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Transforming Growth Factor-β Inhibits Lipopolysaccharide-Stimulated Expression of Inflammatory Cytokines in Mouse Macrophages through Downregulation of Activation Protein 1 and CD14 Receptor Expression

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The septic shock that occurs in gram-negative infections is caused by a cascade of inflammatory cytokines. Several studies showed that transforming growth factor-β1 (TGF-β1) inhibits this septic shock through suppression of expression of the lipopolysaccharide (LPS)-induced inflammatory cytokines. In this study, we investigated whether TGF-β1 inhibition of LPS-induced expression of inflammatory cytokines in the septic shock results from downregulation of LPS-stimulated expression of CD14, an LPS receptor. TGF-β1 markedly inhibited LPS stimulation of CD14 mRNA and protein levels in mouse macrophages. LPS-stimulated expression of CD14 was dramatically inhibited by addition of antisense, but not sense, c-*fos* and c-*jun* oligonucleotides. Since TGF-β1 pretreatment inhibited LPS-stimulated expression of c-*fos* and c-*jun* genes and also the binding of nuclear proteins to the consensus sequence of the binding site for activation protein 1 (AP-1), a heterodimer of c-Fos and c-Jun, in the cells, TGF-β1 inhibition of CD14 expression may be a consequence of downregulation of AP-1. LPS-stimulated expression of interleukin-1β and tumor necrosis factor alpha genes in the cells was inhibited by addition of CD14 antisense oligonucleotide. Also, TGF-β1 inhibited the LPS-stimulated production of both inflammatory cytokines by the macrophages. In addition, TGF-β1 inhibited expression of the two cytokines in several organs of mice receiving LPS. Thus, our results suggest that TGF-β1 inhibition of LPS-stimulated inflammatory responses resulted from downregulation of CD14 and also may be a possible mechanism of TGF-β1 inhibition of LPS-induced septic shock.

Transforming growth factor-β1 (TGF-β1) acts as negative regulator in inflammatory responses. In fact, several investigators (4, 27, 38) have demonstrated that targeted destruction of the mouse TGF-β1 gene causes an excessive inflammatory response. It was also shown that TGF-β1 inhibits lipopolysaccharide (LPS)-induced septic shock in the mouse (33, 43). Although LPS-induced septic shock is mediated by endogenous inflammatory cytokines such as tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1), the inhibitory mechanism of TGF-β1 for the septic shock is not well known in detail. Thus, our interest was to elucidate the mechanism of TGF-β1-mediated inhibition of inflammatory responses induced by LPS.

CD14 is a 55-kDa glycoprotein that binds to LPS via the lipid A moiety of the latter. Therefore, many investigators (9, 11, 13, 44, 47, 50) had suggested that CD14 serves as an LPS receptor and contributes to the LPS-stimulated responses of CD14-positive cells such as macrophages and neutrophils. Interestingly, several studies (10, 14, 15, 45) demonstrated that a peptidoglycan located in the cell walls of all bacteria also is able to bind to CD14 and can reproduce the multiple biological activities of LPS, including septic shock, fever, and inflammation. In addition, several components of the bacterial cell surface such as lipoteichoic acid and mycobacterial lipoarabinomannan stimulate the expression of inflammatory cytokines via binding to CD14 (5, 25, 28, 37, 39). In view of these data, CD14 is considered to play an important role in the first event occurring in host infection by bacteria. Therefore, we wished to investigate the regulation of CD14 expression in the receptor-positive cells.

We investigated in this study the mechanism of TGF-β1-mediated inhibition of LPS-stimulated expression of inflammatory cytokines in mouse macrophages. As a result, we demonstrated that TGF-β1 inhibited LPS-stimulated expression of inflammatory cytokines in the macrophages through downregulation of activation protein 1 (AP-1)-mediated CD14 expression.

