INFECTION AND IMMUNITY, June 1998, p. 2447–2452 Vol. 66, No. 6
0019-9567/98/$04.00+0

Lipopolysaccharide-Related Stimuli Induce Expression of the Secretory Leukocyte Protease Inhibitor, a Macrophage-Derived Lipopolysaccharide Inhibitor

FENYU JIN,[1]† CARL F. NATHAN,[1] DANUTA RADZIOCH,[2] AND AIHAO DING[1]*

Beatrice and Samuel A. Seaver Laboratory, Department of Medicine, Cornell University Medical College, New York, New York 10021,[1] and Department of Medicine, McGill University, Montreal, Quebec H3G 1A4, Canada[2]

Received 12 November 1997/Returned for modification 8 January 1998/Accepted 13 March 1998

Mouse secretory leukocyte protease inhibitor (SLPI) was recently characterized as a lipopolysaccharide (LPS)-induced product of macrophages that antagonizes their LPS-induced activation of NF-κB and production of NO and tumor necrosis factor (TNF) (F. Y. Jin, C. Nathan, D. Radzioch, and A. Ding, Cell 88:417–426, 1997). To better understand the role of SLPI in innate immune and inflammatory responses, we examined the kinetics of SLPI expression in response to LPS, LPS-induced cytokines, and LPS-mimetic compounds. SLPI mRNA was detectable in macrophages by Northern blot analysis within 30 min of exposure to LPS but levels peaked only at 24 to 36 h and remained elevated at 72 h. Despite the slowly mounting and prolonged response, early expression of SLPI mRNA was cycloheximide resistant. Two LPS-induced proteins—interleukin-10 (IL-10) and IL-6—also induced SLPI, while TNF and IL-1β did not. The slow attainment of maximal induction of SLPI by LPS in vitro was mimicked by infection with *Pseudomonas aeruginosa* in vivo, where SLPI expression in the lung peaked at 3 days. Two LPS-mimetic molecules—taxol from yew bark and lipoteichoic acid (LTA) from gram-positive bacterial cell walls—also induced SLPI. Transfection of macrophages with SLPI inhibited their LTA-induced NO production. An anti-inflammatory role for macrophage-derived SLPI seems likely based on SLPI's slowly mounting production in response to constituents of gram-negative and gram-positive bacteria, its induction both as a direct response to LPS and as a response to anti-inflammatory cytokines induced by LPS, and its ability to suppress the production of proinflammatory products by macrophages stimulated with constituents of both gram-positive and gram-negative bacteria.

Human secretory leukocyte protease inhibitor (SLPI), an 11.7-kDa cysteine-rich protein, has long been known to be an epithelial cell product found in saliva, seminal plasma, and cervical, nasal, and bronchial mucus, and it was named for what was then its only known function, the inhibition of serine proteases released by leukocytes (3, 26, 43). Recently, using differential display of mRNA, we identified the gene encoding mouse SLPI as one of two genes overexpressed by a lipopolysaccharide (LPS)-hyporesponsive macrophage cell line from the C3H/HeJ mouse (Lps^d) compared to expression in an LPS-normoresponsive macrophage cell line from the C3H/HeN strain (Lps^n) (32). Further investigation revealed that macrophages and neutrophils are rich sources of SLPI, that its expression is induced in primary macrophages by LPS and suppressed by gamma interferon (IFN-γ), and that SLPI antagonizes LPS-induced signaling and secretion (32).

LPS is among the most potent molecular stimuli of the immune system. Macrophage products released after LPS challenge protect the host from infection but, at high levels, contribute to systemic inflammatory response syndrome and death (16, 36). Among the most important cellular targets of LPS-induced, macrophage-derived secretory products is the macrophage itself. The generally proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF) exert positive feedback (20), whereas the generally anti-inflammatory cytokines IL-10 and transforming growth factor β (TGF-β) are autoinhibitory (14, 21, 25); IL-6 has both effects (50). Cytokines from sources other than macrophages enhance (IFN-γ) (5, 9, 41) or suppress (IL-4) (13) the macrophage's response to LPS. Thus, the net outcome of the host response to LPS depends on a complex network of positive and negative influences set in motion by infection. Responses to the analogous cell wall constituent of gram-positive bacteria, lipoteichoic acid (LTA), are similarly complex.

To further explore the role of SLPI in the regulatory networks of inflammation and innate immunity, we asked the following questions. Is SLPI induced by LPS directly or via an LPS-induced cytokine? When does SLPI expression peak during LPS challenge? Can SLPI be induced in macrophages by stimuli other than LPS, such as products of gram-positive bacteria? Here, we show that SLPI is an LPS-inducible immediate-early gene, yet, paradoxically, its expression is slow to peak and recede. Induction of SLPI during LPS challenge may be sustained by two other LPS-induced macrophage products, IL-10 and IL-6. LTA, which binds the CD14 LPS receptor (18), and taxol, a CD14-independent, microtubule-binding diterpene that mimics many actions of LPS (23, 35), also induce SLPI. Finally, transfection of macrophages with SLPI abolishes their response to LTA as it does their response to LPS (32). During inflammation induced by bacteria or their products, SLPI may be induced both directly and by anti-inflammatory cytokines, some of whose actions it may mediate.

