Dimethyl Sulfoxide Modulates NF- κ B and Cytokine Activation in Lipopolysaccharide-Treated Murine Macrophages

KATHERINE A. KELLY,^{1,2} MOLLY R. HILL,^{1,3,4} KELLE YOUKHANA,¹ FRANK WANKER,¹ AND JEFFREY M. GIMBLE^{1,5,6*}

Immunobiology and Cancer Program, Oklahoma Medical Research Foundation, Oklahoma City, Oklahoma 73104¹; Department of Orthodontics, College of Dentistry,² Department of Clinical Laboratory Sciences, College of Allied Health,³ and Department of Microbiology and Immunology⁴ and Department of Pathology,⁶ College of Medicine, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma 73190; and Department of Natural Sciences, Oklahoma Christian University, Oklahoma City, Oklahoma 73136⁵

Received 24 January 1994/Returned for modification 22 March 1994/Accepted 6 May 1994

Antioxidants are protective against septic shock in animal models. Recently, free radical scavengers have been found to inhibit the activation of the NF- κ B protein in a number of cell lines. This transcriptional regulatory protein binds to the promoters of the proinflammatory cytokines tumor necrosis factor, interleukin-6, and the macrophage inflammatory proteins. The current work examined lipopolysaccharide-induced NF- κ B activation in the J774 macrophage-like cell line and primary peritoneal macrophages from lipopolysaccharide-responsive (C3HeB/Fej) and -nonresponsive (C3H/HeJ) murine strains. The DNA-binding activity of the NF- κ B protein directly correlated with mRNA expression for the genes encoding the proinflammatory cytokines and the free radical scavenging enzyme, superoxide dismutase. Both the p50 and p65 NF- κ B subunits were detected on gel supershift assays. Minimal NF- κ B activity was observed following exposure of C3H/HeJ macrophages to lipopolysaccharide. The antioxidant dimethyl sulfoxide decreased the level of NF- κ B activation in the J774 cells. This correlated with decreased expression of cytokine mRNAs and tumor necrosis factor bioactivity. These results suggest that modulation of NF- κ B activation may provide a mechanism through which antioxidants protect against endotoxemia in murine models.

The host response to lipopolysaccharide (LPS) during gram-negative infection involves a cascade of events which can culminate in septic shock. The systemic release of proinflammatory cytokines plays a major role in initiating this immune defense. Recent advances have been made in understanding the mechanism of transcriptional regulation of cytokines such as tumor necrosis factor (TNF). The NF- κ B protein was originally described as a transcriptional factor recognizing a specific DNA sequence motif within the immunoglobulin κ gene enhancer (15, 17, 42). Since that time, NF- κ B-binding sites have been found to regulate transcription initiation from a wide variety of gene promoters. These include the proinflammatory cytokines TNF (8, 12, 16, 43, 50), interleukin-6 (IL-6) (3, 19, 29, 44), and macrophage inflammatory protein (MIP-1 α , MIP-1 β) of the IL-8/intercrine family (9, 18, 33, 46). The NF- κ B protein complex consists of homo- or heterodimers of the p50 and p65 subunits (17). In resting cells, NF- κ B resides in the cytoplasm associated with an inhibitor, I κ B (17). Following cell activation by cytokines, LPS, or chemical agents, the I κ B protein is proteolytically degraded, thereby activating the DNA-binding ability of NF- κ B and freeing it for transport into the nucleus (22). Rapid changes in the oxidation state of the cell may provide the mechanism underlying these events. Hydrogen peroxide exposure alone can activate NF- κ B in resting cells (17, 41). Moreover, the presence of antioxidants such as *N*-acetylcysteine or pyrrolidine dithiocarbamate (PDTC) can inhibit LPS and cytokine induction of NF- κ B in lymphocytes and other cells (17, 21, 26, 27, 30, 31, 37, 40, 41, 45, 53, 59). These findings indicate that NF- κ B may serve as a

common target for antioxidant compounds known to influence cellular functions associated with immune defense.

Recent investigations with human macrophages have demonstrated that LPS-induced TNF expression was regulated by a free radical scavenger, PDTC, and was directly correlated with the level of NF- κ B activation (59). Free radical scavengers are known to protect against LPS-induced septic shock in rodent models (4, 20, 34). These actions are associated with reduced levels of macrophage-derived proinflammatory cytokines (34). Dimethyl sulfoxide (DMSO) is among the most effective of these agents (4, 9, 28, 36, 54). The current work examines the LPS response in murine macrophages by using both the established J774 cell line (35) and primary peritoneal macrophages from LPS-responsive and -nonresponsive strains of mice (1, 2, 5, 14, 23, 38, 47-49, 55, 56). The expression of mRNA transcripts for proinflammatory cytokines (IL-6, MIP-1 α , MIP-1 β , and TNF) and a free radical scavenging enzyme (superoxide dismutase [57, 58]) was determined in correlation with NF- κ B activation. The murine macrophage response was partially inhibited by pretreatment with DMSO. These results indicate that antioxidants can modulate LPS-induced cytokine production in macrophages through an NF- κ B-mediated mechanism.

