INFECTION AND IMMUNITY, Jan. 1994, p. 119–125
0019-9567/94/$04.00+0

Vol. 62, No. 1

Lipoteichoic Acid Induces Secretion of Interleukin-8 from Human Blood Monocytes: a Cellular and Molecular Analysis

THEODORE J. STANDIFORD,[1]* DOUGLAS A. ARENBERG,[1] JEAN M. DANFORTH,[1] STEVEN L. KUNKEL,[2] GLENN M. VANOTTEREN,[1] AND ROBERT M. STRIETER[1]

Division of Pulmonary and Critical Care Medicine, Departments of Medicine[1] and Pathology,[2] University of Michigan Medical School, Ann Arbor, Michigan

Received 9 August 1993/Returned for modification 30 September 1993/Accepted 27 October 1993

Invasion by gram-positive and gram-negative bacterial organisms is characterized immunopathologically by the activation of mononuclear phagocytic cells, leading to the elaboration of macrophage-derived regulatory and chemotactic factors, and the resultant influx of inflammatory leukocytes. Little is known regarding the mechanisms by which gram-positive organisms initiate macrophage activation and subsequent inflammation. In this investigation, we postulated that lipoteichoic acid (LTA) purified from two different gram-positive bacterial species was an important signal for the expression of chemotactic cytokines from human peripheral blood monocytes (PBM). In initial experiments, we demonstrated that cell-associated interleukin-8 (IL-8) was expressed by mononuclear phagocytes present in inflamed areas of endocardium in cases of acute *Staphylococcus aureus* endocarditis. We next demonstrated that LTA purified from either *Staphylococcus aureus* or *Streptococcus pyogenes* induced the time- and dose-dependent expression of IL-8 mRNA and protein from human PBM. The expression of IL-8 mRNA from LTA- but not lipopolysaccharide (LPS)-treated PBM was superinduced by concomitant treatment with cycloheximide, indicating that the expression of IL-8 mRNA from LTA-treated PBM was negatively controlled by repressor proteins. Furthermore, mRNA stability studies indicated that IL-8 mRNA was less stable in the presence of LTA than in the presence of LPS. Our findings indicate that LTA can induce the secretion of the polymorphonuclear leukocyte chemotactic factor IL-8 and that LTA may be an important cellular mediator of inflammatory cell recruitment that characterizes immune responses to gram-positive bacterial infections.

Invasion by gram-positive and gram-negative bacterial organisms triggers a rather complex and dynamic host response, which is characterized by the recruitment and activation of inflammatory leukocytes. The elicitation of inflammatory cells to a site of infection is dependent upon the coordinated expression of adhesion molecules on leukocytes and endothelial cells, as well as the establishment of chemotactic gradients via the local generation of chemotactic factors (22). The resident mononuclear phagocytes (Mϕ) are believed to be critically involved in the process of leukocyte recruitment, since these cells are the predominant cellular source of several proinflammatory mediators, including tumor necrosis factor alpha (TNF-α) (30), interleukin-1 (IL-1) (8), and a variety of leukocyte chemotactic factors. Leukocyte chemotactic factors produced by endotoxin-challenged Mϕ include C5a, leukotriene B_4, and platelet-activating factor (13, 20). In addition, activated Mϕ express several potent leukocyte chemotactic proteins referred to as chemokines (16, 18).

Two closely related families of chemotactic cytokines, the C-C and C-X-C chemokine families, have been increasingly recognized as important mediators in a variety of inflammatory disease states (1, 16, 18). The C-X-C chemokine family, which includes IL-8, platelet factor 4, GRO, macrophage inflammatory protein 2 (MIP-2), CTAP-III, NAP-2, ENA-78, and IP-10, is made up of homologous 8- to 10-kDa peptides with predominant neutrophil stimulatory and chemotactic activities. The C-C chemokine family, which includes MCP-1, RANTES, and MIP-1, differ from the C-X-C family in that the C-C chemokines selectively augment Mϕ effector cell activities (16, 18). One member of the chemokine C-X-C family, IL-8, is now believed to be a major mediator of acute inflammation in response to infection. Several lines of evidence would suggest that IL-8 is critically involved in host defense. For example, IL-8 has been shown to enhance the antimicrobial activities of polymorphonuclear leukocytes (PMN) by inducing PMN degranulation, respiratory burst, and 5-lipoxygenase activity (19, 24). In functional assays, IL-8 enhances PMN growth-inhibitory activity against *Candida albicans*, which is mediated by the release of azurophilic enzymes (7). In addition, a number of infectious agents or components of these agents have been shown to induce the production of IL-8, including lipopolysaccharide (LPS), *Mycobacterium tuberculosis* (9), and influenza A virus (5). Last, increased amounts of IL-8 have been detected in cases of acute bacterial infections, such as nosocomial pneumonia and bacteremia (10, 21).

While LPS, a cell surface component of gram-negative organisms, has been shown to be a potent inducer of cytokine production from Mϕ, little is known regarding the Mϕ-activating effects of cellular components of gram-positive organisms. Peptidoglycan, which is the major structural component of the gram-positive bacterial cell wall, has previously been shown to induce localized inflammatory responses, augment cell-mediated and humoral immune responses, and enhance Mϕ effector cell function (17, 27). Lipoteichoic acid (LTA) represents another immunologically active constituent of the gram-positive bacterial cell

* Corresponding author. Mailing address: University of Michigan Medical School, 3916 Taubman, 1500 Medical Center Dr., Ann Arbor, MI 48109-0360. Fax: (313) 936-5048.

wall. Specifically, LTA has important Mϕ-enhancing effects, such as stimulation of Mϕ respiratory burst and antitumoricidal activities. In addition, LTA induces the secretion of TNF-α, IL-1β, and IL-6 from various Mϕ populations (3, 12, 31). In this study, we sought to determine whether the chemotactic cytokine IL-8 was expressed in cases of localized gram-positive bacterial infection. Second, we assessed whether LTA isolated from two different gram-positive bacterial species could serve as primary inducers of IL-8 production from human peripheral blood monocytes (PBM).

