The Local Effects of Cachectin/Tumor Necrosis Factor on Wound Healing

GARY D. SALOMON, M.D., ATTAN KASID, PH.D., DOUGLAS T. CROMACK, M.D., ELAINE DIRECTOR, B.S., THOMAS L. TALBOT, M.S., ANTHONY SANK, M.D., and JEFFREY A. NORTON, M.D.

Previous experimental studies have suggested that tumor necrosis factor (TNF) may have either a beneficial or a detrimental role in wound healing. Control and doxorubicin-treated (6 mg/kg, intravenously) rats underwent paired dorsal 5-cm linear wounds and had either vehicle or recombinant (r)TNF (0.5, 5, or 50 μg) applied locally to the wound. Paired wounds were harvested at 7 and 14 days after wounding and analyzed for wound-bursting strength (WBS) and activity of the gene for type 1 collagen and TNF. Doxorubicin treatment decreased WBS at 14 days but not at 7 days after wounding. Local application of 50 μg of rTNF decreased WBS in saline-treated rats and concentrations of 5 and 50 μg decreased WBS in doxorubicin-treated rats when measured 7 days after wounding. These effects dissipated when WBS was measured 14 days after wounding. Doxorubicin decreased wound collagen gene expression and local TNF treatment decreased wound collagen gene expression in saline-treated rats and further decreased it in doxorubicin-treated rats. The decrement in collagen gene expression induced by rTNF increased as the local dose of rTNF increased. The gene for TNF was not detectable in wounds from normal or doxorubin-treated rats at 3, 7, 10, or 14 days after wounding. These data suggest that the gene for TNF is not expressed in wounds and that the local application of TNF is detrimental to wound healing as it decreases WBS and activity of the gene for collagen.

From the Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

TUMOR NECROSIS FACTOR-α (TNF), a 17-kd polypeptide produced by macrophages in response to endotoxin, is a highly conserved molecule that appears to play a major role in the pathogenesis of gram-negative shock.[1] It is also named cachectin because it has many metabolic effects that may result in the wasting syndromes that accompany chronic disease states.[2] In addition studies have shown that TNF has potent, highly specific effects on many different types of cells.[3-8]

Tumor necrosis factor stimulates fibroblast proliferation,[3] prostaglandin production, and collagenase gene expression.[4] The ability of TNF to stimulate fibroblast proliferation may be due, in part, to its ability to promote production of interleukin-6 (IL-6) by fibroblasts[5] because IL-6 or B2-interferon is known to function as an autocrine mediator of cell proliferation in fibroblasts. Tumor necrosis factor stimulates the expression of endothelial cell surface antigens, including intercellular adhesion molecule-1 (ICAM-1), that modulate the adherence of inflammatory leukocytes to endothelial cells.[6] Tumor necrosis factor is chemotactic for macrophages,[7] and has been shown to activate macrophages *in vitro*.[8] It stimulates angiogenesis when implanted in rat cornea and when applied to developing chick chorioallantoic membrane.[9] It has been shown to induce the release of growth factors, including platelet-derived growth factor, known to be involved in the early phases of wound healing.[10]

In vitro the action of TNF has been elucidated at the molecular level. Tumor necrosis factor appears to increase protein kinase C activity in fibroblasts, resulting in prolonged activation of jun gene expression, increased activator protein-1 (AP-1) activity, and induction of AP-1 responsive genes, including collagenase, resulting in a marked increase in collagenase transcription.[11] The ability of TNF to stimulate fibroblasts to repopulate a wound space, attract macrophages and other inflammatory cells to a site of tissue injury, and to promote angiogenesis makes it a prime candidate for a role as a mediator of the cellular response to tissue injury.[12] However contrary to the *in vitro* evidence supporting a role for TNF in normal wound healing is the fact that TNF inhibits collagen gene expression in cultured fibroblasts.[13] Although increased collagenase gene expression and decreased collagen synthesis induced by TNF may have a role in tissue remodeling,[14] this combination is not likely to be beneficial as

Address reprint requests to Jeffrey A. Norton, M.D., Head, Surgical Metabolism Section, Surgery Branch, National Cancer Institute/NIH, Building 10, Room 2B07, Bethesda, MD 20892.

