Journal of Cellular Biochemistry 56:52–61 (1994)

Rationale for Using TNFα and Chemotherapy in Regional Therapy of Melanoma

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Abstract Recombinant tumor necrosis factor-alpha (rTNFα) has potent antitumor activity in experimental studies on human tumor xenografts. However, in humans, the administration of rTNFα is hampered by severe systemic side-effects. The maximum tolerated dose ranges from 350 to 500 mg/m^2, which is at least 10-fold less than the efficient dose in animals. Isolation perfusion of the limbs (ILP) allows the delivery of high dose rTNFα in a closed system with acceptable side-effects. A protocol with a triple-drug regimen was based on the reported synergism of rTNFα with chemotherapy, with interferon-γ, and with hyperthermia. In melanoma-in-transit metastases (stage IIIA or AB) we obtained a 91% complete response, compared with 52% after ILP with melphalan alone. Release of nanograms levels of TNFα in the systemic circulation was evident but control of this leakage and appropriate intensive care resulted in acceptable toxicity. Angiographic, immunohistological, and immunological studies suggest that the efficacy of this protocol is due to a dual targeting: rTNFα activates and electively lyses the tumor endothelial cells while melphalan is mainly cytotoxic to the tumor cells. ILP with rTNFα appears to be a useful model for studying the biochemotherapy of cancer in man.

Key words: melanoma, TNFα, isolation perfusion, melphalan, interferon-γ

INTRODUCTION

Tumor necrosis factor-α (TNFα) was the first cytokine able to produce very fast and effective necrosis of tumors [Carswell et al., 1975; Beutler and Cerami, 1986], in an even more efficient way than chemotherapy itself. Therefore, efforts were made to clone the gene. In 1983 [Aggarwal et al., 1985] the human TNFα gene was cloned and expressed in *Escherichia coli*. Walter Fiers [Fransen et al., 1985] and Pennica [Pennica et al., 1985], in the same year, cloned the gene of murine TNFα [Fransen et al., 1985]. It is commonly accepted that human TNFα structure is a nonglycosylated trimer of 157 amino acids with several cysteine bonds. The trimer has three receptor binding sites apparently situated between each part of the trimer [Aggarwal et al., 1985b].

The recombinant TNFα (rTNFα) was made available for clinical trials. Unfortunately, it was found at that time that the fatal outcome of septic shock in humans was due to TNFα. It is not surprising, therefore, that phase I and II studies in humans were hampered by high levels of toxicity and seldom showed antitumor effects [Spriggs et al., 1988; Creaven et al., 1987; Blick et al., 1987; Kimura et al., 1987; Chapman et al., 1987; Feinberg et al., 1988; Sherman et al., 1988; Robertson et al., 1989].

In 1988, we had developed the practice of isolation perfusion of the limbs (ILP), a method that allows us to isolate the diseased limb, connect it to a heart-lung machine and administer a high dose of chemotherapy. In fact, ILP with melphalan was the best treatment for melanoma-in-transit-metastases, with a 50% complete remission rate, compared with a rate of less than 1% when the same drug was administered systemically. We were then prepared to try the highly toxic TNFα molecule in ILP. We designed a protocol with an effective dose of rTNFα that is 10 times the maximum tolerated dose (MTD) in humans, and is equivalent to the effective dose in animals [Old, 1985, 1990], for melanoma and sarcoma.

Received April 12, 1994; accepted April 13, 1994.

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RECOMBINANT TNFα AS AN EFFECTIVE ANTITUMOR AGENT IN THE HUMAN WHEN ADMINISTERED BY ILP FOR IN TRANSIT MELANOMA METASTASES

The efficient dose of rTNFα in mice, either in syngeneic tumors or in nude mice carrying human tumor xenografts, is around 50 mg/kg [Old, 1990]. The MTD in humans is 350 mg/m^2, or 5 mg/kg. This 10 times lower dose in humans produced only partial responses, sometimes accompanied by severe side-effects. We decided to administer a dose equal to the one efficient in animals by ILP.

In oncology, there are three types of tumors that can be located on the limbs but are irresectable, either because of multiplicity or large volume and tissue invasion: melanomas, soft-tissue sarcomas, and carcinomas. The ILP with chemotherapy only, was shown to produce 50% complete remissions in stage IIIA or IIIAB melanoma [Schraffordt Koops et al., 1990, 1994]. In contrast, for sarcoma the results were no higher than after systemic treatment.

The first three cases were treated with rTNFα only, at total doses of 2, 3, and 4 mg in the perfusate. This pilot study was intended to indicate the pharmacokinetics in the perfusate and to verify whether the side-effects observed in the systemic setting could be abrogated. The isolation perfusion system consists of a circuit made of a heart-lung machine which provides for the circulation of a total of around 2 liters of perfusate, under hyperthermic conditions. The perfusate is heated and oxygenated by a membrane oxygenator; cutaneous tissues of the perfused limb are given extra heat from a heating blanket, with the aim of reaching hyperthermia without any gradient. The pharmacokinetics of rTNFα showed a plateau for the whole 90 minutes, when there was no significant leakage into the systemic circulation.

The plateau levels found both by immunoassay [Liénard et al., 1992b] and by bioassay [Gérain et al., 1992] were around 2 mg/ml, which is the optimal concentration observed to be efficient in experimental models, both in vivo and in vitro.

This feasibility study showed that ILP allows the application of rTNFα in conditions where saturation of the receptors can be expected, because of the high dose. Moreover, these conditions mimic the in vitro systems. That is not the case with chemotherapies, which usually show a bimodal disappearance curve, the first phase representing distribution within the vascular bed, and the second, extraction from the tissue together with hydrolysis.

