Induction of Mn SOD in human monocytes without inflammatory cytokine production by a mutant endotoxin

LICHENG TIAN,[1] JULIE E. WHITE,[1] HUNG-YUN LIN,[1,2] VISA S. HARAN,[1] JOSEPH SACCO,[2,3] G. CHIKKAPPA,[2,3] FAITH B. DAVIS,[2,3] PAUL J. DAVIS,[2,3] AND MIN-FU TSAN[1,2,4]
[1]*Research and* [3]*Medical Services, Stratton Veterans Affairs Medical Center, and Departments of* [2]*Medicine and* [4]*Physiology and Cell Biology, Albany Medical College, Albany, New York 12208*

Tian, Licheng, Julie E. White, Hung-Yun Lin, Visa S. Haran, Joseph Sacco, G. Chikkappa, Faith B. Davis, Paul J. Davis, and Min-Fu Tsan. Induction of Mn SOD in human monocytes without inflammatory cytokine production by a mutant endotoxin. *Am. J. Physiol.* 275 (*Cell Physiol.* 44): C740–C747, 1998.—Endotoxin selectively induces monocyte Mn superoxide dismutase (SOD) without affecting levels of Cu,Zn SOD, catalase, or glutathione peroxidase. However, little is known about the structure-activity relationship and the mechanism by which endotoxin induces Mn SOD. In this study we demonstrated that a mutant *Escherichia coli* endotoxin lacking myristoyl fatty acid at the 3′ *R*-3-hydroxymyristate position of the lipid A moiety retained its full capacity to coagulate *Limulus* amoebocyte lysate compared with the wild-type *E. coli* endotoxin and markedly stimulated the activation of human monocyte nuclear factor-κB and the induction of Mn SOD mRNA and enzyme activity. However, in contrast to the wild-type endotoxin, it failed to induce significant production of tumor necrosis factor-α and macrophage inflammatory protein-1α by monocytes and did not induce the phosphorylation and nuclear translocation of mitogen-activated protein kinase. These results suggest that *1*) lipid A myristoyl fatty acid, although it is important for the induction of inflammatory cytokine production by human monocytes, is not necessary for the induction of Mn SOD, *2*) endotoxin-mediated induction of Mn SOD and inflammatory cytokines are regulated, at least in part, through different signal transduction pathways, and *3*) failure of the mutant endotoxin to induce tumor necrosis factor-α production is, at least in part, due to its inability to activate mitogen-activated protein kinase.

lipopolysaccharide; tumor necrosis factor; nuclear factor-κB; mitogen-activated protein kinase

ENDOTOXIN, a lipopolysaccharide (LPS) of the cell wall of gram-negative bacteria, is responsible for a host of toxic effects that occur in patients infected with these microorganisms, including fever, disseminated intravascular coagulation, and hemodynamic changes that may lead to multiple organ failure characteristics of septic shock (19, 24). On the other hand, LPS exhibits immunostimulatory effects (19, 24) and induces the antioxidant enzyme Mn superoxide dismutase (SOD) (1, 27), which are beneficial to the host. Evidence suggests that induction of Mn SOD may be responsible for LPS-induced protection against pulmonary oxygen toxicity (31, 33). However, the serious toxicities of LPS limit any potential clinical use for its beneficial effects.

The endotoxic effects of LPS are caused indirectly through the activation of monocytes and macrophages, leading to the release of toxic cytokines, such as tumor necrosis factor (TNF), interleukin (IL)-1, IL-6, and macrophage inflammatory proteins (MIPs) (4, 5, 19, 24). The endotoxic principle of LPS resides in its lipid A component. For full endotoxic activity in humans, a lipid A structure containing two β(1—6)-linked D-glucosamine residues, two phosphoryl groups, and six fatty acids in a defined arrangement as present in *Escherichia coli* lipid A is required (19, 24, 25).

The mechanism and the structural requirement for the induction of Mn SOD by LPS are not clear. Recently, Somerville et al. (29) reported a 1,000- to 10,000-fold reduction in the ability of a mutant *E. coli* LPS lacking the myristoyl fatty acid moiety at the 3′ *R*-3-hydroxymyristate position of lipid A (nonmyristoyl LPS, nmLPS) to stimulate E-selectin expression by human endothelial cells and TNF-α production by adherent monocytes compared with the wild-type LPS (wtLPS). In the current study we demonstrated that nmLPS at a concentration as high as 1 μg/ml failed to significantly induce TNF-α and MIP-1α production by human monocytes, whereas it markedly induced the activation of nuclear factor-κB (NF-κB) and the induction of Mn SOD mRNA and enzyme activity. In addition, nmLPS failed to activate mitogen-activated protein kinase (MAPK), a principal component of the signal transduction pathway known to be involved in the wtLPS-induced production of TNF-α (4, 11, 12, 23, 30). These results suggest that lipid A myristoyl fatty acid, although it is important for the induction of inflammatory cytokine production by human monocytes, is not necessary for the induction of Mn SOD. This mutant LPS can be used to distinguish the intracellular signal transduction pathways for LPS-induced cytokine production and Mn SOD induction and to study the potential beneficial effect of Mn SOD induction without the toxic effects of inflammatory cytokines.

MATERIALS AND METHODS

Materials. The endotoxins wtLPS (JM83 *E. coli* K-12) and nmLPS (BMS67C12 *E. coli* K-12) were kindly provided by John E. Somerville (Bristol-Myers Squibb Pharmaceutical Research Institute, Princeton, NJ). The nmLPS preparation was free of wtLPS contamination as determined by gas chromatography and mass spectroscopy. Purified Mn SOD (from *E. coli*) and Cu,Zn SOD (from bovine erythrocytes) were obtained from Sigma Chemical (St. Louis, MO). Mn SOD and Cu,Zn SOD had a specific activity of 4,400 U/mg protein as

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

determined according to the method described by McCord and Fridovich (18). Polyclonal goat anti-recombinant human TNF-α antibody [affinity-purified IgG, 1 mg/ml; 50% neutralization dose 0.02–0.04 μg/ml by cell lytic assay using murine L929 fibroblasts] and normal goat IgG were purchased from R & D Systems (Minneapolis, MN). Rabbit polyclonal antiphosphotyrosine and mouse monoclonal anti-rat MAPK antibodies were obtained from Transduction Laboratories (Lexington, KY). Enhanced chemiluminescence Western blotting detection reagents were from Amersham Life Science (Arlington Heights, IL).

