ANNALS OF SURGERY
Vol. 217, No. 6, 668–675

Tumor Necrosis Factor-Induced Mortality Is Reversed with Cyclooxygenase Inhibition

J. Raymond Fletcher, M.D., Ph.D., Jimmie N. Collins, M.D., Ernest D. Graves, III, M.D., Arnold Luterman, M.D., Mark D. Williams, M.D., Seth D. Izenberg, M.D., and Charles B. Rodning, M.D., Ph.D.

From the Department of Surgery, University of South Alabama, College of Medicine, Mobile, Alabama

Objective

The authors hypothesized that TNF would induce eicosanoid synthesis, and a cyclooxygenase inhibitor would attenuate both eicosanoid synthesis and improve survival in an LD90 TNF-induced (150 ng/kg/IV/5 min) mortality model.

Summary Background Data

Tumor necrosis factor is a cardinal mediator in sepsis; however, little is known about its effects on arachidonate metabolism.

Methods

Conscious male rats with carotid arterial and jugular venous catheters were randomized for mortality: group I, TNF alone (150 kg/IV/15 min, n = 30); group II, ibuprofen (30 mg/kg/IV at t = −20 and +240 min), plus TNF, (n = 28); and for hemodynamics, eicosanoid synthesis, blood gases: group III, TNF alone, (n = 8); group IV, ibuprofen + TNF (n = 8); group V, monoclonal antibody to TNF plus TNF (n = 8). Mortality was determined at 4–72 hr. Other parameters determined over 4 hours (0, 5, 60, 120, 240 min).

Results

TNF stimulated synthesis of (a) TXB_2 (71 ± 30 pg/ml, mean ± SE at base vs. 117 ± 18 at 4 hr, $p < 0.02$); (b) PGE_2 (70 ± 6 pg/ml at base vs. 231 ± 68 at 4 hr, $p < 0.02$); (c) 6PGF (52 ± 6 pg/ml at base vs. 250 ± 80 at 4 hr, $p < 0.02$). Ibuprofen significantly ($p < 0.05$) inhibited eicosanoid synthesis from TNF. TNF-induced mortality (87%, 26/30) was dramatically decreased with Ibuprofen (11%, 3/28), at 4, 24, and 72 hr ($p < 0.01$). Monoclonal antibody to TNF prevented all abnormalities and had 100% survival. Hemodynamic events were similar in both groups, but metabolic acidosis was attenuated with ibuprofen.

Conclusions

TNF stimulates arachidonic acid metabolism *in vivo*. A cyclooxygenase inhibitor attenuates eicosanoid synthesis and dramatically improves survival. TNF appears to have different effect on tissues that synthesize certain eicosanoids. Hypotension from TNF is not mediated via the eicosanoids. TNF-induced mortality, like endotoxemia/sepsis may be mediated, in part, via arachidonic acid metabolites. These new findings support the notion that cyclooxygenase inhibitors may be used as adjunctive therapy in clinical sepsis.

That tumor necrosis factor (TNF) occupies a pivotal role in the pathogenesis of endotoxemia/sepsis is supported by an increasing volume of literature.[1-5] The support for the central role of TNF in endotoxemia/sepsis is derived from several lines of evidence: a) TNF is present in the serum of man and animals with endotoxemia/sepsis;[6-9] b) Injection of TNF in animals induces pathophysiological events (hypotension, multiple organ failure, vascular permeability) which are similar to those observed with endotoxemia/sepsis in man;[10-12] and c) anti-TNF antibodies enhance the survival and reduce physiologic abnormalities after endotoxemia/sepsis in animals.[13-14] These reports demonstrate that TNF is an important contributor to endotoxemia/sepsis; however, these studies do not identify the mechanism(s) by which TNF exerts its systemic effects.

The mechanisms by which TNF produces pathophysiologic events is uncertain. *In vitro,* TNF releases interleukin 1,[15] phospholipase A_2,[16] free fatty acids,[17] arachidonic acid,[18] cyclic Amp,[19] changes in signal transduction,[20] and stimulates prolactin synthesis,[21] to enumerate a few of the proposed mechanisms of its effects. These observations suggest that TNF has a myriad of effects depending on the type of cells used, the species of animal chosen, and the insult being evaluated (dose).

