Tumor Necrosis Factor-α Induces Activation of Coagulation and Fibrinolysis in Baboons Through an Exclusive Effect on the p55 Receptor

By Tom van der Poll, Patty M. Jansen, Kimberly J. Van Zee, M. Burress Welborn III, Irma de Jong, C. Erik Hack, Hansruedi Loetscher, Werner Lesslauer, Stephen F. Lowry, and Lyle L. Moldawer

Tumor necrosis factor-α (TNF-α) can bind to two distinct transmembrane receptors, the p55 and p75 TNF receptors. We compared the capability of two mutant TNF proteins with exclusive affinity for the p55 or p75 TNF receptor with that of wild type TNF, to activate the hemostatic mechanism in baboons. Both activation of the coagulation system, monitored by the plasma levels of thrombin-antithrombin III complexes, and activation of the fibrinolytic system (plasma levels of tissue-type plasminogen activator, and plasminogen activator inhibitor type I), were of similar magnitude after intravenous injection of wild type TNF or the TNF mutant with affinity only for the p55 receptor. Likewise, wild type TNF and the TNF p55 specific mutant were equally potent in inducing neutrophil degranulation (plasma levels of elastase-α_1-antitrypsin complexes). Wild type TNF tended to be a more potent inducer of secretory phospholipase A_2 release than the p55 specific TNF mutant. Administration of the TNF mutant binding only to the p75 receptor did not induce any of these responses. We conclude that TNF-induced stimulation of coagulation, fibrinolysis, neutrophil degranulation, and release of secretory phospholipase A_2 are predominantly mediated by the p55 TNF receptor.

TUMOR NECROSIS factor-α (TNF-α) is a pleiotropic cytokine that exerts a large variety of biological effects on multiple cell types.[1] Excessive production of TNF is considered to contribute to the pathogenesis of the sepsis syndrome.[2] In animals, passive immunization against TNF prevents death after intravenous infusion of a lethal dose of live bacteria,[3] and the administration of high doses of recombinant TNF causes a syndrome with all characteristics of septic shock.[4] In normal human volunteers a bolus intravenous injection of recombinant TNF induces a spectrum of systemic inflammatory responses also found during sepsis, including activation of coagulation and fibrinolysis.[5,6]

TNF can interact with two distinct surface receptors with molecular weights of 55 kD and 75 kD, respectively.[7] The intracellular domains of the p55 (type I) and p75 (type II) TNF receptors are highly different, suggesting the usage of different signal transduction pathways. Indeed, using either specific receptor antibodies or TNF mutants that have affinity for only one of the two receptors, distinct functions of the p55 and p75 TNF receptors have been identified. Stimulation of the p55 TNF receptor reproduces TNF activities such as cytotoxicity,[8,9] expression of adhesion molecules on endothelial cells and leukocytes,[10-13] and activation of NF-κB.[10] Stimulation of the p75 TNF receptor results in a proliferative response of mouse thymocytes and cytotoxic T cells,[8] fibroblasts and natural killer cells.[14] Furthermore, the p75 receptor may function to facilitate triggering of the p55 receptor, especially at low TNF concentrations.[7,11,13,15] In vivo investigations have shown that TNF signaling through the p55 TNF receptor is important for tissue injury produced by endotoxin in mice.[16,17] In accordance, administration of a TNF mutant with exclusive affinity for the p55 TNF receptor caused significant systemic toxicity in baboons.[18]

Infusion of endotoxin or bacteria into humans and nonhuman primates results in activation of both the intrinsic and the extrinsic pathway of the coagulation system.[19-21] During endotoxemia and sepsis, activation of the intrinsic route has been implicated in lethal hypotension,[20] while the tissue-factor-mediated extrinsic route is considered to initiate the activation of the common pathway of the coagulation system.[21,22-24] Indeed, antibodies directed against tissue factor, or infusion of tissue factor pathway inhibitor completely prevent coagulation activation in endotoxemic or bacteremic primates.[22-24] Recently, it was shown that TNF upregulates the expression of tissue factor on endothelial cells by an effect on the p55 receptor.[25] Therefore, in the present study we sought to confirm whether TNF elicits a procoagulant response in vivo, through p55 signaling. For this purpose baboons were infused with either wild type recombinant human TNF (wt-TNF), or TNF mutant proteins (p55-TNF and p75-TNF) with exclusive affinity for the p55 or p75 receptor, respectively.