Endotoxin assay. The endotoxin activity of wtLPS and nmLPS was determined with the *Limulus* amoebocyte lysate (LAL) assay (QCL-1000, BioWhittakker, Walkersville, MD).

Isolation of human monocytes. Human mononuclear cells were isolated from venous blood of normal volunteers (after the nature and possible risks of the studies were explained and informed consent was obtained) using Isolymph (Gallard-Schlesinger Industries, Carle Place, NY). Cells (5×10^6/ml in RPMI 1640 plus antibiotics and 5% autologous serum) were allowed to adhere to tissue culture plates for 2 h. Adherent cells, which consisted of ~90% monocytes as judged by a nonspecific esterase stain, were used for the current studies.

Measurements of TNF-α and MIP-1α. Adherent monocytes were treated with or without LPS (0.01 ng/ml–1.0 μg/ml) for 1, 4, or 18 h at 37°C according to Somerville et al. (29). In some experiments, goat anti-recombinant human TNF-α IgG or normal goat IgG (0.16 mg/ml) was added to the incubation medium. The amounts of TNF-α activity and MIP-1α protein released into the medium or present in cell lysates (collected by scraping and sonication) were determined by the anti-TNF-α antibody-inhibitable lysis of murine L929 fibroblasts as described previously (34) and ELISA (R & D Systems), respectively, carried out according to the manufacturer's instructions.

Measurements of SOD activity. Adherent monocytes were treated with or without (as control) LPS (1 μg/ml) for 1, 4, or 18 h at 37°C. Cells were then collected by scraping and sonicated, and protein contents were determined using bicinchoninic acid according to Smith et al. (28). Aliquots of cell extracts (50 μg/lane) were then assayed for SOD activity using nondenaturing PAGE (8%) according to the method described by Beauchamp and Fridovich (2), on the basis of the inhibitory effect of SOD on the reduction of tetrazolium by superoxide generated by photochemically reduced riboflavin, as described previously (35). The SOD activity gels were quantified using a computing densitometer (Molecular Dynamics, Sunnyvale, CA). In each assay, purified *E. coli* Mn SOD and bovine erythrocyte Cu,Zn SOD (25–800 mU) were used to obtain standard curves from which cell extract Mn SOD and Cu,Zn SOD activities, respectively, were derived.

Northern analysis of TNF-α and Mn SOD mRNAs. Northern blot analysis was performed as described previously (38). Briefly, adherent monocytes were treated with or without 1 μg/ml LPS for 4 h at 37°C, and the total cellular RNA was isolated by the single-step method of Chomczynski and Sacchi (7) using Tri Reagent (Molecular Research Center, Cincinnati, OH). For Northern blots, denatured RNA samples (20 μg/lane) were electrophoresed in 1.2% agarose gels, transferred to nylon membrane (Genescreen plus, New England Nuclear, Boston, MA) by capillary blotting, and stained with methylene blue to visualize the quality and size of 18S and 28S ribosomal RNA species. The membrane was then prehybridized as described previously (38). Hybridization was carried out with 100 μg/ml denatured salmon testis DNA and human TNF-α or Mn SOD cDNA probes that had been labeled by random hexanucleotide priming (GIBCO BRL, Gaithersburg, MD) to a specific activity of $>10^9$ cpm/μg DNA. After the samples were washed, autoradiographs were obtained and radioactive signals were quantified using a computing densitometer.

Electrophoresis mobility shift assay for NF-κB. Adherent monocytes were treated with or without 1 μg/ml LPS for 1 or 4 h at 37°C, and the nuclear extracts were obtained according to Osnes et al. (21). For the electrophoresis mobility shift assay (EMSA), 3 μg of nuclear proteins were incubated for 30 min at room temperature with ~100,000 cpm (5 ng) of an oligonucleotide containing NF-κB consensus sequence (5′-AGT TGA GGG GAC TTT CCC AGG C-3′) that had been 5′-end labeled with [γ-^{32}P]ATP using T4 polynucleotide kinase (Promega, Madison, WI). Competition was carried out using a 100-fold excess of the unlabeled oligonucleotide 10 min before addition of the radiolabeled probe. Samples were then electrophoresed in a 6% nondenaturing polyacrylamide gel. Autoradiographs were obtained and radioactive signals were quantified using a computing densitometer.

Immunoprecipitation and immunoblotting for MAPK. Immunoprecipitation and immunoblotting for MAPK were performed as described previously (16). Briefly, adherent monocytes were treated with or without 1 μg/ml LPS for 1 h at 37°C. After hypotonic lysis, cytoplasmic and nuclear extracts were prepared according to the method of Wen et al. (37) and immunoprecipitated by overnight incubation with antiphosphotyrosine at 4°C with rocking. Protein A-agarose was added and rocking continued for 1 h at 4°C. Samples were eluted with 2× sample solubilizer, and protein was separated by discontinuous SDS-PAGE (9%), transferred to Immobilon membranes (Millipore, Bedford, MA) by electroblotting, and blocked with 5% milk in Tris-buffered saline containing 0.1% Tween. Membranes were then incubated with mouse monoclonal anti-MAPK antibody (1:1,000) overnight at room temperature, then with horseradish peroxidase-labeled secondary antibody, rabbit anti-mouse IgG (1:1,000, Dako, Carpenteria, CA) for 1 h. Immunoblots were visualized by chemiluminescence using the enhanced chemiluminescence Western blotting detection system, exposed to X-ray film, and quantified using a computing densitometer.

Statistical analysis. Data from two groups were compared by a two-tailed *t*-test, and those from more than two groups were compared by one-way ANOVA with correction for multiple comparison (10). A difference is considered to be significant at $P < 0.05$.