MATERIALS AND METHODS

Reagents. LTA from two common human pathogens, *Staphylococcus aureus* and *Streptococcus pyogenes*, was purified by a phenolic extraction procedure and was purchased from Sigma Chemical Co. (St. Louis, Mo.). The purified LTA contained less than 10 ng of LPS per ml, as determined by a *Limulus* amebocyte lysate assay (ICN Biomedicals, Costa Mesa, Calif.). Human recombinant IL-8 was purchased from Peprotech (Rocky Hill, N.J.). Human recombinant IL-1β was a generous gift from The Upjohn Co. (Kalamazoo, Mich.). Polyclonal anti-human IL-8 antiserum used in the enzyme-linked immunosorbent assay (ELISA) was produced by immunization of rabbits with recombinant IL-8 in multiple intradermal sites with complete Freund's adjuvant. Stock cycloheximide (Sigma) was prepared at a concentration of 10 mg/ml in dimethyl sulfoxide and used at a concentration of 5 μg/ml. Stock actinomycin D was prepared in dimethyl sulfoxide and used at final concentration of 10 μg/ml. Stock LPS (*Escherichia coli* O111:B4; Sigma) was prepared at a concentration of 200 μg/ml in sterile RPMI 1640 (Whitaker Biomedical Products, Whitaker, Calif.) containing 1 mM glutamine, 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 100 U of penicillin per ml, and 100 μg of streptomycin (Hazelton Research Products, Denver, Pa.) per ml (complete medium).

Recovery and isolation of PBM. Heparinized venous blood samples were collected from healthy volunteers and mixed 1:1 with 0.9% saline, and mononuclear cells were separated by Ficoll-Hypaque density gradient centrifugation. Isolated mononuclear cells were washed twice in complete medium, and PBM were adherence purified for 1 h (37°C) at a concentration of 10^6 cells per ml. Cells were >95% viable, as assessed by trypan blue exclusion, and consisted of >95% monocytes, as determined by morphology and nonspecific esterase staining.

IL-8 ELISA. Extracellular immunoreactive IL-8 was quantitated by a modified version of a double-ligand method as previously described (26). Briefly, flat-bottomed 96-well microtiter plates (Immuno-Plate I 96-F; Nunc, Roskilde, Denmark) were coated with rabbit anti-IL-8 antibody (50 μl per well) purified from immune rabbit serum in a protein A-Sepharose column (1 μg/ml in 0.6 M NaCl–0.26 M H_3BO_4–0.08 N NaOH [pH 9.6]). After 16 h at 4°C, the plates were washed with phosphate-buffered saline (PBS) (pH 7.5)–0.05% Tween 20 (wash buffer). Nonspecific binding sites were blocked with 2% bovine serum albumin in PBS, and the plates were incubated for 90 min at 37°C. The plates were rinsed four times with wash buffer, and diluted (neat and 1:10) cell-free supernatants (50 μl) in duplicate were added to wash buffer and then incubated for 1 h at 37°C. The plates were washed four times, 50 μl of biotinylated rabbit anti-IL-8 antibodies (3.5 ng/ml in PBS [pH 7.5]–0.05% Tween 20–2% fetal calf serum) was added to each well, and the plates were incubated for 30 min at 37°C. The plates were washed four times, streptavidin-peroxidase conjugate (Bio-Rad Laboratories, Richmond, Calif.) was added, and the plates were incubated for 30 min at 37°C. The plates were again washed four times, and chromogen substrate (Bio-Rad) was added. The plates were then incubated at room temperature to the desired extinction, and the reaction was terminated by adding 50 μl of 3 M H_2SO_4 solution to each well. The plates were read at 490 nm in an ELISA reader. Standards were 0.5-log-unit dilutions of recombinant IL-8 from 1 pg/ml to 100 ng/ml. This ELISA consistently detected IL-8 concentrations above 25 pg/ml and did not detect cross-reactivity between IL-8 and TNF, IL-1, IL-2, IL-4, IL-6, or gamma interferon. In addition, the ELISA did not detect cross-reactivity between IL-8 and members of the C-C chemokine family, including MCP-1, RANTES, MIP-1α, and MIP-1β, or with members of the C-X-C chemokine family, including GRO-α, ENA-78, NAP-2, and IP-10.

Immunohistochemical localization of antigenic IL-8. Paraffin-embedded specimens of resected native aortic or mitral valve obtained from patients with acute *S. aureus* endocarditis were cut in 3-μm sections and placed on poly-L-lysine-coated slides for immunohistochemical localization of IL-8 antigen. Paraffin-embedded tissue was processed for immunohistochemical localization of IL-8 protein by a modified version of our previously described technique (26). Briefly, the tissue sections were dewaxed with xylene and rehydrated through graded concentrations of ethanol. Nonspecific binding sites were then blocked with normal goat serum (BioGenex, San Ramon, Calif.). The tissue sections were then washed and incubated with 1:800 dilution of rabbit anti-human IL-8 antibodies or equivalent dilutions of rabbit preimmune serum for 120 min at 37°C. The tissue sections were washed and then incubated for 60 min with goat anti-rabbit biotinylated antibodies (BioGenex). The tissue sections were then washed twice in Tris-buffered saline and incubated for 40 min with alkaline phosphatase conjugated to streptavidin (BioGenex). Fast red chromogenic substrate (BioGenex) was used for localization of IL-8 antigen. After optimal color development, tissue sections were rinsed in sterile water and counterstained with Mayer's hematoxylin, and an aqueous mounting solution was applied, and coverslips were placed on the slides. Human Mϕ populations were identified by utilizing the monoclonal antibody HAM-56 (Enzo Diagnostics) on duplicate histologic sections.