MATERIALS AND METHODS

TNF preparations. The p55-TNF mutant was generated by introducing two point mutations in the human TNF sequence, replacing Arg^{32} by Trp, and S^{86} by Thr; the p75-mutant was generated by replacing Asp^{143} by Asn, and Ala^{145} by Arg.[9] p75-TNF shows no affinity for the p55 TNF receptor, while it binds to the human p75 TNF receptor with approximately one-tenth of the affinity of wt-TNF.[9] The receptor specificity of p55-TNF and p75-TNF is maintained in the baboon.[18] Wt-TNF, p55-TNF and p75-TNF were purified by sequential ion exchange chromatography (Q-Sepharose, Pharmacia, Uppsala, Sweden) and gel filtration (Superose 12, LKB-Pharmacia) to yield an electrophoretically pure protein preparation.[9]

From the Cornell University Medical College, Laboratory of Surgical Metabolism, New York, NY; Academic Medical Center, Department of Internal Medicine, University of Amsterdam; The Department of Autoimmune Diseases and Inflammation, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, the Netherlands; University of Florida College of Medicine, Department of Surgery, Gainesville; F. Hoffmann-La Roche, Basel, Switzerland.

Submitted October 2, 1995; accepted March 20, 1996.

Supported in part by grants GM-40586-07 (L.L.M.) and GM-34695 (to S.F.L.), by a clinical fellowship from the American Cancer Society (to K.J. V. Z.), and by a fellowship of the Royal Dutch Academy for Arts and Sciences (to T. v d P).

Address reprint requests to Stephen F. Lowry, MD, Cornell University Medical College, Laboratory of Surgical Metabolism, 1300 York Ave, Room LC-705, New York, NY 10021.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

0006-4971/96/8803-0027$3.00/0

The identity of the TNF preparations was confirmed by amino acid composition analyses and 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 preparation was <14 EU/mg protein.

Study design. Ten *Papio anubis* baboons (10 to 15 kg) were housed and quarantined for at least 2 weeks before they were studied to confirm that they were in good health and free of transmissible disease. The experimental protocol was approved by the Institutional Animal Care and Use Committees at Cornell University Medical College (New York, NY) and the University of Florida College of Medicine (Gainesville, FL). The present study was performed simultaneously with studies comparing the effects of wt-TNF, p55-TNF, and p75-TNF on hemodynamic changes and cytokine release, of which the results have been reported separately[18] (Buress Welborn et al, manuscript submitted). Clinical responses registered after administration of wt-TNF, p55-TNF or p75-TNF have been reported earlier[18] (Buress Welborn et al, manuscript submitted). Briefly, wt-TNF and p55-TNF caused comparable degrees of cardiovascular collapse, as reflected by similar hypotension and tachycardia. One of the baboons infused with p55-TNF was moribund after 48 hours, and had to be euthanized because of animal welfare concerns. By contrast, infusion of p75-TNF was not associated with hypotension or tachycardia, and all animals survived. Baboons were fasted overnight and immobilized with ketamine hydrochloride (10 mg/kg intramuscularly) before the experiment. During the study anesthesia was maintained using pentobarbital sodium (3 to 5 mg/kg intravenously at hourly intervals). The animals were instrumented for invasive monitoring as described previously.[18] In previous studies we established that the experimental procedures per se do not induce an inflammatory response.[18,26,27] After baseline blood sampling, wt-TNF (n = 4), p55-TNF (n = 3), or p75-TNF (n = 3) was administered as a bolus injection via the femoral vein. Wt-TNF and p55-TNF were given at a dose of 0.1 mg/kg, whereas p75-TNF was administered at a 10-fold higher dose (ie, 1 mg/kg), because of its 10-fold lower affinity for the p75 receptor than wt-TNF.[9] Arterial blood samples were obtained at −1, 0, 0.5, 1, 2, 3, 4, and 8 hours. Plasma was obtained by centrifugation at 4°C for 15 minutes, and stored at −70°C until assays were performed.

Assays. Thrombin-antithrombin III (TAT) complexes, tissue type plasminogen activator (tPA), and plasminogen activator inhibitor type 1 (PAI-1) were measured by enzyme-linked immunosorbent assay.[28,29] Elastase-α_1-antitrypsin complexes were measured with a RIA,[30] and $sPLA_2$ with an ELISA modified[29] from that described by Smith et al.[31] The antibodies used in this assay were kindly donated by Dr Fletcher B. Taylor Jr (Oklahoma Medical Research Foundation, Oklahoma City).

Statistical analysis. All data are expressed as mean ± SE. Differences between groups were analyzed by the nonparametric Kruskal-Wallis test (comparison of group means) and Mann-Whitney U test (two sample comparisons). $P < .05$ was considered to represent a significant difference.