MATERIALS AND METHODS

Cell culture. Reagents were obtained from Sigma Chemical Co., St. Louis, Mo., unless otherwise noted. All cells were maintained in Dulbecco's modified Eagle's medium (Gibco-BRL, Gaithersburg, Md.) supplemented with 10% fetal bovine serum (Defined; Hyclone, Logan, Utah), 1% sodium pyruvate, 100 U of penicillin per ml, and 100 μ g of streptomycin per ml and cultured at 37°C with 7% CO₂. The histiocytic lymphoma cell line J774 (TIB 67) was obtained from the American Type

* Corresponding author. Mailing address: Immunobiology and Cancer Program, Oklahoma Medical Research Foundation, 825 NE 13th St., Oklahoma City, OK 73104. Phone: (405) 271-7905. Fax: (405) 271-8568.

Culture Collection, Rockville, Md., and passaged every 3 days (35). C3H/HeJ and C3HeB/Fej mice (Jackson Laboratory, Bar Harbor, Maine) were given injections of 4% thioglycolate (2 ml intraperitoneally) 4 days prior to the isolation of peritoneal macrophages. After being washed once with phosphate-buffered saline, the cells were suspended in medium (3×10^6 cells per ml) and incubated in 35-mm culture dishes (Corning, Corning, N.Y.) for 2 h; the medium was then replaced. The peritoneal macrophages were then cultured for 4 days prior to each experiment. Cells were either treated directly with LPS (*Escherichia coli* O111:B4; courtesy of R. McCallum, Texas A&M University, College Station, Tex.) for various periods or pretreated with 0 to 2% DMSO for 15 min, 20 mM *N*-acetylcysteine for 24 h, or 0 to 500 μ M PDTC for 2 h prior to induction with LPS (1% DMSO is equivalent to 141 mM; 2% DMSO is equivalent to 282 mM). Inductions were performed in Dulbecco's modified Eagle's medium containing 2% fetal bovine serum. Conditioned medium and cells were harvested for bioactivity assays and RNA isolation, respectively.

RNA analysis. Total-cell RNA was isolated as previously described (6, 11). Northern (RNA) blots were prepared with approximately 10 μ g of total RNA per lane, transferred to MSI-NT nylon membrane (MSI, Westboro, Mass.), and UV cross-linked (11, 52). The following cDNA probes were labeled by the random-primer method (13): actin, IL-6 (courtesy of S. Clark, Genetics Institute, Cambridge, Mass.), MIP-1 α and MIP-1 β (courtesy of P. Olson, Chiron Corp., Emeryville, Calif.), superoxide dismutase (courtesy of D. Goeddel, Genentech, South San Francisco, Calif.), and TNF (courtesy of B. Beutler, University of Texas Southwestern, Dallas, Tex.). Blots were hybridized and washed at a maximum stringency of 55°C (7, 11). Autoradiographs were prepared after exposure for 1 to 7 days at -70°C with an enhancing screen.

TNF bioactivity assay. The TNF-sensitive fibroblast cell line L929 (obtained from J. Murphy, University of Oklahoma Health Sciences Center, Oklahoma City, Okla.) was exposed to macrophage-conditioned medium in the presence of actinomycin D to determine the production of bioactive cytokine (24). Cell viability and proliferation were evaluated spectrophotometrically and quantitated relative to a standard curve of recombinant TNF (32). One unit of TNF activity was defined as the reciprocal of the dilution effecting 50% killing of L929 indicator cells. Bioactivity experiments were conducted in duplicate and assayed in triplicate.

