severe toxicity has been reported with low plasma concentrations of TNF-α [42], while very high concentrations of this cytokine are relatively well tolerated if its presence in the systemic circulation is short-lived. For example, toxicity in a study where r-TNF-α was infused over 24 hours, resulting in peak plasma concentrations of 8000 ng/L [33], was more severe than in a study where a 30 min. infusion of the drug led to peak levels of 300,000 ng/L [36]. Systemic toxicity seems to be determined mainly by the length of time that elevated concentrations of TNF-α can be found in the circulation. This is in keeping with the observation by Pinsky and coworkers that persistence of TNF-α and interleukin-6 concentrations, rather than peak concentrations of cytokines, predicts a poor outcome in septic shock [53]. On the other hand, the promising results of isolated limb perfusion with very high doses of r-TNF-α suggest that the effects of this drug on the tumor are dependent on high levels of TNF-α, and that, with such high levels, an exposure time of 90 min is sufficient to destroy virtually all malignant tissue. It follows that, generally speaking, short courses with high dose r-TNF-α hold most promise for the future, from the point of efficacy as well as from the point of toxicity.

Regional perfusion techniques were originally developed to avoid systemic toxicity. Ideally the drug would not spread beyond the limb that was being perfused. Indeed, perfusions with melphalan alone showed little systemic toxicity. However, it appeared that adding r-TNF-α to the perfusate invariably induced a sepsis syndrome, probably due to leakage of r-TNF-α into the systemic circulation [44, 47, 48, 54, 55]. We were able to demonstrate plasma levels of TNF-α in these patients of up to 267000 ng/L at the end of perfusion [56]. For comparison, in meningococcal disease and/or septicemia all patients with TNF-α levels over 100 ng/L died [57]. In other types of septic shock median TNF-α levels of 120 ng/L have been reported [58]. TNF-α levels in perfused patients were very high initially but fell rapidly: 12 hours postperfusion TNF-α levels had returned to baseline values [48].

It is still unclear whether the very high levels of systemic TNF-α, measured in perfusion patients, represent free TNF-α or neutralized TNF-α, bound to soluble receptors. The latter possibility would explain why clinical signs and symptoms in these patients are less severe than anticipated from the very high serum TNF-α levels. Thom and coworkers have reported a moderate increase in the soluble 55 kd
TNF receptor (TNF-R1) during perfusion with r-TNF-α, possibly representing an upregulation of receptor shedding [54]. With melphalan alone, the rise in serum TNF-R1 was less pronounced. Interleukin 6 (IL-6) concentrations rise significantly during isolated limb perfusion with r-TNF-α and may contribute to toxicity [54]. Interleukin 1 (IL-1), which can act synergistically with TNF-α, seems to be less important in this setting. The role of anti-inflammatory cytokines like interleukin 4 (IL-4) and interleukin 10 (IL-10), which could mitigate the toxic effects of TNF-α, remains to be elucidated.

Toxicity of r-TNF-α treatment is variable and not always dose-dependent. Hepatic and cardiovascular toxicity have been generally found to increase with increasing dose, but constitutional symptoms like fever, chills, rigors and prostration are probably not dose-related. The maximum tolerated dose of r-TNF-α, administered intravenously over 30 min, is reported to be between 100 and 300 µg/m² [7-10, 12, 13, 14, 17, 29, 39]. Signs of late toxicity are notoriously absent in all reports.

Specific signs and symptoms of toxicity in patients treated with r-TNF-α

**Constitutional**
As was stated before, symptoms after treatment with r-TNF-α by any route are variable and individually determined. However, virtually all patients will experience constitutional symptoms. This influenza like syndrome is characterized by fever up to 40°C, chills, rigors, headache, back pain, fatigue, prostration and malaise. These symptoms are probably not dose-related [13, 17, 19, 30-31] and will disappear spontaneously even if treatment with r-TNF-α is continued without dose adjustment.