Northern (RNA) blot analysis. Total cellular RNA from PBM was isolated by a modified version of the procedure described by Chirgwin et al. (4) and Jonas et al. (11). Briefly, cells were overlaid with 1 ml of a solution consisting of 25 mM Tris (pH 8.0) 4.2 M guanidine isothiocyanate, 0.5% Sarkosyl, and 0.1 M 2-mercaptoethanol. After homogenization, the above suspension was added to an equal volume of 100 mM Tris (pH 8.0) containing 10 mM EDTA and 1.0% sodium dodecyl sulfate. The mixture was then extracted with chloroform-phenol and chloroform-isoamyl alcohol. The RNA was alcohol precipitated, and the pellet was dissolved in a solution of DEPC and H_2O. RNA was separated by Northern blot analysis with formaldehyde–1% agarose gels and transblotted to nitrocellulose. The nitrocellulose was then baked, prehybridized, and hybridized with a ^{32}P 5′-end-labeled oligonucleotide probe. The human IL-8 oligonucleotide probe was complementary to nucleotides 262 to 291 and has the sequence 5′-GTT GCC GCA GTG TGG TCC ACT CTC AAT CAC-3′. Blots were washed and autoradiographs were quantitated by laser densitometry (Ultrascan XS; LKB Instruments, INC., Houston, Tex.).

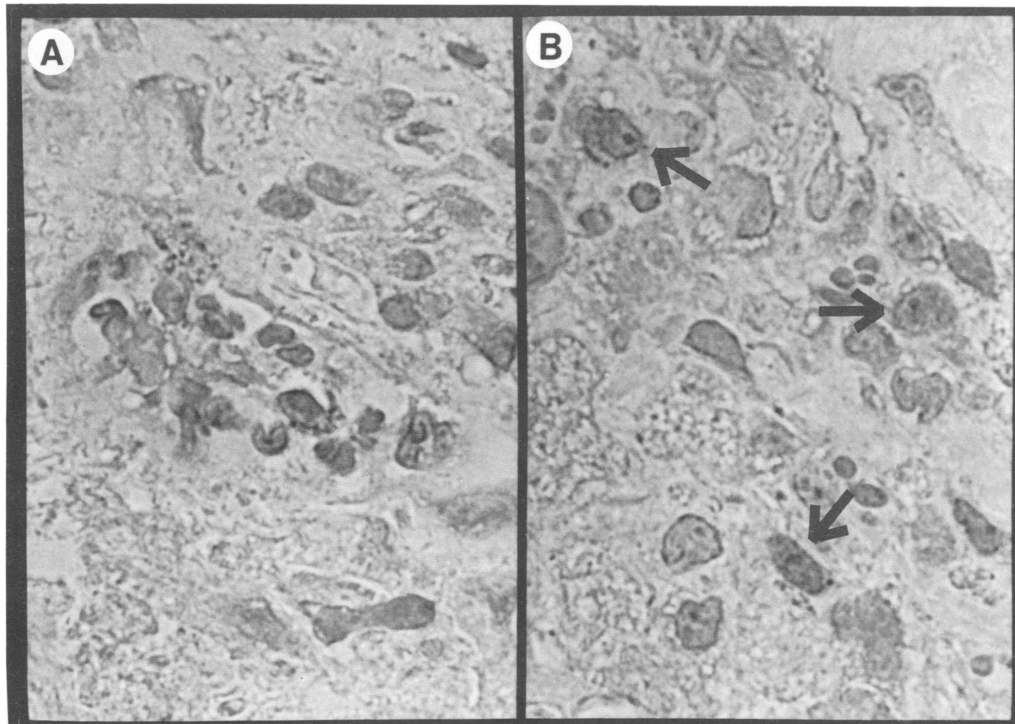

FIG. 1. Immunohistochemical analysis of duplicate sections of a representative endocardial specimen from a patient with acute *S. aureus* endocarditis. Cell-associated IL-8 is detected within Mϕ (as depicted by the arrows) from patients with endocarditis after incubation with immune serum (B) or control serum (A).

Equivalent amounts of total RNA per well were assessed by monitoring 28S and 18S rRNA.

mRNA stability analysis. The rate of IL-8 mRNA decay from LPS- and *S. aureus* LTA-stimulated PBM was determined by mRNA decay analysis. PBM were treated with LPS or LTA (10 μg/ml), RNA synthesis was blocked by adding actinomycin D after 2 h in culture, and then total cellular RNA was isolated at 0.5, 1, 2, 4, and 8 h after the addition of actinomycin D. Duplicate stimulated cell samples were tested in the absence of actinomycin D (10 μg/ml). Northern blot analysis was then performed, and the decay of mRNA was determined by laser densitometry of the autoradiographs.

Statistical analysis. Data were analyzed by a MacIntosh II computer with Statview II statistical package (Abacus Concepts, Inc., Berkeley, Calif.). Data are expressed as means ± standard errors of the means and compared with a two-tailed Student's *t* test. Data were considered statistically significant if *P* values were less than 0.05.

RESULTS

Production of cell-associated IL-8 in acute *S. aureus* endocarditis. In initial experiments, we assessed whether cell-associated IL-8 was produced in inflamed tissue in cases of acute gram-positive bacterial infection. We obtained paraffin-embedded specimens of endocardium from three patients undergoing native aortic or mitral valve replacement for acute *S. aureus* endocarditis. Histologic examination of the biopsy specimens revealed variable amounts of PMN and mononuclear cell infiltration, and gram-positive cocci were identified in one specimen. As shown in a representative section (Fig. 1), substantial quantities of cell-associated IL-8 were detectable in specimens from all three patients with acute staphylococcal endocarditis (Fig. 1A). The predominant source of IL-8 within the inflamed endocardium was cells with the morphologic characteristics of Mϕ. To confirm that the Mϕ were the source of IL-8 within these inflammatory lesions, immunohistochemical analysis on duplicate specimens using the Mϕ-specific anti-human monoclonal antibody HAM-56 was performed and the cells that expressed cell-associated IL-8 expressed the HAM-56 antigen in duplicate sections. Interestingly, no IL-8 was detected within PMN or surrounding stromal cells in the tissue sections examined.