MATERIALS AND METHODS

Reagents. TGF-β1 was purified from human platelets to homogeneity (>98%, determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and high-pressure liquid chromatography analyses (Peprotech EC Ltd., London, England). RPMI 1640 was obtained from Nissui Pharmaceutical Co. (Tokyo, Japan); fetal calf serum was obtained from HyClone (Logan, Utah). *Escherichia coli* O111 B4-derived LPS was from Sigma Chemical Co. (St. Louis, Mo.). Mouse CD14 antibody was purchased from Pharmingen (San Diego, Calif.). 5′-[α-^{32}P]dCTP, megaprime DNA labeling system, and [γ-^{32}P]ATP were purchased from Amersham Pharmacia Biotech (Tokyo, Japan).

Preparation of mouse peritoneal macrophages. BALB/c mice, 7 weeks of age, were injected intraperitoneally with 3 ml of thioglycolate medium (Difco Laboratories, Detroit, Mich.). Peritoneal macrophages were prepared from the mouse peritoneal exudate cells as described earlier (17). The prepared macrophages were treated for selected times with test samples.

Western blot analysis for CD14. Macrophage monolayers in 9-cm-diameter dishes (5×10^6 peritoneal exudate cells) were incubated in the presence or absence of test samples. Thereafter, the cells were solubilized with lysis buffer (20 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 1% [vol/vol] Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM Na_3VO_4, 1 mM β-glycerolphosphate, 1 μg of leupeptin/ml, 1 mM phenylmethylsulfonyl fluoride). The samples (10 μg of protein) were subjected to SDS-PAGE on 10% polyacrylamide gels by using a Tris-glycine buffer system (0.025 M Tris, 0.192 M glycine, 0.1% SDS). The protein was transferred to a polyvinylidene difluoride membrane

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(Millipore Co., Bedford, Mass.) by use of a semidry transblot system (Atto Co., Tokyo, Japan). Blots were blocked for 1 h at room temperature with 5% skim milk in Tris-buffered saline including 0.1% Tween 20 (TBS-T) and washed with TBS-T. Then the membrane was incubated for overnight at 4°C with the primary antibody diluted 1:1,000 in 5% bovine serum albumin in TBS-T. Protein was detected with a Phototope-HRP Western blot detection kit (New England Biolabs), and the blots were exposed to X-Omat film (Eastman Kodak Co., Rochester, N.Y.) for visualization of signals.

cDNA hybridization probe. Plasmids containing mouse CD14 cDNA sequences were provided by S. Yamamoto (Oita Medical University, Oita, Japan); mouse TNF-α, IL-1β, and c-*fos* cDNA sequences were provided by T. Hamilton (Cleveland Clinic Foundation, Cleveland, Ohio). In addition, plasmids bearing c-*jun* and β-actin cDNA sequences were obtained from the Japanese Cell Resource Bank (Tokyo, Japan). The methods used for plasmid preparation were described earlier (30).

Preparation of total RNA and Northern blot analysis. Macrophage monolayers prepared from mouse peritoneal exudate cells (5×10^6 cells) were cultured in RPMI 1640 with 5% fetal calf serum in Falcon 9-cm-diameter plastic plates. The cells were incubated in the presence or absence of test samples at various concentrations. Total cellular RNA in the cells was extracted by the guanidine isocyanate procedure (2). In some experiments, several organs of mice were collected and homogenized in 5 M guanidine isocyanate solution. Total RNA in each test sample was then extracted, and the expression of several kinds of genes in the macrophages was analyzed by Northern blotting as described previously (18). β-Actin was used as an internal standard for the quantification of total mRNA in each lane of the gel.

Measurement of IL-1β and TNF-α. For mouse peritoneal macrophages, the cells in 5-cm-diameter dishes (2×10^6 peritoneal exudate cells) were treated with test samples as indicated in the figure legends, and the cell culture supernatants were harvested. For determination of IL-1β and TNF-α in the sera of LPS-injected mice, LPS at 4 mg/kg of body weight was injected intraperitoneally into mice that had been pretreated or not with TGF-β1 at 20 μg/kg, and then their sera were harvested. IL-1β and TNF-α protein in the culture supernatant and sera were measured with an enzyme-linked immunosorbent assay (ELISA) kit utilizing anti-mouse IL-1β and TNF-α antibody (BioSource International, Inc., Camarillo, Calif.).