**Cardiovascular**
Cardiovascular toxicity is usually dose-limiting. A fall in blood pressure is observed in many patients [7-10, 12, 14-20, 22, 23, 27-28, 30-33, 37-38, 55], sometimes preceded by a short period of hypertension [33]. Hypotension can be severe with systolic blood pressures < 60 mm Hg. Volume resuscitation and inotropic support of the circulation are frequently necessary to maintain acceptable tissue perfusion. Sinus tachycardia is common but other rhythm disturbances are rare [37]. In one patient a transmural myocardial infarction soon after starting treatment with r-TNF-α has been described [7]. Excessive production of nitric oxide, a potent vasodilator, by cytokine-inducible nitric oxide synthase is believed by many to be the underlying mechanism. TNF-α can induce nitric oxide synthase in vascular smooth muscle cells from rat aorta in vitro [59]. In mice the administration of anti-TNF-α antibodies markedly reduces endotoxin-induced shock and nitric oxide synthesis in vivo [60]. Kilbourn and coworkers have induced hypotension in dogs by administering recombinant human TNF-α, N anglais–monomethyl-L-arginine, a competitive inhibitor of nitric oxide formation from L-arginine, completely reversed this fall in blood pressure, which reappeared after the administration of excess L-arginine. The authors conclude that excessive nitric oxide production mediates the hypotensive effect of TNF-α [61]. Whether the same mechanism applies to humans remains to be seen. In a series of 8 patients treated with hyperthermic isolation perfusion with r-TNF-α we have measured nitric oxide metabolites in plasma. All patients developed vasodilation and hypotension, secondary to a pronounced leak of r-TNF-α from the perfusion system to the systemic circulation. However, we were unable to demonstrate any elevation in plasma nitrite or nitrate [56]. Other mechanisms, not involving the nitric oxide pathway, are therefore likely to play a role in the generation of hypotension and septic shock in the setting of r-TNF-α perfusion. The existence of a nitric oxide independent pathway to explain
cytokine-induced vasodilation in humans was already suggested by Beasley and coworkers [62]. Alternatively, cyclooxygenase products like prostacyclin could also play a role [63].

**Respiratory**
Respiratory compromise is common after treatment with r-TNF-α [2, 3, 7-10, 13, 18, 23, 30-33, 39, 64, 65] and may range from slight symptoms of tightness of the chest and tachypnea to severe respiratory distress requiring mechanical ventilation. Administration of r-TNF-α has been shown to affect pulmonary function parameters: it decreases vital capacity, capillary blood volume, diffusing capacity of the alveolo-capillary membrane and transfer capacity for carbon monoxide [64]. It is unclear whether these effects are dose-related [48, 64]. Significant reductions in these parameters are observed 1 week after isolated limb perfusion with r-TNF-α and melphalan. Eight weeks after the perfusion procedure, they have returned to pre-treatment values. Treatment with r-TNF-α also increases pulmonary permeability, which could explain the transient pulmonary infiltrates described in the original publication by Eggimann [47, 66]. A relationship between the Lung Injury Score (Murray) and TNF-α levels has been described [48].

**Renal**
Renal toxicity is surprisingly mild. In most series renal toxicity does not exceed moderate proteinuria and minimal elevation of serum creatinine [7, 9, 13, 17, 20, 31-33, 37-38, 55]. Others have reported more serious renal symptoms, such as a marked reduction in creatinine clearance, oliguria and elevated tubular enzyme excretion [12, 14, 23, 27, 28, 30, 67]. Whether renal toxicity in this setting is a consequence of inadequate perfusion pressures or a direct toxic effect of r-TNF-α remains to be determined [23, 27, 67]. Even in patients who could be kept normotensive throughout the perfusion procedure and during their postoperative stay in the ICU, a temporary decrease in proximal tubular function was observed [67]. This may represent a direct toxic effect of TNF-α.

**Hepatic**
Many patients will develop a significant rise in either bilirubin or ASAT and ALAT or both [7-9, 14-16, 18-20, 22, 23, 27, 28, 30, 32-35, 37-38, 55]. Liver cell damage can be dose-limiting [8-9, 18, 20, 32], but is usually rapidly reversible on discontinuation of the drug. It does not cause clinically significant disturbances of coagulation, due to deficient synthesis of clotting factors.