RESULTS

Coagulation and fibrinolysis. Activation of the coagulation system was monitored by serial measurements of TAT complexes. wt-TNF and p55-TNF induced coagulation activation to a similar extent (Fig 1). Peak concentrations of TAT complexes were 93.7 ± 65.1 ng/mL after injection of wt-TNF, and 138.9 ± 41.3 ng/mL after injection of p55-TNF ($P = .28$ for the difference between groups). Although at 4 and 8 hours postinfusion mean plasma levels of TAT complexes were higher in baboons treated with p55-TNF than in wt-TNF treated animals, two sample comparisons by

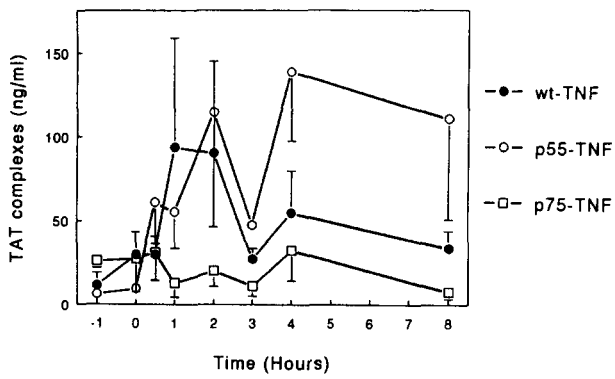

Fig 1. Activation of coagulation. Mean (±SE) plasma concentrations of TAT complexes after intravenous injection at time 0 of either wild type TNF (wt-TNF; 0.1 mg/kg), or TNF mutants (p55-TNF: 0.1 mg/kg; and p75-TNF: 1 mg/kg) with exclusive affinity for the p55 or p75 TNF receptor, respectively, in baboons. The difference between animals treated with wt-TNF or p55-TNF was not significant, whereas in the p75-TNF treated animals the levels of TAT complexes were lower than after either injection wt-TNF or p55-TNF (both $P < .05$).

Mann-Whitney U test did not reveal a significant difference at these time points between groups. The concentrations of TAT complexes remained significantly lower after administration of p75-TNF than after administration of either wt-TNF ($P < .05$ for the difference between groups) or p55-TNF ($P < .001$). Activation of the fibrinolytic system was assessed by measuring tPA and PAI-1 (Fig 2). Administration of wt-TNF and p55-TNF was associated with increases in these parameters, that were not significantly different between groups. Peak levels of tPA registered during the study period, were 8.9 ± 2.1 and 5.3 ± 0.7 ng/mL after injection of wt-TNF and p55-TNF respectively ($P = .98$ for the difference between groups); peak levels of PAI-1 were 1,236.4 ± 217.2 and 1,091.2 ± 249.7 ng/mL, respectively, ($P = .44$). By contrast, after administration of p75-TNF, tPA, and PAI-1 levels remained unchanged, and significantly lower than after injection of either wt-TNF (tPA: $P = .01$; PAI-1: $P = .001$ for the difference between groups), or p55-TNF (tPA: $P = .01$; PAI-1: $P = .01$).

Elastase-α_1-antitrypsin complexes. Both wt-TNF and p55-TNF elicited neutrophil degranulation, as reflected by similar increases in the plasma concentrations of elastase-α_1-antitrypsin complexes (Fig 3). Elastase-α_1-antitrypsin complexes increased continuously until the end of the 8-hour study, at which time levels were 147.5 ± 50.5 ng/mL after injection of wt-TNF, and 174.3 ± 31.6 ng/mL after injection of p55-TNF ($P = .40$ for the difference between groups). p75-TNF did not induce an increase in the levels of elastase-α_1-antitrypsin complexes, which remained significantly lower than after administration of either wt-TNF ($P = .001$ for the difference between groups) or p55-TNF ($P < .0001$).

Secretory phospholipase A_2. The increase in the plasma concentrations of $sPLA_2$ found after injection of wt-TNF was more pronounced than that detected after injection of p55-TNF (Fig 4). Both after injection of wt-TNF and p55-TNF, $sPLA_2$ levels kept rising until the end of the study