Accepted for publication September 26, 1990.

an early promoter of healing. Whether TNF acts as a mediator of the local response to tissue injury and how it affects collagen or collagenase synthesis *in vivo* is still unknown.

Recent work using a sponge matrix model suggested that TNF may be present in normal wound fluids but levels were minimal (1.4 U/mL) and may not have biologic relevance.[15] Furthermore contradictory evidence exists regarding whether local application of TNF to wounds improves healing. One recent study[16] indicates that it does, while another recent study[17] suggests that it does not. In light of *in vitro* evidence for a beneficial effect of TNF on wound healing[3-13] and contradictory *in vivo* evidence,[15-17] we performed the following study to determine if the gene for TNF was expressed in wounds and to identify the effects of local application of recombinant human TNF (rTNF) on normal and doxorubicin-impaired healing.

Materials and Methods

Materials

Recombinant human TNF was a gift of Cetus Corporation (Emeryville, CA) and had a specific activity of 10^7 U/mg, as measured in the L929 bioassay and an endotoxin level of 30 pg/2.5 $\times$ 10^6 U as measured by a standard limulus assay.[18] The human type I α (1)—collagen cDNA probe and the human glyceraldehyde-phosphate dehydrogenase (GAPDH) cDNA probe all have been described previously.[19] The human TNF-α probe also has been described before.[20] Phosphate-buffered saline (PBS) was purchased from Biofluids (Rockville, MD). Doxorubicin (Adriamycin, Adria Laboratories, Dublin, OH) was purchased from Farmitalia Carlo Erba (Milan, Italy).

General Wound Model

Adult Male Fischer 344 rats weighing between 150 to 200 g were anesthetized with 35 mg/kg sodium pentothal (intraperitoneally) and shaved. Five-centimeter paired linear dorsal incisions were made 2 cm from the midline. The wounds were dressed with sterile absorbable collagen sponges (Helistat, US Surgical Corp., Norwalk, CT) into which rTNF (0.5, 5, 50 μg in 0.2 mL PBS with 0.5% bovine serum albumin [BSA]) or vehicle alone (PBS with 0.5% BSA) was placed, and covered with Bioclusive transparent dressings (Johnson and Johnson Products Inc., New Brunswick, NJ) that were held in place with Mastisol, (Ferndale Laboratories, Ferndale, MI) and skin staples (Accustaple, Deknatel, Division of Howmedica, Queens Village, NY). Animals then each received an intravenous injection of doxorubicin (6 mg/kg) or an equivalent volume of 0.9% sodium chloride *via* the dorsal penile vein. Animals were killed at designated intervals by sodium pentothal overdose. The pelts were harvested and a 1 cm $\times$ 3 cm wound specimen was taken from the middle of the wound for wound-bursting strength (WBS), using a standard cutting form. Wound-bursting strength was measured on a Tensiometer (Instron, Canton, MA) at a velocity of 10 cm/min and was expressed in grams. The remaining part of the wound was excised and immediately placed in liquid nitrogen for RNA studies. Wound specimens were stored at −70 C.

In the initial experiment, 51 rats were wounded and randomly allocated to either the doxorubin or saline treatment groups and had one of three doses of rTNF (0.5, 5.0, and 50.0 μg) applied locally to one of the wound pair. These animals were each killed 7 days later. In a second experiment, 40 rats were wounded and allocated to either doxorubicin or saline treatment and two possible doses of rTNF (5.0 and 50 μg) applied locally as before. These animals had WBS measured 14 days later. In a final experiment, 40 rats were wounded and randomly treated with either doxorubicin and saline and one of three possible doses of rTNF applied locally to one of the wound pair. Wounds were harvested for RNA analysis 3, 7, 10, and 14 days after wounding.