RESULTS

Effect of nmLPS on TNF-α and MIP-1α production. To determine whether the reduced capacity of nmLPS to activate human monocytes was restricted to TNF-α production, we measured the production of TNF-α and MIP-1α, a member of the chemokine β-family (39), in response to wtLPS and nmLPS. As shown in Fig. 1*A*, at 4 h after incubation, wtLPS induced a concentration (0.01 ng/ml–1.0 μg/ml)-dependent release of TNF-α and MIP-1α into the medium. In contrast, nmLPS even at a concentration as high as 1 μg/ml failed to induce a significant amount of cytokine release. Time-course experiments using 1 μg/ml of LPS (Fig. 1*B*) demonstrated wtLPS-induced cytokine release within 1 h, reached a near plateau at 4 h, and lasted for 18 h. Production of TNF-α and MIP-1α by nmLPS-treated cells was markedly reduced throughout these time periods. Measurement of TNF-α activity in cell lysates revealed negligible TNF-α in wtLPS- and nmLPS-

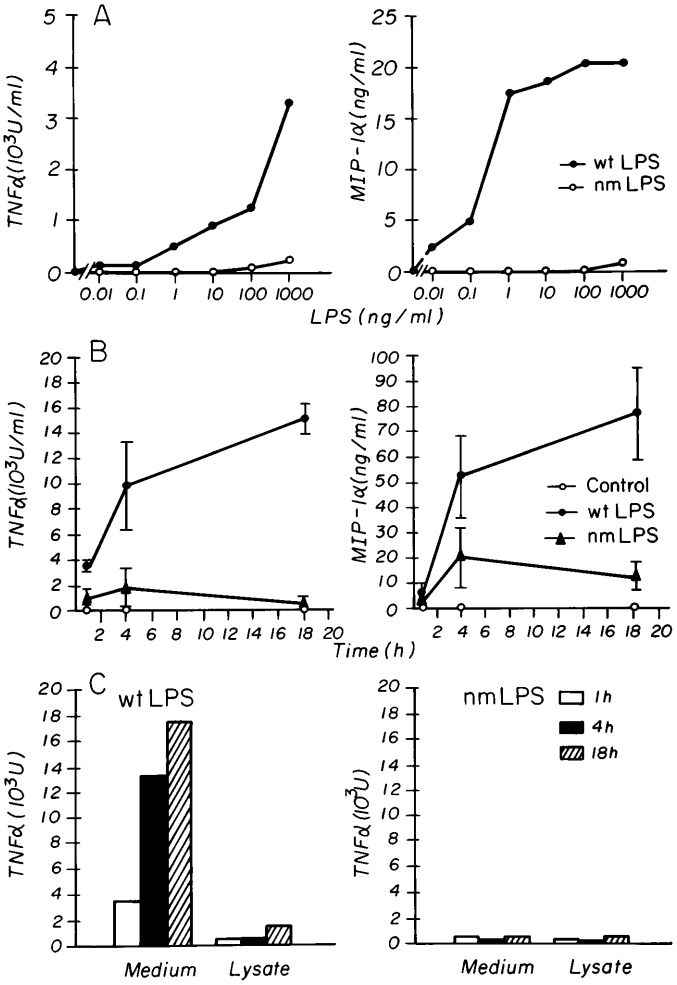

Fig. 1. Effects of wild-type (wtLPS) and nonmyristoyl lipopolysaccharide (nmLPS) on tumor necrosis factor-α (TNF-α) and macrophage inflammatory protein-1α (MIP-1α) production by human monocytes. Adherent monocytes in RPMI 1640 plus 5% autologous serum were treated with or without LPS, and amounts of TNF-α activity and MIP-1α released into media or present in cell lysates were determined. *A*: dose (0.01 ng/ml–1.0 μg/ml)-response experiment, 4 h incubation. *B*: time course experiments, 1 μg/ml wtLPS or nmLPS; values are means ± SE (n = 3). *C*: TNF-α in media and lysates, 1 μg/ml wtLPS or nmLPS.

treated cells (Fig. 1*C*), suggesting that reduced TNF-α release by nmLPS-treated monocytes was in fact due to a decreased production rather than an impaired secretion of the cytokine.

Effect of nmLPS on induction of Mn SOD. We then determined whether nmLPS also had a similarly reduced capacity to induce Mn SOD. LPS has been shown to selectively induce Mn SOD without affecting other antioxidant enzymes, including Cu,Zn SOD, catalase, and glutathione peroxidase (1, 27). The results (Fig. 2) revealed that at 18 h, but not at 1 or 4 h, after incubation, wtLPS induced a marked increase (2.5-fold) in monocyte Mn SOD activity, whereas it had no effect on Cu,Zn SOD activity. nmLPS also induced a significant increase (1.5-fold) in the Mn SOD activity at 18 h, although to a lesser degree than wtLPS.

Figure 3 compares wtLPS and nmLPS in their endotoxin activities as measured by the ability to induce coagulation of the LAL and their capacities to induce monocyte cytokine (TNF-α and MIP-1α) release and SOD (Mn SOD and Cu,Zn SOD) activities. Compared with wtLPS, nmLPS has a comparable LAL clotting ability, consistent with the report of Somerville et al. (29), a markedly reduced capacity to induce monocyte TNF-α and MIP-1α release, and a relatively well preserved ability to induce monocyte Mn SOD. Neither wtLPS nor nmLPS had an effect on Cu,Zn SOD.

The difference in Mn SOD activity between wtLPS- and nmLPS-treated cells, as shown in Fig. 3, was statistically significant. This difference could be due to the effect of TNF-α produced by wtLPS-treated cells, since TNF-α is known to induce Mn SOD (36, 40). To test this possibility, we coincubated monocytes with wtLPS and anti-human TNF-α antibody (anti-TNF-α IgG). As shown in Fig. 4*A*, anti-TNF-α IgG completely neutralized the TNF-α activity induced by wtLPS, whereas control, nonspecific IgG had no effect. In contrast, anti-TNF-α IgG did not reduce Mn SOD activity in wtLPS-treated cells to the level of nmLPS-treated cells (Fig. 4, *B–D*). These results suggested that TNF-α did not contribute to the higher level of Mn SOD activity observed in wtLPS-treated monocytes.

Effect of nmLPS on TNF-α and Mn SOD mRNAs. Northern analysis of TNF-α and Mn SOD mRNAs (Fig. 5) further supported the above-observed effects of wtLPS and nmLPS in the production of TNF-α and the induction of Mn SOD enzyme activity by human monocytes. wtLPS markedly enhanced the levels of TNF-α (17-fold) and Mn SOD (7-fold) mRNAs compared with control, nontreated cells. nmLPS also induced a marked increase (6-fold) in the level of Mn SOD mRNA, whereas it had a markedly reduced effect on TNF-α mRNA (1-fold increase vs. 17-fold increase by wtLPS).

Effect of nmLPS on activation of NF-κB. Considerable evidence suggests that activation of NF-κB is essential for the induction of monocyte and macrophage TNF-α mRNA by LPS (4, 24, 26). Whether induction of monocyte Mn SOD mRNA by LPS is also dependent on NF-κB is not clear. We took advantage of the differential effects of wtLPS and nmLPS observed above to determine the potential role of NF-κB activation in the LPS-induced induction of TNF-α and Mn SOD mRNAs using the EMSA. As shown in Fig. 6, wtLPS and nmLPS markedly activated NF-κB at 1 h after treatment. However, by 4 h the effect was largely gone (data not shown). There was no difference between wtLPS and nmLPS.