Two recent studies *in vivo* suggested that the eicosanoids may have a substantive role in mediating TNF-induced injury. Michie et al.[8] reported an increase in TNF plasma values after intravenous administration of *E. coli* endotoxin in human volunteers. The investigators used ibuprofen pretreatment (800 mg) at 90 minutes before and at the time of injection of endotoxin. The ibuprofen attenuated the symptoms and other responses after endotoxin. The eicosanoids have been reported to have a regulatory role in TNF synthesis.[22,23]

This study specifically determined the effects of TNF on eicosanoid synthesis *in vivo* and the effects of a cyclooxygenase inhibitor on eicosanoid synthesis and on the pathophysiologic events and mortality induced by TNF.

METHODS

Male Sprague-Dawley rats (250 to 300 g) (Charles River Laboratories) were stabilized from 2 to 4 days before experimentation. They were maintained in a 12-hour light-dark sequence and allowed water and antibiotic-free chow *ad libitum.* On the day of the experiment, animals were placed in individual cages. Each animal was lightly anesthetized with halothane/oxygen mixture, had an anterior neck incision, followed by insertion of catheters (PE50) into the left carotid artery and left jugular vein. Catheters were flushed with heparinized saline, tunneled to the dorsal neck of the rat, and secured. The jugular vein catheter was used for injections of saline or pharmacologic agents. Rats were allowed to recover from the effects of anesthesia for at least 1 hour before baseline parameters were measured. At the completion of the experiments, animals were killed.

Hemodynamic measurements were obtained by connecting the carotid catheters to a Gould Bush physiograph (RS2300) via a PE23 transducer (Statham, Oxnard, CA). Thromboxane (TxB_2), PGE_2, and 6-keto-$PGF1_\alpha$ (6PGF) were measured in plasma samples collected at baseline, then at 5, 60, 120, and 240 minutes after TNF infusion in coincidence with hemodynamic measurements. Eicosanoid analyses were performed by radioimmunoassay in batches. Crossreactivity of the antibodies used was less than 3% with other eicosanoid metabolites. Radiolabeled TXB_2, PGE_2, 6PGF were obtained from New England Nuclear (Boston, MA). Authentic eicosanoids were supplied by Dr. John Pike (Upjohn Co., Kalamazoo, MI). Tumor necrosis factor (TNF) was obtained from Peninsula Laboratories (Belmont, CA) and diluted with sterile saline on the day of the experiment. Monoclonal antibody to TNF was obtained as a rabbit anti-murine polyvalent antiserum that contained no endotoxin and no preservative and did not cross react with human TNFα (Biotrans, Inc., Los Angeles, CA). Arterial blood gases were determined using an automated blood gas analyzer (Radiometer, model ABL-30, Copenhagen). Ibuprofen was kindly supplied by the Upjohn Company (Kalamazoo, MI) as a sterile solution, 50 mg/ml.

EXPERIMENTAL DESIGN

All experiments were performed in an American Association for Accreditation for Laboratory Animal Care approved facility and according to the National Institutes of Health guidelines for animal use. Animals were studied in individual cages with free access to food and water. Indwelling catheters were flushed with sterile heparinized saline. There was a 20-minute basal period (−20 to 0 min) and a 4-hour (0–240 min) experimental period during which hemodynamics, eicosanoids, arterial blood gases were determined at designated time intervals. At time 0, the animals received an IV infusion of TNFα over 5 minutes. In studies in which the effects of a cyclooxygenase inhibitor were examined, ibuprofen (30 mg/kg) was administered at t = −20 min and t = +240 min.

Supported in part by NIH grant T32GM08154-05.
Presented in part at the Second International Conference on Shock, Vienna, Austria, 1991.
Address reprint requests to J. Raymond Fletcher, M.D., Department of Surgery, University of South Alabama, College of Medicine, Mobil, AL 36617.
Accepted for publication February 2, 1993.

For the survival studies, rats were randomized into two groups. Group I received TNF alone, 150 ng/kg/IV/5 min (n = 30). Group II received ibuprofen, 30 mg/kg/IV at t = −20 min and t = +240 min, then TNF, 150 ng/kg/IV/5 min at t = −0 min (n = 28). Group III was treated with monoclonal antibody to TNF, 1 mg/kg at t = −60 min, plus TNF, 150 ng/kg/IV/5 min at t = 0 min (n = 8). Mortality rates were determined at 2, 4, 24, 72 hours and reported as a percentage.