Preparation of nuclear extracts. Macrophage monolayers in 15-cm-diameter dishes (10^7 cells) were treated with test samples as indicated in the figure legends. Their nuclei were isolated, and the extracts were prepared as described previously (19). Protein concentration was measured by the method of Bradford (1).

Gel mobility shift assay. The assay was carried out as described previously (19). Binding reactions were performed for 20 min on ice with 10 μg of nuclear protein in 20 μl of binding buffer [2 mM HEPES (pH 7.9), 8 mM NaCl, 0.2 mM EDTA, 12% (vol/vol) glycerol, 5 mM dithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 1 μg of poly(dI-dC)] containing 20,000 cpm of ^{32}P-labeled oligonucleotide in the absence or presence of nonlabeled oligonucleotide. Poly(dI-dC) and nuclear extract were incubated at 4°C for 10 min before addition of the labeled oligonucleotide. Double-stranded oligonucleotides (30-mer) containing the TGACTCA sequence (Oncogene Science, Inc., Manhasset, N.Y.) of the AP-1 binding site were end labeled by the oligonucleotide 5′-end-labeling [γ-^{32}P]ATP method. Reaction mixtures for the binding were incubated for 15 min at room temperature after addition of the labeled oligonucleotide. Unlabeled double-stranded oligonucleotide was used as the competitor. DNA-protein complexes were electrophoresed on native 6% polyacrylamide gels in 0.25× TBE buffer (22 mM Tris, 22 mM boric acid, 0.5 mM EDTA [pH 8.0]). The gels were subsequently vacuumed, dried, and exposed to Kodak X-ray film at −70°C.

Preparation of antisense and sense CD14, c-*fos*, and c-*jun* oligonucleotides. Antisense (5′-AAGCACACGCTCCATGGTCGGTAG-3′) and sense (5′-CTACCGACCATGGAGCGTGTGCTT-3′) CD14 25-mer phosphorothioated oligonucleotides including the translation initiation region were synthesized and purified by Sci-Media Ltd. (Tokyo, Japan). The sense oligonucleotides were used as a control. Also, antisense c-*fos* (5′-TGC-GTT-GAA-GCC-CGA-GAA-3′) and c-*jun* (5′-CGT-TTC-CAT-CTT-TGC-AGT-3′) 18-mer oligodeoxynucleotides were synthesized and purified by Sci-Media Ltd. These nucleotide sequences were complementary to the first 18 bases following the AUG sequence of c-*fos* and c-*jun* mRNAs. The corresponding sense oligonucleotides were also synthesized, purified, and then used as a control.

RESULTS

TGF-β1 inhibits LPS-stimulated expression of the CD14 gene in several organs of mice. First, we examined by Northern blot analysis whether TGF-β1 is able to inhibit LPS-stimulated expression of the CD14 gene in the mouse kidney, liver, and spleen. TGF-β1 at 20 μg/kg was injected intraperitoneally into mice 24 h before LPS at 4 mg/kg was injected intraperitoneally; then expression of the CD14 gene in each organ was analyzed by Northern blot analysis. As shown in Fig. 1, TGF-β1 inhibited dramatically LPS-stimulated expression of the CD14 gene in these organs.

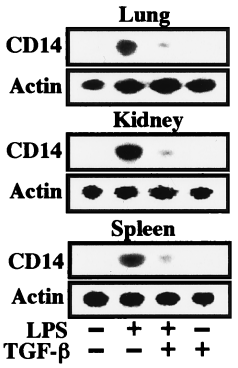

FIG. 1. TGF-β1 inhibits expression of CD14 gene in several organs of LPS-injected mice. Mice were treated or not intraperitoneally for 24 h with TGF-β1 at 20 μg/kg and then injected or not intraperitoneally with LPS at 4 mg/kg. Two hours later, the total RNA in each organ was prepared and used for Northern blot analysis performed with CD14 and β-actin cDNAs used as probes. An identical experiment independently performed gave similar results.