**Digestive tract**
Upper as well as lower digestive tract symptoms are common in this type of treatment. Nausea and vomiting can be distressing [7, 23, 32, 33] and in some cases dose-limiting [35]. Watery diarrhea has been observed in a number of patients [15, 19, 23, 32, 33].

**Blood**
A dose-dependent decrease in platelets is common [20, 22, 23, 29-31, 55]. It can be very pronounced (< 25,000/mm³) and dose-limiting [20, 22]. However, to our knowledge, petechiae and overt bleeding have not been reported.

A decrease in hemoglobin has also been reported, but its cause remains unclear [29]. Hemodynamic instability will often necessitate infusion of large quantities of fluids, which in turn will aggravate any existing low hemoglobin level.
Initially, granulocytes tend to decline in numbers in the peripheral blood, probably because of sequestration. After the infusion of r-TNF-α has been discontinued, a significant increase in numbers is observed [9-10, 15, 18, 22, 23, 28, 31, 39, 55]. Monocytes and lymphocytes also decrease dramatically in number in the early phase but their recovery seems to be less quick [9].

It has been shown by various authors that TNF-α has a procoagulant effect on the hemostatic mechanism in humans through expression of tissue factor and downregulation of thrombomodulin [68-70]. Signs of activated coagulation have been confirmed in a few clinical series [35, 39, 55, 71], but prolonged clotting parameters are rare [48, 55]. A normal clotting profile seems to be most common [17, 22, 23, 31-33]. Fibrinolysis is inhibited by a large increase of plasma activator inhibitor type 1 (PAI-1) and a decrease of tissue plasminogen activator (t-PA) [72, 73]. Inhibition of fibrinolysis has been shown in healthy subjects treated with r-TNF-α [72], in patients treated with intravenous r-TNF-α [73], and in patients treated with isolated limb perfusion with r-TNF-α and melphalan [74].

**Nervous system**

Neurological sequelae of treatment with r-TNF-α have been mentioned by a number of authors. Confusion and hallucinations can be severe but seem to be quickly reversible after discontinuation of the drug [23, 31, 33, 37]. Transient aphasia and diplopia have also been described [22, 30, 32, 33]. Three cases of blindness have been reported, one cortical [7], and two as a consequence of a retinal vein thrombosis [14]. Seizures have been reported in one series [31].

**Miscellaneous**

Wasting, considered to be due to prolonged administration of r-TNF-α has been described [25].

Authors who have studied the effect of r-TNF-α on lipid metabolism have reported a decrease in high-density lipoproteins, as well as increases in triglycerides and very-low-density lipoproteins [22].

In one series, bacteremia and sepsis were considered to be causally related to treatment with r-TNF-α [31].

To our knowledge at least six patients have died as a consequence of treatment with r-TNF-α: one after a cardiac arrest 90 minutes after receiving the first dose of r-TNF-α [8], two following septic episodes during a 5-day continuous infusion [31], one from pulmonary embolism [39], one from intracranial hemorrhage [39] and one from treatment-related pulmonary edema [39].

The most relevant side effects of hyperthermic isolated limb perfusion with r-TNF-α are summarized in Table 1.

<table>
<thead>
<tr>
<th>Clinically relevant side effects of HILP with r-TNF-α</th>
<th>α.</th>
</tr>
</thead>
<tbody>
<tr>
<td>hypotension</td>
<td></td>
</tr>
<tr>
<td>respiratory failure</td>
<td></td>
</tr>
<tr>
<td>liver cell necrosis</td>
<td></td>
</tr>
<tr>
<td>thrombocytopenia</td>
<td></td>
</tr>
<tr>
<td>fever</td>
<td></td>
</tr>
<tr>
<td>rigors</td>
<td></td>
</tr>
<tr>
<td>malaise</td>
<td></td>
</tr>
</tbody>
</table>

Table 1. Side effects.
Some symptoms of r-TNF-α toxicity can be prevented, others can be minimized by adequate treatment (Table 2). There is ample evidence that non-steroidal anti-inflammatory drugs can alleviate constitutional symptoms, probably because they are partially mediated by prostaglandins. Paracetamol [34, 35], indomethacin [28-30], ibuprofen [10] and ketoprofen [16, 30] have been used successfully for this indication. Routine administration of one or more of these drugs should be considered in all patients treated with r-TNF-α. Pethidine (meperidine) is possibly effective against chills and rigor [17, 30, 34, 35, 40]. Steroids are of unproven efficacy [10].

