A Human Tumor Necrosis Factor (TNF) α Mutant That Binds Exclusively to the p55 TNF Receptor Produces Toxicity in the Baboon

By Kimberly J. Van Zee,* Sarah A. Stackpole,* Walton J. Montegut,* Michael A. Rogy,* Steve E. Calvano,* Katharine C. Hsu,‡ Moses Chao,‡ Carol L. Meschter,§ Hansruedi Loetscher,‖ Dietrich Stüber,‖ Urs Ettlin,‖ Beat Wipf,‖ Werner Lesslauer,‖ Stephen F. Lowry,* and Lyle L. Moldawer*‡

*From the *Department of Surgery, and the ‡Department of Cell Biology and Anatomy, Cornell University Medical College, New York 10021; the §Department of Toxicology and Pathology, Hoffmann-La Roche, Inc., Nutley, New Jersey 07110; and the ‖Department of Biology, F. Hoffmann-La Roche AG, CH-4002 Basel, Switzerland*

Summary

A number of recent studies have demonstrated that cellular responses to tumor necrosis factor (TNF) mediated by the p55 and the p75 TNF receptors are distinct. To evaluate the relative in vivo toxicities of wild-type TNFα (wtTNFα) and a novel p55 TNF selective receptor agonist, healthy, anesthetized baboons (*Papio sp.*) were infused with a near-lethal dose of either wtTNFα or a TNFα double mutant (dmTNFα) that binds specifically to the p55, but not to the p75, TNF receptor. Both wtTNFα and dmTNFα produced comparable acute hypotension, tachycardia, increased plasma lactate, and organ dysfunction in *Papio*. However, administration of wt TNFα produced a marked granulocytosis and loss of granulocyte TNF receptors, whereas little if any changes in neutrophil number or cell surface TNF receptor density were seen after dmTNFα mutant administration. Infusion of dmTNFα resulted in a plasma endogenous TNFα response that peaked after 90–120 min. We conclude that selective p55 TNF receptor activation is associated with early hemodynamic changes and the autocrine release of endogenous TNFα. Significant systemic toxicity results from p55 TNF receptor activation, but the role of the p75 TNF receptor in systemic TNF toxicity requires further study.

TNFα is a pleiotropic cytokine with varying immunologic and inflammatory host defense activities. The in vitro actions of TNFα include cytotoxicity of some tumor cell lines, antiviral activity, fibroblast and lymphocytic cell proliferation, and endothelial cell activation (for a review see reference 1). In certain transplantable tumors in mice, TNFα causes hemorrhagic necrosis in vivo (2). Because of its potential as an antineoplastic agent, recombinant TNFα has been administered to patients with malignancies in several clinical trials. Whereas systemic administration of >200 $\mu g/m^2$ of TNFα has not produced the expected potent and general antitumor activity (3), such infusions were found to produce unwelcome fever and hemodynamic changes (4). Similarly, when administered to experimental animals in relatively greater quantities, TNFα induced shock and mortality (5, 6). The continued interest in the antitumor activity of TNFα is supported by the results obtained with high-dose, TNFα perfusions combined with a chemotherapeutic agent (7).

Recent reports on cellular studies have suggested that binding of TNFα to its two cellular receptors, p55 and p75, elicits distinct biological responses. For example, human umbilical vein endothelial cells express both TNF receptor types, but TNFα-induced cell adhesion and expression of intercellular adhesion molecule 1, E-selectin and vascular cell adhesion molecule 1 were found under exclusive p55 receptor control (8). TNF binding to p55 TNF receptor also confers antiviral activity in hepatocytes and IFN-γ primed fibroblasts (9). Tartaglia et al. (10) reported that p55 mediated apoptosis in sensitive cell lines and induced manganese superoxide dismutase, whereas p75 activated thymocyte proliferation and generation of cytotoxic T cells.

Controversy exists whether the shock responses to TNFα in vivo are mediated by binding to p55 or p75. Historically, the relatively low systemic toxicity of human TNFα in mice has been attributed to the fact that human TNFα only binds the mouse p55 TNF receptor (11, 12). The lethal dose of

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/94/04/1185/07 $2.00
Volume 179 April 1994 1185–1191

human TNFα in the mouse is in excess of 1 mg/kg body weight (BW)[1], whereas murine TNFα causes death with doses as low as 10 μg/kg BW (13). Thus, a TNFα agonist that binds to p55 but not p75 in humans might produce less pronounced systemic toxicity than wild-type TNFα (wtTNFα), while retaining antitumor and antiviral activities. Van Ostade et al. (14) have recently shown that a mutant TNFα exclusively binding to the human p55 receptor has antitumor activity against a sensitive human tumor cell line growing as a solid tumor in nude mice without any associated toxicity. However, recent studies using p55-deficient mice showed that TNF binding to p55 was required for its systemic toxicity (15, 16).