SYSTEMIC TOXICITY DURING AND AFTER ILP WITH RTNFα

It is well established that systemic toxicity from isolation perfusion with any drug is the result of leakage. The latter is monitored by the use of radioactive human serum albumin injected into the perfusate. One of the standard methods is to take blood samples from the systemic circulation at 5, 30, 60, and 90 minutes, and to calculate the leakage from the theoretical plasma volume of the patient. A more accurate determination of the leakage is obtained by continuous monitoring using a gamma detector placed above the heart [Schraffordt Koops et al., 1994]. It was claimed by Hoekstra et al. [1992] that excessive changes in a patient's blood pressure or heart rate during perfusion have a great influence on the percentage of leakage. Pump flow is critical in that respect. In Lausanne, increasing perfusion flow from 500 to 1,000 ml/min was accompanied by a dramatic increase in side-effects [Eggiman et al., in press]. Our collaborative experience is that both keeping the flow to 40–45 ml/liter of perfused limb and injecting rTNFα when radioactivity over the heart (see above) reaches a plateau provide the safest perfusion conditions with minimal systemic toxicity.

HEMODYNAMIC MANIFESTATIONS OF RTNFα AFTER LEAKAGE AND/OR RELEASE INTO THE SYSTEMIC CIRCULATION

The maximum side-effect of rTNFα that can be encountered with ILP were demonstrated at the time when workers at Lausanne were using a high pump-flow. There were 19 patients, 13 males and 6 females, with an average age of 58 years (range 38–79 years). The perfusions were of the lower-limb in 15 cases and of the upper limb in 4 cases. Nine patients experienced a pure distributive shock and eight a mixed distributive and cardiogenic shock due to preoperative cardiac pathology, but rTNF perfusion was not contraindicated.

In the two groups of patients, the maximum TNFα concentrations in the peripheral blood were very similar, 113 and 119 ng/ml, respectively. The duration of the high TNFα concentration in the peripheral blood was 60 minutes in the group with distributive shock compared with

120 minutes in the group with mixed shock. In the first group, norepinephrine had to be given for an average of 4 hours (1–11 hours) but no dobutamine was given. In contrast, norepinephrine was given for an average of 6.3 hours (2–10 hours) and dobutamine for an average of 9 hours (1–16 hours) in the second group. These hemodynamic acute effects all evolved into a hyperdynamic state. A complete recovery was obtained on day 3 (days 2–5) with no toxic death and no sequellae [Eggiman et al., in press].

This contrasts with the side-effects reported in the literature after systemic application of rTNFα. We should point out that in most of the phase I and II studies in the literature, there was no report of a systematic protocol for avoiding the side-effects. The fact that our patients were under general anesthesia, were carefully monitored, and were managed by an intensive care specialist, is a strong argument for a multidisciplinary approach to such treatments.

rTNFα PHARMACOKINETICS AND SIDE EFFECTS

The pharmacokinetics of TNFα release show that the patients who did not experience significant side-effects during and after the perfusion did not demonstrate more than 1% leakage. However, no correlations were found between the maximum TNFα concentration in the peripheral blood of an individual and the side-effects, indicating that patients vary in their sensitivity to TNFα. Moreover, the more than 100 ng TNFα levels found in our patients [Gérain et al., 1992] contrast with the current literature on septic shock, which indicates that people dying of multiorgan failure show less than pg levels of TNFα. The difference between septic shock and isolation perfusion for regional cancer is that in the first case the patients are infected and have significant levels of endotoxin, which has been shown to be synergistic with TNFα for toxicity. Another hypothesis to explain why our patients tolerated high plasma concentration of TNFα is that it was shown that there is a very early and efficient production of soluble p75 and p55 receptors in the peripheral blood [Gérain et al., 1994], presumably due to perioperative and even low leakage of TNFα into the systemic circulation.

When we analyzed the pharmacokinetics of TNFα in relation to leakage, it was obvious that even in the absence of detectable leakage, and after intense wash-out (2 to 3 liters) of the intravascular TNFα, there was always a release of TNFα into the systemic circulation. The data can only be explained by the fact that TNFα is extravasated and slowly released from the perfused tissues after restoration of the physiological circulation.

Therefore, the release of TNFα after perfusion is unavoidable. It seems to be well tolerated, although some patients experienced side-effects but they were essentially hemodynamic effects that are easily corrected in the intensive care unit by administration of large amounts of fluids and, if necessary, of dopamine and norepinephrine. Our observations indicate that the acute response to TNFα observed in ILP is different from the one reported in the literature in septic shock. In the latter, TNFα and endotoxin have synergistic effects, and measurements of TNFα are unreliable because of irregular endogenous production. This contrasts with ILP where only one bolus injection of rTNFα is given at a preset time to patients normally devoid of circulating endotoxin. In fact, one patient died of genuine septic shock 3 weeks after perfusion with rTNFα because his tumor was heavily infected and the treatment resulted in a mobilization of bacteria. Since then, we have considered that an infected tumor is a contraindication to isolation perfusion with TNFα.

The side-effects that we encountered with rTNFα ILP are better named systemic inflammatory response syndrome (SIRS), since rTNFα is acting alone and produces clear-cut, predominantly hemodynamic side-effects that are characteristic of an acute reaction to a single application [Bone et al., 1992].

Lung infiltration was claimed to be due to activation of granulocytes and animal data indicate that mice can be protected from lethal rTNFα doses by blocking cyclooxygenase using indomethacin [Takahashi et al., 1993]. We treated about 20 of our patients with an indomethacin schedule, using 50 mg during and for 4 hours after perfusion, followed by an additional 200 mg for a further 20 hours. Although we did see a reduction of chills and fever in the postoperative period, there was no change in the other side-effects. Recently, Sigurdsson et al. [1993] showed that he could counteract the hemodynamic side-effects of TNFα in sheep by using ketoprofen, a cyclo- and lipooxygenase inhibitor.