TGF-β1 inhibits LPS-stimulated expression of CD14 in mouse peritoneal macrophages. Since TGF-β1 inhibition of LPS-stimulated expression of the CD14 gene in several organs as described above may have been caused by macrophages which are the predominant CD14-positive cell type in these tissues, using mouse peritoneal macrophages, we next tested whether TGF-β1 is able to inhibit expression of the CD14 gene in the cells. The cells were pretreated or not for various times with the cytokine and subsequently stimulated or not with LPS. CD14 gene expression was examined 3 h later, because our preliminary data showed that the peak LPS-stimulated expression of the CD14 gene occurred at 3 h after initiation of the treatment, though gene expression started 1 h after LPS treatment (data not shown). As shown in Fig. 2A, TGF-β1 inhibited LPS-stimulated expression of the CD14 gene in a pretreatment

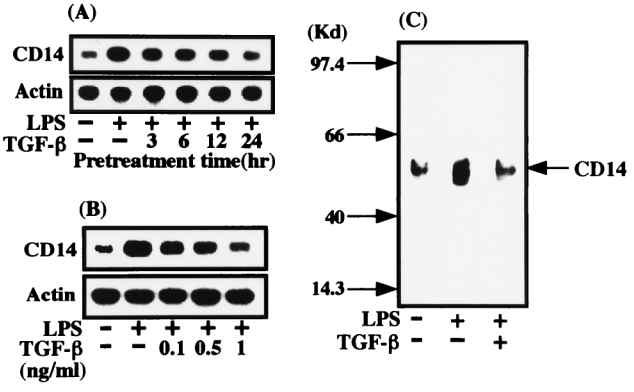

FIG. 2. TGF-β1 inhibits LPS-stimulated expression of CD14 in mouse peritoneal macrophages. (A) The cells from BALB/c mice were pretreated or not for the selected times with TGF-β1 at 1 ng/ml and then treated or not with LPS at 100 ng/ml. Thereafter, their total RNA was prepared 3 h after the LPS addition. Northern blot analysis was performed with CD14 and β-actin cDNAs used as probes. (B) The cells were pretreated or not for 24 h with TGF-β1 at the selected doses and then treated or not for 3 h with LPS at 100 ng/ml. Northern blot analysis was performed with CD14 and β-actin cDNAs used as probes. (C) The cells were pretreated or not for 24 h with TGF-β1 at 1 ng/ml and then treated or not for 3 h with LPS at 100 ng/ml. Thereafter, CD14 in equal amounts of cell lysates was analyzed after SDS-PAGE and with anti-CD14 antibody. Arrows show the positions of proteins used as apparent weight markers. An identical experiment independently performed gave similar results.

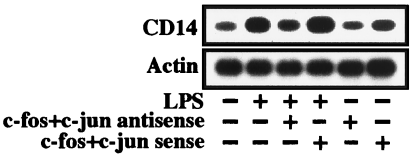

FIG. 3. Antisense c-*fos* and c-*jun* inhibit LPS-stimulated expression of CD14 gene in mouse peritoneal macrophages. The cells were from BALB/c mice incubated in the presence or absence of antisense oligonucleotides for c-*fos* and c-*jun* or sense oligonucleotides of c-*fos* and c-*jun*, each at 2.5 μM. After 3 h, LPS at 100 ng/ml was added, and total RNA was prepared 3 h later. Northern blot analysis was performed with CD14 and β-actin cDNAs used as probes. An identical experiment independently performed gave similar results.

time-dependent fashion. The TGF-β1 inhibition was also dose dependent (Fig. 2B). These results suggested to us that CD14 protein may be inhibited by the cytokine. The Western blot in Fig. 2C clearly shows TGF-β1 inhibition of CD14 protein having a molecular size of 55 kDa. These results showed that TGF-β1 acts as a negative regulator of LPS-stimulated expression of CD14 in mouse macrophages.

LPS-stimulated expression of the CD14 gene in mouse peritoneal macrophages is mediated by AP-1. Since it was demonstrated that the 12-tetradecanoylphorbol-13-acetate-responsive element (TRE) consensus sequence of the transcriptional factor AP-1, a heterodimer of proto-oncoproteins c-Fos and c-Jun, is located in the promoter region of the mouse CD14 gene (31), we investigated using antisense c-*fos* and c-*jun* oligonucleotides whether LPS-stimulated expression of the CD14 gene in the cells was mediated via AP-1. Figure 3 shows that LPS-stimulated expression of the CD14 gene was inhibited by treatment with both antisense oligonucleotides but not with the sense ones. Constitutive expression of the CD14 gene in the cells was inhibited by antisense c-*jun* oligonucleotide alone (data are not shown). These results imply that CD14 expression in the macrophages is AP-1 dependent.