Here we present a study of the relative in vivo toxicity in baboons of human wtTNFα compared with that of a TNFα mutant which binds with wild-type activity to human p55, but does not bind to p75. This TNFα mutant was previously generated by introducing two point mutations replacing Arg^{32} by Trp, and Ser^{86} by Thr (identified as a double mutant TNFα [dmTNFα]) (17). dmTNFα also binds selectively to the baboon p55, but not to p75, whereas wtTNFα binds to both baboon TNF receptors. When administered to healthy *Papio* at a dose of 100 μg/kg BW, dmTNFα and wtTNFα produced comparable cardiovascular disturbances and tissue injuries. These data demonstrate that the p55 TNF receptor has an important role in systemic TNF toxicity.

Materials and Methods

The generation of a dmTNFα mutant specific for the human p55 receptor has been reported (17). wtTNFα and dmTNFα were purified by sequential gel filtration (Q-Sepharose, Pharmacia, Uppsala, Sweden) and ion exchange chromatography (Mono-S; LKB-Pharmacia) as described in reference 17 to yield an electrophoretically pure protein preparation. The identity of the TNFα preparations was confirmed by amino acid composition analyses or ion spray mass spectrometry. Samples were diluted in sterile, endotoxin-free, physiologic saline to a final concentration of 500 μg/ml. Endotoxin content of the final preparations was <14 EU/mg protein.

Solid-phase Radioligand Binding Assay. To demonstrate that receptor type specificities of the human wild-type TNFα and mutant TNFα were maintained in *Papio*, solid-phase radioligand binding studies were performed. HL60 cells were cultured and lysed with Triton X-100 as previously described (18). Baboon buffy coats were obtained from 100 ml of venous blood from *Papio* anticoagulated with EDTA; cells were subsequently pelleted and frozen at −70°C. Cell pellets containing ~10^8 leukocytes were resuspended in 1.0 ml of PBS, pH 7.4, containing a cocktail of protease inhibitors (19) and diluted with an equal volume of the same buffer containing 2% Triton X-100. After extraction overnight at 4°C, the samples were clarified by centrifugation and immediately used for radioligand binding assay or stored at −80°C.

96-well microtiter plates were coated with affinity-purified polyclonal antibodies (10 μg/ml in PBS) raised against recombinant soluble human p55 and p75 (8). After blocking with 1% defatted milk powder in 50 mM Tris, 140 mM NaCl, 5 mM EDTA, 0.001% Kathon MW/WT for 1–2 h at room temperature, HL60 cell extract (2.3 × 10^8 cells/ml) or baboon buffy coat extract (5 × 10^7 leukocytes/ml) were added (100 μl/well) and incubated overnight at 4°C. The wells were then incubated with 15 ng/ml human ^{125}I-TNFα (sp act 0.3–1.0 × 10^8 cpm/μg [19]) in blocking buffer containing 0.1% defatted milk powder in the presence or absence of 3 μg/ml unlabeled human wtTNFα or dmTNFα mutant. After incubation at room temperature for 4 h, the amount of ^{125}I-TNFα bound to each well was determined in a Phosphoimager® (Molecular Dynamics, Inc., Sunnyvale, CA).

Treatment of Experimental Animals. Nine young adult male and female *Papio sp.* baboons were purchased from Southwest Foundation for Biomedical Research (San Antonio, TX). All animals were quarantined for a minimum of 2 wk at the Research Animal Resource Center of Cornell University Medical College (CUMC) to confirm their good health and lack of disease transmissible to humans. The experimental protocol was approved by the Institutional Animal Care and Use Committee at CUMC.

Study Protocol. After an overnight fast, animals were initially anesthetized with ketamine (10 mg/kg intramuscularly) and anesthesia was thereafter maintained by intravenous administration of sodium pentobarbital at 3–5 mg/kg BW/h i.v. The animals were instrumented for invasive monitoring as described previously (20, 21).

After baseline blood sampling and a waiting period of at least an hour to allow equilibration, 100 μg/kg BW of either wtTNFα (n = 3) or dmTNFα (n = 3) were administered via the femoral vein as a bolus injection. An additional three baboons received no injections and served as instrumented controls. Arterial blood samples were obtained at 0.5, 1, 3, 5, 10, 15, 30, 60, 90, and 120 min, at hourly intervals through 8 h, and again at 24, 48, and 96 h for pharmacokinetic and blood chemistry analyses. The investigators caring for the animals were blinded to treatment. Leukocyte and thrombocyte counts were measured on venous blood anticoagulated with EDTA by flow cytometric, light scatter, and Coulter counter analyses, respectively, as previously reported (20). Prothrombin and partial thromboplastin times were measured by the clinical laboratories at the Animal Medical Center (New York). TNF receptors on granulocytes were quantitated by cytofluorometry using biotinylated TNFα and PE-conjugated streptavidin. Briefly, baboon blood was anticoagulated with Na-EDTA. Erythrocytes were lysed with a bicarbonate-buffered (pH 7.2) ammonium chloride solution. Leukocytes were recovered by centrifugation and washed with PBS containing 0.1% sodium azide (PBS-A). Specific staining was determined with 1.0 μg/ml biotinylated, human TNFα whereas nonspecific staining was determined with biotinylated TNFα plus 100-fold excess, unlabeled human TNFα. After incubation on ice for 15 min, cells were washed with PBS-A and incubated with 0.5 μg/ml streptavidin-conjugated PE for 15 min on ice. Cells were then washed and resuspended in PBS-A for flow cytofluorometric analysis. For each experiment, the flow cytometer photomultiplier gain was standardized using a single lot of PE-conjugated beads. Mean channel fluorescence (>570 nm) of forward and side angle light scatter-gated granulocytes was assessed. Data are presented as the difference (linear units) between mean channel fluorescence intensities of specifically and nonspecifically stained cells.