<table>
<thead>
<tr>
<th>Prevention and treatment of r-TNF-α toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>prevention</td>
</tr>
<tr>
<td>invasive monitoring</td>
</tr>
<tr>
<td>volume loading</td>
</tr>
<tr>
<td>leakage monitoring</td>
</tr>
<tr>
<td>low perfusion flow rates</td>
</tr>
<tr>
<td>extensive washout</td>
</tr>
<tr>
<td>treatment</td>
</tr>
<tr>
<td>invasive monitoring</td>
</tr>
<tr>
<td>volume resuscitation</td>
</tr>
<tr>
<td>dopamine</td>
</tr>
<tr>
<td>norepinephrine</td>
</tr>
<tr>
<td>positive pressure ventilation</td>
</tr>
</tbody>
</table>

Table 2. Prevention and treatment of toxicity

Adequate fluid resuscitation is mandatory to prevent r-TNF-α induced hypotension as much as possible. Patients on chronic diuretic therapy are at special risk for this complication. Volume loading with 500-1000 ml of saline is recommended if invasive measurement of filling pressures is not feasible.

In the case of isolated limb perfusion with r-TNF-α meticulous surgical technique can limit leakage to the systemic circulation to a minimum. Low perfusate flow rates (up to 500 ml/min for a lower limb and up to 300 ml/min for an upper limb) have been reported to reduce systemic leakage and attenuate side effects, probably by reducing vascular pressures in the isolated limb [55]. A thorough washout procedure at the end of perfusion with approximately 6-10 liters of washout fluid, may also contribute to a reduction of leakage and systemic side effects [47].

Leakage during the procedure should be monitored continuously with a radioactive marker, viz. I$^{131}$-albumin, as described previously [75]. In this way unacceptably large leaks can be discovered early and corrected before extensive damage is done.

Management in theatre can be facilitated by inserting a Swan-Ganz catheter after induction of anesthesia. Most patients will require extensive fluid administration to maintain adequate filling pressures. Since the main circulatory problem is excessive vasodilation, the majority will also require vasopressors. In our institution all patients who have undergone isolated limb perfusion with r-TNF-α are routinely admitted to the ICU. However, if the patient has been hemodynamically stable during perfusion and leakage is minimal (≤ 1%), the chances to develop severe toxicity are low [54] and postoperative treatment in a 24-hour recovery facility can be considered.

Patients with a substantial leak should definitely be managed in the ICU. In our experience hypotension and respiratory failure due to pulmonary edema are the only side-effects demanding immediate attention. As was pointed out above, other side effects are common, but they are usually selflimiting, do not need treatment and will not delay the patients discharge from the ICU. Hypotension
is treated with colloids and cristalloids to maintain filling pressures. A pulmonary artery wedge pressure of 12 mm Hg should be adequate. If hypotension persists, and it usually will, dopamine or norepinephrine is added to maintain perfusion pressures. Norepinephrine has the advantage of not being a positive chronotrope, as opposed to dopamine, and is a more potent vasopressor. Since most patients will be in sinustachycardia anyway this would favor the use of norepinephrine. In view of the hemodynamic profile of most of these patients, with a high cardiac output and low systemic vascular resistance, norepinephrine is probably the drug of choice. If blood pressure can be maintained renal failure is quite rare. In our own series of 64 patients only one needed continuous veno-venous hemofiltration because of renal failure. In this patient we were unsuccessful in our attempts to maintain adequate blood pressures.