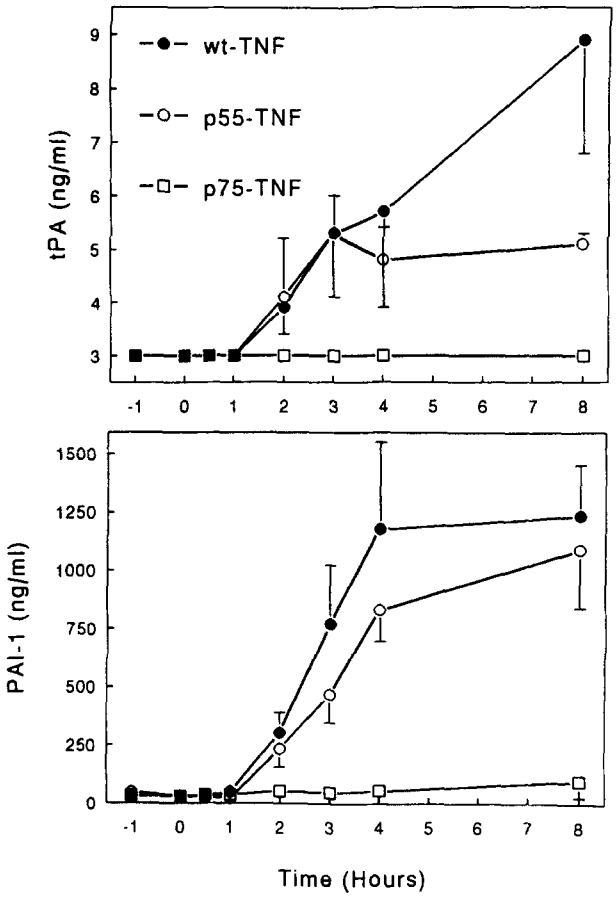

Fig 2. Activation of fibrinolysis. Mean (±SE) plasma concentrations of tPA and PAI-1 after intravenous injection at time 0 of either wild type TNF (wt-TNF; 0.1 mg/kg), or TNF mutants (p55-TNF: 0.1 mg/kg; and p75-TNF: 1 mg/kg) with exclusive affinity for the p55 or p75 TNF receptor, respectively, in baboons. The differences between animals treated with wt-TNF or p55-TNF were not significant, whereas in the p75-TNF treated animals the levels of tPA and PAI-1 were lower than after either injection wt-TNF or p55-TNF (both $P <$ .05).

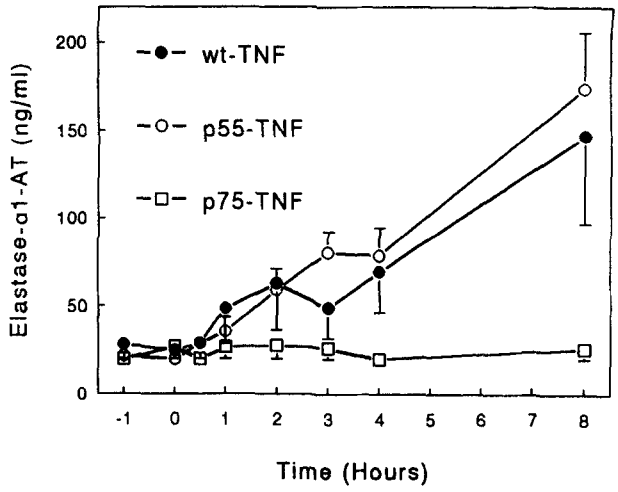

Fig 3. Neutrophil degranulation. Mean (±SE) plasma concentrations of elastase-α_1-antitrypsin complexes after intravenous injection at time 0 of either wild type TNF (wt-TNF; 0.1 mg/kg), or TNF mutants (p55-TNF: 0.1 mg/kg; and p75-TNF: 1 mg/kg) with exclusive affinity for the p55 or p75 TNF receptor, respectively, in baboons. The differences between animals treated with wt-TNF or p55-TNF were not significant, whereas in the p75-TNF treated animals the levels of elastase-α_1-antitrypsin complexes were lower than after either injection wt-TNF or p55-TNF (both $P <$ .05).

period (wt-TNF: 1,173.6 ± 292.3 ng/mL; p55-TNF: 318.5 ± 97.2 ng/mL). Although the overall $sPLA_2$ responses did not differ between wt-TNF and p55-TNF infused animals ($P = 0.10$ by Kruskal-Wallis test), two sample comparisons by Mann-Whitney U test at 4 and 8 hours postinfusion did reveal a significant difference between these two treatment groups (both $P < .05$). p75-TNF did not influence $sPLA_2$ levels, and $sPLA_2$ remained lower than after injection of either wt-TNF ($P < .05$ for the difference between groups) or p55-TNF ($P < .05$).

DISCUSSION

The hemostatic response to an infectious challenge is tightly regulated. Administration of low dose endotoxin to healthy humans results in an early activation of the fibrinolytic system mediated by the release of tPA, which is followed in time by an abrupt inhibition of fibrinolytic activity by the appearance of PAI-1.[32,33] Thereafter, both the contact system[34] and the common pathway of the coagulation system[33] become activated. Qualitatively similar changes have been found in baboons infused with live *Escherichia coli*.[19,20,28,29] Both low dose endotoxin and live bacteria appear to induce activation of the common pathway of the coagulation system by enhancing the activity and/or expres-