Northern Blotting and Processing of Wounds for RNA

Total RNA extraction was a modification of the method of Chirgwin et al.[21] Frozen wound tissue (0.1 to 0.5 g) was ground into a fine powder using a mortar and pestle, homogenized in guanidine isothiocyanate buffer, placed on a cesium chloride gradient, and centrifuged overnight in a SW41 rotor (Beckman Instruments, Palo Alto, CA) at 30,000 RPM. The RNA pellet was taken up in 0.3 mol/L sodium acetate and precipitated with chilled, absolute ethyl alcohol. The resulting pellet was resuspended in autoclaved, deionized, distilled water. RNA concentration and purity was evaluated using a Beckman DU-50 spectrophotometer (Beckman Instruments) and a minigel. Thirty micrograms total RNA was loaded on a 1% Agarose gel containing 2.2 mol/L formaldehyde and run at 20 mV overnight. Gels were stained with ethidium bromide and then transferred to nitrocellulose filters (Bethesda Research Labs, Gaithersburg, MD) by capillary blotting in 10X sodium chloride sodium citrate (SSC) buffer. The nitrocellulose filters were then vacuum baked for 3 hours. The filters were prehybridized for 2 to 4 hours at 42 C in prehybridization solution containing formamide, 5X SSC, TRIS buffer, Denhart's solution, and Salmon Sperm DNA (from 5'3', West Chester, PA) 20 μg/mL. Probes were labeled with P^{32} by the Random Primer labeling method (Lofstrand Labs, Rockville, MD) and filters were then hybridized overnight using 2×10^7 CPM of labeled probe (specific activity 5×10^6 CPM/25 ng) in hybridization solution containing the above prehybridization solution components as well as 10% dextran sulfate. Filters were

then washed extensively with 1X SSC with 0.1% sodium dodecyl sulfate and finally with 0.5% SSC. Filters were then exposed to x-ray film at −70 C with an intensifying screen. The films were processed and scanned using a Beckman DU-8 Spectrophotometer (Beckman Instruments). The collagen mRNA index was calculated as described previously[19] by normalization with GAPDH for loading differences and comparison to FS-4 fibroblast or normal rat skin as a positive control because these cells express mRNA for the type 1 collagen gene. RNA from peritoneal exudative cells stimulated with endotoxin and probed for TNF served as the positive control for the TNF gene.

Cell RNA Samples

FS-4 fibroblasts were a gift from Dr. Jan Vilchek, New York University, New York, NY. Two large flasks of cells grown to confluence were harvested and washed in sterile, autoclaved PBS. Cells were resuspended in guanidium isothiocyanate (GIT) buffer, vortexed for 2 minutes, and then processed for RNA extraction as above and probed as for the tissue specimens.

Rat thioglycollate elicited peritoneal macrophages were harvested as previously described[22] and incubated for 2 hours at 37 C in the presence of lipopolysaccharide (10 μg/mL). Cells were then processed for RNA as described above.

Statistics

Data are presented as mean ± standard error of the mean and compared by dependent and independent Student's t test.

Results

Wound-bursting Strength

Recombinant TNF decreases WBS at 7 days in saline-treated animals when rTNF was applied locally at a dose of 50 μg ($p < 0.05$) (Fig. 1). There was no effect on WBS in saline-treated animals at local rTNF doses of 0.5 or 5 μg. In the doxorubicin-treated animals, there was a significant decrease in WBS at local rTNF doses of 5 and 50 μg ($p < 0.05$, Fig. 1). Seven days after wounding there were no differences in WBS of control wounds from saline-treated rats compared to the same wounds of doxorubicin-treated rats (Fig. 1). However locally applied TNF at a dose of 5 μg significantly decreased WBS in doxorubicin-treated rats compared to saline-treated animals that received the same dose of local rTNF ($p < 0.05$) (Fig. 1). At 14 days after wounding there were no significant differences in WBS of wounds treated locally with either dose of rTNF compared to paired control wounds treated with buffered vehicle (Fig. 2). At 14 days rats previously given intravenous doxorubicin had significantly reduced