Gel retention analysis. Complementary oligonucleotides were prepared by Ken Jackson, Oklahoma Center for Molecular Medicine, for the following sequences (the protein recognition motifs are underlined): murine immunoglobulin κ enhancer NF- κ B site, 5'-ATCTCAACAGAGGGGACTTTCCGAGAGCCA-3' (17); murine IL-6 promoter NF- κ B site, 5'-AAATGTGGGATTTTCCCATGAG-3' (29); and murine lipoprotein lipase promoter octamer 1 site, 5'-TGAGTCTTATTTGCATATTTCCAG-3' (25). Nuclear extracts were prepared by the method of Dignam et al. (10). Protein concentrations were determined by using the bicinchoninic acid reagent as specified by the manufacturer (Pierce, Rockford, Ill.). All assays were conducted with equivalent amounts of nuclear extract protein (5 μ g) and performed as previously described (25, 50). In competition studies, unlabeled oligonucleotides (immunoglobulin NF- κ B, IL-6, NF- κ B, or OCT-1) were added to the incubation mixture. In supershift assays, polyclonal antibodies were present throughout the incubation period at 1:20 dilutions; these included rabbit anti-NF- κ B p65 (Santa Cruz Biotechnology, Santa Cruz, Calif.); anti-NF- κ B p50 and anti-relB (courtesy of R. Bravo, Bristol-Myers Squibb,

FIG. 1. LPS induction of gene expression in J774 cells. Total RNA was isolated from J774 cells 2 h following induction with increasing concentrations of LPS (0 to 100 ng/ml). Identical Northern blots prepared with 10 μ g of total RNA per lane were hybridized successively with probes for TNF, MIP-1 α or MIP-1 β , IL-6, superoxide dismutase (SOD), and actin. The locations of the 18S and 28S rRNAs are indicated. The figure illustrates the results of one of two experiments.

Princeton, N.J.) (39, 51); and goat anti-mouse C3 (Cappel-Organon-Teknika, West Chester, Pa.).

RESULTS AND DISCUSSION

LPS induction of J774 cells. In initial studies we used the established murine histiocytic cell line, J774, to investigate the kinetics of the LPS response. Because of the importance of the free radical-scavenging enzyme, superoxide dismutase, in setting the redox state of the cell, its mRNA level was determined, together with those of the NF- κ B-regulated cytokines IL-6, MIP-1 α , MIP-1 β , and TNF, at various times following exposure to LPS (0 to 100 ng/ml). Each gene exhibited a dose-dependent induction pattern. As shown in Fig. 1 at 2 h, maximum mRNA expression levels were achieved at LPS concentrations of 10 to 30 ng/ml for all genes except IL-6, which required concentrations of 30 to 100 ng/ml. Peak mRNA levels were observed for all genes within 4 to 6 h following induction with 10 ng of LPS per ml (data not shown). Measurements of TNF bioactivity exhibited a similar dose and time dependence. At 4 h after exposure to LPS, the TNF released into the cell-conditioned medium increased 20-fold over control levels. The TNF activity rose from a baseline of <20 U/ml to a maximum of $16,150 \pm 2,200$ U/ml at LPS concentrations of 10 ng/ml. By 24 h following induction with 10 ng of LPS per ml, the TNF bioactivity rose an additional twofold ($39,800 \pm 13,150$ U/ml).

Since the NF- κ B protein is involved in transcriptional regulation of the cytokine genes, its activity was assessed by gel retention assays with a radiolabeled oligonucleotide encoding its DNA sequence recognition motif (based on the classic

FIG. 2. Gel retention analysis of NF- κ B activity in J774 cells. (A) LPS response. Nuclear extracts were prepared from J774 cells induced for various times (0 to 2 h) with LPS (0 to 30 ng/ml). Gel retention assays detected NF- κ B activity based on binding to a radiolabeled oligonucleotide encoding the classic immunoglobulin κ enhancer NF- κ B site. The unbound or free (F) oligonucleotide and the major LPS-inducible gel retention complex (arrowhead 1) are indicated. (B) Unlabeled-competitor studies. Gel retention assays were performed in the absence or presence of the following unlabeled oligonucleotide competitors at a twofold (2X) or fivefold (5X) molar excess of unlabeled oligonucleotides relative to the labeled probe: NF- κ B, immunoglobulin (Ig) enhancer NF- κ B site; IL-6, IL-6 promoter NF- κ B site; OCT-1 = lipoprotein lipase octamer 1 site. (C) Antibody (Ab) supershift studies. Gel retention studies were performed in the presence of antibodies directed against the NF- κ B p50 or p65 subunit or a control antibody. The supershifted complexes for the anti-p50 and anti-p65 antibodies are indicated by arrowheads 2 and 3, respectively. Experiments in panels B and C were performed with LPS-induced (10 ng/ml, 2 h) J774 extracts. Each assay used 5 μ g of nuclear extract protein. Each panel illustrates one of two or more experiments.