The conclusion of this study is that rTNFα can safely be administered by ILP but that this procedure does not abrogate the subsequent re-

lease of TNFα into the systemic circulation, indicating that the prophylaxis of systemic side-effects is important.

rTNFα, COMBINED WITH INTERFERON-γ AND MELPHALAN: RATIONALE FOR ITS USE IN ISOLATION PERFUSION FOR MELANOMA

If we consider the various publications on experimental models using either syngeneic tumors or human tumor xenografts, it appears that rTNFα alone is rarely able to induce tumor regression of long duration and with no regrowth.

In experimental conditions in vivo, it has been shown that the combination of rTNFα and recombinant interferon-γ (rIFNγ) is highly synergistic [Balkwill et al., 1986; Fiers et al., 1986; Sohmura, 1988; Soehnlen et al., 1985]. At a rTNFα dose that induced minimal tumor growth retardation, the addition of a small amount of rIFNγ could completely inhibit tumor growth. Moreover, IFNγ was shown to upregulate TNFα receptors [Fiers et al., 1986; Aggarwal et al., 1985a; Ruggiero et al., 1986].

TNFα treatment can also be synergistically combined with chemotherapy. Synergism was found on various human tumor xenografts using different sorts of chemotherapy, including alkylating agents, such as platinum and cyclophosphamide [Haranaka et al., 1987; Mutch et al., 1989; Regenass et al., 1987]. The results were similar to the synergy obtained with rIFNγ.

In our protocol, we decided to combine rTNFα with rIFNγ and with melphalan (Fig. 1), since no additional toxicity has been reported with combined rIFNγ and chemotherapy. However, synergistic toxicity has been reported for rTNFα and rIFNγ. We decided to use the gold standard, melphalan, a bifunctional alkylating agent, since it has been shown to produce a 50% complete response as a single agent, at high concentrations, in ILP for in-transit metastases of melanoma [Schraffordt Koops et al., 1990, 1994].

We chose to perform the isolation perfusion under mildly hyperthermic conditions since hyperthermia has been shown to potentiate the activity of both rTNFα and melphalan [Watanabe et al., 1988; Honess et al., 1984].

PILOT PROTOCOL OF RTNFα, RIFNγ, AND MELPHALAN ILP FOR MELANOMA IN TRANSIT METASTASES

Recombinant human TNFα (a gift from Boehringer Ingelheim, Germany) was obtained as a lyophilized powder in aliquots of 0.2 mg, which were reconstituted using sterile water provided by the company. Recombinant IFNγ (a gift from Boehringer Ingelheim, Germany) was obtained, for the initial studies, in vials containing a solution of 0.2 mg rIFNγ in 1 ml of saline. More recently, it was obtained in 1 ml vials containing 0.1 mg rIFNγ. Melphalan was purchased from Burroughs Wellcome (UK), as a sterile powder in 100 mg lots, which have to be solubilized with an acidic alcoholic solution, followed by dilution in a buffer provided by the company. More recently, 50 mg ampoules of lyophilized powder, which could be directly solubilized in the buffer solution, were supplied.

The patients, who had given informed consent, received rIFNγ by subcutaneous injection of 0.2 mg in the evening, of days 1 and 2,

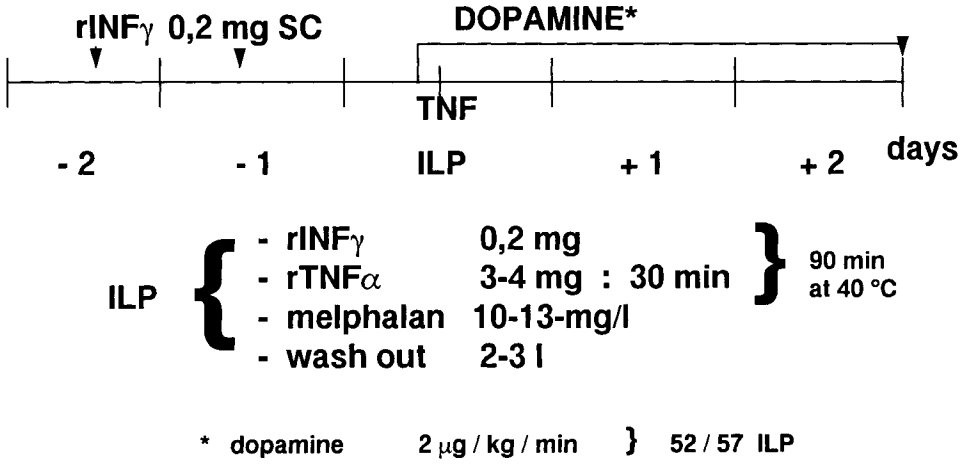

Fig. 1. Protocol (melanoma pilot study).

preceded by the administration of 500 mg of acetaminophen. The volume of the limb to be perfused was measured according to Wieberdink by water displacement [Wieberdink et al., 1982].

ILP was performed under general anesthesia. Before the procedure, an arterial and a pulmonary (Swan-Ganz®) catheter were installed by the anesthesiologist. Typically, dopamine infusion with 3 μg/kg/minute was started just before the administration of rTNFα. The operating procedure has been described elsewhere [Schraffordt Koops et al., 1990, 1994; Lejeune et al., 1987].