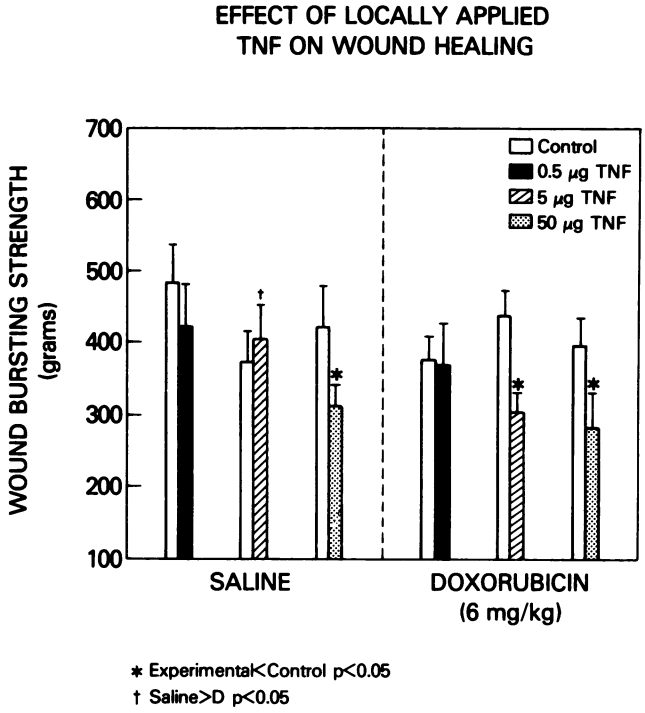

FIG. 1. Effect of locally applied TNF on wound-bursting strength (WBS) of 7-day wounds. Rats were treated with either saline (left panel) or doxorubicin (right panel) and wounded. Either vehicle or rTNF (0.5, 5, or 50 μg) was applied locally to paired wounds on the day of wounding. Wound bursting strength in grams was measured 7 days after wounding. *The local application of 50 μg of rTNF in saline-treated rats significantly reduced WBS compared to vehicle-treated paired wounds ($p < 0.05$). *The local application of either 5 or 50 μg of TNF in doxorubicin-treated rats significantly reduced WBS compared to vehicle-treated paired wounds ($p < 0.05$). †Doxorubicin administration significantly decreased WBS in wounds treated locally with 5 μg of TNF ($p < 0.05$).

WBS compared to animals given saline (Fig. 2). The same doxorubicin-induced reduction in WBS was present with or without locally applied rTNF (Fig. 2).

Wound RNA

Northern blots of RNA from FS-4 fibroblasts (Fig. 3A) and normal rat skin (Fig. 3B) hybridized against a specific cDNA probe for type I collagen demonstrate the presence of message and serve as a positive control. The collagen message is not expressed by macrophages that serve as a negative control (Fig. 3B). Wounds from doxorubicin-treated rats (dox) have decreased levels of collagen gene expression compared to wounds from saline-treated animals (con) (dox 7 days *versus* con 7 days, Fig. 3A and dox 7 days *versus* con 7 days, Fig. 3B). When normalized for inadvertent loading variability by comparing it to expression of the gene for GAPDH (collagen index), the reduction in collagen gene expression by doxorubicin is approximately 40%. Local rTNF treatment decreased collagen gene expression in wounds from saline-treated animals at doses of 0.5, 5.0, and 50 μg (sal 7 days/TNF 50, sal 7 days/TNF 5, and sal 7 days/TNF 0.5 *versus* CON