The animals used for hemodynamic, eicosanoid, and blood gas analyses were randomized in two groups after catheter insertion. Group IV received TNF alone, 150 ng/kg/IV/5 min, (n = 8) and group V was administered ibuprofen, (30 mg/kg/IV, at t = −20 min and t = +240 min, plus TNF, 150 ng/kg/IV/5 min, (n = 8). Blood samples for eicosanoid analyses were collected in heparinized tubes containing indomethacin (100 ug); the blood was centrifuged, the plasma was removed and stored at −80 C until assayed. Arterial blood gas samples were collected in heparinized tubes and immediately analyzed. For each milliliter of blood removed, 1 ml of sterile 0.9% saline (w/v) was returned to the animal via the arterial catheter.

The selection of the dose of TNF utilized and the observation time for mortality was determined in a series of pilot dose response studies in our laboratory. Prior to these experiments, control studies in animals not given TNF were performed in rats with the following groups: anesthesia alone (n = 4), anesthesia plus incision and ligation of vessels (n = 5); anesthesia, incision, catheter placement and vehicle administration (saline) for TNF (n = 5). Since none of these groups had any significant perturbations on either the hemodynamic or eicosanoid changes, these studies were not repeated.

Data analysis was accomplished using ANOVA for repeated measures within and between groups with a least squares difference test (Sheffe) as a follow up test. Differences in survival were determined by the chi squared method. A value of $p < 0.05$ was considered significant.

RESULTS

TNF-induced hemodynamic events in conscious rats are seen in Table 1. TNF alone significantly decreased MAP by 240 minutes when compared with the baseline values. Even with ibuprofen pretreatment, there was a significant decrease in MAP at 240 minutes, when compared with the baseline; however, this decrease was significantly less than with TNF alone. Monoclonal antibody to TNF prevented any significant changes in MAP. There were no significant differences in the observed heart rates between the groups at any observation point during the study.

Arterial blood gases and serum bicarbonate values are shown for the groups studies in Table 2. Animals that received TNF alone had a significantly greater metabolic acidosis than did the other groups by 240 minutes. Even though the animals decreased their pCO_2 to compensate for the changed metabolic events, animals that received TNF alone had the greater difficulty in respiratory compensation and the greatest decrease in serum bicarbonate. Interestingly, the lowest pCO_2 values were observed at 240 minutes in the TNF group and the lowest pO_2 values were seen in the Ibu/TNF group at 60 and at 240 minutes. Both of these values were significantly less than the baseline and significantly less than the corresponding values in the other groups. Serum bicarbonate values at 240 minutes in both the TNF alone and the Ibu/TNF group were significantly less than the baseline values; however, Ibu/TNF values of HCO_{3-} were greater than TNF alone.

Table 1. EFFECTS OF TNF ON MEAN ARTERIAL PRESSURE AND HEART RATE WITH AND WITHOUT CYCLOOXYGENASE INHIBITION

Mean arterial blood pressure, torr				
Time/min	0	60	120	240
Control	110 ± 5	125 ± 7	110 ± 7	108 ± 8
TNF alone	108 ± 5	127 ± 5	110 ± 8	40 ± 4*
Ibu/TNF	118 ± 10	123 ± 4	113 ± 8	86 ± 6*†
Mab/TNF	110 ± 7	112 ± 10	106 ± 9	114 ± 6
Heart rate, beats/min				
Time/min	0	60	120	240
Control	378 ± 13	404 ± 20	418 ± 22	440 ± 18
TNF alone	372 ± 7	378 ± 10	447 ± 21	440 ± 30
Mab/TNF	380 ± 20	410 ± 15	400 ± 14	420 ± 30

* Significantly different from baseline $p < 0.01$.
† Significantly different from TNF alone $p < 0.05$.
Mean ± SEM, n = 8.