TGF-β1 inhibits LPS-stimulated AP-1 in mouse peritoneal macrophages. AP-1 dependence of CD14 expression in the macrophages suggested that TGF-β1 inhibition of LPS-stimulated CD14 expression may have resulted from AP-1 suppression by the cytokine. Therefore, we examined whether TGF-β1 was able to inhibit LPS-stimulated expression of AP-1 in the macrophages. As shown in Fig. 4A, LPS-stimulated expression of both proto-oncogenes in the cells was clearly inhibited by pretreatment for 24 h with TGF-β1 at 1 ng/ml. These data suggest that the cytokine was able to inhibit LPS-stimulated AP-1 expression in the cells. In fact, using the gel mobility shift assay, we observed that LPS-stimulated AP-1 binding to its consensus sequence was markedly inhibited when the cells were pretreated for 24 h with the cytokine (Fig. 4B). These results indicate that TGF-β1 is a negative regulator of LPS-stimulated expression of AP-1 in the macrophages.

Endogenous CD14 contributes to LPS-stimulated expression of IL-1β and TNF-α genes in mouse peritoneal macrophages. Since IL-1β and TNF-α are potent mediators of LPS-induced septic shock, using CD14 antisense oligonucleotide, we addressed the contribution of endogenous CD14 to LPS-stimulated expression of IL-1β and TNF-α genes in the macrophages. Figure 5 shows that the stimulated expression of both cytokine genes was dramatically inhibited by treatment with the CD14 antisense oligonucleotide but with the sense one. These results show that endogenous CD14 plays an important role in LPS-stimulated expression of IL-1β and TNF-α genes in the macrophages.

TGF-β1 inhibits LPS-stimulated expression of IL-1β and TNF-α genes in vitro and in vivo. Next we wanted to demonstrate both in vitro and in vivo TGF-β1 inhibition of LPS-stimulated expression of IL-1β and TNF-α genes, major mediators of LPS-induced septic shock. For the in vitro experiment, we tested the effect of TGF-β1 on LPS-stimulated expression of both cytokines in mouse peritoneal macrophages. As shown in Fig. 6A and B, TGF-β1 clearly inhibited LPS-stimulated expression of these cytokine genes in the cells in a pretreatment time- and dose-dependent manner. Furthermore, production of these cytokines by the cells also was inhibited by the TGF-β1 pretreatment (Fig. 6C).

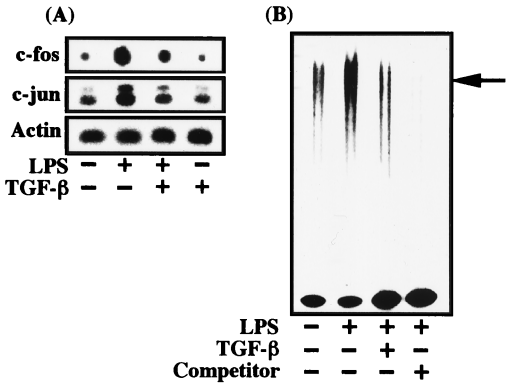

FIG. 4. TGF-β1 inhibits LPS-stimulated expression of AP-1 in mouse peritoneal macrophages. (A) Cells from BALB/c mice were pretreated or not for 24 h with TGF-β1 at 1 ng/ml and then treated or not with LPS at 100 ng/ml. Thereafter, their total RNA was prepared at 1 h after the start of incubation. Northern blot analysis was performed with c-*fos*, c-*jun*, and β-actin cDNAs used as probes. (B) Cells from BALB/c mice were pretreated or not for 24 h with TGF-β1 at 1 ng/ml and then treated or not with for 1 h LPS at 100 ng/ml. Then the nuclear proteins were prepared for the gel mobility shift assay, which was performed with ^{32}P-labeled oligonucleotide containing the AP-1 consensus sequence or it plus unlabeled oligonucleotide as the competitor, in the presence of the nuclear proteins. The arrow indicates the position of the DNA and nuclear protein complexes. An identical experiment independently performed gave similar results.