Effect of nmLPS on activation of MAPK. The MAPK signal transduction pathway plays an important role in the LPS-induced production of TNF-α (4, 11, 12, 23, 30). We determined whether failure of nmLPS to induce TNF-α production was due to its inability to activate MAPK. Cytoplasmic and nuclear fractions were immunoprecipitated with antiphosphotyrosine, then immunoblot analysis of phosphorylated MAPK was performed. As shown in Fig. 7, wtLPS markedly increased the nuclear content of tyrosine-phosphorylated MAPK. In

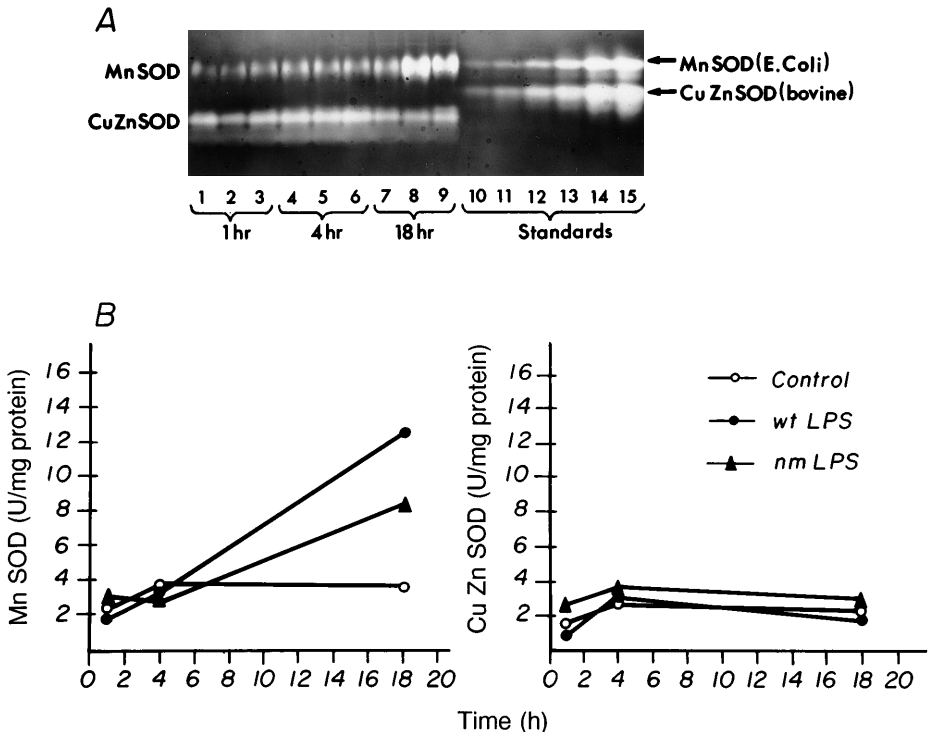

Fig. 2. Effects of wtLPS and nmLPS on monocyte superoxide dismutase (SOD) activity. Adherent monocytes were treated with or without 1 μg/ml wtLPS or nmLPS for 1, 4, or 18 h, and SOD activities of cell extracts (50 μg/lane) were assayed using activity gel. Purified *Escherichia coli* Mn SOD and bovine erythrocyte Cu,Zn SOD (25–800 mU) were used as standards. *A*: SOD activity gel. *Lanes 1, 4,* and *7,* control; *lanes 2, 5,* and *8,* wtLPS; *lanes 3, 6,* and *9,* nmLPS; *lanes 10–15,* standards. *B*: densitometric quantification of monocyte Mn SOD and Cu,Zn SOD activities. Results were expressed as U/mg protein according to McCord and Fridovich (18).

contrast, nmLPS failed to induce tyrosine phosphorylation and nuclear translocation of MAPK. No tyrosine-phosphorylated MAPK was detectable in the cytoplasmic fractions of wtLPS- or nmLPS-treated monocytes (data not shown).

DISCUSSION

The data presented in this study demonstrated that deletion of myristoyl fatty acid at the 3′ *R*-3-hydroxymyristate position of the *E. coli* lipid A moeity (nmLPS) resulted in a markedly reduced ability to induce the production of not only TNF-α (29), but also MIP-1α, by human monocytes, suggesting a more generalized phenomenon of impaired induction of inflammatory cytokine production by nmLPS. Furthermore, the impaired induction of inflammatory cytokine production by nmLPS was associated with an impaired activation (tyrosine phosphorylation and nuclear translocation) of MAPK but a normal activation of NF-κB.

The production of TNF-α by monocytes is regulated at the transcriptional and translational levels (4, 14, 15). The exact signal transduction pathway(s) by which LPS induces TNF-α production has not been fully established. However, LPS stimulates TNF-α gene transcription and translation, and it requires the activation of the protein tyrosine kinase (PTK)/ras/raf-1/MEK/MAPK signal transduction pathway and NF-κB (4, 11, 12, 14, 15, 23, 30). Thus the inability to activate MAPK by nmLPS may explain the observed impairment in TNF-α production by nmLPS-treated human monocytes. The exact location and mechanism by which nmLPS fails to activate MAPK are not clear. It is likely to be at the level of PTK activation (receptor-PTK interaction) or ras activation, since other members of

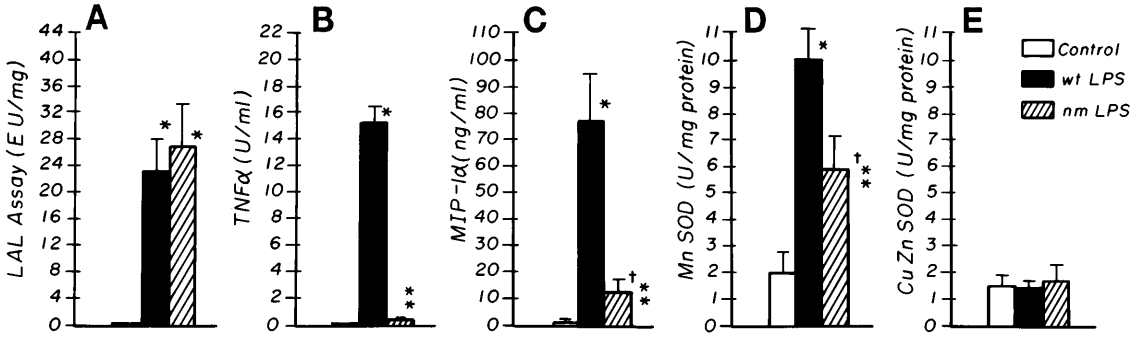

Fig. 3. Comparison of wtLPS and nmLPS endotoxin activities and their effects on monocyte cytokine release and SOD activities. Endotoxin activities were assayed using *Limulus* amoebocyte lysate (LAL) and expressed as endotoxin units (EU). For cytokine release and SOD activity, adherent monocytes were treated with or without 1 μg/ml wtLPS or nmLPS for 18 h, and amounts of TNF-α activity and MIP-1α protein in media and Mn SOD and Cu,Zn SOD activities of cell extracts were determined. Values are means ± SE of 3–6 experiments. $*P < 0.001$; $\dagger P < 0.05$, treatment vs. control. $**P < 0.01$ vs. wtLPS.

the MAPK pathway do not have access to the plasma membrane at which wtLPS or nmLPS is acting. Further studies are necessary to clarify this point.