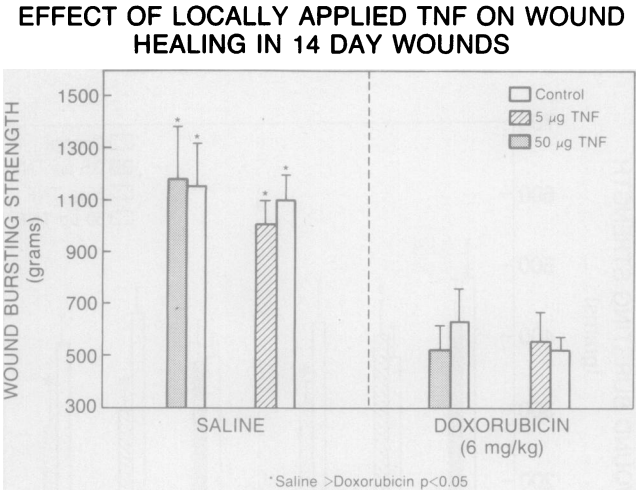

FIG. 2. Effect of locally applied TNF on WBS of 14-day wounds. Rats were treated as in Figure 1 except that only two doses of local rTNF were used, either 5 or 50 μg. Local application of either dose of rTNF at wounding did not affect WBS measured 14 days later. Systemic administration of doxorubicin (6 mg/kg IV) significantly reduced 14-day WBS whether the wound was treated locally with vehicle or rTNF ($p < 0.05$).

7 days, Fig. 3B). When adjusted for loading differences, the negative effect of rTNF on collagen gene expression appeared to be dose related: as the dose of rTNF increases the amount of collagen gene expression decreases (Fig. 3b). In doxorubicin-treated rats there is also a dose-related decrease in collagen gene expression with local rTNF treatment at doses of 5 (dox 7 days/TNF 5 *versus* dox 7 days, Fig. 3B) and 50 μg (dox 7 days/TNF 50 *versus* dox 7 days, Fig. 3A). The reduction in collagen gene expression with the combination of 50 μg of local TNF and intravenous doxorubicin was so profound that collagen gene expression is barely detectable 7 days after treatment at a time when control wounds have a collagen index greater than 2 (Fig. 3A).

Northern blot analysis for the expression of the TNF gene (Fig. 4) is positive only for the endotoxin-stimulated macrophages that are known to produce TNF[22] and serve as a positive control. RNA from wounds of saline-treated or doxorubicin-treated rats demonstrate no evidence for the expression of the gene for TNF (Fig. 4).

Discussion

Despite the previous reports of *in vitro* (fibroblast proliferation, chemotaxis, and angiogenesis)[3-10,12] and *in vivo*[16] data that suggest that TNF may improve wound healing, the present data suggest that exogenous rTNF applied locally to wounds impairs both WBS and collagen gene expression. The detrimental effects of TNF on WBS were apparent in both saline- and doxorubicin-treated animals 7 days after wounding. In doxorubicin-treated rats a decrease in WBS was seen with a lower dose of rTNF than in saline-treated rats (Fig. 1), which suggests that the deleterious effects of local TNF on WBS may be even more substantial during conditions that are known to impair healing. This observation further indicates that local application of rTNF will not be useful to correct healing in conditions associated with impaired healing. In fact the use of it in these conditions may have the opposite effect and further worsen the healing process.

Local application of TNF had no apparent effect on bursting strength 14 days after wounding in either saline- or doxorubicin-treated rats, suggesting that the initial reduction in WBS seen with locally applied TNF may be reversible with time (Fig. 2). The results of northern blot analysis for the gene for type-1 collagen also indicated