For the in vivo experiment, TGF-β1 at 20 μg/kg was injected intraperitoneally into mice at 24 h before LPS at 4 mg/kg was injected by the same route. Then expression of IL-1β and TNF-α genes in each organ was analyzed by Northern blot assay. Figure 7A shows that TGF-β1 inhibited LPS-stimulated expression of these cytokine genes in each organ tested. In addition, the serum levels of these cytokines elevated by LPS treatment were also dramatically lowered by the TGF-β1 pretreatment (Fig. 7B).

DISCUSSION

Many studies (5, 10, 14, 15, 25, 28, 37, 39, 45) demonstrated that several components of the bacterial cell surface such as

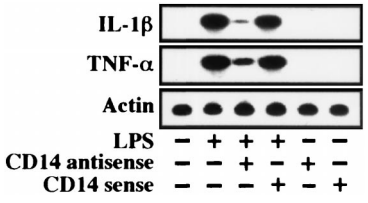

FIG. 5. Antisense CD14 oligonucleotide inhibits LPS-stimulated expression of IL-1β and TNF-α genes in mouse peritoneal macrophages. Cells from BALB/c mice were incubated in the presence or absence of 2 μM antisense oligonucleotide for CD14 or 2 μM sense oligonucleotide of CD14. After 3 h, LPS at 100 ng/ml was added, and total RNA was prepared 3 h later. Northern blot analysis was performed with IL-1β, TNF-α, and β-actin cDNAs used as probes. An identical experiment independently performed gave similar results.

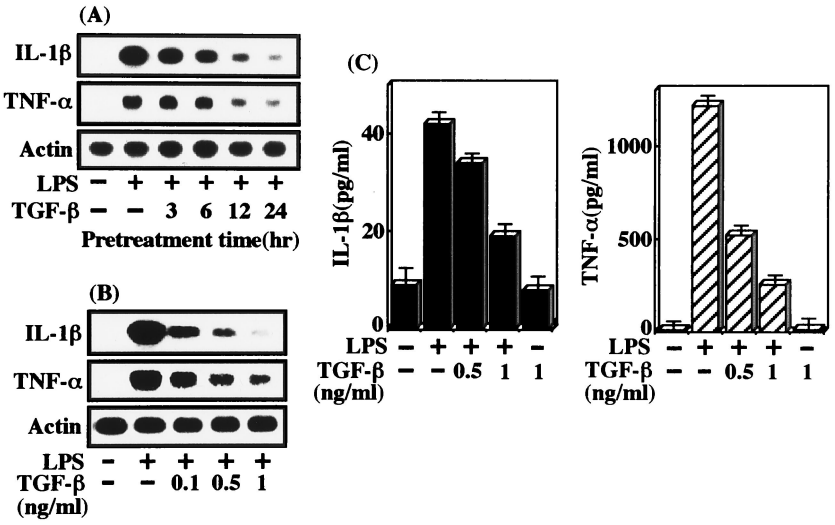

FIG. 6. TGF-β1 inhibits LPS-stimulated expression of IL-1β and TNF-α genes in mouse peritoneal macrophages. (A) Cells from BALB/c mice were pretreated or not for the selected times with TGF-β1 at 1 ng/ml and then treated or not with LPS at 100 ng/ml; their total RNA was prepared 3 h after LPS addition. Northern blot analysis was performed with IL-1β, TNF-α, and β-actin cDNAs used as probes. (B) Cells were pretreated or not for 24 h with TGF-β1 at the selected doses and then treated or not for 3 h with LPS at 100 ng/ml. Northern blot analysis was performed with IL-1β, TNF-α, and β-actin cDNAs used as probes. (C) Cells were pretreated or not for 24 h with TGF-β1 at the selected doses and then treated or not for 12 h with LPS at 100 ng/ml. Thereafter, IL-1β and TNF-α in their culture supernatant was measured by ELISA. The results are expressed as the means ± standard deviations for triplicate cultures. An identical experiment independently performed gave similar results.