Time-dependent expression of IL-8 mRNA and protein from LPS-, staphylococcal LTA-, or streptococcal LTA-stimulated PBM. Having demonstrated that IL-8 is produced in cases of gram-positive bacterial infection, we next wanted to determine whether LTA purified from either *S. aureus* or *S. pyogenes* could induce IL-8 gene expression from PBM in vitro. Human PBM (10^6 cells per ml) were challenged with either LPS, staphylococcal LTA, or streptococcal LTA (10 μg/ml) at time zero, and then IL-8 mRNA and protein levels were determined at various time points thereafter for 24 h. LPS induced a time-dependent increase in IL-8 mRNA, with substantial expression persisting to 24 h posttreatment (Fig. 2). In contrast, steady-state levels of IL-8 mRNA induced by streptococcal and staphylococcal LTA were maximal at 4 and 8 h poststimulation, respectively, with rapid decline in IL-8 mRNA levels by 24 h (Fig. 2). LPS-induced IL-8 protein production paralleled message levels, as extracellular IL-8 levels continued to increase for 24 h (93.3 ± 11.2 ng/ml significantly different [$P < 0.01$] from the value ob-

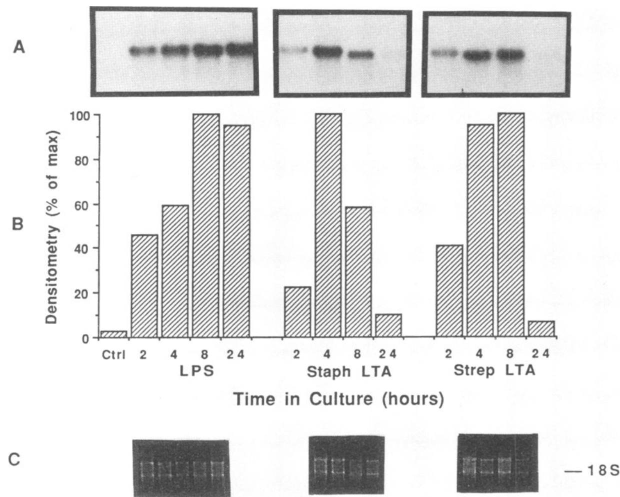

FIG. 2. Time-dependent expression of IL-8 mRNA from LPS- and LTA-treated PBM. The results of composite Northern blot analysis are shown and depict the effects of LPS, *S. aureus* LTA, (Staph LTA), and *S. pyogenes* LTA (Strep LTA) (10 μg/ml) on IL-8 mRNA expression from PBM. (A) Autoradiographs of Northern blots. (B) Densitometry of autoradiographs. max, maximum; Ctrl, Control. (C) 18S and 28S rRNA. Data shown are representative of two separate experiments.

tained for the 24-h control [Fig. 3]). The production of IL-8 from PBM treated with streptococcal and staphylococcal LTA plateaued after 8 h and was significantly less than that induced by LPS at 24 h (49.9 ± 2.9 and 50.9 ± 2.1 ng/ml, respectively, significantly different [$P < 0.05$] from the value with LPS treatment). In addition to stimulation of IL-8 secretion, staphylococcal and streptococcal LTA also in-

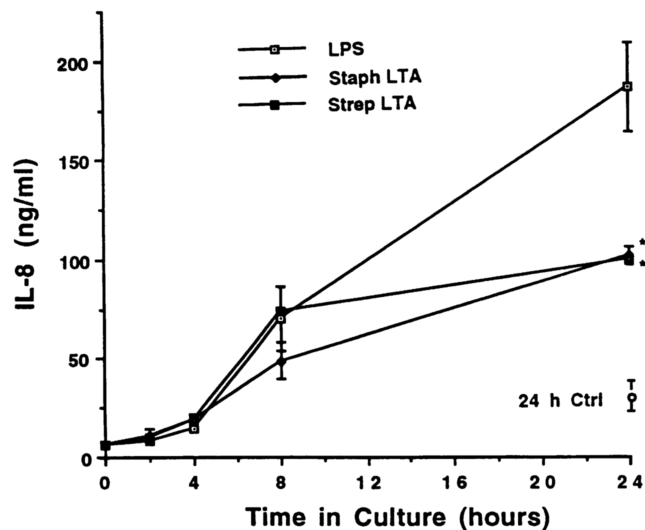

FIG. 3. Time-dependent expression of IL-8 from LPS- and LTA-treated PBM. PBM were treated with either LPS, *S. aureus* LTA (Staph LTA), or *S. pyogenes* LTA (Strep LTA) (10 μg/ml), and supernatant IL-8 was assessed at various time points. Values significantly different ($P < 0.05$) from the 24-h unstimulated control (24 h Ctrl) are indicated by asterisks. Data shown are from five separate experiments.