The plasma fraction of additional EDTA and heparinized samples was separated by centrifugation at 4°C and stored at −70°C until assayed for TNFα and sTNFR I (p55) immunoactivity, as previously described (20, 21). TNFα bioactivity was determined using

[1] *Abbreviations used in this paper:* BW, body weight; dmTNFα, double mutant TNFα; PBS-A, PBS and 0.1% sodium azide; wtTNFα, wild-type TNFα.

the murine WEHI clone 13 cytotoxicity assay (21). IL-6 bioactivity (B.9 hybridoma proliferation) and IL-8 immunoactivity were also assayed (20, 21). At the end of an 8-h monitoring period, all catheters were removed, and animals were awakened and returned to their cages. Blood was sampled at 24, 48, and 96 h. After the last blood sampling, baboons were sacrificed by the intravenous administration of 65 mgs/kg sodium pentobarbital. Necropsy was performed at death, and tissues were fixed in 10% buffered formalin for light microscopy.

Statistical Analyses. Values are presented as the mean ± standard error. Differences in responses between baboons administered wild-type and mutant TNFα were analyzed by two-way analysis of variance. In some cases, differences from baseline were analyzed by one-way analysis of variance, and Dunnett's multiple range test. Significance was determined at the 95% level of confidence employing a one-tailed test.

Results

Specificity of TNFα Mutant in Papio. To test whether receptor-type selectivity for p55 of dmTNFα was maintained in baboons, the competitive binding of wtTNFα and dmTNFα to p55 and p75 TNF receptors derived from a human cell line and from *Papio* leukocytes was compared. Detergent-extracted receptors from both species were immobilized on a solid-phase coated with receptor-type–specific polyclonal antibodies that had been raised in rabbits against recombinant human receptors and that cross-reacted with the respective baboon receptors. As shown in Fig. 1, binding of ^{125}I-labeled wtTNFα to the immobilized human and *Papio* p55 TNF receptors was competitively inhibited by unlabeled wtTNFα or dmTNFα, whereas binding to the p75 receptors of both species was competed by wtTNFα but not by dmTNFα.

Papio Cardiovascular and Physiologic Responses. Administration of 100 μg/kg BW of human wtTNFα produced significant hypotension and tachycardia in the anesthetized baboon (Fig. 2). Cardiac output declined (data not shown) and

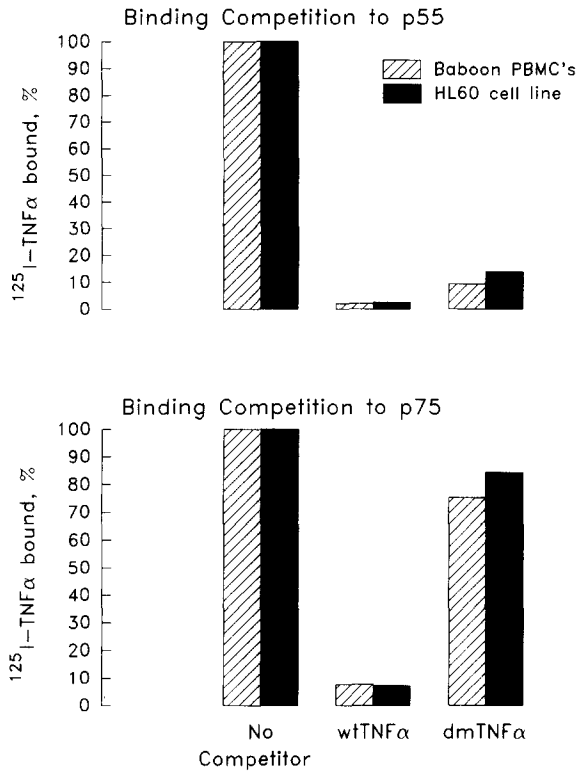

Figure 1. Competitive binding of human wtTNFα and dmTNFα mutant to human and baboon p55 and p75. Solubilized human and baboon TNF receptors were captured on microtiter plates by coating the wells with polyclonal antibodies specific for human p55 and p75. Binding of human wild-type ^{125}I-TNFα to the TNF receptors was determined in the presence or absence of excess unlabeled wtTNFα or dmTNFα mutant. The amount of radioactivity bound in the absence of competitor is taken as 100% and was 24,911 and 74,383 counts for the p55 from baboon PBMC and HL60 cells, respectively, and 11,077 and 20,587 counts for the p75 from baboon PBMC and HL60 cells, respectively. (*Top*) Binding competition to p55 (receptors from both species immobilized by antihuman p55 antibodies); (*bottom*) binding competition to p75 (receptors from both species immobilized by antihuman p75 antibodies).