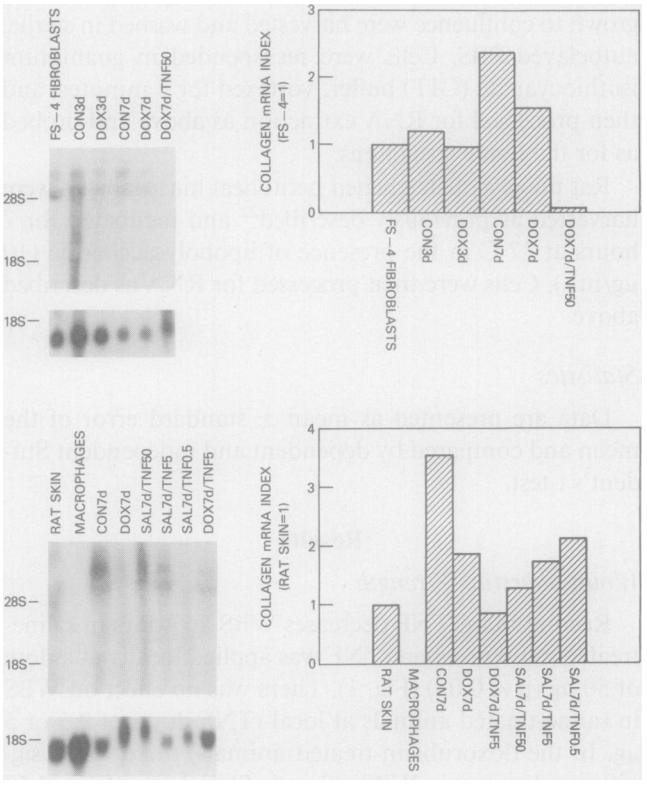

FIG. 3A and 3B. Collagen gene expression in wounds. A, (top) Gel (left) and Calculated collagen mRNA index (right) for wounds harvested at either 3 or 7 days after wounding normalized to FS-4 fibroblasts (positive control) by calculation of the collagen mRNA index. Gels probed with cDNA for type-1 collagen and glyceraldehyde-phosphate dehydrogenase GAPDH. Collagen mRNA expression is presented as an index by normalization with GAPDH for loading differences and comparison with FS-4 cells (index = 1). Abbreviations used: CON = control, 3d or 7d postwounding day 3 or 7, respectively, DOX = doxorubicin, TNF 50 = 50 μg tumor necrosis factor applied to the wound. B, (bottom). Same as A except rat skin is positive control and macrophages are negative control. Collagen mRNA expression is presented as an index by normalization with GAPDH for loading differences and comparison with normal rat skin (index = 1). Abbreviations used: same as A except SAL = saline-treated IV, TNF 0.5, 5, and 50 = 0.5, 5, and 50 μg of TNF applied to the wound.

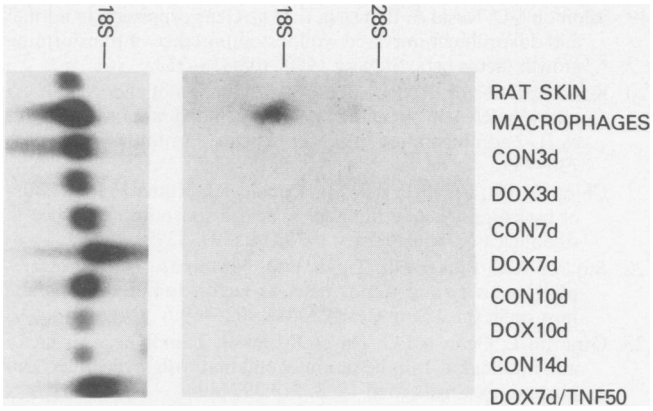

FIG. 4. Tumor necrosis factor gene expression in wounds. Gel demonstrating TNF-α gene expression in rat skin, macrophages treated with endotoxin and various treated wounds 3, 7, 10, and 14 days after wounding. Gels probed with cDNA for TNF and cDNA for GAPDH. Despite the fact that each lane had RNA present (blot) with GAPDH probe), only macrophages had evidence of the gene for TNF (positive control). The TNF gene was not expressed in normal rat skin or in any wound tested. Abbreviations used: CON = control, DOX = doxorubicin treated, 3, 7, 10, 14 d = 3, 7, 10, 14 days after wounding, and TNF 50 = wound treated locally with 50 μg TNF.

that the local application of rTNF to wounds reduced the expression of the gene for collagen in a dose-dependent manner (Fig. 3). This effect was again additive with doxorubicin in that doxorubicin also decreases the expression of this gene but the application of rTNF decreased it further. This finding suggests that one mechanism of rTNF's reduction in WBS is decreased synthesis of collagen, the basic protein element in healing. The application of TNF to fibroblast cultures *in vitro* also has resulted in decreased collagen gene expression.[13] In other words rTNF appears to have a direct inhibitory effect on the gene that controls new collagen synthesis.