immunoglobulin κ enhancer) (Fig. 2). At 1 h following exposure to 3 to 30 ng of LPS per ml, proteins isolated from J774 nuclei caused a major gel-shifted DNA complex (Fig. 2A, arrowhead 1). Although additional, less-intense complexes were present, these were not specifically induced by LPS; they appeared in the absence of LPS at the 1-h time point (Fig. 2A). The proteins of the major inducible complex (arrowhead 1) bound specifically to oligonucleotides containing the NF- κ B DNA motif (Fig. 2B). Excess unlabeled oligonucleotides encoding the NF- κ B recognition motif (NF- κ B, IL-6) (17, 29) competed for the binding proteins in LPS-induced J774 nuclear extracts, whereas an unrelated oligonucleotide encoding the octamer 1 recognition site (OCT-1) did not (25). Gel supershift assays with polyclonal antibodies recognizing NF- κ B proteins p50 and p65 (39, 51) confirmed the identity of the protein components of the gel-shifted complex (Fig. 2C). Both

antibodies partially shifted the major LPS-induced complex (Fig. 2C, arrowheads 2 and 3), whereas an unrelated polyclonal antibody did not. A similar gel supershift was observed with a polyclonal antibody directed against the NF- κ B-related protein, c-Rel (data not shown). Together, these data indicate that LPS activates the NF- κ B p50 and p65 proteins in J774 cells.

LPS induction of primary peritoneal macrophages. Further studies examined mRNA expression and NF- κ B activation in primary peritoneal macrophages from LPS-responsive (C3HeB/Fej) and LPS-nonresponsive (C3H/HeJ) mice. These strains differ in their sensitivity to LPS by more than 2 orders of magnitude (14, 23, 38, 47–49, 55, 56). These experiments would establish whether there was a link between the LPS response and NF- κ B activation.

The mRNA response of C3HeB/Fej macrophages to LPS exhibited a pattern similar to that observed with J774 cells (Fig.

FIG. 3. LPS response of primary peritoneal macrophages. (A) Northern blot analysis. Total RNA from C3HeB/Fej and C3H/HeJ macrophages induced with LPS for various times was hybridized on Northern blots with the probes used in the experiment in Fig. 1. The C3H/HeJ blot included a control lane of activated J774 total RNA. (B) Gel retention analysis. The NF-κB activity in cell extracts from resting and LPS-activated (10 ng/ml, 2 h) macrophages was determined as described in the legend to Fig. 2. The free oligonucleotide (F) and major inducible DNA complex (arrowhead 1) are indicated. Each panel illustrates one of two experiments.

3A). For the cytokine genes, maximum responses were achieved within 2 to 4 h at 10 ng of LPS per ml. Both the 4- and 1-kb transcripts of superoxide dismutase were induced in a sequential manner, with persistence of the mRNA for up to 24 h (57, 58). This was paralleled by the TNF bioactivity, which increased from a baseline of 210 ± 20 to $21,150 \pm 1,860$ U/ml following induction with LPS (10 ng/ml). The same LPS concentrations failed to elicit a significant response from C3H/HeJ macrophages over a 24-h period; only a minimal induction of TNF mRNA was observed after a 4-h treatment

with 10 ng of LPS per ml (Fig. 3A). As a control, the C3H/HeJ Northern blots included a lane of activated J774 total RNA to confirm that probe hybridization had occurred. Likewise, the level of TNF bioactivity in C3H/HeJ cell-conditioned medium was not significantly induced in response to LPS (150 ± 30 U/ml baseline versus 150 ± 10 U/ml at 10 ng/ml). LPS activation of NF-κB in the primary macrophages paralleled that of the cytokine genes (Fig. 3B). In untreated C3HeB/Fej nuclear extracts, a constitutive DNA-binding complex was observed on gel retention assays; this was absent in

FIG. 4. Effect of DMSO. J774 cells pretreated (for 15 min) with DMSO (0 to 2%) prior to induction with LPS (0 to 3 ng/ml) for 1, 2, or 4 h were examined. (A and B) On Northern blot analysis, DMSO pretreatment decreased the mRNA levels of TNF, MIP-1α, and MIP-1β at 2 h of LPS induction (A) and decreased the mRNA levels of IL-6 and superoxide dismutase (SOD) at 4 h of LPS induction (B). (C) Gel retention assay for NF-κB activation after 1 h of LPS exposure. Each panel illustrates one of two experiments.