Table 2. EFFECTS OF TNF ON ARTERIAL BLOOD GAS AND SERUM BICARBONATE WITH AND WITHOUT CYCLOOXYGENASE INHIBITOR

	Time/min		
	0	60	240
pH			
Control	7.34 ± 0.09	7.33 ± 0.03	7.36 ± 0.04
TNF alone	7.32 ± 0.01	7.30 ± 0.01	7.1 ± 0.01*
Ibu/TNF	7.33 ± 0.01	7.27 ± 0.01	7.20 ± 0.01
Mab/TNF	7.35 ± 0.03	7.36 ± 0.02	7.32 ± 0.01
*p < 0.01 vs. control and Mab/TNF.			
pCO_2 torr			
Control	21.2 ± 1.2	23 ± 2	22 ± 1.6*
TNF alone	20.7 ± 1.4	25.9 ± 2	17.8 ± 2.6†
Ibu/TNF	19.8 ± 1.9	21 ± 2	20.7 ± 1.2
Mab/TNF	19.6 ± 1.4	21 ± 2	20.7 ± 1.2
*p < 0.005 vs. TNF; †p < 0.05 vs. baseline.			
pO_2 torr			
Control	98 ± 4	110 ± 3	108 ± 6
TNF alone	104 ± 10	119 ± 10	137 ± 8
Ibu/TNF	102 ± 6	90 ± 13	81 ± 9*
Mab/TNF	100 ± 6	106 ± 4	102 ± 7
*p < 0.05 vs. TNF.			
HCO_{3^-} units/ml			
Control	10 ± 0.05	11 ± 0.4	9 ± 0.3
TNF alone	9.3 ± 0.06	10.8 ± 0.7	6.8 ± 0.8†
Ibu/TNF	10.2 ± 0.6	8.5 ± 1.1	7.9 ± 1.5*†
* p <0.05 vs. TNF; †p < 0.05 vs. baseline.			

Mean ± SEM, n = 7.

The effect of TNF on synthesis of the eicosanoids is shown in Table 3. TNF stimulated the synthesis and/or release of thromboxane (TxB_2), prostaglandin E_2, and prostacyclin gradually, but significantly during the 4-hour observation period. The values of prostacyclin (PGF) and PGE_2 increased 3–5-fold at 240 minutes, whereas thromboxane values had increased significantly but modestly. Ibuprofen significantly attenuated the synthesis/release of TXB_2, 6PGF, and PGE_2 after TNF infusion although there was a gradual rise in both PGF and PGE_2 over time.

The dose response effects of TNF on mortality are shown in Table 4. The dose of 150 ng/kg/IV was chosen for this study versus the 600 ng/kg/IV dose to preclude

Table 3. EFFECTS OF TNF ON TXB_2, PGF, PGE_2 SYNTHESIS/RELEASE WITH AND WITHOUT CYCLOOXYGENASE INHIBITOR *IN VIVO* (PG/ML)

	Time/Min				
	0	5	60	120	240
TXB_2					
TNF alone	71 ± 17	102 ± 10	102 ± 28	158 ± 65	117 ± 18*
Ibu/TNF	148 ± 20	46 ± 5	63 ± 7	92 ± 7	41 ± 6†
6-Keto-$PGF_{1\alpha}$					
TNF alone	54 ± 18	166 ± 93	77 ± 47	151 ± 72	250 ± 80*
Ibu/TNF	35 ± 6	37 ± 5	32 ± 5	79 ± 3†	71 ± 17†
PGE_2					
TNF alone	70 ± 6	84 ± 3	76 ± 5	210 ± 85	230 ± 68*
Ibu/TNF	35 ± 6	37 ± 5	32 ± 5	79 ± 3†	71 ± 19†

* p < 0.02 vs. baseline; †p < 0.01 vs. TNF alone.
Mean ± SEM, n = 8.

Table 4. EFFECTS OF TNF ON MORTALITY WITH OR WITHOUT CYCLOOXYGENASE INHIBITOR OR WITH MONOCLONAL ANTIBODY TO TNF

	Mortality %			
	2 hr	4 hr	24 hr	72 hr
TNF 60 ng/kg/IV	0 (0/6)	0 (0/6)	0 (0/6)	0 (0/6)
150 ng/kg/IV	70 (21/30)	87 (26/30)	100	—
600 ng/kg/IV	70 (4/6)	85 (5/6)	100	—
Ibu/TNF	11 (3/28)*	11 (3/28)*	11 (3/28)*	11 (3/28)*
Mab/TNF 1 mg/kg/IV	0 (0/8)*	0 (0/8)*	0 (0/8)*	0 (0/8)*

* $p < 0.01$ vs. TNF alone.

an overwhelmingly lethal model. Even with the 150 ng/kg dose, 87% of the animals died in 4 hours. Cyclooxygenase inhibition dramatically decreased the mortality as early as 2 hours after TNF infusion and produced permanent effects. There were no deaths with the use of a TNF monoclonal antibody as pretreatment.