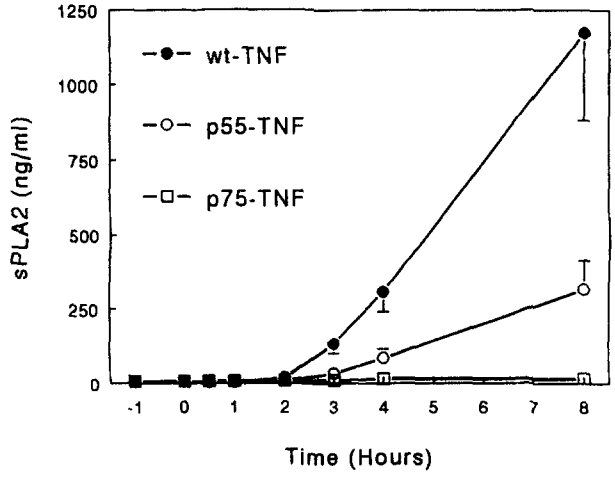

Fig 4. Mean (±SE) plasma concentrations of secretory phospholipase A_2 ($sPLA_2$) after intravenous injection at time 0 of either wild type TNF (wt-TNF; 0.1 mg/kg), or TNF mutants (p55-TNF: 0.1 mg/kg; and p75-TNF: 1 mg/kg) with exclusive affinity for the p55 or p75 TNF receptor, respectively, in baboons. The difference between animals treated with wt-TNF or p55-TNF was not significant ($P = .10$), whereas in the p75-TNF treated animals the levels of $sPLA_2$ were lower than after either injection wt-TNF or p55-TNF (both $P <$ .05).

sion of tissue factor.[22-24] Administration of TNF to healthy humans causes similar changes in the fibrinolytic and coagulation systems as endotoxin.[5,6] In vitro, TNF has a net procoagulant effect on endothelial cells by enhancing the synthesis and surface expression of tissue factor, by downregulating thrombomodulin and protein S secretion, and by inhibiting the fibrinolytic response via inducing the synthesis and secretion of PAI-1.[25,35-38] The present results indicate that TNF elicits coagulation activation in vivo by an effect on the p55 receptor. In vitro data suggest that both TNF receptor species may be involved in the induction of tissue factor on endothelial cells.[39,40] Indeed, antagonistic antibodies against either the p55 or p75 receptor have been found to attenuate TNF-induced tissue factor expression on human endothelial cells, although blockade of the p55 receptor had the most potent inhibiting effect.[39,40] Further, agonistic antibodies against either the p55 or p75 receptor were able to stimulate tissue factor expression.[40] However, using the same TNF mutant proteins as in the present investigation, Kirchhofer et al[25] found that tissue factor mediated fibrin depositions on endothelial cells in a human ex vivo native blood flow system could only be induced by p55-TNF, not by p75-TNF. Together, it seems reasonable to conclude that the p55 receptor is the predominant receptor mediating TNF-induced tissue factor expression by endothelial cells in vitro. Because in this experimental model fibrinolytic activation can not be considered a mere reaction to coagulation activation,[21] our results also suggest that TNF activates fibrinolysis through an effect on the p55 receptor.

TNF can induce neutrophil degranulation in vitro, and in healthy humans in vivo.[41,42] Recent in vitro studies have established that the p55 TNF receptor is responsible for TNF-induced stimulation of respiratory burst activity of neutrophils,[12,13,43] and TNF-induced potentiation of neutrophil elastase release triggered by FMLP.[43] The present data indicate that TNF-induced neutrophil degranulation in vivo, is mediated by the p55 TNF receptor, as reflected by similar increases in the plasma concentrations of elastase-α_1-antitrypsin complexes after injection of wt-TNF and p55-TNF, which contrasts with the previous finding that p55-TNF is unable to induce leukocytosis.[18] Indexes of contact system activation were not measured in the present investigation. Thus, a possible role of contact system products in the initiation of neutrophil degranulation and fibrinolytic activation elicited by wt-TNF and p55-TNF in vivo can not be excluded by our results. Administration of p75-TNF did not result in a rise in plasma elastase-α_1-antitrypsin levels, which is in line with the in vitro finding that stimulation of the p75 TNF receptor does not influence FMLP-induced elastase release.[43]

$sPLA_2$ is a regulatory enzyme that controls the synthesis of eicosanoids and platelet activating factor. The plasma concentrations of $sPLA_2$ are elevated in sepsis, in which it may contribute to tissue injury.[44] TNF can stimulate PLA_2 activation by renal mesangial cells in vitro,[45] and intravenous injection of recombinant TNF into normal humans elicits a rapid release of prostacyclin, a major circulating end product of the arachidonic acid pathway that is initiated by PLA_2.[46] We now confirm that TNF can induce release of $sPLA_2$ in vivo. The increase in $sPLA_2$ was more pronounced after administration of wt-TNF than after injection of p55-TNF, whereas p75-TNF failed to induce an increase in $sPLA_2$ levels. Conceivably, optimal stimulation of $sPLA_2$ release occurs when both the p55 and p75 TNF receptors are triggered. In support of this explanation is the in vitro finding that simultaneous stimulation of the p75 TNF receptor potentiates some neutrophil and endothelial cell responses that are mediated by the p55 TNF receptor.[13,39]