TABLE 1. TNF bioactivity following LPS induction in the presence of DMSO^a

LPS concn (ng/ml)	DMSO concn (%)	Mean TNF activity (U/ml of medium) ± SD after LPS induction for:	
		2 h	4 h
0.3	0	350 ± 190	570 ± 270
0.3	1	60 ± 30	<20
0.3	2	<20	<20
3	0	6,110 ± 1,590	8,090 ± 4,880
3	1	850 ± 280	1,980 ± 450
3	2	140 ± 60	620 ± 280

^a The J774 cells were pretreated with DMSO (0 to 2%) for 15 min prior to induction with LPS. TNF bioactivity was assayed by using conditioned medium containing the L929 indicator cell line and purified TNF; treatment of J774 cells with DMSO in the absence of LPS did not induce detectable TNF activity (<20 U/ml [data not shown]).

extracts from C3H/HeJ cells. Within 2 h of LPS stimulation, a major inducible DNA-binding complex (arrowhead 1) appeared in C3HeB/Fej extracts, equivalent in size to that observed in the J774 cells. Although induced C3H/HeJ nuclear extracts exhibited the same complex, the level of activation was greatly reduced relative to that in the C3HeB/Fej controls. Oligonucleotide competition experiments and antibody supershift assays confirmed the specificity of the induced C3HeB/Fej-binding complex and the presence of both p50 and p65 proteins (data not shown). These data directly link NF- κ B activation with cytokine gene induction by LPS in primary peritoneal macrophages. In addition, they confirm the validity of the J774 cell line as an *in vitro* model for primary macrophages.

Modulation of the LPS response by DMSO. Because NF- κ B activation can be regulated by the redox state of the cell (17), we investigated the effect of the antioxidant DMSO on the J774 cell LPS response. Cells were pretreated with DMSO (0 to 2%) for 15 min prior to exposure to LPS (0 to 3 ng/ml) (Fig. 4). The period of DMSO pretreatment was chosen on the basis of the effects observed in previous studies with this agent *in vivo* (4). DMSO alone had no stimulatory effects on cytokine mRNA levels, NF- κ B activation, or TNF bioactivity levels (data not shown) and no effect on cell viability as measured by vital dye exclusion (trypan blue). At 2 h after LPS induction in the presence of 2% DMSO, induction of the MIP-1 α , MIP-1 β , and TNF cytokine mRNAs was decreased but not abolished; lower DMSO concentrations (1%, vol/vol) had no inhibitory effect at the RNA level (Fig. 4A). DMSO (2%) inhibition of mRNA levels of IL-6 and superoxide dismutase was seen after 4 h of LPS stimulation (Fig. 4B). However, 1 or 2% DMSO reduced LPS-induced TNF bioactivity between 4- and 20-fold relative to that in controls (Table 1). This apparent variation in DMSO effects on TNF mRNA and protein may reflect differences in their sensitivity. The DMSO may act more rapidly on TNF protein translation and/or stability relative to TNF mRNA transcription and/or stability.

The effect of DMSO on NF- κ B activation paralleled that observed with the cytokine mRNAs (Fig. 4C). In the presence of 2% DMSO, the intensity of the LPS-inducible NF- κ B complex was reduced but not abolished. These data do not rule out the possibility that free radical scavengers also influence the rate of TNF protein translation or stability. Similar results were obtained with the antioxidant *N*-acetylcysteine, which, at 50 mM, partially reduced cytokine mRNA levels and NF- κ B activation (data not shown); this chemical is metabolized into glutathione, increasing the endogenous free radical scavenging

capacity of the cell. However, the metal chelator antioxidant PDTC, at concentrations up to 500 μ M, did not consistently reduce NF- κ B activation or cytokine mRNA levels (data not shown). Thus, some free radical scavengers, at sufficiently high concentrations, can inhibit NF- κ B activation and diminish the LPS-induced cytokine response in murine macrophage-like cells.

Overall, antioxidant-induced inhibition of NF- κ B activation in murine macrophages was less pronounced than that observed in other murine cell lines (lymphocytes or fibroblasts) or human macrophages (17, 41, 59). This may be accounted for by differences between mechanisms of the cell types for maintaining intracellular redox homeostasis. Nevertheless, NF- κ B activation plays a central role in initiating the endotoxic response. The current work suggests that the protective mechanism of DMSO and other agents against endotoxemia in murine models may occur partially through the modulation of NF- κ B-mediated events (4, 20, 34).

ACKNOWLEDGMENTS

In addition to the individuals identified in Materials and Methods as the generous sources of reagents, we acknowledge K. Buchanan, P. W. Kincade, L. Thompson, and C. Webb for helpful discussions; the staff of the Oklahoma Medical Research Foundation OASIS for editorial assistance; and S. Wasson for secretarial assistance.

This work was supported in part by Public Health Service grant CA-50898 from the National Cancer Institute (to J.M.G.) and by grants from American Heart Association Oklahoma Affiliate (OK-93-GS-14) (to J.M.G.) and the Oklahoma Center for Advancement of Science and Technology (HR9-073) (to M.R.H.). K.K. is supported by NIH Training Grant T32-GM-08237.

