ABSTRACT

Multiple administration of sublethal doses of recombinant murine tumor necrosis factor (TNF), e.g., 2 μg i.p. twice daily for 4 to 6 consecutive days, produces tachyphylaxis to the anorectic effects of TNF and tolerance towards a lethal challenge with recombinant murine TNF. The objective of this study was to examine whether the antitumor efficacy of TNF was retained in mice made tolerant. We treated tolerant and nontolerant C57BL/6 mice bearing a syngeneic B16BL6 melanoma tumor with repeated administrations of recombinant murine TNF (5 to 12.5 μg/injection) alone or in combination with recombinant murine γ-interferon (5,000 to 50,000 units/injection). When the paralesional administration route was used, the tolerance-inducing pretreatment protected mice against the lethal outcome of both the single and the combination treatments (100% versus 40% survival in the former case; 80% versus 0% survival in the latter case) and still allowed complete regression of the tumor. When the i.p. route of administration was used, the final outcome was less positive; nevertheless, a significant protection against the lethal effects of the treatment was achieved without reduction of the antitumor efficacy. It is concluded that the toxic and antitumor activities of TNF are not inevitably linked and that their separation is an achievable research and perhaps a clinical goal.

INTRODUCTION

TNF is a cytokine which is selectively cytotoxic to many transformed cell lines in vitro and possesses antitumor activity against a variety of murine as well as human tumors in vivo (1, 2). It is currently applied to human cancer patients in clinical trials. The major problem hampering its successful clinical application is toxicity. TNF is also a cardinal mediator in septic shock (3), and the dose required to exert therapeutic antitumor effects almost equals the lethal dose in animals (4, 5). In clinical trials, hypotension is the dose-limiting side effect (6).

Various strategies to diminish toxicity while optimizing antitumor potency have been followed. The cyclooxygenase inhibitor indomethacin can protect rodents against the lethality induced by the administration of recombinant TNF, at least to a certain extent (7), and in a subrenal capsule assay, it was shown that indomethacin does not affect the antitumor effects of TNF (8). However, when we applied indomethacin in regimens involving daily injections of TNF and IFN-γ similar to those described previously (5), we observed that the protective effect of indomethacin was no longer present and that its coadministration eventually even enhanced the toxicity.4 IFN-γ, which has a synergistic effect with TNF both in vitro (9) and in vivo (5, 10), also enhances toxicity, although the former effect is considerably more pronounced than the latter. However, an intrinsic relationship between the toxicity and the antitumor effect of TNF has never been demonstrated or disproven.

Development of tolerance to the anorectic effect of TNF by repeated injections of TNF has been observed frequently in a number of laboratories (11–14). Also reported are tolerance to the gastrointestinal toxicity (15) and the lethal effect (16). There are some indications that tolerance to TNF is selective, implicating that several independent mechanisms are involved in the wide range of TNF actions. Hyperlipidemia (17) and prostaglandin synthesis by splenocytes (18) are TNF-induced effects that persist in animals rendered tolerant to the anorectic effect of TNF.

The purpose of the experiments described in this paper was to determine whether the antitumor efficacy of TNF in combination with IFN-γ was maintained in animals made tolerant to the lethal effects of TNF. By doing so, we could obtain evidence that the lethal and antitumor effects of TNF are not inevitably linked and that their separation remains a realistic research goal.

For this study, we chose the B16BL6 syngeneic melanoma model in which we previously reported an unambiguous antitumor effectiveness resulting from treatment with a combination of TNF and IFN-γ (5); the positive outcome was, however, overshadowed by the severe toxicity of the treatment.

MATERIALS AND METHODS

Animals. Specific-pathogen-free, female C57BL/cmb mice and C57BL/6 mice, 8 to 10 weeks old at the beginning of the experiments, were obtained from SCK, Mol, Belgium. The former strain has been used throughout the TNF toxicity studies in our laboratory. We switched to the latter to be able to use a syngeneic tumor model. The animals were housed in a temperature-controlled environment with 12-h light/dark cycles and received food and water ad libitum.