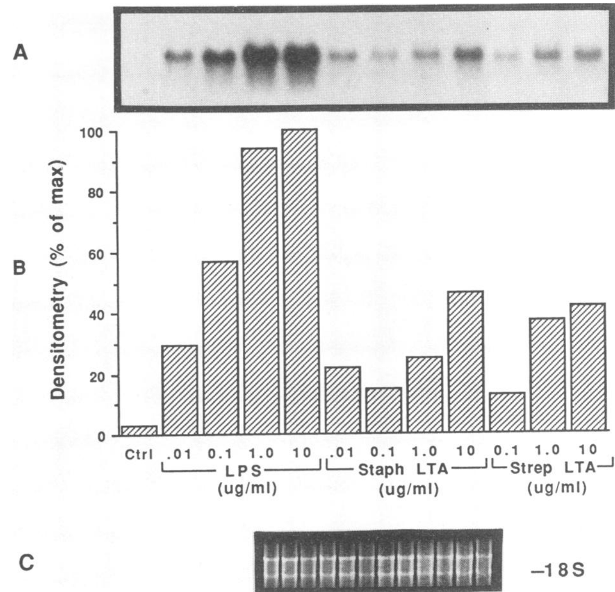

FIG. 4. Dose-dependent expression of IL-8 mRNA from LPS- and LTA-treated PBM. The results of Northern blot analysis are shown and depict the effects of LPS, *S. aureus* LTA, (Staph LTA), and *S. pyogenes* LTA (Strep LTA) on IL-8 mRNA expression from PBM after 6 h in culture. (A) Autoradiograph of the Northern blot. (B) Densitometry of the autoradiograph. Max, maximum; Ctrl, control. (C) 18S and 28S rRNA. Data shown are representative of two separate experiments.

duced the secretion of the monocyte chemotactic and activating protein MIP-1α from PBM (data not shown).

Dose-dependent expression of IL-8 mRNA and protein from PBM challenged with LPS, staphylococcal LTA, or streptococcal LTA. To determine whether LTA induced IL-8 gene expression from PBM in a dose-dependent fashion, we challenged human PBM (10^6 cells per ml) with graded doses of LPS, staphylococcal LTA, or streptococcal LTA and assessed IL-8 mRNA levels at 6 h and antigenic IL-8 protein levels at 24 h poststimulation. As shown in Fig. 4, LPS induced increases in IL-8 mRNA in a dose-dependent fashion, with maximal IL-8 mRNA levels seen after treatment with 10 μg of LPS per ml. Similarly, both staphylococcal LTA and streptococcal LTA stimulated dose-dependent increases in IL-8 mRNA, although the maximal values for IL-8 mRNA expression were only 46 and 41% of the maximal IL-8 mRNA levels induced by LPS. The induction of IL-8 mRNA was paralleled by dose-dependent increases in the expression of IL-8 protein, as maximal LPS-, staphylococcal LTA-, and streptococcal LTA-induced IL-8 levels were 6.9-, 3.3-, and 2.6-fold greater than that produced by unstimulated cells, respectively (Fig. 5).

Effects of cycloheximide on the expression of IL-8 from LPS, staphylococcal LTA-, and streptococcal LTA-challenged PBM. To assess whether the induction of IL-8 mRNA by LTA was dependent upon de novo protein synthesis, LPS- and staphylococcal LTA-treated PBM were incubated in the presence or absence of the protein synthesis inhibitor cycloheximide (5 μg/ml) and RNA was extracted after 4 h in culture. As shown in Fig. 6, treatment of PBM (10^6 cells per ml; 5×10^6 cells total) with cycloheximide alone resulted in modest superinduction of IL-8 mRNA expression. Treatment of PBM with staphylococcal LTA resulted in upregu-

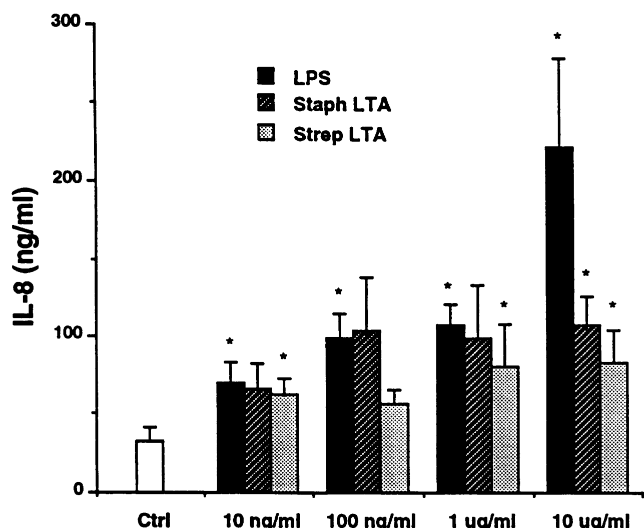

FIG. 5. Dose-dependent expression of IL-8 from LPS- and LTA-treated PBM. PBM were treated with graded doses of either LPS, *S. aureus* LTA (Staph LTA), or *S. pyogenes* LTA (Strep LTA), and IL-8 levels were assessed after 24 h in culture. Values significantly different ($P < 0.05$) from the 24-h unstimulated control (Ctrl) are indicated by asterisks. Data shown are from seven separate experiments.

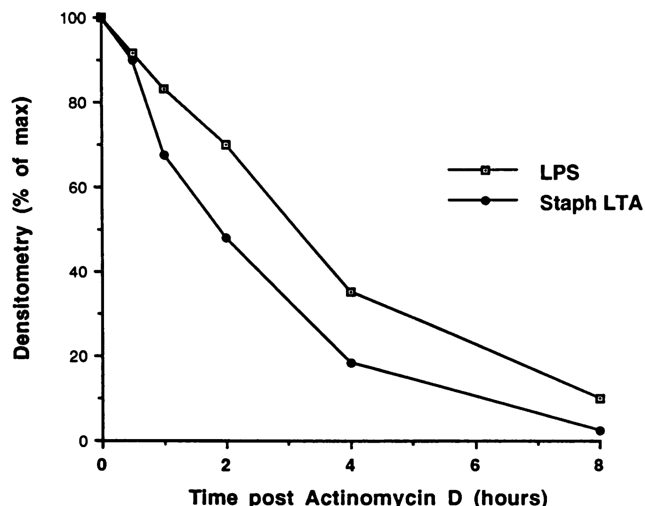

FIG. 7. Effects of LPS and staphylococcal LTA on IL-8 mRNA stability. The half-life of IL-8 mRNA from PBM treated with either LPS or *S. aureus* LTA (10 μg/ml) was determined by Northern blot analysis after treatment with actinomycin D (10 μg/ml, time zero). Steady-state levels of IL-8 mRNA were standardized to densitometric value of 28S rRNA. Data shown are representative of two separate experiments.