RESULTS OF ILP WITH RTNFα, RIFNγ, AND MELPHALAN IN MELANOMA IN TRANSIT METASTASES

This pilot study started in 1989 and was completed at the end of 1993. The objective was to improve upon the 50% response rate predicted for ILP with melphalan alone, and to evaluate the duration of the response. The first report [Liénard, 1992b] was followed by several full reports of interim analysis [Liénard, 1992a, 1993, in press; Lejeune et al., 1992]. In this updated analysis, the responses of 53 patients are evaluated. In order to compare the results of this pilot study with the standard melphalan ILP, a database was built up of 103 patients, with the same stage III regionally recurrent melanomas, who were treated between 1980 and 1988 by the same four teams [Klaase, 1993] (Schmitz et al., in preparation).

Table I shows that melphalan ILP alone produced the predicted 52% complete response compared with the 91% with the combined rTNFα, rINFγ, and melphalan treatment. When subgroups are analyzed, the difference remains highly significant. When the cases presenting only with in-transit-metastases without lymph-node involvement (stage IIII A) are considered, the complete remission rate is 62% with mephalan alone, compared with 100% for the combination treatment. However, when the patients with both in-transit and lymph-node metastases are analyzed (stage III AB), the difference remains highly significant, that is, 41% compared with 87%. The difference between the treatments remained highly significant ($P < 0.001$) when the bivariate logistic model was used for analysis after adjusting for sex, age, stage, site, number of lesions, and time since primary (Schmitz et al., in preparation).

In systemic chemotherapy of cancer, when combination therapy is used to increase the response rate, it can be found that the response duration diminishes. To address that question, the durations of complete response for melphalan ILP and for the combined rTNFα, rINFγ, and melphalan treatments were compared. The median duration has not yet been reached, but the means are 3.9 years and 3.6 years, respectively, a difference that is not significant (log-rank test: $P = 0.35$). These results confirm that the quality of this never previously reached high complete remission rate, obtained with the triple combination, is equal to that of the 50% complete remission obtained with melphalan alone ILP. With a median follow-up time of 26 months, there were only 12 (23%) regional recurrences, 15 (29%) distant metastases, and 9 cases (17%) of both regional and distant recurrence. The overall median survival time has been 28 months.

These results show that ILP with high-dose rTNFα, rIFNγ, and melphalan is a highly efficient therapy of in-transit-melanoma metastases. However, it has its limitations. First of all, it is a regional therapy which can influence patient survival only when the patient is devoid of distant micrometastases at the time of ILP. This is well illustrated by the fact that the survival rate does not seem to be higher than that expected in regionally advanced melanoma [Liénard, 1994]. A reliable comparison of survivals is difficult to obtain because more than half of our patients had been previously treated, sometimes several times, with surgery and chemotherapy, and had a heavy tumor bulk. Because of the technical difficulty of the procedure, most of the patients could only benefit from one treatment course, while it is well established that a single procedure is rarely definitive in the treatment of cancer. However, the high limb-sparing rate (87%) and the very good survival of patients experiencing complete response [Liénard, 1994] indicate that this treatment has an overall value

TABLE 1. Complete Response in Malignant Melanoma With in Transit Metastases *

Stage	ILP with melphalan alone (%)	P	ILP with TNFα + melphalan + IFNγ (%)
AII	54/103 (52)	<0.001	48/53 (91)
III A + III B	48/89 (54)	<0.001	46/48 (96)
III A	34/55 (62)	<0.001	33/33 (100)
III AB	13/34 (41)	<0.003	13/15 (87)

*Pilot study results adapted from Schmitz et al., in preparation.

in the armentarium against malignant melanoma.

IS IFNγ SYNERGISTIC WITH RTNFα IN ILP?

The rationale for the triple regimen was based on experimental data, including human tumor xenografts (see above). However, neither the effect of IFNγ on TNFα receptors nor its antitumor effect has been proven in humans. In contrast, IFNγ was found to be synergistic for toxicity with TNFα [Abbruzzese et al., 1989; Demetri, 1989]. Moreover, since IFNγ upregulates TNFα receptors (see above), it is possible that it might also increase soluble receptors, resulting in the inhibition of TNFα efficacy. Since the triple-drug regimen contains two experimental drugs, it does not allow us to reach definitive conclusions about the impact of TNFα alone, in combination with chemotherapy. Therefore, we designed a randomized phase II trial, to establish whether withdrawal of IFNγ diminishes the complete remission rate and response duration observed with the triple-drug regimen. This trial was started in spring of 1992, but it is too early to reach conclusions about the response rate. However, we can already rule out toxicity problems, since no difference between the two arms has yet been seen. Patients receiving rIFNγ did not express more circulating TNFα receptors (Gérain J, in preparation). There has been no sign of tumor enhancement with IFNγ.

MECHANISMS INVOLVED IN TUMOR NECROSIS AFTER ILP WITH RTNFα AND MELPHALAN

Experimental models have indicated that only tumors with organized microvascularization can be intensively necrotized by rTNFα, as is the case for tumors implanted in the subcutaneous tissues, as observed by Old [1987]. This condition is fulfilled in melanoma-in-transit metastases that are located either in the subcutaneous tissue or in the dermis, and invade the epidermis. The 90% complete response rate obtained in this tumor condition is higher than the 40 or 50% seen in soft-tissue sarcomas that are more deeply located. However, we observed dramatic necrosis of soft-tissue sarcomas that were invading subcutaneous or cutaneous tissues.