Tumor. The B16BL6 melanoma subline, selected from a spontaneous melanoma B16F10 line by I. Hart (19) and syngeneic with C57BL/6 mice, was a generous gift of Dr. M. Mareel (by courtesy of Dr. I. Fidler). Cells were expanded in vitro in Dulbecco’s MEM containing 10% fetal calf serum, 3.4 mM L-glutamine, 1 mM sodium pyruvate, MEM-nonessential amino acids, MEM vitamins, 100 units/ml penicillin, and 100 μg/ml streptomycin. On day 0 (the day of inoculation), cells were detached from the culture flask by a short EDTA treatment, rinsed in 0.1 ml PBS, and injected s.c. in the back just in front of the hind limb. All the mice used in a single experiment were inoculated on the same day with cells of the same suspension.

Agents. rmTNF produced by Escherichia coli containing an appropriate expression plasmid (20) was purified to apparent homogeneity. Two different preparations from the same origin were used. Preparation a had a specific activity of 1.5 × 10⁶ units/mg protein as determined by a cytotoxicity assay on L929 cells (21); the endotoxin content was 5.4 ng/mg protein. Preparation b had a specific activity of 4.7 × 10⁶ units/mg and contained 7.1 ng endotoxin/mg protein. In each single experiment, the identification of the preparation used is indicated.

rmIFN-γ, produced by transformed Chinese hamster ovary cells and purified to homogeneity, was a kind gift from Y. Guisez. Its specific
activity, determined in a cytopathic reduction assay using L929 cells challenged with vesicular stomatitis virus, was 1.3 × 10^7 units/mg. Reference rmIFN-γ (code Gg02-901-533) was from the NIH, Bethesda, MD; the endotoxin content amounted to 1.8 ng/mg.

The endotoxin contents were assessed by the chromogenic Limulus amebocyte lysate assay (Coatest; KabiVitrum, Stockholm, Sweden).

Tumor Treatment and Monitoring. Treatment of TB mice started either on day 4 or 9 after tumor inoculation. At that time, the s.c. tumor began to form a visible nodule. Treatment consisted of rmTNF, rmIFN-γ, or a combination of both agents as described here. Control groups received no treatment. The total volume of an injection was either 0.2 ml i.p. (i.e., a s.c. injection near the site of the tumor, but outside the nodule) or 0.5 ml i.p.

The tumor size was assessed at least every 5 days and was expressed as tumor size index, the product of the largest, perpendicular diameters (22).

Statistics. Data are presented as mean ± SD, unless otherwise stated. Student's t test was used to determine any significant difference in daily change of body weight from the appropriate control group. The significance of the observed difference in lethality was analyzed using the χ^2 test, with Yates' correction for small samples. The significance of the difference in survival time was analyzed using the Mantel modification of Gehan's generalized Wilcoxon test. The significant difference in tumor growth was assessed by regression coefficient analysis (23) or by applying Student's t test to the tumor size index on the days of measurement.

RESULTS

Induction of Tolerance in NTB Mice to Lethality Caused by TNF. C57BL/cnb mice were randomly grouped (n = 5). Mice were given i.p. injections of 2 μg rmTNF (a) dissolved in 0.2 ml PBS twice daily for 4 consecutive days. Control mice received the same volume of PBS twice daily for the same period. Mice receiving rmTNF showed a sharp decline of weight within 24 h (P < 0.001 as compared with the PBS control), but the daily weight change quickly returned to normal levels on day 2 in spite of continued administration (Fig. 1). The profile of tachyphylaxis to TNF-caused weight loss in this experiment was comparable with that in rats treated with rhTNF (16). We will refer to TNF-caused weight loss as an anorectic effect because it is a reflection of reduced food and water intake (17).

In the following experiments mice were challenged with a 100% lethal dose of rmTNF i.v. on day 5 or day 6, i.e., 1 or 2 days after the treatment as mentioned above (Table 1). The 100% lethal dose of rmTNF was 20 μg. Mice pretreated with rmTNF were completely protected against the lethal challenge with rmTNF on days 5 and 6 (P < 0.025). Mice were still 100% protected against rmTNF when challenged 5 days after the last injection (day 9), but they were no longer protected when challenged 10 days after the last injection (data not shown). A sufficiently long duration of tolerance allowed us to apply this procedure to an experimental tumor model in order to examine its effects on the therapeutic efficacy of rmTNF.