Northern blots for collagen also demonstrated that 7 days after wounding doxorubicin-treated animals had decreased levels of collagen mRNA expression relative to saline-treated controls (Figs. 3A and B), despite the fact that the WBS were comparable (Fig. 1). This reduction in new collagen synthesis resulted in a decrease in WBS of doxorubicin-treated rats (compared to saline-treated controls) 14 days after wounding (Fig. 2).

When the present observations that local application of rTNF can reduce WBS and collagen gene expression are combined with the fact that the message for TNF is not present in either normal or doxorubicin-treated wounds (Fig. 4), it is difficult to suggest that the paracrine action of TNF may be used as a strategy to improve impaired healing. Rather these results suggest that TNF is not a local factor in normal or chemotherapy-impaired wound healing and that conditions resulting in high local levels of TNF may cause reduced collagen gene expression and impaired healing. Pathologic conditions that are known to result in elevated serum levels of TNF also are associated with impaired wound healing. These include gram-negative endotoxic shock,[23,24] acquired immune deficiency syndrome,[25] meningococcemia,[26] and cancer.[27] In addition, during recent investigations in our laboratory, we administered sublethal systemic doses of rTNF to rats and measured WBS. As expected from the current results, we found that systemically administered rTNF significantly reduced WBS when administered twice daily for 7 days after wounding (unpublished observations). This finding is consistent with the hypothesis that disease states associated with elevated circulating levels of TNF may mediate a healing impairment through the presence of systemic levels of TNF. However further investigations will be necessary to determine unequivocally if the wound healing impairment associated with these conditions is, in fact, mediated through an effect of TNF.

Despite previous reports of *in vitro*[3-10,12] and *in vivo*[16] evidence that suggests that tumor necrosis factor may be an important acute mediator of healing[12] and that the addition of exogenous TNF may improve impaired healing,[16] the present results suggest that the gene for TNF is not expressed in either normal or doxorubicin-impaired wounds and that when exogenous TNF is added to wounds it reduces the expression of the gene for type 1 collagen and it reduces the force required to disrupt the wound. These changes appear to be amplified by the concurrent administration of doxorubicin, a substance known to inhibit healing. Our results suggest that the local application of TNF will not be useful to improve impaired healing.

References

1. Tracey KJ, Fong Y, Hesse DG, et al. Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. Nature 1987; 330:662–664.
2. Tracey KJ, Wei H, Manogue KR, et al. Cachectin/tumor necrosis factor induces cachexia, anemia, and inflammation. J Exp Med 1988; 167:1211–1227.
3. Sugarman BG, Aggarwal BB, Hass PE, et al. Recombinant human tumor necrosis factor-α: effects on proliferation of normal and transformed cells *in vitro*. Science 1985; 230:943–945.
4. Dayer JM, Beutler B, Cerami A. Cachectin/TNF stimulates collagenase and prostaglandin E2 production by human synovial cells and dermal fibroblasts. J Exp Med 1985; 162:2163–2168.
5. Kohase M, Henrikson-Destefano D, May LT, et al. Induction of β 2-interferon by tumor necosis factor: a homeostatic mechanism in the control of cell proliferation. Cell 1986; 45:659–666.
6. Pober JS, Gimbrone MA Jr, Lapierre LA, et al. Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. J Immunol 1986; 136: 1680–1687.
7. Ming WJ, Bersan L, Mantovani A. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. J Immunol 1987; 138:1469–1474.
8. Philip R, Epstein LB. Tumor necrosis factor as immunomodulator and mediator of monocyte cytotoxicity induced by itself, Γ-interferon and interleukin-1. Nature (Lond) 1986; 323:86–89.
9. Leibovitch SJ, Polverini PJ, Shepard HM, et al. Macrophage-induced