Soft-tissue sarcomas are appropriate tumors for studying tumor vascularization. Angiographies performed before rTNFα ILP and 1 week to 10 days later have shown, as was predicted from animal models, an extensive and fast destruction of the hypervascularization associated with the tumor, leaving intact the normal small vessels in the limb, including the small vessels surrounding the tumors. Using histology and immunohistochemistry, we were able to demonstrate that the first target of rTNFα is the tumor endothelial cells [Lejeune et al., 1993; Renard et al., 1994a,b]. rIFNγ is able to upregulate adhesion molecules, but they are further increased a few hours after rTNFα, especially ELAM-1 (Endothelial Leucocytes Adhesion Molecule-1 or E-selectin) and VCAM-1 (Vascular Cell Adhesion Molecule-1). Moreover, signs of endothelial cell activation appear only in the tumor endothelial cells. They become swollen and are eventually lysed at a time when the tumor cells appear histologically normal, as do the normal tissue endothelial cells. Tumor endothelial cell destruction is preceded by polymorphonuclear cell sequestration and activation within the tumor vessels, and this is followed by an intense infiltration of the tumor [Ashkenazi et al., 1991]. Platelet aggregation on the tumor endothelium associated with an increase of Von Willebrand factor was also an immunohistological finding [Renard et al., 1994b].

The angiographic, histological, and immunological observations allow us to conclude that the double or triple combination protocols work through a dual-targeting system (Fig. 2). The first target is represented by the tumor vessels. rTNFα, with or without rIFNγ, activates and uses the tumor endothelial cells. The second target is the tumor cells themselves, which are, at a later stage, subjected to melphalan.

ILP WITH rTNFα: A MODEL FOR BIOCHEMOTHERAPY OF CANCER IN MAN

From our experience with rTNFα ILP, there is evidence for biological and immunological systemic effect of rTNFα. TNFα was detected at ng levels in the blood for up to 6 hours after ILP. It was leaked from the ILP and/or released from the perfused tissues after the treatment. Induction of IL-6, as measured in the peripheral blood, indicates that the cytokine cascade is triggered systemically [Gérain et al., 1994]. The 100-fold increase in plasma elastase as a result of the granulocyte activation and degranulation suggests that an important part of the granulocyte systemic pool is activated and degranulated, resulting in an intense but short leucopenia followed, after 24 hours, by a burst of immature polymorphs [Eggiman et al., in press; Van der Auwera et al., 1990].

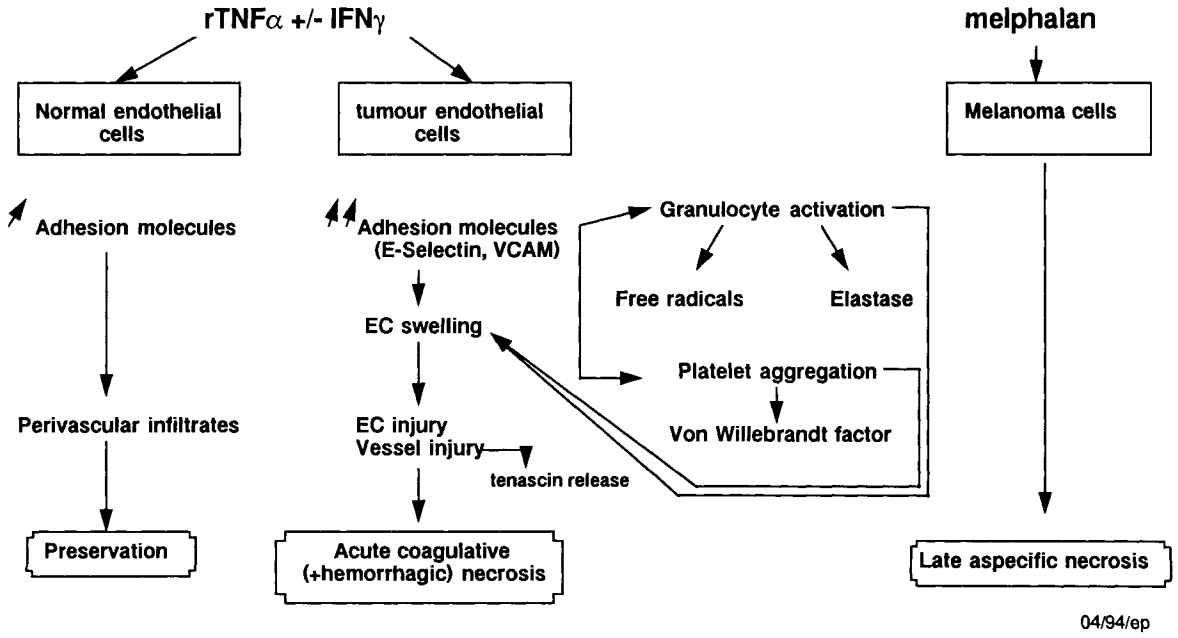

Fig. 2. Mechanisms involved in tumor necrosis by TNFα and chemotherapy.

Granulopenia and thrombopenia seem also to result from the upregulation of adhesion molecules and the sequestration of those cells in the perfused area, as shown by the immunohistological studies. As a result, there is an increase of acute-phase proteins [Swaak et al., 1993]. Overall, the perfused tumors appear to be the elective site of acute inflammatory reaction.

The fact that our patients had a long-lasting complete response prompted us to study some immunological parameters. Preliminary results on the systemic lymphocyte phenotyping indicate that eight out of ten patients showed a sustained increase of HLADR-positive T-lymphocytes and of CD38, which is a sign of T-lymphocyte activation [Blum et al., 1994]. It remains to be seen whether this T-lymphocyte activation correspond to the induction of specific cytotoxic T-lymphocytes.