Introduction of Tumor Cells into Mice in the Course of the Tolerance-inducing Pretreatment and Subsequent Antitumor Treatment with rmTNF and IFN-γ. We examined whether inoculation of tumor cells into animals undergoing tolerance-inducing treatment had any influence on the acquisition of tolerance, since it is known that a tumor burden usually increases the susceptibility of animals to the toxic effect of TNF (24). Mice were pretreated with 2 μg rmTNF (b) given twice daily from day -2 to day 3 (day 0 being the day of tumor inoculation). Control mice received PBS according to the same schedule and are designated as nontolerant groups. The period during which tolerance was induced was prolonged in order to allow tumor growth before initiation of the treatment. The same protection effect as obtained with 4 days of pretreatment was observed when NTB mice were pretreated for 6 consecutive days (data not shown). On day 0, B16BL6 melanoma cells were inoculated as described in “Materials and Methods.” The antitumor treatment was started on day 4 and involved injections with 12.5 μg rmTNF in combination with 5000 units rmIFN-γ i.p. As a result of this treatment, arrest of tumor growth was apparent as early as on day 5 (P < 0.0005 in the nontolerant group as compared with the untreated control) or day 6 (P < 0.01 in the group made tolerant as compared with the untreated control; complete regression was observed on day 7, both in the group made tolerant and in the nontolerant control. No inhibition of tumor growth was seen in the tolerant, untreated group as compared with the nontolerant, untreated control (Fig. 2A). The treatment, however, proved to be highly toxic; 80% of the nontolerant mice died before day 7, while undergoing tumor regression. Remarkably, 80% of the tolerant mice survived the treatment (Fig. 2B). The tolerance-inducing procedure itself had no influence on the survival time of untreated mice. Subsequently, in order to prevent relapse of the primary tumor, all the surviving mice received a maintenance treatment with 5 μg rmTNF and 5000 units rmIFN-γ. One more mouse died on day 39. The survivors (60%) lived more than 100 days and were thus considered completely cured.

In this experiment, no reduction of the therapeutic efficacy of the rmTNF and rmIFN-γ treatment was seen by the induction of tolerance, nor did the procedure influence an untreated tumor. The induction of tolerance protected mice against the lethal effect of the combination therapy (P < 0.05 in terms of survival time compared with the nontolerant treated control).

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Table 1 Lethality in TNF-tolerant mice challenged with i.v. TNF
Effect of pretreatment on survival after an i.v. challenge of 20 μg rmTNF/mouse on day 5 (A), day 6 (B), or day 9 (C). C57BL/cnb mice were pretreated with 2 μg rmTNF i.p. twice daily for 4 consecutive days prior to an i.v. challenge. Control mice received PBS i.p. twice daily during the same period.

<table>
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* P < 0.025 versus control.

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Fig. 1. Effect of rmTNF on the body weight of NTB mice. Mice were randomly grouped (n = 5). Treatment began on day 0 and lasted until day 3. Two μg rmTNF were given i.p. twice daily (arrows). TNF was dissolved in 0.2 ml PBS. The control group received 0.2 ml of the vehicle solution i.p. twice daily. The daily change of the body weight is plotted versus time (with day 0 as the start of the treatment). o, PBS-treated group; O, TNF-treated group. *, P < 0.001 as compared with the control.
Induction of Tolerance in TB Mice and Its Effect on Single-Agent Treatment with rmTNF or rmIFN-γ. The previous experiment showed that the antitumor effect of the combined treatment with rmTNF and rmIFN-γ was not affected by the induction of tolerance. Although our earlier results (5) indicated little, if any, effect of rmIFN-γ alone on B16BL6 melanoma, we could not exclude the possibility that prior induction of tolerance potentiates the effect of rmIFN-γ to lead to antitumor activity. To address this question, the effect of the tolerance-inducing process on single-agent treatment with either rmTNF or rmIFN-γ was assessed. Since an early tumor burden did not interfere with the acquisition of tolerance, we proceeded to make TB mice tolerant in order to develop a more relevant model for tumor therapy. B16BL6 melanoma cells were inoculated on day 0, followed by a tolerance-inducing regimen with 2 μg rmTNF(b) injected i.p. twice daily from day 3 to day 8 after tumor inoculation. The p.l. treatment with 10 μg rmTNF or 5000 units rmIFN-γ began on day 9 and lasted for 8 days. Each group consisted of 5 mice. Following the treatment with rmTNF, both tolerant and nontolerant groups exhibited a statistically significant suppression of tumor growth on day 12 (P < 0.02 in tolerant and P < 0.001 in nontolerant mice, as compared with the respective controls) and the tumor regressed completely by day 15 (in the nontolerant group) and by day 17 (in the group made tolerant) (Fig. 3A). Treatment with rmIFN-γ had no effect on the tumor growth in the tolerant or in the nontolerant groups. The induction of tolerance had no impact on the survival of the tumor in the untreated animals. Therefore, the tolerance-inducing process had neither negative nor positive effects on treatment with rmIFN-γ, which in itself was ineffective.