angiogenesis is mediated by tumour necrosis factor-α. Nature (Lond) 1987; 329:630–632.
10. Hajjar KA, Hajjar DP, Silverstein RL, Nachman RL. Tumor necrosis factor-mediated release of platelet-derived growth factor from cultured endothelial cells. J Exp Med 1987; 166:235–245.
11. Brenner DA, O'Hara M, Angel P, et al. Prolonged activation of jun and collagenase genes by tumor necrosis factor-α. Nature 1989; 337:661–663.
12. Sherry B, Cerami A. Cachectin/tumor necrosis factor exerts endocrine, pararine and autocrine control of inflammatory responses. J Cell Biol 1988; 107:1269–1277.
13. Solis-Herruzo JA, Brenner DA, Chojkier M. Tumor necrosis factor inhibits collagen gene transcription and collagen synthesis in cultured human fibroblasts. J Biol Chem 1988; 263:5841–5845.
14. Vlassara H, Brownless M, Manogue KR, et al. Cachectin/TNF and IL-1 induced by glucose-modified proteins: role in normal tissue remodelling. Science 1988; 240:1546–1548.
15. Ford HR, Hoffman RA, Wing EJ, Magee DM, et al. Characterization of wound cytokines in the sponge matrix model. Arch Surg 1989; 124:1422–1428.
16. Mooney DP, Gamelli RL, O'Reilly M. Improved wound healing through the local delivery of tumor necrosis factor. Surgical Forum 1988; 39:77–79.
17. Steenfos HH, Hunt TK, Scheuenstuhl H, Goodson WH. Selective effects of tumor necrosis factor-alpha on wound healing in rats. Surgery 1989; 106:171–176.
18. Fraker DL, Stovroff MC, Merino MJ, Norton JA. Tolerance to tumor necrosis factor in rats and the relationship to endotoxin tolerance and toxicity. J Exp Med 1988; 168:95–105.
19. Salomon GD, Kasid A, Bernstein E, et al. Gene expression in normal and doxorubicin-impaired wounds: importance of transforming growth factor-beta. Surgery 1990; 108:318–323.
20. Kasid A, Director EP, Rosenberg SA. Induction of endogenous cytokine-mRNA in circulating peripheral blood mononuclear cells by IL-2 administration to cancer patients. J Immunol 1989; 143: 736–739.
21. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979; 18:5294–5299.
22. Stovroff MC, Fraker DL, Travis WD, Norton JA. Altered macrophage activity and tumor necrosis factor: tumor necrosis and host cachexia. J Surg Res 1989; 46:462–469.
23. Girardin E, Georges EG, Dayer JM, et al. Tumor necrosis factor and interleukin-1 in the serum of children with severe infectious purpura. N Engl J Med 1988; 319:397–400.
24. Hesse DG, Tracey KJ, Fdong Y, et al. Cytokine appearance in human endotoxemia and primate bacteremia. Surg Gynecol Obstet 1988; 166:147–153.
25. Lahdevirta J, Maury CP, Teppo AM, Repo H. Elevated levels of circulating cachectin/tumor necrosis factor in patients with acquired immunodeficiency syndrome. Am J Med 1988; 85:289–291.
26. Waage A, Halstensen A, Espevik T. Association between tumour necrosis factor in serum and fatal outcome in patients with meningococcal disease. Lancet 1987; i:355–357.
27. Balkwill F, Burke F, Talbot D, et al. Evidence for tumor necrosis factor/cachectin production in cancer. Lancet 1987; ii:1229–1232.