Despite the fact that ILP is aimed at obtaining an essentially regional response, our experience indicates that it is accompanied and followed by strong systemic effects. Therefore, we propose that ILP with high-dose rTNFα is a useful model for studying cancer biochemotherapy in man, since it provides a means

1. of following the physiopathological changes in an area, and in the whole body, after a single bolus of rTNFα;
2. of controlling the systemic inflammatory response syndrome manifestations when present, since there was no toxic death and no sequellae;
3. of identifying endothelial-cell alterations in the tumors by histological methods and by angiography;
4. of determining the cytokines level and TNFα receptors in the perfusate, and in the systemic circulation;
5. of evaluating lymphocyte activation and cytotoxicity; and
6. of evaluating the inflammatory parameters.

ARE THERE ANY PROSPECTS FOR THE USE OF rTNFα IN MEDICAL ONCOLOGY?

The experience with rTNFα ILP has been very rewarding, not only because of its performance as a highly effective therapy for in-transit melanoma metastases, irresectable sarcomas and carcinomas, but also as a model for biochemotherapy. Indeed, the concept of associating cytokines to chemotherapy has been tried in other settings, including systemic treatment, using, for example, interleukin-2 and chemotherapy, or rIFNα and chemotherapy.

Since we have demonstrated that the effect of the combination of rTNFα and chemotherapy is based on dual targeting, that is, on the tumor microvascularization on one hand, and on the tumor cells themselves on the other hand, and that a better understanding of the side-effects

and an improvement in the prevention of side-effects has been achieved, we can tentatively suggest that there is a future for the combination of rTNFα and chemotherapy in a systemic setting. Two recent findings may help in achieving that purpose. First, the fact that our patients with nanogram levels of TNFα tolerated it well and had circulating soluble receptors [Gérain 1994] indicate that the latter may be extremely useful for buffering TNFα in the systemic blood. In fact, a construct made of Fc fragments of human immunoglobulins with two TNF receptors was shown to provide a 20-fold protection of animals receiving rTNFα, compared with TNF antibodies [Lesslauer et al., 1991; Ashenazi et al., 1991]. What is unknown is the potential neutralization of the antitumor effect of rTNFα, when such constructs are used, but it will be worthwhile to design pilot studies, based on the protection achieved in patients with septicemia. Another approach, which has been successful in an experimental model, is the use of mutant TNFα where one or two amino-acids were changed in the area of receptor binding, rendering the mutant TNFα less toxic. This approach is based on the observation made by Fiers' group [Brouckaert, 1992b] that human TNFα is much less toxic in mice than is mouse TNFα, and that human TNFα does not bind to the mouse p75 receptors. Therefore, the computer-assisted design of the new mutants was aimed at removing the p75 receptor binding sites from the new molecules. The achievement of this aim has been reported by the groups of Fiers and Leslauer [Van Ostade et al., 1993], who showed that mutants with 100-fold less p75 activity could bind to p55 and were still able to destroy human tumor xenografts. Before embarking on human studies, results from experimental animal toxicities are awaited.

Our experience has been that the systemic side-effects of TNFα can only be counteracted by appropriate intensive care management. New drugs, such as lipo- and cyclooxygenase inhibitors [Sigurdsson et al., 1993], NO synthetase inhibitors, or platelet aggregation inhibitors, will be tried; but it will be important to verify that these inhibitors do not interfere with the antitumor effects of TNFα.

Further research is needed to understand and predict individual susceptibility to TNFα toxicity [Brouckaert et al., 1992a].

ACKNOWLEDGMENTS

We thank Mrs. Evelyne Parent for secretarial help.

REFERENCES

Abbruzzese JL, Levin B, Ajanai J, et al. (1989): Phase I trial of recombinant human gamma-interferon and recombinant human tumor necrosis factor in patients with advanced gastrointestinal cancer. Cancer Res 49:4057–4061.

Aggarwal BB, Eessalu TE, Hass PE (1985a): Characterization of receptors for human tumor necrosis factor and their regulation by gamma-interferon. Nature 318:665–667.

Aggarwal BB, Kohr WJ, Hass PE, et al. (1985b): Human tumor necrosis factor: Production, purification and characterization. J Biol Chem 260:2345–2354.

Asher AL, Mule JJ, Reichert CM, Shilone E, Rosenberg SA (1987): Studies of the antitumor efficacy of systematically administered recombinant tumor necrosis factor against several murine tumors in vivo. J Immunol 138:963–974.

Ashkenazi A, Marsters SA, Capon DJ, Chamow SM, Figari IS, Pennica D, Goeddel DV, Palladino MA, Smith DH (1991): Protection against endotoxic shock by a tumor necrosis factor receptor immunoadhesion. Proc Natl Acad Sci USA 88:10535–10539.

Balkwill F, Lee A, Aklom G, et al. (1986): Human tumor xenografts treated with human TNF alone or in combination with interferon. Cancer Res 46:3990–3993.

Beutler B, Cerami A (1986): Cachectin and tumor necrosis factor as two sides of the same biological coin. Nature 320:585–588.

Blick MB, Sherwin SA, Rosenblum M, et al. (1987): A phase I trial of recombinant human tumor necrosis factor in cancer patients. Cancer Res 47:2986–2989.

Blum S, Liénard D, Cerottini JC, Lejeune F, Von Fliedner V (1994): Phenotypic changes occurring in peripheral blood T lymphocytes from patients treated by high dose rTNFalpha in isolation limb perfusion. Eur Cytokine Netw 5:235 (Abstract).

Bone RC, Balk RA, Cerra FB, Dellinger R Ph, Fein AM, Knaus WA, Schein RM, Sibbald WJ (1992): Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Chest 101:1644–1655.

Brouckaert PGG, Fiers W, Lejeune FJ (1992a): Coley's vaccine and TNF therapy. Nature 358:630.