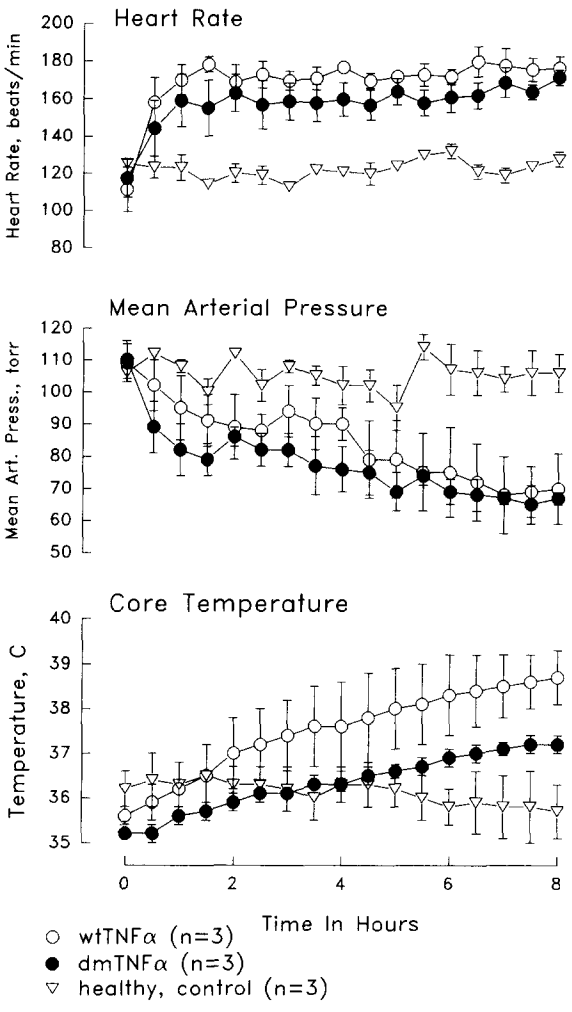

Figure 2. Hemodynamic and body temperature changes in baboons treated with 100 μg/kg BW of either wtTNFα or dmTNFα. (*Top*) Heart rate (*middle*), mean arterial pressure, and (*bottom*) core temperature were all measured as described in Materials and Methods. Administration of both TNFα molecules resulted in a significant tachycardia and falls in mean arterial pressure and cardiac output. The difference in increase in core temperature in baboons treated with wtTNFα and dmTNFα was statistically significant.

a significant rise in blood lactate concentrations was also evident (Table 1). The animals also developed a pyrexic response. Although none of the animals administered wtTNFα expired over the subsequent 4 d, significant organ dysfunction was observed. For example, serum creatinine and blood urea nitrogen (BUN) concentrations markedly increased over 48 h in the TNFα-treated animals indicating impairment of renal function (Table 1). In addition, concomitant increases in hepatic enzyme levels (serum glutamate-oxaloacetate transaminase [SGOT], serum glutamate-pyurate transaminase [SGPT]) were consistent with acute hepatocellular damage. The baboons receiving wtTNFα also developed a significant plasma IL-6 and IL-8 response.

Administration of dmTNFα at 100 μg/kg BW produced comparable degrees of hypotension and tachycardia; however, the pyrexic response was significantly less pronounced (Fig. 2). The elevation of blood lactate and presence of renal and hepatic dysfunction were similar between the animals treated with wtTNFα and with dmTNFα in the initial phase of the study, but these dysfunctions tended to be more protracted in the animals treated with dmTNFα. One of the three animals treated with dmTNFα became unresponsive and moribund after 48 h, and was euthanized because of animal welfare concerns.

Sham infusions had no effect on hemodynamic responses in healthy baboons (Fig. 2), nor were there any observed changes in measures of organ function (data not shown).

Hematopoietic Responses. Administration of the wtTNFα produced a significant granulocytosis ($p < 0.05$ at 1, 3, 4, and 5 h versus baseline; Fig. 3). Blood monocyte and lymphocyte numbers rapidly declined (data not shown). Despite this granulocytosis, there was an almost complete loss of total cellular TNF receptors from blood granulocytes (Fig. 3). Unfortunately, because of the lympho- and monocytopenia induced by the TNFα administration, there were insufficient numbers to perform flow cytofluorometric analysis of TNF receptors upon these cells. Platelet counts declined from about 300,000/μl^3 to 100,000–150,000/μl^3 24–48 h after wtTNFα treatment, although prothrombin and partial thromboplastin times were only modestly affected (data not shown).

In baboons administered dmTNFα, granulocytosis was not observed. In addition, TNF receptors on granulocytes declined only transiently and then rapidly returned to levels comparable to baseline, suggesting an important role for p75 TNF receptor in receptor shedding. Baboons treated with the dmTNFα exhibited a similar monocytopenia and lymphopenia as seen in animals treated with wtTNFα. The soluble TNF p55 receptor concentrations increased in baboons treated with both wtTNFα and dmTNFα, although concentrations were marginally higher in baboons treated with wtTNFα. Adminis-