DISCUSSION

The findings in this investigation are that a) TNF produces significant hypotension, metabolic acidosis, increased eicosanoid synthesis, and dramatic mortality in 4 hours; b) cyclooxygenase inhibition attenuates the TNF-induced hypotension, metabolic acidosis and substantively reverses TNF-induced mortality; c) monoclonal antibody to murine TNF prevented the effects of the synthetic TNF and demonstrates the recognition of the TNF by the antibody *in vivo;* d) the variability of TNF on eicosanoid synthesis indicates that some cells are more sensitive than others to the effects of TNF in inflammatory mediator release.

That TNF induces hypotension, metabolic derangements and death similar to that which occurs with endotoxemia/sepsis is consistent with results reported by others.[24–27] This study supports the previous *in vitro* findings that show that TNF stimulates eicosanoid synthesis and release[28,29] and suggests a major role TNF has in inflammatory mediator release. Most *in vitro* studies have determined prostaglandin E_2 (PGE_2) concentrations and have not reported TNF effects on the synthesis of other arachidonic acid metabolites. The rationale for determining other eicosanoids was that vascular endothelial cells (prostacyclin) and platelets (thromboxane), in addition to macrophages (PGE_2), are rich sources of these mediators and might indicate a generalized inflammatory response *in vivo* rather than a limited one.

It is well known that the eicosanoids participate in the inflammatory response of sepsis/endotoxemia.[30] If TNF occupies a central role in the pathophysiology of sepsis, then it could enhance eicosanoid synthesis and release as demonstrated in this study. The reasons that TNF had greater effects on PGE and PGF synthesis when compared with TXB_2 synthesis are speculative, but suggest vascular endothelial cells and macrophages in this model may be more sensitive to TNF than the rat platelet. Feuerstein et al. demonstrated little change in rat platelet counts after TNFα injection.[31]

The decision to administer the nonsteroidal anti-inflammatory drug (NSAID) ibuprofen, was based on observations reported by Michie et al.,[8] Kettlebut et al.,[32] Talmadge et al.,[33] Evans et al.,[34] and Carey et al.[35] These investigators demonstrated beneficial aspects of NSAIDS therapy related to TNF actions. Michie et al. showed the NSAIDS attenuated the constitutional and other systemic effects in human endotoxemia. Kettlebut reported improved survival in TNF-induced mortality similar to the present study. Evans and Talmadge demonstrated beneficial effects of ibuprofen on other effects of TNF. That ibuprofen attenuated the respiratory burst from neutrophils and decreased TNF levels in a pig model of bacteremia was shown by Carey, et al. In addition to these studies, a large body of evidence that supports the efficacy of NSAID in endotoxemia/sepsis exists.[35–39] The above findings suggest that the eicosanoids are likely to be a major effector of TNF *in vivo* and that nonsteroidal anti-inflammatory drugs will be useful in the therapy of disease states in which TNF has a putative role.

TNF has been heralded as the cardinal mediator of sepsis/endotoxemia. A review of pertinent literature on the possible mechanisms by which it exerts its effects is relevant.[5] TNF alters procoagulant activity,[40] induces expressions of specific antigens on endothelial cells,[41] increases the expression of intracellular adhesive molecules,[41] induces synthesis of platelet-activating factor,[42] and induces superoxide anion(O_2-) generation.[43] These findings indicate that TNF has a multitude of effects on the cell, including our findings of effects on eicosanoid synthesis. The complexity of these scientific inquires and

interrelationships are uncertain at the present time and remain a challenge for further investigation.