On the other hand, the induction of tolerance had a clear influence on the survival of TB mice treated with rmTNF (Fig. 3B). All the mice made tolerant survived the rmTNF treatment, while only 40% of the nontolerant control mice survived the same treatment. The protection obtained by the induction of tolerance was significant in terms of survival time (P < 0.01) as compared with the nontolerant controls treated with rmTNF. IFN-γ in itself has no lethal effect and the induction of tolerance did not influence its outcome. Hence one can conclude that the tolerance-inducing process has no negative impact on the antitumor efficacy of rmTNF, whereas it sufficiently protected TB mice treated with that agent. However, some mice, namely 2 of 5 in the group made tolerant and 1 of 2 in the nontolerant group, developed a relapse of the primary tumor soon after the treatment was ended. These animals were not further treated, and the experiment was terminated on day 23, i.e., 1 week after the last treatment.

Induction of Tolerance in TB Mice and Therapeutic Potency of a Combined Treatment with rmTNF and rmIFN-γ. As the previous experiment indicated a limited efficacy of rmTNF alone with a high incidence of early relapse, an optimum treatment regimen was pursued with the combination of rmTNF and rmIFN-γ on TB mice. The first experiment (Fig. 2) achieved a successful cure of a small tumor by the combination treatment. Mice were made tolerant by the same regimen as described before. The antitumor treatment, consisting of p.l. injections of 10 μg rmTNF and 5000 units rmIFN-γ, began on day 9 and lasted 8 days. Groups that received treatment consisted of 10 mice, while those receiving no treatment had 5 mice per group. Group regression of the tumors occurred rapidly in both the tolerant (P < 0.025 on day 10) and the nontolerant groups (P < 0.05 on day 10); on day 14, the primary tumor had regressed completely in both groups. Again, the tolerance-inducing procedure did not retard the subsequent tumor growth in the untreated animals (Fig. 4A). Among the treated groups, 70% of the mice made tolerant survived the antitumor treatment schedule, while none of the nontolerant mice survived, thus achieving a significant protection (P < 0.025). The survival time was also significantly prolonged in the tolerant mice compared with the nontolerant control (P < 0.005; Fig. 4B). For the untreated groups, the
induction of tolerance had no statistically significant influence on the survival rate or the survival time. These results are in full agreement with those obtained in the previous experiment. The daily weight change monitored through the experimental period showed that tolerance to the anorectic effect was overcome when treatment with the high dose of rmTNF was started on day 9; the mice made tolerant also lost weight ($P < 0.001$, both in the tolerant and nontolerant mice as compared with the untreated controls; Fig. 4C). Yet there was an essential difference between the tolerant and nontolerant groups ($P < 0.05$). On day 11, the body weight declined further in the nontolerant mice ($P < 0.001$ as compared with the untreated control), followed by death during the following days. In contrast, a reversal of the negative weight change took place in the mice treated with rmTNF.

![Fig. 3. Induction of tolerance and single-agent treatment of B16BL6 TB mice. Effect of the tolerance-inducing process on therapeutic efficacy (A) and survival time (B). Tumor cells were inoculated on day 0 in all groups (n = 5), after which the mice were made tolerant (small arrows) from days 3 to 8 (regimen as in the legend to Fig. 2). Treatment p.l. was started on day 9 either with 10 ng rmTNF or 5000 units rmlFN-γ as indicated (medium arrows): Δ, nontolerant, untreated group; ○, nontolerant, treated with rmTNF; △, nontolerant, treated with rmlFN-γ; Δ, tolerant, untreated group; ○, tolerant, treated with rmTNF; △, tolerant, treated with rmlFN-γ.](image1)