lation of IL-8 message compared with that of control, with substantial superinduction (1.7-fold increase) of IL-8 mRNA observed after concomitant treatment with cycloheximide. In contrast, the induction of IL-8 mRNA by LPS was not appreciably altered by concomitant treatment with cycloheximide. These data indicated that the induction of IL-8 mRNA by staphylococcal LTA was not dependent upon the synthesis of a protein intermediate and that steady-state levels of IL-8 mRNA expressed constitutively or in response to LTA, but not LPS, were under regulation by repressor proteins.

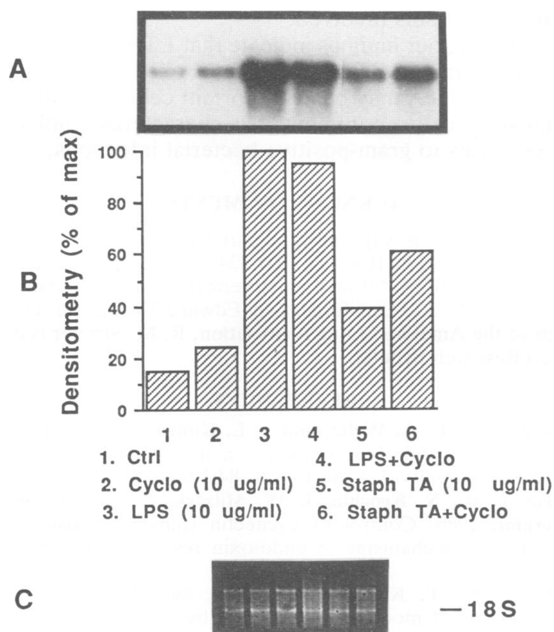

FIG. 6. Effects of cycloheximide on the expression of IL-8 mRNA from LPS- and *S. aureus* LTA-treated PBM. The results of Northern blot analysis are shown and depict the effects of cycloheximide (Cyclo) on the induction of IL-8 mRNA from PBM stimulated by LPS (10 μg/ml) and staphylococcal LTA (Staph TA) (10 μg/ml) after 6 h in culture. (A) Autoradiograph of the Northern blot. (B) Densitometry of the autoradiograph. (C) 18S and 28S rRNA. Data shown are representative of three separate experiments.

Effects of LPS and staphylococcal LTA on IL-8 mRNA decay. Our previous studies indicate that LPS and staphylococcal LTA induced distinctly different temporal patterns of IL-8 mRNA and protein expression from PBM. In addition, IL-8 message induced by LTA, but not LPS, appears to be negatively controlled by labile proteins. To assess whether differences in the regulation of IL-8 mRNA induced by LTA and by LPS were attributable to differences in mRNA stability, we performed mRNA stability studies. PBM (10^6 cells per ml; 5×10^6 cells total) were treated with either LPS (10 μg/ml) or staphylococcal LTA (10 μg/ml), actinomycin D was added 2 h poststimulation, and then IL-8 mRNA levels assessed in a time-dependent fashion. The half-life of IL-8 mRNA from PBM treated with LPS was approximately 3.2 h (Fig. 7). However, treatment of PBM with LTA resulted in an IL-8 mRNA half-life of 1.9 h, representing an approximately 41% reduction in the half-life compared with that observed after LPS stimulation.

DISCUSSION

Gram-negative bacterial infections are often characterized by marked inflammatory responses that can culminate in significant tissue injury and end-organ failure. LPS is believed to be the predominant trigger of immune cell activation, leading to the release of host-derived proinflammatory cytokines and further amplification of the inflammatory response. Like gram-negative bacterial infections, gram-positive bacterial infections can be associated with rather vigorous inflammatory responses both clinically and experimentally (10, 25, 32). For example, infusion of heat-killed

Staphylococcus epidermidis into rabbits has been shown to induce complement activation, TNF and IL-1 release, and shock, although to a lesser degree than that seen with infusion of a comparable number of *E. coli* (32). Likewise, Hack and colleagues found increased levels of IL-8 in sera of 89% patients with clinical sepsis (10). Interestingly, these researchers found no difference in serum IL-8 levels in patients with gram-positive bacteremia and those with gram-negative bacteremia. In this investigation, we found significant expression of macrophage-derived IL-8 in patients with localized gram-positive infections, specifically acute *S. aureus* endocarditis. We cannot be certain that the expression of IL-8 in staphylococcal endocarditis is directly attributable to the staphylococcal organism and/or its cell surface components, rather than endogenously produced cytokines such as TNF or IL-1. However, our observations that purified LTA can directly induce the expression of IL-8 from human PBM in the absence of de novo protein synthesis suggests that direct induction of IL-8 in gram-positive bacterial infection is likely to occur. Immunohistochemical analysis of inflamed endocardium indicates that IL-8 protein is localized to the Mϕ. While we have previously demonstrated that PMN can synthesize IL-8 in vitro and in vivo (14, 29), the Mϕ, rather than the PMN, appear to be the major cellular source of IL-8 in subacute bacterial endocarditis and chronic pulmonary inflammation (14).

LTA from two different species of gram-positive bacteria triggered the expression of IL-8 from PBM in both a time- and dose-dependent fashion. IL-8 cDNA was initially identified by Schmid and Weissmann as an inducible protein produced by staphylococcal enterotoxin A-challenged human blood leukocytes (23). Although it is plausible that the induction of IL-8 by purified preparations of *S. aureus* LTA could be due to enterotoxin A contamination, the presence of such a contaminant in *S. pyogenes* LTA preparations is highly unlikely. Furthermore, the induction of IL-8 by LTA was not a result of endotoxin contamination, since we were unable to detect the presence of endotoxin in either of the LTA preparations.