REFERENCES

1. Adi, S., A. S. Pollock, J. K. Shigenaga, A. H. Moser, K. R. Feingold, and C. Grunfeld. 1992. Role of monokines in the metabolic effects of endotoxin. Interferon-gamma restores responsiveness of C3H/HeJ mice *in vivo*. *J. Clin. Invest.* **89**:1603-1609.
2. Beutler, B., N. Krochin, I. Milsark, C. Luedke, and A. Cerami. 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. *Science* **232**:977-979.
3. Brach, M. A., H.-J. Gruss, T. Kaisho, Y. Asano, T. Hirano, and F. Herrman. 1993. Ionizing radiation induces expression of interleukin 6 by human fibroblasts involving activation of nuclear factor- κ B. *J. Biol. Chem.* **268**:8466-8472.
4. Brackett, D. J., M. R. Lerner, and M. F. Wilson. 1991. Dimethyl sulfoxide antagonizes hypotensive, metabolic, and pathologic responses induced by endotoxin. *Circ. Shock* **33**:156-163.
5. Chedid, L. M., M. Parant, C. Damais, F. Parant, D. Juy, and A. Galelli. 1976. Failure of endotoxin to increase nonspecific resistance to infection of lipopolysaccharide low-responder mice. *Infect. Immun.* **13**:722-727.
6. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* **162**:156-159.
7. Church, G., and W. Gilbert. 1984. Genomic sequencing. *Proc. Natl. Acad. Sci. USA* **81**:1991-1995.
8. Collart, M. A., P. Baeuerle, and P. Vassalli. 1990. Regulation of tumor necrosis factor α transcription in macrophages: involvement of four κ B-like motifs and of constitutive and inducible forms of NF- κ B. *Mol. Cell. Biol.* **10**:1498-1506.
9. DeForge, L. E., J. S. Fantone, J. S. Kenney, and D. G. Remick. 1992. Oxygen radical scavengers selectively inhibit interleukin 8 production in human whole blood. *J. Clin. Invest.* **90**:2123-2129.
10. Dignam, J. D., R. M. Lebovitz, and R. G. Roeder. 1983. Accurate transcription initiation by RNA polymerase II in a soluble extract isolated from mammalian nuclei. *Nucleic Acids Res.* **11**:1475-1489.
11. Dorheim, M.-A., M. Sullivan, V. Dandapani, X. Wu, J. Hudson, P. R. Segarini, D. M. Rosen, A. L. Aulthouse, and J. M. Gimble. 1993. Osteoblastic gene expression during adipogenesis in hema-