![Fig. 4. Induction of tolerance and combined treatment of B16BL6 TB mice p.l. Effect of the tolerance-inducing process on therapeutic efficacy and tumor growth (A), survival time (B), and daily body weight change (C). Tumor cells were inoculated on day 0 in all groups (n = 10 for treated groups; n = 5 for untreated groups), after which the mice were made tolerant (small arrows) from day 3 to day 8 (regimen as in the legend to Fig. 2). The p.l. treatment was commenced on day 9 with 10 μg rmTNF and 5000 units rmlFN-γ as indicated (medium arrows). After complete tumor regression, the schedule was shifted to that of maintenance (large arrows) every 5 days up to the 36th day (cf. legend to Fig. 2). Δ, nontolerant, untreated group; ○, nontolerant, treated group; Δ, tolerant, untreated group; ○, tolerant, treated group. *, $P < 0.05$ as compared with the control; **, $P < 0.01$ as compared with the control.](image2)
made tolerant (yet still different from the normal level; \( P < 0.02 \)). The difference between the tolerant and nontolerant group was more apparent on this day \( (P < 0.01) \). Later on, both groups returned to the normal level. Thus further deaths occurred without being associated with weight loss.

Induction of Tolerance in TB Mice Followed by Treatment i.p. When TB mice were treated i.p. with a single-dose schedule, the antitumor efficacy was limited (data not shown). Then we introduced a gradually escalating dose schedule involving one additional injection of rmIFN-\( \gamma \). Mice were made tolerant by the same regimen between days 3 and 8 as described before, followed by i.p. treatment from day 9 on. The treatment schedule consisted of rmTNF and rmIFN-\( \gamma \) given every 24 h and every 12 h, respectively. The initial dose of rmIFN-\( \gamma \) was 10,000 units, which was increased to 20,000 units and to 50,000 units for each second successive day, then maintained at 50,000 units throughout. The dose of rmTNF initially amounted to 5 \( \mu \)g; it was raised to 7.5, 10, and 12.5 \( \mu \)g at the beginning of each new cycle consisting of 6 consecutive days. Two days of recovery were introduced between two cycles, except in the first transition. Although a virtually complete suppression of further tumor growth was observed in both treated groups \( (P < 0.005 \) in the tolerant mice; \( P < 0.025 \) in the nontolerant mice as compared with the corresponding controls), no actual regression was achieved (Fig. 5A). No difference in tumor growth rate was seen when tolerant and nontolerant groups were compared among the treated as well as the untreated groups. The protective effect of the tolerance-inducing procedure was evident only in terms of prolonged survival time \( (P < 0.025) \), without resulting in long-term curing (Fig. 5B). When the weight loss curve was analyzed, there was again a decrease in weight 1 day after starting the treatment (day 11), but the difference between the tolerant and nontolerant mice was not pronounced (Fig. 5C), unlike the previous experiment involving p.l. treatment.

Therefore, compared with the p.l. route of administration, both efficacy of therapy and protection by the induction of tolerance were moderate in the i.p. treatment. Yet, in accordance with the results obtained by p.l. treatment, the tolerance-inducing process clearly protected mice from the toxic effects of treatment without inhibiting its antitumor effect. Presumably the escalating dose was too severe in this experiment, and schedules may be found which combine efficacious inhibition of tumor growth and complete survival.

DISCUSSION

The main problem hampering a successful application of TNF as an anticancer drug in patients is its toxicity (6). In mice we were able to obtain a clear antitumor effect, but also in these model systems treatment toxicity appeared to be the main problem (5). As detailed under “Introduction,” we investigated whether the selective state of tolerance occurring after repetitive low-dose administrations of TNF (16) could be useful to broaden the therapeutic margin.

First, our results show that the induction of tolerance also protected against lethality in TB animals. Although a tumor burden sensitizes animals to the toxic effect of TNF (24), it did not interfere with the acquisition of tolerance.

Secondly, the tolerance-inducing procedure did not retard the tumor growth in the untreated animals, ruling out the possibility that the pretreatment itself may be responsible for the antitumor activity in the mice made tolerant. Nor did it have any influence on the survival time of the untreated TB animals.