Care should be taken that infusion of fluids does not aggravate non-cardiogenic pulmonary edema, which in a subclinical form is present in the majority of patients [48]. If this can be avoided most patients can be extubated within 24 hours after the perfusion procedure. Of the 64 patients treated in our institution the median duration of post-operative ventilation was between 24 and 48 hours; only 2 patients needed mechanical ventilation for longer than 48 hours. As soon as the patient has become hemodynamically stable, a diuretic can be useful to excrete the surplus of fluids that had to be administered in the acute stages of the sepsis syndrome. This will usually improve oxygenation by reducing extravascular lung water. Once symptoms in the acute phase have resolved, the patients can be safely sent to a general ward for further care; delayed toxicity is not a feature of treatment with r-TNF-α.

It would appear that a sepsis syndrome, that occurs as side effect of a medical intervention, is easier to treat than sepsis from an infectious source. In the treatment of naturally occurring septic shock, many attempts to stop the relentless propagation of the cascade of inflammatory mediators with blocking agents have been unsuccessful, simply because they could not be given early enough in the process. Obviously, timing of interventions could be much more favorable in iatrogenically-induced sepsis. The set-up of isolated limb perfusion with r-TNF-α would seem ideally suited for prophylactic treatment with a monoclonal antibody against TNF-α. Continuous systemic infusion of such an antibody during perfusion could theoretically neutralize all r-TNF-α that would leak from the perfusion circuit to the general circulation. If production of IL-1 could be shown to substantially increase the toxicity of r-TNF-α, continuous systemic infusion of an IL-1 receptor antagonist could also be of benefit. In theory, such an approach could prevent virtually all side effects of perfusion with r-TNF-α. However, before such treatment can be attempted, it is of crucial importance to make certain that the anti-tumor effect of r-TNF-α is in no way abrogated by anti-TNF-α antibodies, delivered systemically. A different approach is the development of mutant TNF's, with, ideally, anti-tumor activity similar to native TNF but reduced toxicity [76].

Finally, if nitric oxide does play a role in TNF-α induced hypotension in this setting, prophylactic treatment with competitive inhibitors of nitric oxide formation from L-arginine, like N\(^G\)-monomethyl-L-arginine, could block nitric oxide production and subsequently prevent excessive vasodilation. In naturally occurring septic shock this approach has had limited success [77], but, again, it could be more successful in a situation that permits early administration and timely discontinuation of the drug.

**Concluding remarks**
TNF-α has a remarkable capacity to kill tumor cells in vitro, while largely sparing normal cells. Apart from direct cytotoxic/cytostatic effects, multiple indirect processes induced by TNF-α as a "biological response modifier", are possibly involved in regression of in vivo tumor. These include potent immunomodulatory effects of TNF-α, in recruiting and activating immune cells, augmenting the expression of cell surface molecules, and inducing the production of intermediate cytokines. These characteristics make TNF potentially useful as an anti-cancer drug in vivo. Early clinical trials with recombinant human TNF have been disappointing: at best a small clinical response has been obtained at the cost of serious toxicity. Recently, encouraging results have been reported in patients with irresectable extremity sarcomas and melanomas with a combination of high dose r-TNF-α, IFN-γ and an alkylating drug, administered into a system of hyperthermic isolated regional perfusion [45, 46]. Due to leakage of r-TNF-α from the perfusion system to the general circulation, this type of treatment is also complicated by considerable clinical toxicity, mainly hypotension and respiratory failure. However, as we and others have shown, modern intensive care is able to cope with this, providing there is sufficient knowledge of the clinical syndrome of TNF toxicity. Our experience with this technique so far has led us to believe that even higher doses can be tolerated by the fully monitored, sedated and ventilated patient. In our series, serum concentrations of TNF-α with isolated limb perfusion are higher than the levels that have been documented with the intravenous administration of 300 µg/m² r-TNF-α, the dose traditionally considered to be maximal. The majority of our patients recovered within 24 hours and could leave the ICU on the day following perfusion. Accordingly we believe that there may be a place for new trials with intravenously administered r-TNF-α in dosages exceeding what has been considered the maximum tolerable dose for bolus iv. administration.

References


Side effects of treatment with TNF-α


45. Eggermont AMM, Schraffordt Koops H, Liénard D, Kroon BBR, van Geel AN, Hoekstra HJ, Lejeune FJ. Isolated limb perfusion with high dose tumor necrosis factor α in combination with interferon γ and