Activation of NF-κB, although essential, is not sufficient for the induction of TNF-α mRNA and production of TNF-α by LPS in monocytes/macrophages. Peritoneal macrophages from LPS-resistant, C3H/HeJ mice are able to respond to LPS by activating NF-κB normally (9) but are unable to induce TNF-α mRNA and produce TNF-α (3). The TNF-α synthetic pathway in C3H/HeJ macrophages is intact, since they are able to produce TNF-α normally on costimulation with interferon-γ and LPS (6). Our observation that nmLPS activated monocyte NF-κB normally but failed to induce TNF-α mRNA and TNF-α production is consistent with this concept. Recent studies suggest that concerted participation of *cis*-acting regulatory elements at the Egr-1 (42), AP-1/CRE-like (20), NF-IL6 (C/EBPβ) (22), and κB3 sites is required for optimal induction of the TNF-α promoter by LPS in human monocytes.

Binding of LPS to a circulating LPS-binding protein, which in turn binds to the monocyte LPS membrane receptor mCD14, is essential in the efficient cellular response to low concentrations (<100 ng/ml) of LPS (4, 30, 32, 41). However, the concentration of LPS used in the current study (1 μg/ml) is not dependent on mCD14 binding to induce TNF-α production by human monocytes (4, 30). Somerville et al. (29) demonstrated that the effect of nmLPS can be inhibited by a monoclonal anti-CD14 antibody, MY4. In addition, at high concen-

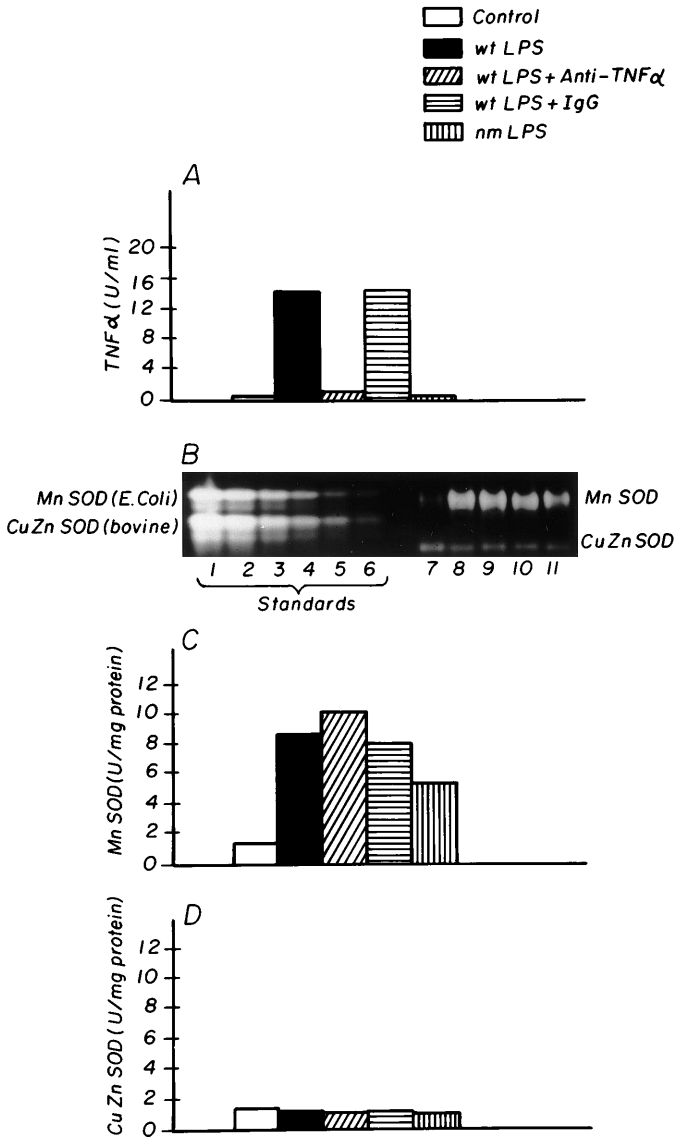

Fig. 4. Role of TNF-α on wtLPS-induced increase in Mn SOD activity. Adherent monocytes were treated with or without 1 μg/ml wtLPS in presence or absence of goat anti-human TNF-α IgG (0.16 mg/ml), 0.16 mg/ml goat nonspecific IgG, or 1 μg/ml nmLPS for 18 h, and TNF-α activities in media and SOD activities of cell extracts were determined. *A*: TNF-α activity. *B*: SOD activity gel. *Lanes 1–6*, standards; *lane 7*, control; *lane 8*, wtLPS; *lane 9*, wtLPS + anti-TNF-α IgG; *lane 10*, wtLPS + IgG; *lane 11*, nmLPS. *C*: Mn SOD activity. *D*: Cu,Zn SOD activity.