Table 1. *Biochemical Parameters in Baboons Treated with 100 μg/kg BW wtTNFα or dmTNFα*

	wtTNFα				dmTNFα			
	0	8	24	48 h	0	8	24	48 h*
Lactate mg/dl	4.2 ± 0.7	40.6 ± 14.5‡	35.5 ± 7.8‡	24.0 ± 0.6‡	5.5 ± 0.9	27.9 ± 1.4‡	118 ± 43.7‡	95 ± 45.3‡
BUN, mg/dl	14.2 ± 0.7	15.8 ± 2.1	32.6 ± 9.1‡	37.4 ± 12.5‡	15.8 ± 4.8	13.6 ± 3.2	34.0 ± 8.8‡	41.8 ± 8.2‡
Creatinine, mg/dl	0.8 ± 0.1	1.2 ± 0.1‡	2.0 ± 0.5‡	1.7 ± 0.4‡	1.0 ± 0.1	1.3 ± 0.1	2.7 ± 0.4‡	3.3 ± 1.2‡
SGOT, IU/ml	28 ± 6	100 ± 24‡	332 ± 31‡	156 ± 34‡	30 ± 4	57 ± 11	244 ± 39‡	366 ± 165‡
SGPT, IU/ml	28 ± 8	56 ± 17	274 ± 36‡	227 ± 44‡	29 ± 11	36 ± 14	139 ± 13‡	197 ± 56‡
IL-6, B.9 ng/ml	0 ± 0	26.9 ± 4.7‡	ND	ND	0 ± 0	21.9 ± 2.1‡	ND	ND
IL-8, ng/ml	0 ± 0	17.8 ± 3.0	ND	ND	0 ± 0	19.9 ± 3.5	ND	ND

* One baboon (No. 92-150) that received the dmTNF was euthanized at 48 h because of animal welfare concerns. The animal was unresponsive, and could not adequately eat or drink.
‡ $p < 0.05$ versus baseline (time zero) by one-way analysis of variance, and Dunnett's multiple range test.

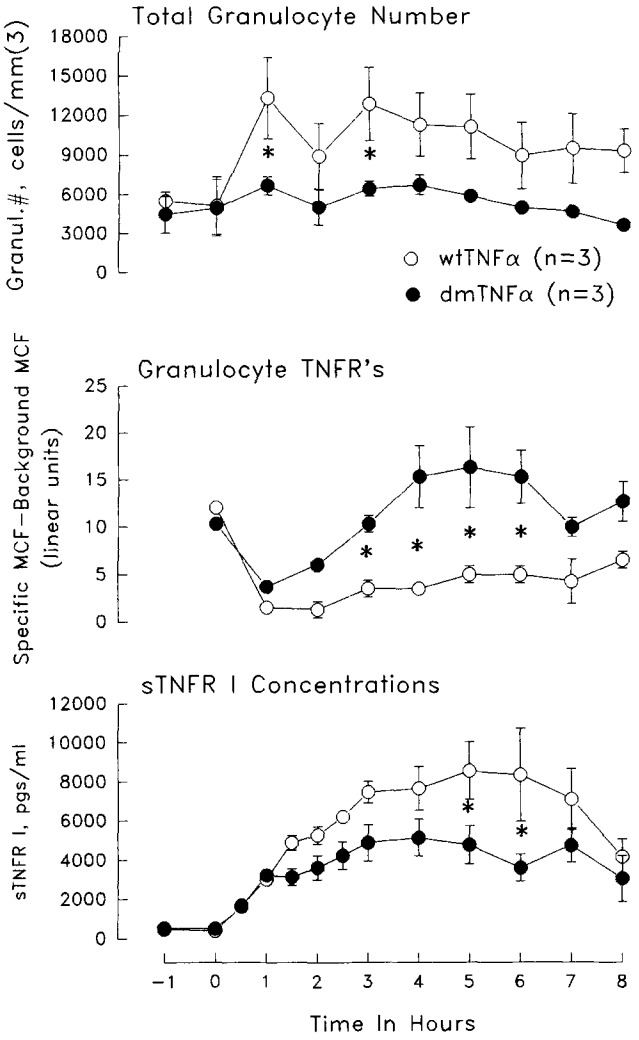

Figure 3. (*Top*) Total granulocyte numbers, (*middle*) granulocyte TNF receptor activity, and (*bottom*) soluble TNF receptor I concentrations. Administration of either wtTNFα or dmTNFα resulted in increased soluble TNF p55 receptor concentrations, as determined by immunoassay. Infusion of wtTNFα, but not dmTNFα, resulted in a sustained loss of granulocyte cellular TNF receptors (*, $p < 0.05$, one-sided test).