As reported previously, the administration of p55-TNF led to a modest endogenous TNF response with peak levels after 2 hours that were 1,000-fold lower than those measured after injection of wt-TNF.[18] Although we can not exclude completely that this endogenously produced TNF mediated part of the systemic inflammatory responses detected after injection of p55-TNF, we consider this possibility unlikely, because not only the magnitude of the wt-TNF- and p55-TNF-induced responses were similar, but also their kinetics (ie, the changes after administration of p55-TNF were not delayed).

Activation of the hemostatic mechanism represents major component of the early response to sepsis. The present data shows that TNF induces activation of the coagulation system and the fibrinolytic system in the baboon by an effect on the p55 receptor. In addition, TNF-induced neutrophil degranulation and $sPLA_2$ in vivo are also predominantly mediated by the p55 TNF receptor. Earlier investigations in mice have indicated that the p55 TNF receptor may be the predominant receptor mediating TNF effects in vivo, ie, mutant mice that are deficient for the p55 receptor were resistant to endotoxin-induced death after sensitization with D-galactosamine, but susceptible to infection with Listeria monocytogenes,[16,17] thereby reproducing murine studies that used neutralizing anti-TNF antibodies.[2] Further, p55-TNF produced similar hemodynamic changes and organ dysfunction as wt-TNF in baboons.[18] Taken together, these data suggest that the p55 receptor is the major TNF receptor involved in TNF-induced inflammatory responses in vivo.

REFERENCES

1. Vassalli P: The pathophysiology of tumor necrosis factors. Annu Rev Immunol 10:411, 1992

2. van der Poll T, Lowry SF: Tumor necrosis factor in sepsis: Mediator of multiple organ failure or essential part of host defense? Shock 3:1, 1995

3. Tracey KJ, Fong Y, Hesse DG, Manogue KR, Lee AT, Kuo GC, Lowry SF, Cerami A: Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. Nature 330:662, 1987

4. Tracey KJ, Beutler B, Lowry SF, Merryweather J, Wolpe S, Milsark IW, Hariri RJ, Fahey TJ, Zentella A, Albert JD, Shires GT, Cerami A: Shock and tissue injury induced by recombinant human cachectin. Science 234:470, 1986

5. van der Poll T, Büller HR, ten Cate H, Wortel CH, Bauer KA, van Deventer SJH, Hack CE, Sauerwein HP, Rosenberg RD, ten Cate JW: Activation of coagulation after administration of tumor necrosis factor to normal subjects. N Engl J Med 322:1622, 1990

6. van der Poll T, Levi M, Büller HR, van Deventer SJH, de Boer JP, Hack CE, ten Cate JW: Fibrinolytic response to tumor necrosis factor in healthy subjects. J Exp Med 174:729, 1991

7. Tartaglia LA, Goeddel DV: Two TNF receptors. Immunol Today 13:151, 1992

8. Tartaglia LA, Weber RF, Figari IS, Reynolds CMA, Palladino J, Goeddel DV: Two different receptors for tumor necrosis factor mediate distinct cellular responses. Proc Natl Acad Sci USA 88:9292, 1991

9. Loetscher H, Stueber D, Banner D, Mackay F, Lesslauer W: Human tumor necrosis factor α (TNFα) mutants with exclusive specificity for the 55-kDa or 75-kDa TNF receptors. J Biol Chem 268:26350, 1993

10. Mackay F, Loetscher H, Stueber D, Gehr G, Lesslauer W: Tumor necrosis factor α (TNF-α)-induced cell adhesion to human endothelial cells is under dominant control of one TNF receptor type, TNF-R55. J Exp Med 177:1277, 1993

11. Slowik MR, De Luca LG, Fiers W, Pober JS: Tumor necrosis factor activates human endothelial cells through the p55 tumor necrosis factor receptor but the p75 receptor contributes to activation at low tumor necrosis factor concentration. Am J Pathol 143:1724, 1993

12. Menegazzi R, Cramer R, Patriarca P, Scheurich P, Dri P: Evidence that tumor necrosis factor α (TNF)-induced activation of neutrophil respiratory burst on biologic surfaces is mediated by the p55 TNF receptor. Blood 84:287, 1994

13. Barabara JAJ, Smith WB, Gamble JR, van Ostade X, Vandenabeele P, Tavernier J, Fiers W, Vadas MA, Lopez AF: Dissociation of TNF-α cytotoxic and proinflammatory activities by p55 receptor- and p75 receptor-selective TNF-α mutants. EMBO J 13:843, 1994