Brouckaert P, Libert C, Everaerdt B, Fiers W (1992b): Selective species specificity of tumor necrosis factor for toxicity in the mouse. Lymphokine Cytokine Res 11:193–196.

Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975): An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 72: 3666–3670.

Chapman PB, Lester TJ, Casper ES, et al. (1987): Clinical pharmacology of recombinant human tumor necrosis factor in patients with advanced cancer. J Clin Oncol 5:1942–1951.

Creaven PJ, Plager JE, Dupere S, et al. (1987): Phase I trial of recombinant human tumor necrosis factor. Cancer Chemother Pharmacol 20:317–144.

Demetri GD, Spriggs DR, Sherman ML, et al. (1989): A phase I trial of recombinant human tumor necrosis factor and interferon-gamma: Effects of combination cytokine administration in vivo. J Clin Oncol 10:1545–1553.

Eggiman P, Chioléro R, Chassot PG, Revelly JP, Liénard D, Gérain J, Heumann D, Lejeune F (in press): Systemic and hemodynamic effects of high-dose recombinant tumor necrosis factor alpha in isolation perfusion of the limbs for melanoma and malignant soft tissue tumors. Chest.

Feinberg B, Kurzrock T, Talpaz M, et al. (1988): A phase I trial of intravenously administered recombinant tumor necrosis factor alpha in cancer patients. J Clin Oncol 6:1328–1334.

Fiers W, Brouckaert P, Guisez Y (1986): Recombinant interferon gamma and its synergism with tumor necrosis factor in the human and mouse systems. In Schellekens H, Stewart WE (eds): "The Biology of the Interferon System." Amsterdam: Elsevier Science.

Fransen L, Muller R, Marmenout A, Tavernier J, Van Der Heyden J, Kawashima E, Chollet E, Tizard R, Van Heuverswyn H, Van Vliet A, Ruysschaert M, Fiers W (1985): Molecular cloning of mouse tumor necrosis factor cDNA and its eukaryotic expression. Nucleic Acids Res 13:4417–4429.

Gérain J, Liénard D, Ewalenko P, Lejeune F (1992): High serum levels of TNF-alpha after its administration for isolation perfusion of the limbs. Cytokine 4:585–591.

Gérain J, Liénard D, Buurman W, Lejeune FJ (1994): Systemic release of soluble tumor necrosis factor alpha receptors (sTNFr) after isolated limb perfusion with high dose TNFα (Abstract). 3rd International Conference on cytokines: Basic principles and practical applications, Firenze.

Haranaka K, Satomi N, Sakurai A, Haranaka R (1987): Antitumour effects of tumour necrosis factor: Cytotoxic or necrotizing activity and its mechanism. In "Tumour Necrosis Factor and Related Cytokines." Ciba Foundation Symposium 131. Chichester: Wiley.

Hoekstra HJ, Naujocks T, Schraffordt Koops H, Beekhuis H, van Groningen a Stuling R, ter Veen H, Oldhoff J (1992): Continuous leakage monitoring during hyperthermic isolated regional perfusion of the lower limb: Techniques and results. Reg Cancer Treat 4:301–304.

Honess DJ, Bleehen NM (1984): Heat potentiation of melphalan: Studies on melphalan transport systems. In Overgaard J (ed): "Hyperthermic Oncology," vol 1. London: Taylor & Francis.

Kimura K, Taguchi T, Urushizaki I, et al. (1987): Phase I study of recombinant human tumor necrosis factor. Cancer Chemother Pharmacol 20:223–229.

Klaase JM (1993): "Regional Isolated Perfusion for Melanoma of the Extremities." MD thesis, University of Amsterdam.

Lejeune F, Ghanem G (1987): A simple and accurate new method for cytostatics dosimetry in isolation perfusion of the limbs based on exchangeable blood volume determination. Cancer Res 47:639–643.

Lejeune F, Liénard D, Leyvraz S, Mirimanoff RO (1992): Regional therapy of melanoma. Eur J Cancer 29A:606–612.

Lejeune F, Liénard D, Eggermont AMM, Renard N, Gérain J (1993): Evidence for early intratumoral endothelium activation, IL-6 production and high systemic TNFalpha levels after isolation perfusion with high dose rTNFα associated with Interferon-gamma. In Fiers W, Buurman WA (eds): "Tumor Necrosis Factor: Molecular and Cellular Biology and Clinical Relevance." Basel: Karger.

Lesslauer W, Tabuchi H, Gentz R, Schaeger EJ, Brockhaus M, Grau G, Piguet PF, Pointaire P, Vassali P, Loetscher H (1991): Bioactivity of recombinant human TNF receptor fragments. Cytokines and their receptors—from clonal to clinical investigation. J Cell Biochem Suppl 15F:115.

Liénard D, Lejeune F, Ewalenko P (1992a): In transit metastases of malignant melanoma treated by high dose rTNF-alpha in combination with interferon-gamma and melphalan in isolation perfusion. World J Surg 16:234–240.

Liénard D, Delmotte JJ, Renard N, Ewalenko P, Lejeune F (1992b): High doses of rTNF-alpha in combination with IFN-gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. J Clin Oncol 10, 1:52–60.

Liénard D, Eggermont AMM, Schraffordt Koops H, Lejeune FJ (1993): High dose of rTNF-alpha, rIFN-gamma, and melphalan in isolation perfusion produces 90% CR in melanoma in transit metastases. Melanoma Res 3 (Suppl 1):30.

Liénard D, Eggermont AMM, Schraffordt Koops H, Kroon BBR, Rosenkaimer F, Autier P, Lejeune FJ (1994): Isolated Perfusion of the limb with high dose TNFalpha, IFNgamma and melphalan for melanoma stage III: results of a multicentric pilot study. Melanoma Res 4 (S1): 21–26.