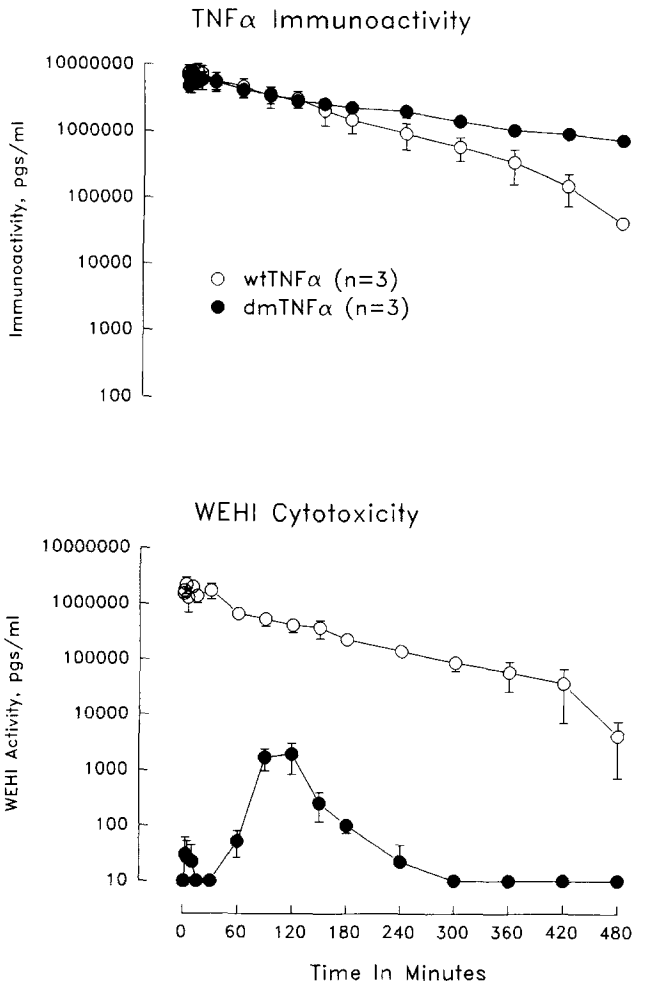

Figure 4. Plasma TNFα immunoactivity and bioactivity. (*Top*) TNFα immunoactivity was determined by ELISA employing monoclonal and polyclonal antibodies raised against recombinant human wtTNFα. TNFα bioactivity was assessed by (*bottom*) WEHI clone 13 cytotoxicity assay. In baboons treated with dmTNFα, a monophasic peak of TNFα bioactivity was observed 90–120 min after the infusion suggesting endogenous production of TNFα.

tration of the dmTNFα produced a similar thrombocytopenia as seen with wtTNFα, and had no significant effect on either prothrombin or partial thromboplastin times (data not shown).

Instrumentation and the sham procedure had no effect on hematologic parameters (data not shown).

Histological Examination. Treatment of baboons with wtTNFα produced only modest histopathologic changes, including hepatocellular edema in one animal. In the three baboons treated with dmTNFα, mild hepatocellular edema was also noted. Furthermore, in the one dmTNFα-treated baboon that was euthanized for animal welfare concerns, moderate necrosis and hemorrhage of the spleen and adrenals was also observed, as well as some neutrophil margination and pooling in the lungs.

TNFα Pharmacokinetics. The apparent β-phase half-life wtTNFα immunoactivity in *Papio* was 61 min (Fig. 4). The half-life of wtTNFα bioactivity, as determined by the WEHI cytotoxicity assay, was comparable. In contrast, the dmTNFα mutant had an apparent β-phase half-life that was significantly longer, ~169 min. Previous in vitro studies have shown that this dmTNFα does not bind to the murine p55, although its affinity for human p55 is not different than wtTNFα's affinity for p55 (16). Thus, incubation of dmTNFα with the murine WEHI clone 13 cell does not induce cytotoxicity. However, when dmTNFα was infused into otherwise healthy baboons and plasma from such animals was coincubated with WEHI clone 13 cells, cytotoxicity was observed in plasma 90–120 min later (Fig. 4, *bottom*). Since dmTNFα is not bioactive in this assay, cytotoxicity must have resulted from another source. Coincubation of these plasma samples with either a mAb directed against human TNFα (mAb 18.1.2) or a chimeric p55, human IgG fusion protein (22), completely

eliminated this cytotoxicity (data not shown), confirming that the plasma appearance of WEHI cytotoxicity was endogenous TNFα.

Discussion

In this study, administration of recombinant human TNFα at a dose of 100 μg/kg BW to the healthy baboon (*Papio*) produced a 35% fall in mean arterial pressure, tachycardia, and evidence of both renal and hepatic dysfunction. These responses are key elements of systemic TNF toxicity and have also been observed in cancer patients receiving TNFα as an antitumor agent (4). In dogs receiving comparable doses of TNFα, irreversible tissue damage and mortality have also been reported (6).

Healthy baboons treated with the same dose of the p55-specific dmTNFα responded similarly in several respects. For example, the hemodynamic changes and organ damage were of similar magnitude in both groups of animals. In fact, one mutant TNFα-treated baboon had to be euthanized, because the animal was unresponsive and unable to eat and drink. The binding studies confirm that dmTNFα competes with wtTNFα for *Papio* p55, but not for p75. Thus, it must be concluded that the systemic toxicity of dmTNFα in *Papio* is a result of its binding to the baboon p55 receptor exclusively.

The more protracted signs of organ dysfunction and the histological changes in the animals treated with dmTNFα could be the consequence of its longer pharmacologic half-life which translates into a higher persistent dose at the later phases of the experiment. The reasons for the different half-lives of wtTNFα and dmTNFα are not understood, but the present results are consistent with a significant role for p75 and its soluble form in TNFα elimination, which might be correlated with the role of p75 in TNF receptor shedding.