That a cyclooxygenase inhibitor would have such dramatic effect on survival in this severe model was unexpected. The dose of cyclooxygenase inhibitor used was determined from studies in which this dose inhibited eicosanoid synthesis from endotoxin injection. The dose of TNF used was selected from the dose-response curve presented in the results. The TNF used was synthetic TNF, which contains the highly conserved amino acids[114–130] that are common to rabbit, rat, and human TNF. A rabbit raised murine monoclonal antibody to TNF was administered to ensure the antibody recognized the TNF and prevented the effects of the synthetic TNF. Others have questioned the benefit of cyclooxygenase inhibition on survival[44] in TNF-induced mortality. There are several studies that report that passive immunization with monoclonal antibody will improve survival in systemic sepsis.[1,11,13,14,32] In addition, cyclooxygenase inhibition in studies related to TNF effects/plasma values TNF produce variable results depending on the models used and species selected.[18,20,23,29,33,34]

This study and others support the notion that TNF stimulates the synthesis/release of the cyclooxygenase end products. Further, the effects of cyclooxygenase inhibition on TNF-induced mortality indicate there may be a close relationship between TNF effects and the cyclooxygenase products. These findings are consistent with the effects of cyclooxygenase inhibitors on eicosanoid release and mortality in endotoxemia/sepsis. Additional studies are needed to determine the significance of these observations.

Acknowledgment

The authors thank Ms. Carolyn Clark for preparing this manuscript.

References

1. Beutler B, Milsark IW, Cerami AC. Passive immunization against cachectin/tumor necrosis factor protects mice from lethal effects of endotoxin. Science 1985; 229:869–871.
2. Remick DG, Strieter RM, Eskandari MK, et al. Role of tumor necrosis factor-a in lipopolysaccharide-induced pathologic alterations. Am J Pathol 1990; 136:49–60.
3. Waage A. Production and clearance of tumor necrosis factor in rats exposed to endotoxin and dexamethasone. Clin Immunol Immunopathol 1987; 45:348–355.
4. Mathison JC, Wolfson E, Ulevitch RJ. Participation of tumor necrosis factor in the mediation of gram negative bacterial lipopolysaccharide-induced injury in rabbits. J Clin Invest 1988; 81:1925–1937.
5. Simpson SW, Casey L. Role of tumor necrosis factor in sepsis and acute lung injury. Crit Care Clin 1989; 5:27–47.
6. Bauss F., Drage W, Männel DN. Tumor necrosis factor mediates endotoxic effects in mice. Infect Immunol 1987; 55:1622–1625.
7. Hesse DG, Tracey KJ, Fong Y, et al. Cytokine appearance in human endotoxemia and primate bacteremia. Surg Gynecol Obstet 1988; 166:147–53.
8. Michie HR, Manogue KR, Spriggs DR, et al. Detection of circulating tumor necrosis factor after endotoxin administration. N Engl J Med 1988; 318:1481–1486.
9. Damas P, Reuter A, Gysen P, et al. Tumor necrosis factor and interleukin-1 serum levels during severe sepsis in humans. Crit Care Med 1989; 17:975–978.
10. Tracey KJ, Beutler B, Lawry SF, et al. Shock and tissue injury induced by recombinant human cachectin. Science 1986; 234:470–474.
11. Remick DG, Kunkel RG, Larrick JW, Kunkel SL. Acute *in vivo* effects of human recombinant tumor necrosis factor. Lab Invest 1987; 56:583–590.
12. Tracey KJ, Lowry SF, Fahey TJ III, et al. Cachectin/TNF induces lethal shock and stress hormone responses in the dog. Surg Gynecol Obstet 1987; 164:415–422.
13. Tracey KJ, Fong Y, Hesse DG, et al. Anti cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteremia. Nature 1987; 330:662–664.
14. Hinshaw LB, Tekamp-Olson P, Chang AC, et al. Survival of primates in LD_{100} septic shock following therapy with antibody to tumor necrosis factor (TNF_α). Circ Shock 1990; 30:279–292.
15. Bachwich PR, Chensue Sw, Lanick JW, Kunkel SL. Tumor necrosis factor stimulates Interleukin-1 and Prostaglandin E_2 production in resting macrophages. Biochem Biophys Res Comm 1986; 136:94–101.
16. Suffys P, Byaert R, deValck D, et al. Tumor-necrosis factor mediated cytotoxicity is correlated with phospholipase-A_2 activity, but not arachidonic acid release per se. Eur J Biochem 1991; 195:465–475.
17. Feingold KR, Doerrler W, Dinarella CA, Fiers W, Grunfeld C. Stimulation of lipolysis in cultured fat cells by tumor necrosis factor, interleukin-1, and the interferons is blocked by inhibition of Prostaglandin synthesis. Endocrinology 1992; 130:10–16.
18. Matthews N, Neale ML, Jackson SK, Stark JM. Tumor cell killing by tumor necrosis factor: inhibition by aerobic conditions, free radical scavengers and inhibitors of arachidonic metabolism. Immunology 1987; 62:153–155.
19. Breed L, Perez J, Friedlander G, Ardaillous J. Tumor necrosis factor stimulate prostaglandin production and cyclic AMP levels in cultured mesangial cells. FEBS Lett 1988; 239:50–54.
20. Haliday EM, Ramesha CS, Ringold G. TNF induces c-*fos* via a novel pathway requiring conversion of arachidonic acid to a lipoxygenase metabolite. EMBO J 1991; 10:109–115.
21. Koike, K, Hirota K, Ohmici M, Kadowaki K, et al. Tumor necrosis factor-α increases release of arachidonate and prolactin from rat anterior pituitary cells. Endocrinology 1991; 128:2791–2798.
22. Kunkel SL, Spengler M, May MA, et al. Prostaglandin E_2 regulates macrophage-derived tumor necrosis factor gene expression. J Biol Chem 1988; 263:5380–5384.
23. Ertel W, Morrison MH, Wang P, et al. The complex pattern of cytokines in sepsis. Ann Surg 1991; 214:141–148.
24. Ciancio MJ, Hunt J, Jones SB, Filkins JP. Comparative and interactive *in vivo* effects of tumor necrosis factor-α and endotoxin. Cir Shock 1991; 33:108–120.
25. Natanson C, Eichenholz PW, Danner RL, Eichacher PQ, et al. Endotoxin and tumor necrosis factor challenges in dogs stimulate the cardiovascular profile of human septic shock. J Exp Med 1989; 169:823–832.
26. Turner CR, Esser KM, Wheeldon EB, Slivak M, Smith EF. Cardiovascular and pulmonary effects of human recombinant tumor necrosis factor in the conscious rat. Cir Shock 1989; 28:369–384.
27. Warren RS, Starnes HF, Alcock N, Calvaro S, Brennan MF. Hor-