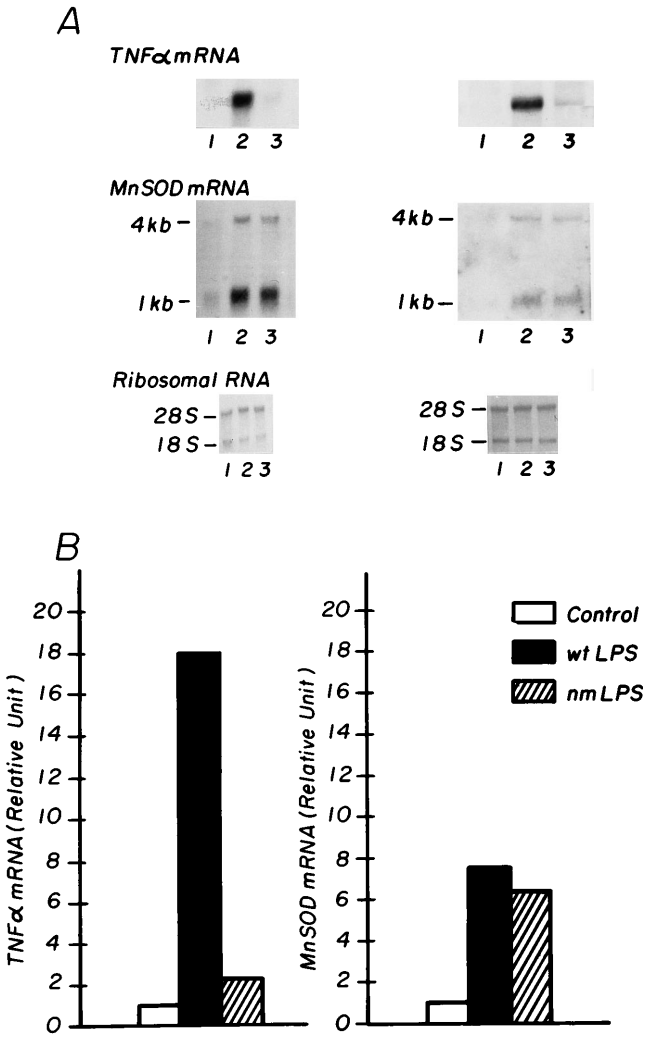

Fig. 5. Effects of wtLPS and nmLPS on steady-state levels of TNF-α and Mn SOD mRNAs. Adherent monocytes were treated with or without 1 μg/ml wtLPS or nmLPS for 4 h. Cellular RNAs were extracted, and aliquots (20 μg/lane) were subjected to Northern blot analysis for TNF-α and Mn SOD mRNAs. *A*: Northern blots from 2 experiments. *Lane 1*, control; *lane 2*, wtLPS; *lane 3*, nmLPS. *B*: densitometric quantification. Results are means of 2 experiments and are expressed as relative units with mean values of controls normalized to 1 relative unit.

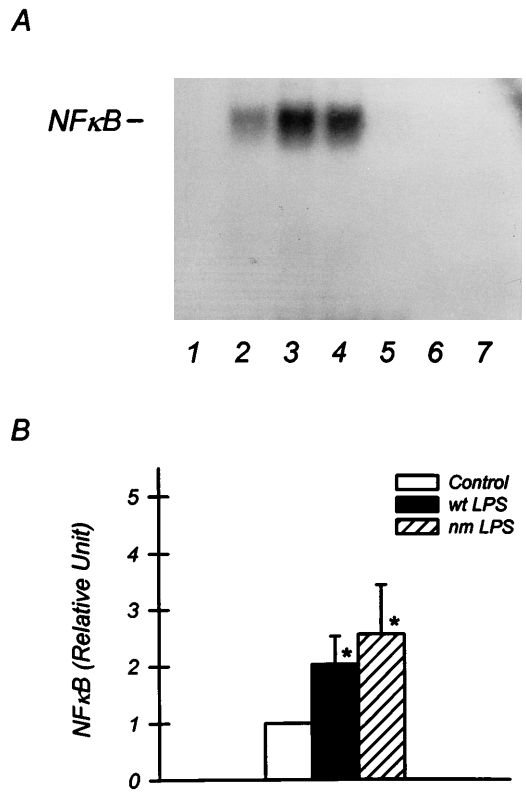

Fig. 6. Activation of nuclear factor-κB (NF-κB) by wtLPS and nmLPS. Adherent monocytes were treated with or without 1 μg/ml wtLPS or nmLPS for 1 h. Nuclear extracts were obtained, and aliquots (3 μg/lane) were probed for NF-κB using electrophoresis mobility shift assay. *A*: representative electrophoresis mobility shift assay. *Lane 1*, probe alone; *lane 2*, control; *lane 3*, wtLPS; *lane 4*, nmLPS; *lane 5*, control + competition; *lane 6*, wtLPS + competition; *lane 7*, nmLPS + competition. *B*: densitometric quantification. Results are expressed as relative units with mean values of controls normalized to 1 relative unit. Values are means ± SE ($n = 3$). *$P <$ 0.05 vs. control.

trations, nmLPS competitively inhibits wtLPS-induced E-selectin induction in human endothelial cells. It was, therefore, suggested that nmLPS could serve as an LPS antagonist through competitive binding to mCD14 (29). A recent study by Cunningham et al. (8) reveals that nmLPS, compared with wtLPS, binds normally to immobilized soluble CD14 (sCD14) and a panel of 23 different point-mutated sCD14 molecules. Thus failure of nmLPS to induce TNF-α production by human monocytes is unlikely due to an impaired binding to CD14.

Little is known about the regulation of Mn SOD gene expression and the signal transduction pathway(s) responsible for the LPS-mediated induction of Mn SOD. Our results suggest that inductions of Mn SOD and TNF-α by LPS are mediated through different signal transduction pathways, since nmLPS was able to induce Mn SOD mRNA and enzyme activity without inducing TNF-α mRNA and activity. These results are consistent with previous observations in LPS-resistant C3H/HeJ mice. Peritoneal macrophages from C3H/HeJ mice are unable to produce TNF-α in response to LPS (3); however, LPS is able to induce Mn SOD normally in these macrophages compared with macrophages from LPS-sensitive, C3H/HeOuJ mice (13). Although LPS induction of Mn SOD in human monocytes, as demonstrated in the current study and in peritoneal macrophages from C3H/HeJ mice (9, 13), is associated with the activation of NF-κB, the role of NF-κB activation in the induction of Mn SOD by LPS cannot be conclusively ascertained.