S. aureus and *S. pyogenes* LTA induced modest differences in the temporal expression of IL-8 mRNA. However, the magnitudes of IL-8 production induced by these two stimuli were quite similar. These findings differ somewhat from those reported in previous investigations, which have found significant differences in the macrophage-activating effects of various LTAs, depending upon the bacterial species from which the LTA was isolated. Keller and associates reported that the LTA isolated from *S. aureus* or *Enterococcus faecalis* was a potent inducer of macrophage TNF and nitrite production, respiratory burst, and tumoricidal activity, whereas LTA recovered from *S. pyogenes* stimulated TNF and nitrite synthesis but had no effect on reductive capacity or tumoricidal activity (12). Bhakdi and colleagues found that *E. faecalis* LTA was a potent inducer of TNF, IL-1, and IL-6 from human monocytes, while LTA isolated from either *S. aureus* or *Streptococcus pneumoniae* exerted much less effect on cytokine production from these cells (3). However, these investigators were unable to identify any apparent relationship between the macrophage-activating effects of LTA and chain length, glycosyl substitution, or glycolipid content of LTA. Our findings suggest that the ability of various LTAs to induce IL-8 synthesis and secretion is less discriminatory than the induction of other LTA-mediated macrophage effector cell activities.

Although LTA can induce the secretion of IL-8 from PBM, the concentration of LTA at which IL-8 production occurs is approximately 100-fold greater than that of LPS. Furthermore, LPS at the maximal doses tested induces approximately twice as much IL-8 mRNA and protein than did comparable concentrations of staphylococcal and streptococcal LTA. The greater potency and efficacy of LPS compared with those of LTA may be partially explained by the enhanced stability of IL-8 mRNA in the presence of LPS. It has previously been shown that increases in IL-8 mRNA can be attributable to transcriptional activation, mRNA stabilization, or a combination of these two mechanisms (18, 28). Although the predominant effect of LPS on TNF mRNA expression is induction of mRNA synthesis, studies suggest that LPS may also stabilize TNF mRNA transcripts (2). Similar to the induction of IL-8 by IL-1, LPS may lead to increases in IL-8 message both by transcriptionally activating the IL-8 gene and by stabilizing IL-8 mRNA transcripts (28). Conversely, IL-8 mRNA produced by LTA-treated PBM appears to be less stable than that induced by LPS. In total, these findings indicate that the release of IL-8 from PBM challenged with LTA is more tightly controlled than is IL-8 expression observed in response to LPS.

Interestingly, we observed the superinduction of IL-8 mRNA from unstimulated and LTA-challenged PBM when coincubated with cycloheximide, suggesting that repressor proteins are produced under basal conditions which inhibit mRNA transcription (6) or accelerate the rate of mRNA decay (15), and these proteins may be inducible in the presence of LTA. In contrast, cycloheximide failed to superinduce IL-8 message from PBM after LPS treatment. These results indicate either that tonically produced repressor proteins are not induced by LPS or that the stimulatory effects of LPS simply overwhelm the ability of these proteins to suppress IL-8 mRNA synthesis and/or accelerate mRNA decay. Studies are ongoing to further characterize the molecular mechanisms involved.

In summary, our findings indicate that LTA can induce the secretion of the PMN-activating and chemotactic protein IL-8 and that LTA may be an important cellular mediator of inflammatory cell recruitment that characterizes inflammatory responses to gram-positive bacterial infections.

ACKNOWLEDGMENTS

This research was supported in part by National Institutes of Health grants (1P50HL46487, HL02401, HL31693, HL35276, HL02701, and DK38149) and an American Lung Association research grant. T. J. Standiford is an Edward Livingston Trudeau Scholar of the American Lung Association. R. M. Strieter is a RJR Nabisco Research Scholar.

REFERENCES

1. **Baggiolini, M., A. Waltz, and S. L. Kunkel.** 1989. Neutrophil-activating peptide-1/interleukin-8, a novel cytokine that activates neutrophils. J. Clin. Invest. **84:**1045–1049.
2. **Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami.** 1986. Control of cachectin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. Science **232:** 977–979.
3. **Bhakdi, S., T. Klonisch, P. Nuber, and W. Fischer.** 1991. Stimulation of monokine production by lipoteichoic acids. Infect. Immun. **59:**4614–4620.
4. **Chirgwin, J. M., A. E. Przybyla, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry **18:**5294–5299.
5. **Choi, A. M. K., and D. B. Jacoby.** 1992. Influenza virus A infection induces interleukin-8 gene expression in human airway epithelial cells. FEBS Lett. **309:**327–329.
6. **Collart, M. A., D. Belin, J. Vassalli, S. de Kossodo, and P.**

Vassalli. 1986. γ Interferon enhances macrophage transcription of the tumor necrosis factor/cachectin, interleukin 1, and urokinase genes, which are controlled by short-lived repressors. J. Exp. Med. **164:**2113–2118.