- topoietic supporting murine bone marrow stromal cells. *J. Cell. Physiol.* **154**:317–328.
12. Economou, J. S., K. Rhoades, R. Essner, W. H. McBride, J. C. Gasson, and D. L. Morton. 1989. Genetic analysis of the human tumor necrosis factor α /cachectin promoter region in a macrophage cell line. *J. Exp. Med.* **170**:321–326.
 13. Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* **132**:6–13.
 14. Flebbe, L. M., S. K. Chapes, and D. C. Morrison. 1990. Activation of C3H/HeJ macrophage tumoricidal activity and cytokine release by R-chemotype lipopolysaccharide preparations. Differential effects of IFN- γ . *J. Immunol.* **145**:1505–1511.
 15. Gimble, J. M., and E. E. Max. 1987. Human immunoglobulin κ gene enhancer: chromatin analysis at high resolution. *Mol. Cell. Biol.* **7**:15–25.
 16. Goldfeld, A. E., C. Doyle, and T. Maniatis. 1990. Human tumor necrosis factor α gene regulation by virus and lipopolysaccharide. *Proc. Natl. Acad. Sci. USA* **87**:9769–9773.
 17. Grimm, S., and P. A. Baeuerle. 1993. The inducible transcription factor NF- κ B: structure-function relationship of its protein subunits. *Biochem. J.* **290**:297–308.
 18. Grove, M., and M. Plumb. 1993. C/EBP, NF- κ B, and c-Ets family members and transcriptional regulation of the cell-specific and inducible macrophage inflammatory protein 1 α immediate-early gene. *Mol. Cell. Biol.* **13**:5276–5289.
 19. Gruss, H.-J., M. A. Brach, and F. Hermann. 1992. Involvement of nuclear factor- κ B in induction of the interleukin-6 gene by leukemia inhibitory factor. *Blood* **80**:2563–2570.
 20. Hamburger, S. A., and P. B. McCay. 1989. Endotoxin-induced mortality in rats is induced by nitrones. *Circ. Shock* **29**:329–334.
 21. Hayashi, T., Y. Ueno, and T. Okamoto. 1993. Oxidative regulation of nuclear factor κ B. Involvement of a cellular reducing catalyst thioredoxin. *J. Biol. Chem.* **268**:11380–11388.
 22. Henkel, T., T. Machleidt, I. Alkalay, M. Kronke, Y. Ben-Neriah, and P. A. Baeuerle. 1993. Rapid proteolysis of I κ B- α is necessary for the activation of transcription factor NF- κ B. *Nature (London)* **365**:182–185.
 23. Hogan, M. M., and S. N. Vogel. 1988. Production of tumor necrosis factor by rIFN- γ -primed C3H/HeJ (Lps^d) macrophages requires the presence of lipid A-associated proteins. *J. Immunol.* **141**:4196–4202.
 24. Hogan, M. M., and S. N. Vogel. 1991. Measurements of tumor necrosis factor α and β , p. 6.10.1–6.10.5. In J. E. Coligan, A. M. Kruisbeek, D. H. Margulies, E. M. Shevach, and W. Strober (ed.), *Current protocols in immunology*. John Wiley & Sons, Inc., New York.
 25. Hua, X., S. Enerback, J. Hudson, K. Youkhana, and J. M. Gimble. 1991. Cloning and characterization of the promoter of the murine lipoprotein lipase-encoding gene: structural and functional analysis. *Gene* **107**:247–258.
 26. Israel, N., M.-A. Gougerot-Pocidallo, F. Aillet, and J.-L. Virelizier. 1992. Redox status of cells influences constitutive or induced NF- κ B translocation and HIV long terminal repeat activity in human T and monocytic cell lines. *J. Immunol.* **149**:3386–3393.
 27. Ivanov, V., M. Merkenschlager, and R. Ceredig. 1993. Antioxidant treatment of thymic organ cultures decreases NF- κ B and TCF1(α) transcription factor activities and inhibits $\alpha\beta$ T cell development. *J. Immunol.* **151**:4694–4704.
 28. Kharasch, N., and B. S. Thyagarajan. 1983. Structural basis for biological activities of dimethyl sulfoxide. *Ann. N. Y. Acad. Sci.* **411**:391–402.
 29. Liebermann, T. A., and D. Baltimore. 1990. Activation of interleukin-6 gene expression through the NF- κ B transcription factor. *Mol. Cell. Biol.* **10**:2327–2334.
 30. Mathews, J. R., N. Wakasugi, J.-L. Virelizier, J. Yodoi, and R. T. Hay. 1992. Thioredoxin regulates the DNA binding activity of NF- κ B by reduction of a disulphide bond involving cysteine 62. *Nucleic Acids Res.* **20**:3821–3830.
 31. Molitor, J. A., D. W. Ballard, and W. C. Greene. 1991. κ B-specific DNA binding proteins are differentially inhibited by enhancer mutations and biological oxidations. *New Biol.* **3**:987–996.
 32. Mosmann, T. 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **65**:55–63.
 33. Oppenheim, J. J., C. O. C. Zachariae, N. Mukaida, and K. Matsushima. 1991. Properties of the novel proinflammatory supergene “intercrine” cytokine family. *Annu. Rev. Immunol.* **9**:617–648.
 34. Pogrebniak, H., M. J. Merino, S. M. Hahn, J. B. Mitchell, and H. L. Pass. 1992. Spin trap salvage from endotoxemia: the role of cytokine down-regulation. *Surgery* **112**:130–139.
 35. Ralph, P., M. A. S. Moore, and K. Nilsson. 1976. Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. *J. Exp. Med.* **143**:1528–1533.
 36. Repine, J. E., J. W. Eaton, M. W. Anders, J. R. Hoidal, and R. B. Fox. 1979. Generation of hydroxyl radicals by enzymes, chemicals, and human phagocytes in vitro. Detection with the anti-inflammatory agent, dimethyl sulfoxide. *J. Clin. Invest.* **64**:1642–1651.
 37. Roederer, M., F. J. T. Staal, P. A. Raju, S. W. Ela, L. A. Herzenberg, and L. A. Herzenberg. 1990. Cytokine-stimulated human immunodeficiency virus replication is inhibited by N-acetyl-L-cysteine. *Proc. Natl. Acad. Sci. USA* **87**:4884–4888.
 38. Rosenstreich, D. L., S. N. Vogel, A. R. Jacques, L. M. Wahl, and J. J. Oppenheim. 1978. Macrophage sensitivity to endotoxin: genetic control by a single codominant gene. *J. Immunol.* **121**:1664–1670.
 39. Ryseck, R.-P., P. Bull, M. Takamiya, V. Bours, U. Siebenlist, P. Dobrzanski, and R. Bravo. 1992. RelB, a new Rel family transcription activator that can interact with p50-NF- κ B. *Mol. Cell. Biol.* **12**:674–684.
 40. Schreck, R., B. Meier, D. N. Mannel, W. Droge, and P. A. Baeuerle. 1992. Dithiocarbamates as potent inhibitors of nuclear factor κ B activation in intact cells. *J. Exp. Med.* **175**:1181–1194.
 41. Schreck, R., P. Rieber, and P. A. Baeuerle. 1991. Reactive oxygen intermediates as apparently widely used messengers in the activation of the NF- κ B transcription factor and HIV-1. *EMBO J.* **10**:2247–2258.
 42. Sen, R., and D. Baltimore. 1986. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* **46**:705–716.
 43. Shakhov, A. N., M. A. Collart, P. Vassalli, S. A. Nedospasov, and C. V. Jongeneel. 1990. κ B-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.
 44. Shimizu, H., K. Mitomoto, T. Watanabe, S. Okamoto, and K.-I. Yamamoto. 1990. Involvement of a NF- κ B-like transcription factor in the activation of the interleukin-6 gene by inflammatory lymphokines. *Mol. Cell. Biol.* **10**:561–568.
 45. Staal, F. J. T., M. Roederer, L. A. Herzenberg, and L. A. Herzenberg. 1990. Intracellular thiols regulate activation of nuclear factor κ B and transcription of human immunodeficiency virus. *Proc. Natl. Acad. Sci. USA* **87**:9943–9947.
 46. Stein, B., and A. S. Baldwin, Jr. 1993. Distinct mechanisms for regulation of the interleukin-8 gene involve synergism and cooperativity between C/EBP and NF- κ B. *Mol. Cell. Biol.* **13**:7191–7198.
 47. Sultzter, B. M. 1968. Genetic control of leukocyte responses to endotoxin. *Nature (London)* **219**:1252–1254.
 48. Sultzter, B. M. 1969. Genetic factors in leukocyte responses to endotoxin: further studies in mice. *J. Immunol.* **103**:32–38.
 49. Sultzter, B. M. 1972. Genetic control of host responses to endotoxin. *Infect. Immun.* **5**:107–113.
 50. Sung, S.-S. J., J. A. Walters, J. Hudson, and J. M. Gimble. 1991. Tumor necrosis factor- α mRNA accumulation in human myelomonocytic cell lines. Role of transcriptional regulation by DNA sequence motifs and mRNA stabilization. *J. Immunol.* **147**:2047–2054.
 51. Tewari, M., P. Dobrzanski, K. L. Mohn, D. E. Cressman, J.-C. Hsu, R. Bravo, and R. Taub. 1992. Rapid induction in regenerating liver of RL/IF-1 (an I κ B that inhibits NF- κ B, RelB-p50, and c-Rel-p50) and PHF, a novel κ B site-binding complex. *Mol. Cell. Biol.* **12**:2898–2908.
 52. Thomas, P. S. 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. *Proc. Natl. Acad. Sci. USA* **77**:5201–5205.