The reason for the reduced increase of Mn SOD activity in nmLPS-treated moncytes in the face of an almost comparable increase in Mn SOD mRNA, compared with wtLPS-treated cells, is not clear. However, we demonstrated that neutralization of TNF-α activity did not reduce Mn SOD activity of wtLPS-treated cells to the level of nmLPS-treated monocytes, suggesting that TNF-α produced by wtLPS-treated cells could not account for the observed difference in the levels of Mn SOD activity. Because nmLPS is unable to activate MAPK, it is possible that the MAPK pathway via cross talk contributes to the signal transduction cascade involved in the induction of Mn SOD by LPS and that the inability of nmLPS to activate MAPK is responsible for the reduced increase of Mn SOD activity in nmLPS-treated monocytes. Further studies are necessary to determine the role of MAPK in the induction of Mn SOD by LPS. In addition, IL-1, which is known to be produced by wtLPS-treated monocytes, can also induce

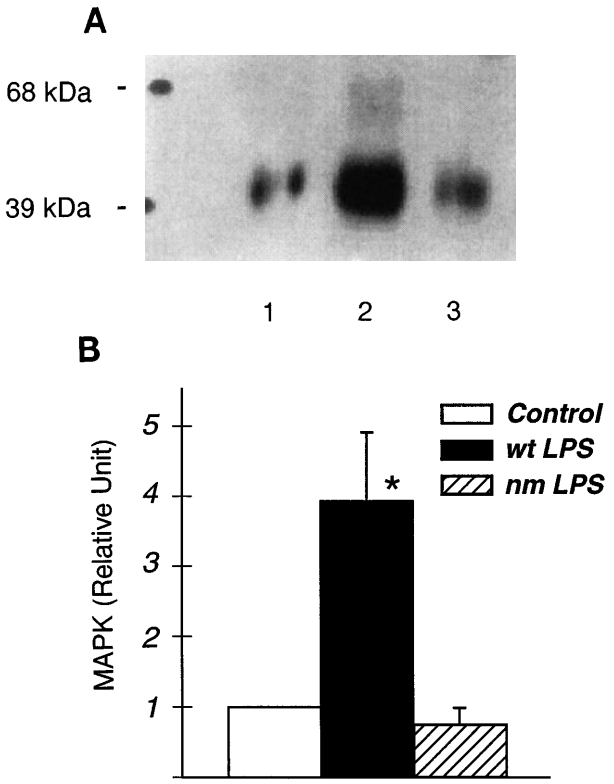

Fig. 7. Effect of wtLPS and nmLPS on phosphorylation and nuclear translocation of mitogen-activated protein kinase (MAPK). Adherent monocytes were treated with or without 1 μg/ml wtLPS or nmLPS for 1 h. Nuclear proteins were extracted, immunoprecipitated with antiphosphotyrosine, and immunoblotted for MAPK. *A*: representative immunoblot. *Lane 1*, control; *lane 2*, wtLPS, *lane 3*, nmLPS. *B*: densitometric quantification. Results are expressed as relative units with mean values of controls normalized to 1 relative unit. Values are means ± SE ($n = 3$). *$P < 0.05$ vs. control or nmLPS.

Mn SOD (17). It is not clear whether nmLPS induces monocytes to produce IL-1 and whether IL-1 produced by wtLPS-treated monocytes can account for the observed difference in the levels of MnSOD activity between wtLPS- and nmLPS-treated cells.

In summary, we have demonstrated that induction of Mn SOD and TNF-α production by LPS in human monocytes can be dissociated, suggesting that induction of Mn SOD and TNF-α is regulated by different signal transduction pathways. The ability of nmLPS to induce Mn SOD without inflammatory cytokine production provides an opportunity to study the potential benefits of an LPS-induced increase in Mn SOD without the associated toxic effects of inflammatory cytokines.

This work was supported by the Department of Veterans Affairs, Office of Research and Development, Medical Research Service.

Address for reprint requests: M.-F. Tsan, Research Service (151), Stratton VA Medical Center, 113 Holland Ave., Albany, NY 12208.

Received 13 March 1998; accepted in final form 2 June 1998.