One novel observation of this study was that infusion of dmTNFα induced an endogenous, circulating TNFα response. It is unlikely that the minute contamination of dmTNFα with endotoxin (14 EU/mg protein) can explain the endogenous TNFα response. The baboon is the most endotoxin-resistant of all nonhuman primates (23), and our previous studies have demonstrated that 500 μg/kg BW of *Salmonella typhosa* LPS is required to produce a 1 ng/ml TNFα plasma response (24), whereas the current animals received only ~150 pg/kg BW.

However, the present studies cannot exclude entirely the possibility that toxicity induced by dmTNFα was secondary to the endogenous TNFα response. The toxicity seen is clearly due to the p55 agonist, but it cannot be distinguished whether these responses are the direct result of dmTNFα binding to p55 or are the result of the induced endogenous TNFα that subsequently binds to both p55 and p75. The onset of hypotension and hemodynamic disturbances was not delayed in animals treated with dmTNFα, relative to those animals receiving wtTNFα, as might have been expected if the changes were due to endogenous TNFα production. Nevertheless, the present studies do not distinguish rigorously between responses due to exogenous dmTNFα administration and endogenous TNFα because of the variability in the onset of clinical symptoms or signs. Furthermore, if p55 receptors were fully occupied by dmTNFα, then endogenous baboon TNFα is competed at p55 and would bind with some preference to the p75 receptor. Thus, the relatively low concentrations of endogenous TNFα might have disproportionate activities and elicit toxic host responses equivalent to higher TNFα concentrations.

Based on in vitro studies, distinct functions have been attributed to TNFα binding to these two TNF receptor types in various cells. However, the significance of TNFα in vivo, like that of other cytokines, cannot be easily deduced from individual tissue responses, but rather, should be understood from its integrated actions on the complex assembly of cells and organ systems in a living organism. The systemic toxicity of TNFα, as reflected in hemodynamic changes and organ dysfunction, is undoubtedly the sum of responses by different cells in various organs.

It has been proposed from the differing toxicities of mouse and human TNFα in mice that p55 selective agonists can be expected to have a lower systemic toxicity than wild-type TNFα. This hypothesis has not been confirmed in this study. In fact, the results of this study are more consistent with the conclusion of Pfeffer et al. (15) who demonstrated that p55-deficient mice sensitized with D-galactosamine were tolerant of endotoxic shock. Furthermore, Rothe et al. (16) demonstrated that p55-deficient mice are insensitive to TNF toxicity, although they remain susceptible to the lethal effects of LPS in the absence of D-galactosamine sensitization. Finally, the fact that TNFα induction of endothelial cell adhesion molecules, which must be considered part of systemic toxicity, is under dominant p55 control (8) also argues that p55 selective agonists cannot be completely devoid of systemic toxicity.

This work was supported in part by grants GM-40586 and CA-50281 (both to L. L. Moldawer), and GM-34695 (to S. F. Lowry), from the National Institutes of Health, U.S. Public Health Service, and by a clinical fellowship from the American Cancer Society (to K. J. Van Zee).

Address correspondence to Dr. Lyle L. Moldawer, Department of Surgery, Box 100286, University of Florida College of Medicine, Gainesville, FL 32610.

Received for publication 27 September 1993 and in revised form 3 January 1994.