LPS, peptidoglycan, lipoteichoic acid, and mycobacterial lipoarabinomannan stimulate the expression of inflammatory cytokines via binding to CD14. Therefore, CD14 is considered to play an important role as a key receptor molecule of these bacterial cell components in the first event occurring in host infections caused by bacteria.

Recent interesting studies (3, 22, 26, 34, 49) suggested that a Toll-like receptor is a signaling component of a cellular receptor for LPS. However, CD14 plays an important role as a trigger of LPS-induced biological responses in monocytes/macrophages and neutrophils, because CD14 transgenic mice that overexpress human CD14 are highly responsive to LPS (12) whereas CD14 knockout mice are dramatically less sensitive to LPS (20, 21). Therefore, to examine the inhibitory mechanism of TGF-β1 in LPS-induced septic shock, we focused here whether TGF-β1 is able to inhibit LPS-stimulated expression of CD14 in mice. Our present study demonstrated that TGF-β1 acts as a potent inhibitor of the LPS-stimulated expression of CD14 in mice and consequently inhibits LPS-stimulated expression of inflammatory cytokines such as IL-1β and TNF-α, cytokine mediators of septic shock.

LPS binding to CD14 on macrophages induces the synthesis

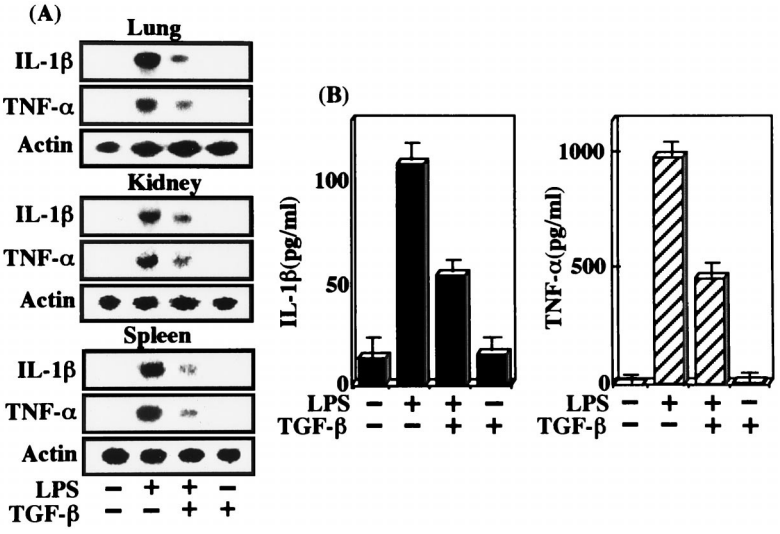

FIG. 7. TGF-β1 inhibits LPS-stimulated expression of IL-1β and TNF-α in several organs of LPS-injected mice. (A) The mice were treated or not intraperitoneally for 24 h with TGF-β1 at 20 μg/kg and then injected or not intraperitoneally with LPS at 4 mg/kg. Two hours later, the total RNA in each organ was prepared and used for Northern blot analysis performed with IL-1β, TNF-α, and β-actin cDNAs used as probes. (B) Serum was prepared from mice treated under the same experimental conditions as for panel A. Then IL-1β and TNF-α in sera were measured by ELISA. The results are expressed as the means ± standard deviations for triplicate cultures. An identical experiment independently performed gave similar results.

of inflammatory mediators involved in septic shock. Macrophage CD14 is thought to play a crucial role in the pathogenesis of septic shock due to gram-negative bacteria. However, since it was not known whether TGF-β1 inhibits CD14 expression in mouse macrophages, we first examined the effect of TGF-β1 pretreatment on expression of the CD14 gene in several organs (kidney, spleen, and lung) of LPS-treated mice. The cytokine dramatically inhibited LPS-stimulated expression of the gene in these organs. Since the predominant cells bearing CD14 in these organs are of the macrophage lineage, we assessed the inhibitory action of TGF-β1 on CD14 expression of mouse peritoneal macrophages. As shown by Northern and Western blot assays, the cytokine clearly inhibited LPS-stimulated expression of CD14 in mouse macrophages. The TGF-β1 inhibition of LPS-stimulated expression of CD14 in the cells was pretreatment time and dose dependent. In addition, we observed that the cytokine was able to inhibit constitutive (basal) CD14 expression in the macrophages (unpublished data). These observations demonstrated that TGF-β1 acts as a potent inhibitor of CD14, a key molecule triggering LPS-induced septic shock.