REFERENCES

1. **Asayama, K., R. L. Janco, and I. M. Burr.** Selective induction of manganous superoxide dismutase in human monocytes. *Am. J. Physiol.* 249 (*Cell Physiol.* 18): C393–C397, 1985.
2. **Beauchamp, C., and I. Fridovich.** Superoxide dismutase: improved assay and an assay application to acrylamide gels. *Anal. Biochem.* 44: 276–282, 1971.
3. **Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami.** Control of cachetin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science* 232: 977–980, 1986.
4. **Beutler, B., and V. Kruys.** Lipopolysaccharide signal transduction, regulation of tumor necrosis factor biosynthesis, and signaling by tumor necrosis factor itself. *J. Cardiovasc. Pharmacol.* 25: S1–S8, 1995.
5. **Beutler, B., I. W. Milsark, and A. Cerami.** Passive immunization against cachetin/tumor necrosis factor (TNF) protects mice from the lethal effect of endotoxin. *Science* 229: 869–871, 1985.
6. **Beutler, B., V. Tkacenko, I. Milsark, N. Krochin, and A. Cerami.** Effect of γ interferon on cachetin expression by mononuclear phagocytes. *J. Exp. Med.* 164: 1791–1796, 1986.
7. **Chomczynski, P., and N. Sacchi.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162: 156–159, 1987.
8. **Cunningham, M. D., J. Bajorath, J. E. Somerville, and R. P. Darveau.** *Escherichia coli* and *Porphyromonase gingivalis* interactions with CD14: implications for myeloid and non-myeloid cell activation. *J. Clin. Infect. Dis.* In press.
9. **Ding, A., S. Hwang, H. M. Lander, and Q.-W. Xie.** Macrophages derived from C3H/HeJ (Lps^d) mice respond to bacterial lipopolysaccharide by activating NF-κB. *J. Leukoc. Biol.* 57: 174–179, 1995.
10. **Einot, I., and K. R. Gabriel.** A study of power of several methods of multiple comparison. *J. Am. Stat. Assoc.* 70: 574–583, 1975.
11. **Geng, Y., B. Zhang, and M. Lotz.** Protein tyrosine kinase activation is required for lipopolysaccharide induction of cytokines in human monocytes. *J. Immunol.* 151: 6692–6700, 1993.
12. **Geppert, T. D., C. E. Whitehurst, P. Thompson, and B. Beutler.** LPS signals activation of TNF biosynthesis through the RAS/RAF-1/MEK/MAPK pathway. *Mol. Med.* 1: 93–103, 1994.
13. **Gibbs, L. S., P. J. Del Vecchio, and J. B. Shaffer.** Mn and Cu/Zn SOD expression in cells from LPS-sensitive and LPS-resistant mice. *Free Radic. Biol. Med.* 12: 107–111, 1992.
14. **Han, J., T. Brown, and B. Beutler.** Endotoxin-responsive sequences control cachetin/tumor necrosis factor biosynthesis at the translational level. *J. Exp. Med.* 171: 465–475, 1990.
15. **Han, J., G. Huez, and B. Beutler.** Interactive effects of the tumor necrosis factor promoter and 3′-untranslated regions. *J. Immunol.* 146: 1843–1848, 1991.
16. **Lin, H. Y., F. B. Davis, J. K. Gordinier, L. J. Martino, and P. J. Davis.** Thyroid hormone induces phosphorylation and nuclear translocation of mitogen-activated protein kinase. *J. Immunol.* In press.
17. **Masuda, A., D. L. Longo, Y. Kobayashi, E. Appella, J. J. Oppenheim, and K. Matsushima.** Induction of mitochondrial manganese superoxide dismutase by interleukin 1. *FASEB J.* 2: 2087–2091, 1988.
18. **McCord, J. M., and I. Fridovich.** Superoxide dismutase. An enzymatic function for erythrocuprein (hemocuprein). *J. Biol. Chem.* 244: 6049–6055, 1969.
19. **Morrison, D. C., and J. L. Ryan.** Bacterial endotoxins and host immune responses. *Adv. Immunol.* 28: 293–450, 1979.
20. **Newell, C. L., A. B. Deisseroth, and G. Lopez-Berestein.** Interaction of nuclear proteins with an AP-1/CRE-like promoter sequence in the human TNF-α gene. *J. Leukoc. Biol.* 56: 27–35, 1994.
21. **Osnes, L. T. N., K. B. Foss, G. B. Joo, C. Okkenhaug, A.-B. Westvik, R. Ovstebo, and P. Kierulf.** Acetylsalicylic acid and sodium salicylate inhibit LPS-induced NF-κB/c-Rel nuclear translocation, and synthesis of tissue factor (TF) and tumor necrosis factor-α (TNF-α) in human monocytes. *Thromb. Haemost.* 76: 970–976, 1996.
22. **Pope, R. M., A. Leutz, and S. A. Ness.** C/EBPβ regulation of the tumor necrosis factor-α gene. *J. Clin. Invest.* 94: 1449–1455, 1994.
23. **Reiman, T., D. Buscher, R. A. Hipskind, S. Krautwald, M.-L. Lohmann-Matthes, and M. Baccarini.** Lipopolysaccharide induces activation of the Raf-1/MAPK kinase pathway. A putative role for Raf-1 in the induction of the IL-1β and TNF-α genes. *J. Immunol.* 153: 5740–5749, 1994.
24. **Rietschel, E. T., and H. Brade.** Bacterial endotoxins. *Sci. Am.* 267: 54–61, 1992.
25. **Rietschel, E. T., T. Kirikae, F. U. Shade, A. J. Ulmer, O. Holst, H. Brade, G. Schmidt, U. Mamat, H.-D. Grimmecke, S. Kusumoto, and U. Zahringer.** The chemical structure of bacterial endotoxin in relation to bioactivity. *Immunobiology* 187: 169–190, 1993.
26. **Shakhov, A. N., M. A. Collart, P. Vassalli, S. A. Nedospasov, and C. V. Jongeneel.** κB-type enhancers are involved in lipopolysaccharide-mediated transcriptional activation of the tumor necrosis factor-α gene in primary macrophages. *J. Exp. Med.* 171: 35–47, 1990.
27. **Shiki, Y., B. O. Meyerick, K. L. Brigham, and I. M. Burr.** Endotoxin increases superoxide dismutase in cultured bovine pulmonary endothelial cells. *Am. J. Physiol.* 252 (*Cell Physiol.* 21): C436–C440, 1987.
28. **Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk.** Measurement of protein using bicinchoninic acids. *Anal. Biochem.* 150: 76–85, 1985.
29. **Somerville, J. E., Jr., L. Cassiano, B. Bainbridge, M. D. Cunningham, and R. P. Darveau.** A novel *Escherichia coli* lipid A mutant that produces an anti-inflammatory lipopolysaccharide. *J. Clin. Invest.* 97: 359–365, 1996.
30. **Sweet, M. J., and D. A. Hume.** Endotoxin signal transduction in macrophages. *J. Leukoc. Biol.* 60: 8–26, 1996.
31. **Tang, G., J. T. Berg, J. E. White, P. D. Lumb, C. Y. Lee, and M. F. Tsan.** Protection against oxygen toxicity by tracheal insufflation of endotoxin: role of Mn SOD and alveolar macrophages. *Am. J. Physiol.* 266 (*Lung Cell. Mol. Physiol.* 10): L38–L45, 1994.
32. **Tobias, P. S., K. Soldau, and R. J. Ulevich.** Isolation of a lipopolysaccharide-binding acute phase reactant from rabbit serum. *J. Exp. Med.* 164: 777–793, 1986.
33. **Tsan, M. F.** Superoxide dismutase and pulmonary oxygen toxicity. *Proc. Soc. Exp. Biol. Med.* 214: 107–113, 1997.
34. **Tsan, M. F., D. Lawrence, and J. E. White.** Erythrocyte insufflation-induced protection against oxygen toxicity: role of cytokines. *J. Appl. Physiol.* 71: 1751–1757, 1991.

35. **Tsan, M. F., N. J. Tacy, B. A. Lindau-Shepard, and J. E. White.** Protection of rats against oxygen toxicity by tracheal administration of plasmid DNA: role of endogenous tumor necrosis factor. *Proc. Assoc. Am. Physicians* 109: 409–419, 1997.
36. **Tsan, M. F., J. E. White, C. Treanor, and J. B. Shaffer.** Molecular basis for tumor necrosis factor-induced increase in pulmonary superoxide dismutase activities. *Am. J. Physiol.* 259 (*Lung Cell. Mol. Physiol.* 3): L506–L512, 1990.
37. **Wen, Z., Z. Zhong, and J. E. Darnell, Jr.** Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. *Cell* 82: 241–250, 1995.
38. **White, J. E., and M. F. Tsan.** Induction of pulmonary Mn superoxide dismutase mRNA by interleukin-1. *Am. J. Physiol.* 266 (*Lung Cell. Mol. Physiol.* 10): L664–L671, 1994.
39. **Wolpe, S. D., and A. Cerami.** Macrophage inflammatory proteins 1 and 2: members of a novel superfamily of cytokines. *FASEB J.* 3: 2565–2573, 1989.
40. **Wong, G. H. W., and D. V. Goddel.** Induction of manganous superoxide dismutase by tumor necrosis factor. Possible protective mechanism. *Science* 242: 941–944, 1988.
41. **Wright, S. D., R. A. Ramos, P. S. Tobias, R. J. Ulevich, and J. C. Mathison.** CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* 249: 1431–1433, 1990.
42. **Yao, J., N. Mackman, T. S. Edgington, and S.-T. Fan.** Lipopolysaccharide induction of the tumor necrosis factor-α promoter in human monocytic cells. *J. Biol. Chem.* 272: 17795–17801, 1997.