Mutch DG, Powell CB, Kao MS et al. (1989): In vitro analysis of the anticancer potential of tumor necrosis factor in combination with cisplatin. Gynecol Oncol 34: 328–333.

Old LJ (1985): Tumor necrosis factor (TNF). Science 230: 630–632.

Old LJ (1987): General discussion 11. In Old LJ (ed): "Tumor Necrosis Factor and Related Cytotoxins." Ciba Foundation Symposium 131. New York: John Wiley & Sons.

Pennica D, Hayflick JS, Bringman TS, Palladino MA, Goeddel DV (1985): Cloning and expression in E. Coli of the cDNA for murine tumor necrosis factor. Proc Natl Acad Sci USA 82:6060–6064.

Regenass U, Müller M, Curschellas E, et al. (1987): Antitumor effects of tumor necrosis factor in combination with chemotherapeutic agents. Int J Cancer 39:266–273.

Renard N, Liénard D, Lespagnard L, Eggermont A, Heimann R, Lejeune FJ (1994a): Early endothelium activation and polymorphonuclear cell invasion precede specific necrosis of human melanoma and sarcoma treated by intravascular high dose tumor necrosis factor alpha. Int J Cancer 57:656–663.

Renard N, Nooijen P, Schalkwijk L, de Waal R, Eggermont A, Liénard D, Kroon B, Lejeune F, Ruiter D (1994b): Intravascular thrombocyte aggregation and erythrostasis are associated with necrosis in melanoma metastases after perfusion with a high dose of tumor necrosis factor alpha. Eur Cytokine Netw 5:219 (Abstract).

Robertson PA, Ross HJ, Figlin RA (1989): Tumor Necrosis Factor induces hemorrhagic necrosis of a sarcoma. Ann Int Med (111)8:682–684.

Ruggiero V, Tavernier J, Fiers W, et al. (1986): Induction of the synthesis of tumor necrosis factor receptors by interferon-gamma. J Immunol 136:2445–2450.

Schraffordt Koops H, Kroon BBR, Lejeune FJ (1990): Isolated regional perfusion in the treatment of local recurrence, satellites and in transit metastases of extremity melanomas. In Rümke P (ed): "Therapy of Advanced Malignant Melanoma," vol. 10, Pigment Cell Series. Basel: Karger.

Schraffordt Koops H, Kroon BBR, Lejeune FJ (1994): Management of local recurrences, satellites and in transit metastases of the limbs with isolation perfusion. In Lejeune F, Chaudhuri PK, Das Gupta T (eds): "Malignant Melanoma: Medical and Surgical Management." New York: McGraw-Hill.

Sigurdsson GH, Youssef HAF, Banic A (1993): Effects of ketoprofen on respiratory and circulatory changes in endotoxic shock. Intensive Care Med 19:333–339.

Sherman ML, Spriggs DR, Arthur KA, et al. (1988): Recombinant human tumor necrosis factor administered as a five day continuous infusion in cancer patients: Phase I toxicity and effects on lipid metabolism. J Clin Oncol 6:344–350.

Soehnlen B, Liu R, Salmon S (1985): Recombinant TNF exhibit antitumor activity against clonogenic human tumor cells and synergism with gamma interferon. Proc Am Assoc Cancer Res 26:303.

Sohmura Y (1988): Antitumor effect of recombinant human tumor necrosis factor alpha and its augmentation in vitro and in vivo. In Bonavida B, Gifford GE, Kirchner H, Old LJ (eds): "Tumor Necrosis Factor/Cachectin and Related Cytokines." Basel: Karger.

Spriggs DR, Sherman ML, Michie H, et al. (1988): Recombinant human tumor necrosis factor administered as a 24 h intravenous infusion. A phase I and pharmacologic study. J Natl Cancer Inst 80:1039–1044.

Swaak AJG, Liénard D, Schraffordt Koops H, Lejeune FJ, Eggermont AMM (1993): Effects of recombinant tumor necrosis factor (rTNFalpha) in cancer. Observations on the acute phase protein reaction and immunoglobulin synthesis after high dose recombinant TNFalpha administration in isolated limb perfusions in cancer patients. Eur J Clin Invest 23:812–818.

Takahashi N, Brouckaert P, Fiers W (1993): Cyclooxygenase inhibitors prevent the induction of tolerance to the toxic effects of tumor necrosis factor. J Immunother 14:16–21.

Van der Auwera P, Bonnet M, Duchateau V, Ewalenko P, Liénard D, Lejeune F (1990): Influence of rTNFalpha and rIFNgamma given by isolation perfusion (ILP) on neutrophil (PMN) chemiluminescence and plasma elastase concentration in patients (pts) with malignant melanoma. 12th international RES congress—27th annual meeting of the society for leukocyte biology—20th leukocyte culture conference. Heraklion, Greece, Oct. 14–18.

Van Ostade X, Vandenabeele P, Everaerdt B, Loetscher H, Gentz R, Brockhaus M, Lesslauer W, Tavernier J, Brouckaert P, Fiers W (1993): Human TNF mutants with selective activity on the p55 receptor. Nature 361:266.

Watanabe N, Niitsu Y, Umeno H, et al. (1988): Synergistic cytotoxic and antitumor effects of recombinant human tumor necrosis factor and hyperthermia. Cancer Res 48: 650–653.

Wieberdink J, Benckhuizen C, Braat RP et al. (1982): Dosimetry in isolation perfusion of the limbs by assessment of perfused tissue volume and grading of toxic tissue reactions. Eur J Cancer Clin Oncol 18:905–910.