monal and metabolic response to recombinant human tumor necrosis factor in rat: *In vitro* and *in vivo*. Am J Physiol 1988; 255:E206–E212.
28. Dayer JM, Beutler B, Cerami A. Cachectin/tumor necrosis factor stimulates collagenase and prostaglandin E_2 production by human synovial cells and dermal fibroblasts. J Exp Med 1985; 162:2163–2168.
29. Burch RM, Tiffany CW. Tumor necrosis factor causes amplification of arachidonic acid metabolism in response to Interleukin-1, bradykinin and other agonists. J Cell Physiol 1989; 141:85–89.
30. Fletcher JR. The role of prostaglandins in sepsis. Scand J Infect Dis 1982; 31:55–60.
31. Feuerstein G, Hallenbeck JM, Vanatta B, et al. Effects of gram-negative endotoxin on levels of serum corticosterone, TNFα circulating blood cells, and the survival of rats. Cir Shock 1990; 30:265–278.
32. Kettelbut IC, Fiers W, Goldberg AL. The toxic effects of tumor necrosis factor *in vivo* and their prevention by cyclooxygenase inhibitors. Proc Natl Acad Sci 1987; 84:4273–4277.
33. Talmadge JE, Bowersox O, Tribble H. Toxicity of tumor necrosis factor is synergistic with gamma interferon and can be reduced with cyclooxygenase inhibition. Am J Pathol 1987; 128:410–426.
34. Evans DA, Jacobs DO, Revhang A, et al. The effects of tumor necrosis factor and their selective inhibition by ibuprofen. Ann Surg 1989; 209:312–321.
35. Hinshaw LB, Soloman LA, Erdös EG, Reins BJ. Effects of acetylsalicylic acid on the canine response to endotoxin. J Pharmacol Exp Ther 1967; 157:665–671.
36. Fletcher JR, Ramwell PW. Lidocaine on indomethacin improves survival in baboon endotoxin shock. J Surg Res 1978; 24:154–160.
37. Wise WC, Cook JA, Eller T, Haluska PV. Ibuprofen improves survival from endotoxic shock in the rat. J Pharmacol Exp Ther 1980; 215:160–164.
38. Short BL, Miller MK, Fletcher JR. Improved survival in the suckling rat model of Group B streptococcal sepsis after treatment with non-steroidal anti inflammatory drugs. Pediatrics 1982; 70:343–347.
39. Jacobs ER, Soulsby ME, Bone RC, Wilson FJ, et al. Ibuprofen in canine endotoxin shock. J Clin Invest 1982; 536–541.
40. Bevilacqua MP, Pober JS, Wheller ME, Cotran RS, Gimrone MA. Interleukin-1 activation of vascular endothelium effects on procoagulant activity and leukocyte adhesion. Am J Pathol 1985; 121:393–403.
41. Pober JS, Bevilacqua MP, Lapierre LA, Fiers DL, et al. Two distinct monokines, Interleukin-1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells. J Immunol 1986; 136:1680–1687.
42. Camussi G, Bussolino F, Salvideo G, Baghioni G. Tumor necrosis factor/cachectin stimulates peritoneal macrophages, polymorphonuclear neutrophils, and vascular endothelial cells to synthesize and release platelet-activating factor. J Exp Med 1987; 166:1390–1404.
43. Larrick JW, Graham D, Toy K, et al. Recombinant tumor necrosis factor causes activation of human granulocytes. Blood 1987; 69:640–644.
44. Myers AK, Roby JW, Price RM. Relationships between tumor necrosis factor, eicosanoids, and platelet activating factor as mediators of endotoxin shock in mice. Br J Pharmacol 1990; 99:499–502.