7. **Djeu, J. Y., K. Matsushima, J. J. Oppenheim, K. Shiotsuki, and D. K. Blanchard.** 1990. Functional activation of human neutrophils by recombinant monocyte-derived neutrophil chemotactic factor/IL-8. J. Immunol. **144:**2205–2210.
8. **Elias, J. A., A. D. Schrieber, and K. Gustilo.** 1985. Differential interleukin 1 elaboration by unfractionated and density fractionated human alveolar macrophages and blood monocytes: relationship to cell maturity. J. Immunol. **135:**3198–3204.
9. **Friedland, J. S., D. G. Remick, R. Shattock, and G. E. Griffin.** 1992. Secretion of interleukin-8 following phagocytosis of mycobacterium tuberculosis by human monocyte cell lines. Eur. J. Immunol. **22:**1373–1378.
10. **Hack, C. E., M. Hart, R. J. M. Strack van Schijndel, A. J. M. Eerenberg, J. H. Nuijens, L. G. Thijs, and L. A. Aarden.** 1992. Interleukin-8 in sepsis: relation to shock and inflammatory mediators. Infect. Immun. **60:**2835–2842.
11. **Jonas, E., T. D. Sargent, and I. B. Davis.** 1985. Epidermal keratin-gene expressed in embryos of xenopus laevis. Proc. Natl. Acad. Sci. USA **82:**5413–5416.
12. **Keller, R., W. Fischer, R. Keist, and S. Bassetti.** 1992. Macrophage response to bacteria: induction of marked secretory and cellular activities by lipoteichoic acids. Infect. Immun. **60:**3664–3672.
13. **Lee, T. C., and F. Snyder.** 1985. Function, metabolism, and regulation of platelet activating factor and related ether lipids, p. 1–39. *In* J. F. Kuo (ed.), Phospholipids and cellular regulation. CRC Press, Inc., Boca Raton, Fla.
14. **Lynch, J. P., III, T. J. Standiford, M. W. Rolfe, S. L. Kunkel, and R. M. Strieter.** 1992. Neutrophilic alveolitis in idiopathic pulmonary fibrosis: the role of interleukin-8. Am. Rev. Respir. Dis. **145:**1433–1439.
15. **Malter, J. S.** 1987. Identification of an AUUUA-specific messenger RNA binding protein. Science **246:**664–666.
16. **Matsushima, K., and J. J. Oppenheim.** 1989. Interleukin 8 and MCAF: novel inflammatory cytokines inducible by IL 1 and TNF. Cytokine **1:**2–13.
17. **Nauciel, C., J. Fleck, M. P. Martin, M. Mock, and H. Nguyen-Huy.** 1974. Adjuvant activity of bacterial peptidogycans on the production of delayed hypersensitivity and on antibody response. Eur. J. Immunol. **4:**352–356.
18. **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:**617–647.
19. **Peveri, P., A. Walz, B. Dewald, and M. Baggiolini.** 1988. A novel neutrophil-activating factor produced by human mononuclear phagocytes. J. Exp. Med. **167:**1547–1559.
20. **Rankin, J. A., I. Sylvester, S. Smith, T. Yoshimura, and E. J. Leonard.** 1990. Macrophages cultured in vitro release leukotriene B_4 and neutrophil attractant/activation protein (interleukin 8) sequentially in response to stimulation with lipopolysaccharide and zymosan. J. Clin. Invest. **86:**1556–1564.
21. **Rodriguez, J. L., C. G. Miller, L. E. DeForge, L. Kelty, C. J. Shanley, R. H. Bartlett, and D. G. Remick.** 1992. Local production of interleukin-8 is associated with nosocomial pneumonia. J. Trauma **33:**74–81.
22. **Rot, A.** 1990. The role of leukocyte chemotaxis in inflammation, p. 263–296. *In* S. L. Kunkel, I. Lindley, and J. Warwick (ed.), Chemotactic cytokines. S. Karger, Basel.
23. **Schmid, J., and C. Weissman.** 1987. Induction of mRNA for a serine protease and a β-thromboglobulin-like protein in mitogen-stimulated human leukocytes. J. Immunol. **139:**250–256.
24. **Schroder, J. M.** 1989. The monocyte-derived neutrophil activating peptide (NAP/interleukin 8) stimulates neutrophil arachidonate-5-lipoxygenase, but not the release of cellular arachidonate. J. Exp. Med. **170:**847–863.
25. **Sheagren, J. N.** 1984. Staphylococcus aureus. The persistent pathogen. N. Engl. J. Med. **310:**1437–1442.
26. **Standiford, T. J., R. M. Strieter, S. W. Chensue, J. Westwick, K. Kasahara, and S. L. Kunkel.** 1990. IL-4 inhibits the expression of IL-8 from stimulated human monocytes. J. Immunol. **145:**1435–1439.
27. **Stewart-Tull, D. E. S.** 1980. The immunological activities of bacterial peptidoglycans. Annu. Rev. Microbiol. **34:**311–340.
28. **Stoeckle, M. Y.** 1991. Post-transcriptional regulation of *gro*α, β, γ, and IL-8 mRNAs by IL-1β. Nucleic Acids Res. **19:**917–920.
29. **Strieter, R. M., K. Kasahara, R. A. Allen, T. J. Standiford, M. W. Rolfe, F. S. Becker, S. W. Chensue, and S. L. Kunkel.** 1992. Cytokine-induced neutrophil-derived interleukin-8. Am. J. Pathol. **141:**397–407.
30. **Strieter, R. M., D. G. Remick, J. P. Lynch III, M. Genord, C. Raiford, R. Spengler, and S. L. Kunkel.** 1989. Differential regulation of tumor necrosis factor-alpha in human alveolar macrophages and peripheral blood monocytes: a cellular and molecular analysis. Am. J. Respir. Cell Mol. Biol. **1:**57–64.
31. **Usami, H., A. Yamamoto, Y. Sugawara, S. Hamada, T. Yamamoto, K. Kato, S. Kokeguchi, H. Takada, and S. Kotani.** 1987. A nontoxic tumour necrosis factor induced by streptococcal lipoteichoic acids. Br. J. Cancer **56:**797–799.
32. **Wakabayashi, G., J. A. Gelfand, W. K. Jung, R. J. Connolly, J. F. Burke, and C. A. Dinarello.** 1991. *Staphylococcus epidermidis* induces complement activation, tumor necrosis factor and interleukin-1, a shock-like state and tissue injury in rabbits without endotoxemia comparison to *Escherichia coli*. J. Clin. Invest. **87:**1925–1935.