Last, but undoubtedly most important, the efficacy of the antitumor treatment of either rmTNF alone or in combination with rmIFN-\( \gamma \) was not reduced by prior induction of tolerance. With respect to the two different administration routes, p.l.
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While it fails to reach an optimal local concentration at the tumor site, the efficacy of TNF is affected by the induction of tolerance. This result is presumably due to a pharmacokinetic effect rather than to the intrinsic nature of the tolerance-inducing phenomenon, because the difference was only quantitative in that a clear protection against lethality was obtained and no inhibition of the antitumor potency was seen in the i.p. route experiment. The systemic administration route is known to be more toxic (5) and less effective against a solid tumor (22). The level of TNF in circulation may be too high, while it fails to reach an optimal local concentration at the tumor site.

Taken together, our results lead to the conclusion that the antitumor activity of TNF is maintained in the mice made tolerant to its lethal effects. These results provide a rationale for an attempt to reduce toxicity, while optimizing the beneficial antitumor effect of TNF. Tolerance allowed administration of high doses of TNF in the treatment protocol, which led to a complete regression of the tumor in all mice. Addition of IFN-γ did not abrogate tolerance. Since IFN-γ strongly synergizes in the antitumor effect of TNF (5, 9) and has been reported to be a valuable agent against experimental metastasis (25), it is of considerable therapeutic and prognostic interest. The less dramatic results obtained thus far with i.p. treatment protocols should be improved in order to achieve more clinical relevance. Both the protection by the induction of tolerance and the antitumor efficacy were dose and schedule dependent, suggesting that such an optimization may be possible.

The mechanisms underlying induction of tolerance are unknown at present. Regarding the selective effects of the tolerance treatment, it is quite unlikely that tolerance reflects an altered absorption or clearance of TNF. In rats, the pharmacokinetics of TNF was not influenced by the tolerance-inducing process (16). Possibly, receptor down-regulation may mediate the refractory state of target cells. There exist at least two types of TNF receptor (26). Therefore, it is conceivable that the different types of receptor are modulated differently, leading to a selective abrogation of some TNF actions in particular cell types.

Alternatively, the selective regulation may operate at a later stage, such as secondary signaling transmitted by messenger molecules in target cells. Other cytokines and various inflammation-related mediators are believed to play a role in such a network. A single-bolus injection of IL-1 desensitizes mice to the subsequent challenge with TNF (27), while it considerably enhances the lethal effects of TNF when given at the same time (28). Desensitization by IL-1 is likely to be mediated by the liver (29) and can also be achieved with TNF. This kind of desensitization presumably operates through the boosting of endogenous protective mechanisms which are at present largely undefined but which may include acute phase proteins such as antiproteases. Although there may be some overlap of secondary effectors or processes, the desensitization phenomenon (27, 29) is clearly different from the induction of tolerance as described here. In the latter process, TNF cannot be replaced with IL-1 at the same dose range, it takes many days to establish tolerance, and the latter, but not the former, process is accompanied by a transient anorectic effect.

During the final preparation of our paper, we became aware of a recently published study by Fraker et al. (30) which addresses the same question and presents results seemingly contradictory to ours. Indeed, their results suggest that the antitumor efficacy of TNF is affected by the induction of tolerance. There are, however, several important differences between both systems used: the aforementioned authors used rhTNF, and not rmTNF; the tumors studied as well as the treatment regimens applied differed from ours. Consequently, several mechanisms can account for the discrepancy observed between both studies. Thus the possibility of antibody formation against rhTNF in their model cannot be excluded. Alternatively, different mechanisms (direct versus indirect ones) might contribute to the antitumor activity against B16BL6 melanoma (this report) versus MCA-106 and MCA-102 sarcomas studied by Fraker et al. (30). In this respect it should be noted that B16BL6 is resistant to rhTNF given alone but becomes sensitive after addition of rmIFN-γ (5). Furthermore, the reported finding that 40 μg rhTNF caused 100% lethality in NTB mice is surprising and contradictory to previous studies (31–34): rhTNF does not cause lethality in healthy mice (except at doses over 200 μg) unless relatively small amounts of endotoxin or other sensitizing factors are added.

To solve this paradox and to improve further the TNF treatment protocols based on protection by the induction of tolerance, further elucidation of the mechanisms of tolerance, antitumor activity, and toxicity will be required. Such data are needed to design rational approaches to the modulation of TNF activities and to define more in detail the requirements for the induction of a selective tolerance as observed by us. This may provide the basis for the development of clinically applicable cancer treatment protocols.

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Induction of Tolerance Allows Separation of Lethal and Antitumor Activities of Tumor Necrosis Factor in Mice

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