53. **Toledano, M. B., and W. J. Leonard.** 1991. Modulation of transcription factor NF- κ B binding activity by oxidation-reduction *in vitro*. *Proc. Natl. Acad. Sci. USA* **88**:4328–4332.
54. **Ward, P. A., G. O. Till, R. Kunkel, and C. Beauchamp.** 1983. Evidence for role of hydroxyl radical in complement and neutrophil-dependent tissue injury. *J. Clin. Invest.* **72**:789–801.
55. **Watson, J., and R. Riblet.** 1974. Genetic control of responses to bacteria lipopolysaccharides in mice. I. Evidence for a single gene that influences mitogenic and immunogenic responses to lipopolysaccharides. *J. Exp. Med.* **140**:1147–1161.
56. **Watson, J., R. Riblet, and B. A. Taylor.** 1977. The response of recombinant in-bred strains of mice to bacterial lipopolysaccharides. *J. Immunol.* **118**:2088–2093.
57. **Wong, G. H. W., J. H. Elwell, L. W. Oberley, and D. V. Goeddel.** 1989. Manganous superoxide dismutase is essential for cellular resistance to cytotoxicity of tumor necrosis factor. *Cell* **58**:923–931.
58. **Wong, G. H. W., and D. V. Goeddel.** 1988. Induction of manganous superoxide dismutase by tumor necrosis factor: possible protective mechanism. *Science* **242**:941–943.
59. **Ziegler-Heitbrock, H. W. L., T. Sternsdorf, J. Liese, B. Belohradsky, C. Weber, A. Wedel, R. Schreck, P. Baeuerle, and M. Strobel.** 1993. Pyrrolidine dithiocarbamate inhibits NF- κ B mobilization and TNF production in human monocytes. *J. Immunol.* **151**:6986–6993.