The sequence of the murine CD14 gene promoter has been reported (31). The promoter contains TRE, which binds the transcription factor AP-1. Since many studies (8, 24, 35, 42, 48) have demonstrated LPS-stimulated expression of c-*fos* and c-*jun* in macrophages, our next interest was to determine whether LPS stimulates CD14 expression in macrophages via AP-1 and, if so, whether TGF-β1 is able to inhibit both proto-oncogenes stimulated by the endotoxin. We showed here that LPS-stimulated expression of the CD14 gene was markedly inhibited by addition of the antisense oligonucleotides of both c-*fos* and c-*jun* genes to the cells. These observations suggested to us that LPS stimulated CD14 expression in the cells via AP-1 and also the possibility that TGF-β1 inhibition of the toxin-stimulated expression of CD14 may have resulted from down-regulation of c-*fos* and c-*jun* expression. As expected, TGF-β1 inhibited the stimulated expression of both proto-oncogenes in cells. In fact, our gel mobility shift assay showed that the cytokines inhibited the stimulated AP-1 binding to its consensus sequence TRE in the cells. In view of all of the data taken together, we believe that LPS stimulation of CD14 is AP-1 dependent, though it is well known that LPS also is able to stimulate transcriptional activity of NF-κB and NF-IL-6 in cells of the macrophage lineage.

It is well known that IL-1β and TNF-α are potent mediators of LPS-induced septic shock. We observed that TGF-β1 inhibited the LPS-stimulated expression of these cytokine genes in mouse macrophages in a pretreatment time- and dose-dependent manner. On the other hand, as shown in this study, LPS-stimulated expression of these cytokine genes in the cells was markedly inhibited by treatment of CD14 antisense oligonucleotide. These observations imply that endogenous CD14 plays an important role in LPS-stimulated expression of these genes in the cells. However, we do not know whether LPS receptor molecules in addition to CD14 are partially involved in LPS-stimulated expression of these genes. In addition, in order to define in vivo the TGF-β1 inhibition of CD14-mediated expression of IL-1β and TNF-α of LPS-induced septic shock, it was necessary to address whether TGF-β1 could also inhibit LPS-stimulated expression of these inflammatory cytokines in several organs in vivo. We observed that TGF-β1 markedly inhibited LPS-stimulated expression of these cytokines in these tissues. Based on these results, we can conclude that TGF-β1 acts as a potent inhibitor of LPS-stimulated expression of IL-1β and TNF-α via downregulation of CD14, thus suggesting a possible mechanism of TGF-β1 inhibition for LPS-induced septic shock.

Several recent studies (6, 7, 16, 29, 32, 36, 41, 46) showed that LPS activates three separate mitogen-activated protein kinases, i.e., c-Jun N-terminal kinase (JNK), extracellular signal-regulated kinase, and p38, in a macrophage cell line. LPS may activate these kinases via a CD14-dependent pathway. In particular, it is well known that JNK regulates the transcription of many genes by activating AP-1 with relative high specificity. In fact, a recent study (40) showed the direct involvement of JNK in the biosynthesis of TNF-α, a major mediator of septic shock, in macrophages. Therefore, it is of interest to address whether TGF-β1 inhibits LPS-stimulated activity of JNK in the macrophages through downregulation of CD14 expression. Our previous study showed that TGF-β1 is indeed able to do so (23). These observations suggested the possibility that TGF-β1 acts as a potent inhibitor of LPS-stimulated expression of TNF-α and IL-1β in the macrophages via downregulation of CD14-mediated JNK activation.

In conclusion, this study demonstrates that TGF-β1 acts as a negative regulator of CD14, a receptor that plays an important role as a key molecule in the initiation stage of LPS-induced septic shock, and suggests that by this mechanism TGF-β1 acts as an inhibitor of LPS-induced septic shock.

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