References

1. Vassalli, P. 1992. The pathophysiology of tumor necrosis factors. *Annu. Rev. Immunol.* 10:411.
2. Carswell, E.A., L.J. Old, S. Green, N. Fiore, and B. Williamson. 1975. An endotoxin induced serum factor that causes necrosis of tumors. *Proc. Natl. Acad. Sci. USA.* 72:3666.
3. Taguchi, T., and Y. Sohmura. 1991. Clinical studies with TNF. *Biotherapy.* 3:177.
4. Warren, R.S., H.F. Starnes, Jr., J.L. Gabrilove, H.F. Oettgen, and M.F. Brennan. 1987. The acute metabolic effects of tumor necrosis factor administration. *Arch. Surg.* 122:1396.
5. Tracey, K.J., B. Beutler, S.F. Lowry, J. Merryweather, S. Wolpe, I.W. Milsark, R.J. Hariri, T.J. Fahey III, A. Zentella, J.D. Albert, et al. 1986. Shock and tissue injury induced by recombinant human cachectin. *Science (Wash. DC).* 234:470.
6. Tracey, K.J., S.F. Lowry, T.J. Fahey III, J.D. Albert, Y. Fong, D. Hesse, B. Beutler, K.R. Manogue, S. Calvano, H. Wei, et al. 1987. Cachectin/tumor necrosis factor induces lethal shock and stress hormone responses in the dog. *Surg. Gynecol. & Obstet.* 164:415.
7. Lienard, D., P. Ewalenko, J.J. Delmotte, N. Renard, and F.J. Lejeune. 1992. High-dose recombinant factor alpha in combination with interferon gamma and melphalan in isolation perfusion of the limbs for melanoma and sarcoma. *J. Clin. Oncol.* 10:52.
8. Mackay, F., H. Loetscher, D. Stueber, G. Gehr, and W. Lesslauer. 1993. Tumor Necrosis Factor α (TNFα)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. *J. Exp. Med.* 177: 1277.
9. Wong, G.H., L.A. Tartaglia, M.S. Lee, and D.V. Goeddel. 1992. Antiviral activity of tumor necrosis factor (TNF) is signaled through the 55 kDa receptor, type I TNF. *J. Immunol.* 149:3350.
10. Tartaglia, L.A., R.F. Weber, I.S. Figsri, C. Reynolds, M.A. Palladino, Jr., and D.V. Goeddel. 1991. The two different receptors for tumor necrosis factor mediate distinct cellular responses. *Proc. Natl. Acad. Sci. USA.* 88:9292.
11. Lewis, M., L.A. Tartaglia, A. Lee, G.L. Bennett, G.C. Rice, G.H.W. Wong, E.Y. Chen, and D.V. Goeddel. 1991. Cloning and expression of cDNAs for two distinct murine tumor necrosis factor receptors demonstrate one receptor is species-specific. *Proc. Natl. Acad. Sci. USA.* 88:2830.
12. Kramer, S.W., B.B. Aggarwal, T.E. Eessalu, S.M. McCabe, B.L. Ferraiolo, I.S. Figari, and M.A. Palladino, Jr. 1988. Characterization of the in vitro and in vivo species preference of human and murine tumor necrosis factor-alpha. *Cancer Res.* 48:920.
13. Broukaert, P.G., B. Everaerdt, C. Libert, N. Takahashi, and W. Fiers. 1989. Species specificity and involvement of other cytokines in endotoxic shock action of recombinant tumor necrosis factor in mice. *Agents Actions.* 26:196.
14. Van Ostade, X., P. Vandenabeele, B. Everaerdt, H. Loetscher, R. Gentz, M. Brockhaus, W. Lesslauer, J. Tavernier, P. Brouckaert, and W. Fiers. 1993. Human TNF mutants with selective activity on the p55 receptor. *Nature (Lond.).* 361:266.
15. Pfeffer, K., T. Matsuyama, T.M. Kündig, A. Wakeham, K. Kishihara, A. Shahinian, K. Wiegmann, P.S. Ohashi, M. Krönke, and T.W. Mal. 1993. Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock yet succumb to L. monocytogenes infection. *Cell.* 73:457.
16. Rothe, J., W. Lesslauer, H. Loetscher, Y. Lang, P. Koebel, F. Kontgen, A. Althage, R. Zinkernagel, M. Steinmetz, and H. Bluethmann. 1993. Mice lacking the tumor necrosis factor 1 are resistant to TNF-mediated toxicity, but highly susceptible to infection by *Listeria monocytogenes*. *Nature (Lond.).* 364:798.
17. Loetscher, H., D. Stueber, D. Banner, F. Mackay, and W. Lesslauer. Human tumor necrosis factor α (TNFα) mutants with exclusive specificity for the 55 kDa or 75 kDa TNF receptors. 1993. *J. Biol. Chem.* 268:26350.
18. Loetscher, H., R. Gentz, M. Zulauf, A. Lustig, H. Tabuchi, E.J. Schlaeger, M. Brockhaus, H. Gallati, M. Manneberg, and W. Lesslauer. 1991. Recombinant 55 kDa tumor necrosis factor (TNF) receptor. Stoichiometry of binding to TNFα and TNFβ and inhibition of TNF activity. *J. Biol. Chem.* 266:18324.
19. Loetscher, H., E.J. Schlaeger, H.W. Lahm, Y.C.E. Pan, W. Lesslauer, and M. Brockhaus. 1990. Purification and partial amino acid sequence analysis of two distinct tumor necrosis factor receptors from HL60 cells. *J. Biol. Chem.* 265:20131.
20. Van Zee, K.J., E. Fischer, A.S. Hawes, C.A. Hébert, T.G. Terrell, J.B. Baker, S.F. Lowry, and L.L. Moldawer. 1992. The sequellae of IL-8 administration in nonhuman primates. *J. Immun.* 148:1746.
21. Van Zee, K.J., T. Kohno, E. Fischer, C.S. Rock, L.L. Moldawer, and S.F. Lowry. 1992. TNF soluble receptors protect against excessive TNFα during infection and injury. *Proc. Natl. Acad. Sci. USA.* 89:4845.
22. Ashkenasi, A., S.A. Marsters, D.J. Capon, S.M. Chamow, I.S. Figari, D. Pennica, D.V. Goeddel, M.A. Palladino, and D.H. Smith. 1991. Protection against endotoxic shock by a tumor necrosis factor immunoadhesin. *Proc. Natl. Acad. Sci. USA.* 88:10535.
23. Zurovsky, Y., H. Laburn, D. Mitchell, and A.P. MacPhail. 1987. Responses of baboons to traditionally pyrogenic agents. *Can. J. Physiol. Pharmacol.* 65:1402.
24. Fischer, E., M.A. Marano, A. Barber, A. Hudson, K. Lee, C. Rock, A.S. Hawes, R.C. Thompson, T.V. Hayes, T.D. Anderson, et al. 1991. A comparison between the effects of interleukin-1α administration and sublethal endotoxemia in primates. *Am. J. Physiol.* 261:R442.