14. Espevik T, Brockhaus M, Loetscher H, Nonstad U, Shalaby R: Characterization of binding and biological effects of monoclonal antibodies against a human tumor necrosis factor receptor. J Exp Med 171:415, 1990

15. Tartaglia LA, Pennica D, Goeddel DV: Ligand passing: The 75-kDa tumor necrosis factor (TNF) receptor recruits TNF for signaling by the 55-kDa TNF receptor. J Biol Chem 268:18542, 1993

16. Pfeffer K, Matsuyama T, Kündig TM, Wakeham A, Kishihara K, Shahinian A, Wiegmann K, Ohashi PS, Krönke M, Mak TW: Mice deficient for the 55 kd tumor necrosis factor receptor are resistant to endotoxic shock, yet succumb to L. monocytogenes infection. Cell 73:457, 1993

17. Rothe J, Lesslauer W, Lötscher H, Lang Y, Koebel P, Köntgen F, Althage A, Zinkernagel R, Steinmetz M, Bluethmann H: Mice lacking the tumour necrosis factor receptor 1 are resistant to TNF-mediated toxicity but highly susceptible to infection by Listeria monocytogenes. Nature 364:798, 1993

18. Welborn MB III, Van Zee KJ, Edwards PD, Pruitt JH, Kaibara A, Vauthey JN, Castelman WL, Lowry SF, Kenney JS, Stuber D, Ettlin U, Wipf B, Loetscher H, Copeland EM III, Lesslauer W, Moldawer LL: A human tumor necrosis factor (TNF) p75 agonist stimulates *in vitro* T-cell proliferation but does not produce inflammation or shock in the baboon. J Exp Med 1996 (in press)

19. Pixley RA, DeLa Cadena RA, Page JD, Kaufman N, Wyshock EG, Colman RW, Chang A, Taylor FB Jr: Activation of the contact system in lethal hypotensive bacteremia in a baboon model. Am J Pathol 140:897, 1992

20. Pixley RA, De La Cadena R, Page JD, Kaufman N, Wyshock EG, Chang A, Taylor FB Jr, Colman RW: The contact system contributes to hypotension but not to disseminated intravascular coagulation in lethal bacteremia. J Clin Invest 91:61, 1993

21. Levi M, ten Cate H, van der Poll T, van Deventer SJH: Pathogenesis of disseminated intravascular coagulation in sepsis. JAMA 270:975, 1993

22. Taylor FB Jr, Chang A, Ruf W, Morrissey JH, Hinshaw L, Catlett R, Blick K, Edgington TS: Lethal *E. coli* septic shock is prevented by blocking tissue factor with monoclonal antibody. Circ Shock 33:127, 1991

23. Creasey AA, Chang ACK, Feigen L, Wün TC, Taylor FB Jr, Hinshaw LB: Tissue factor pathway inhibitor reduces mortality from *Escherichia coli* septic shock. J Clin Invest 91:2850, 1993

24. Levi M, Ten Cate H, Bauer KA, van der Poll T, Edgington TS, Büller HR, Van Deventer SJH, Hack CE, Ten Cate JW, Rosenberg RD: Inhibition of endotoxin-induced activation of coagulation and fibrinolysis by pentoxifylline or by a monoclonal anti-tissue factor antibody in chimpanzees. J Clin Invest 93:114, 1994

25. Kirchhofer D, Tschopp TB, Hadvary P, Baumgartner HR: Endothelial cells stimulated with tumor necrosis factor-α express varying amounts of tissue factor resulting in inhomogenous fibrin deposition in a native blood flow system. Effects of thrombin inhibitors. J Clin Invest 93:2073, 1994

26. Fischer E, Marano MA, Barber AE, Hudson A, Lee K, Rock CS, Hawes AS, Thompson RC, Hayes TJ, Anderson TD, Benjamin WR, Lowry SF, Moldawer LL: Comparison between effects of interleukin-1α administration and sublethal endotoxemia in primates. Am J Physiol 261:R442, 1991

27. van der Poll T, Fischer E, Coyle SM, van Zee KJ, Pribble JP, Stiles DM, Barie PS, Buurman WA, Moldawer LL, Lowry SF: Interleukin 1 contributes to increased concentrations of soluble tumor necrosis factor receptor type I in sepsis. J Infect Dis 172:577, 1995

28. De Boer JP, Creasy AA, Chang A, Roem D, Brouwer MC, Eerenberg AJM, Hack CE, Taylor FB Jr: Activation patterns of coagulation and fibrinolysis in baboons following infusion with lethal or sublethal dose of *Escherichia coli.* Circ Shock:39, 59, 1993