Discussion

DR. EDWARD M. COPELAND, III (Gainesville, Florida): In your study, the majority of animals died in the initial 2 hours after the administration of TNF, yet the measured maximal effects of TNF occurred at the four-hour time point or later. Why were not death and the maximal effects of TNF more closely correlated? TNF induces arachidonic acid which is metabolized to eicosanoids via the cyclooxygenase pathway as you told us. You chose to block this pathway with ibuprofen. Has selective blockade been done to dissect out the relative importance of the various end products of the cyclooxygenase pathway, in other words, thromboxane, prostaglandins, and prostacyclin? The other pathway for the metabolism of arachidonic acid is via the 5-lipoxygenase pathway, which leads to the production of leukotrienes. Can you block this pathway, and if so, speculate on the results as related to your experiments. TNF directly induces the gene for nitric oxide synthase resulting in nitric oxide production, which likely was responsible for the hypotension in your model. What role would nitric oxide synthase inhibitors have in this model? Finally, have you used ibuprofen in a more relevant model of sepsis such as cecal ligation and puncture or endotoxemia to see if eicosanoids can be blocked and the outcome improved?

DR. ALDEN H. HARKEN (Denver, Colorado): A number of mediators like endotoxin, Dr. Fletcher has now indicated that TNF can also do it, many of the neuroendocrine hormones like norepinephrine, epinephrine, adenosine can activate a receptor right here. Schema reperfusion can also do it. Multiple separate second messengers can then activate the phospholipases. The endothelial cell membrane is made up of phospholipids, therefore when you activate a phospholipase, that is a big problem. The phospholipase A2s act on the second position in the glycerol backbone with a bunch of fatty acids hanging off it. When the fatty acid is a 20-carbon fatty acid, it then comes down as arachidonate and is metabolized by cycloxoygenase into eicosanoids. That is part of the problem. The other problem is that lysolipids are an obligate byproduct of this reaction and these lysolipids when acted on by *in situ* acetyltransferase produce platelet-activating factor from endothelial cells. There is both a calcium-dependent and calcium-independent stimulator of nitric oxide development from NO synthase, but how can blockade of arachidonate into eicosanoids by cyclooxygenase at this point feed back on a PLA2 system that may well then promote PAF elaboration? We are looking at multiple different opportunities for TNF activation of a cell surface receptor to play through intracellular calcium then onto NO synthase activating PLA2, either relating through eicosanoids or PAF to produce the septic MOF kind of picture. What is the mechanism? Why do the eicosanoids impact either directly on foreign elements in the blood to produce ectopic organ injury, or do they relate to the production of PAF nitric oxide themselves in producing this syndrome?

DR. J. RAYMOND FLETCHER (Closing Discussion): This is a complex area. Our work has been in the eicosanoids for a long period, and our work in the past 5 years has been trying to look at the relationship of platelet-activating factor, tumor necrosis factor, and the eicosanoids. Therefore it is not easy to explain the observations. We hope that will come down the pike. If death occurred early and the maximum effects of TNF were at 4 hours, why couldn't we see a more closely related effect? Interestingly enough, in those animals that died early, it ap-