29. Jansen PM, Boermeester MA, Fischer E, de Jong IW, van der Poll T, Moldawer LL, Hack CE, Lowry SF: Contribution of interleukin-1 to activation of coagulation and fibrinolysis, neutrophil degranulation and the release of secretory type phospholipase A_2 in sepsis: Studies in non-human primates following interleukin-1α administration and during lethal bacteremia. Blood 86:1027, 1995

30. Nuijens JH, Abbink JJ, Wachtfogel YT, Colman RW, Eerenberg AJM, Dors D, Kamp AM, Strack van Schijndel RJM, Thijs LG, Hack CE: Plasma elastase-α_1-antitrypsin and lactoferrin in sepsis: Evidence for neutrophils as mediators of fatal sepsis. J Lab Clin Med 119:159, 1992

31. Smith GM, Ward RL, MacGuigan L, Rajkovic A, Scott KF: Measurement of phospholipase A_2 in arthritic plasma using a newly developed sandwich ELISA. Br J Rheumatol 31:175, 1992

32. Suffredini AF, Harpel PC, Parrillo JE: Promotion and subsequent inhibition of plasminogen activation after administration of intravenous endotoxin to normal subjects. N Engl J Med 320:1165, 1989

33. Van Deventer SJH, Büller HR, ten Cate JW, Aarden LA, Hack CE, Sturk A: Experimental endotoxemia in humans: Analysis of cytokine release and coagulation, fibrinolytic and complement pathways. Blood 76:2520, 1990

34. DeLa Cadena RA, Suffredini AF, Page JD, Pixley RA, Kaufmann N, Parillo JE, Colman RW: Activation of the kallikrein-kinin system after endotoxin administration to normal human volunteers. Blood 81:3313, 1993

35. Nawroth PP, Stern DM: Modulation of endothelial cell hemostatic properties by tumor necrosis factor. J Exp Med 163:740, 1986

36. Scarpati EM, Sadler JE: Regulation of endothelial cell coagulant properties. Modulation of tissue factor, plasminogen activator inhibitors and thrombomodulin by phorbol 12-myristate 13-acetate and tumor necrosis factor. J Biol Chem 264:20705, 1989

37. Schleef RR, Bevilacqua MP, Sawdey M, Gimbrone MA Jr, Loskutoff DJ: Cytokine activation of vascular endothelium. Effects on tissue-type plasminogen activator and type 1 plasminogen activator inhibitor. J Biol Chem 263:5797, 1988

38. Hooper WC, Philips DJ, Ribeiro MJA, Benson JM, George VG, Ades EW, Evatt BL: Tumor necrosis factor-α downregulates

protein S secretion in human microvascular and umbilical vein endothelial cells but not in the HepG-2 hepatoma cell line. Blood 84:483, 1994

39. Paleolog EM, Delasalle SAJ, Buurman WA, Feldmann M: Functional activities of receptors for tumor necrosis factor-α on human vascular endothelial cells. Blood 84:2578, 1994

40. Schmid EF, Binder K, Grell M, Scheurich P, Pfizenmaier K: Both tumor necrosis factor receptors, TNFR60 and TNFR80, are involved in signaling endothelial cell tissue factor expression by juxtacrine tumor necrosis factor α. Blood 86:1836, 1995

41. Burnett D, Chamba A, Hill SL, Stockley RA: Effects of plasma, tumour necrosis factor, endotoxin and dexamethasone on extracellular proteolysis by neutrophils from healthy subjects and patients with emphysema. Clin Sci 77:35, 1989

42. van der Poll T, van Deventer SJH, Hack CE, Wolbink GJ, Aarden LA, Büller HR, ten Cate JW: Effects on leukocytes following injection of tumor necrosis factor into healthy humans. Blood 79:693, 1992

43. Abe Y, Osuka Y, Nakata T, Kashu Y, Kimura S: The functional role of 55- and 75-kDa tumour necrosis factor receptors in human polymorphonuclear cells in vitro. Cytokine 7:39, 1995

44. Anderson BO, Moore EE, Banerjee A: Phospholipase A_2 regulates critical inflammatory mediators of multiple organ failure. J Surg Res 56:199, 1994

45. Pfeilschifter J, Leighton J, Pignat W, Marki F, Vosbeck K: Cyclic AMP mimics, but does not mediate, interleukin-1- and tumour necrosis factor-stimulated phospholipase A_2 secretion from rat renal mesangial cells. Biochem J 273:199, 1991

46. van der Poll T, van Deventer SJH, Büller HR, Sturk A, ten Cate JW: Comparison of the early dynamics of systematic prostacyclin release after administration of tumor necrosis factor and endotoxin to healthy humans. J Infect Dis 164:599, 1991