* Corresponding author. Mailing address: Beatrice and Samuel A. Seaver Laboratory, Department of Medicine, Cornell University Medical College, New York, NY 10021. Phone: (212) 746-2986. Fax: (212) 746-8536. E-mail: ahding@med.cornell.edu.

† Present address: Millennium Pharmaceuticals, Inc., Cambridge, MA 02139-4815.

MATERIALS AND METHODS

Materials. Reagents and supplies were obtained as follows: LPS prepared by phenol extraction from *Escherichia coli* O111:B4 from List Biological Laboratories (Campbell, Calif.); *Staphylococcus aureus* LTA (LPS content in 10 μg of LTA per ml = 0.1 ng/ml as determined by the *Limulus* amebocyte lysate assay

[BioWhittaker, Walkersville, Md.] but possibly attributable to cross-reaction), dexamethasone, cyclohexamide, and taxol from Sigma (St. Louis, Mo.); purified recombinant mouse TNF (protein concentration, 0.98 mg/ml; specific activity, 1.2×10^7 U/mg; LPS content, <52 pg/ml) from Genentech (South San Francisco, Calif.); purified recombinant murine IL-10 from Bachem Bioscience (Philadelphia, Pa.) (protein concentration, 0.1 mg/ml; LPS content in 100 ng of IL-10 per ml = 0.08 ng/ml); human TGF-β (LPS content in 50 μg TGF per ml < 10 pg/ml) from Amgen, Inc. (Thousand Oaks, Calif.); recombinant murine IL-1β from Upstate Biotechnology, Inc. (Lake Placid, N.Y.); crude recombinant human IL-6 and IL-6 receptor (IL-6R) mixture from Selina Cheng-Kiang (Cornell University Medical College, N.Y.); oligonucleotide primers from Oligos, Etc., Inc. (Guilford, Conn.); G418 from Gibco Life Technologies (Grand Island, N.Y.); AmpliTaq DNA polymerase, deoxynucleoside triphosphates, and PCR buffer solutions from Perkin-Elmer Cetus (Foster City, Calif.); [^{35}S]methionine-cysteine protein-labeling mix and [α-^{32}P]dCTP from NEN Life Science Products (Boston, Mass.); guanidinium isothiocyanate, formaldehyde, and formamide from Fluka Chemica-Biochemica (Ronkonkoma, N.Y.); plasmid DNA preparation columns from Qiagen (Chatsworth, Calif.); and tissue culture dishes from Corning Glass Works (Corning, N.Y.). Plasmid vector p463-neo was a gift from JianXun Li, Nashville, Tenn.

Mice. Adult female C3H/HeN mice were purchased from Charles River Breeding Laboratories (Wilmington, Mass.). C57BL/6 mice were from Harlan Sprague Dawley (Indianapolis, Ind.).

Cells. Primary mouse macrophages were collected from the peritoneal cavity 4 days after intraperitoneal injection with 2 ml of 4% Brewer's thioglycollate broth (Difco, Detroit, Mich.). HeNC2 and GG2EE cells are bone marrow-derived, v-*myc*- and v-*raf*-transformed macrophage cell lines (11). HeNC2 cells stably transfected with p463-neo-SLPI or p463-neo vectors as described previously (32) were maintained in medium containing G418 at 500 μg/ml. The RAW 264.7 macrophage cell line was from the American Type Culture Collection, Manassas, Va. Cells were maintained in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (HyClone, Logan, Utah), 2 mM L-glutamine, 200 U of penicillin per ml, and 200 μg of streptomycin per ml at 37°C in 5% CO_2 and 95% air. Complete culture medium was routinely monitored for LPS contamination as described above and found to contain <25 pg of LPS/ml.

Northern blot. Total RNA (20 or 25 μg/lane) was electrophoresed on a 1% agarose gel with 20 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS, pH 7.0), 50 mM sodium acetate, 1 mM EDTA (1× MOPS), and 2% formaldehyde, and equal loading was confirmed by ethidium bromide staining. RNA was transferred in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) onto a nylon membrane (NEN Research Products, Boston, Mass.). The membrane was hybridized for 18 h at 42°C with probe (10^6 cpm/ml) labeled with the Prime-a-Gene kit from Promega (Madison, Wis.) in 5× SSC, 5× Denhardt solution, 50% formamide, and 1% sodium dodecyl sulfate (SDS) plus 100 μg of sperm DNA per ml. Membranes were then washed twice with 1× SSC–0.1% SDS (10 min at room temperature) and with 0.25× SSC–0.1% SDS (10 min at 55°C) before autoradiography. Control probes for β-actin and glyceraldehyde-3-phosphate dehydrogenase (G3PDH) cDNA were amplified with the manufacturer's templates and amplimers (Clontech, Palo Alto, Calif.). The membranes were then exposed to X-Omat autoradiography film (Kodak, Rochester, N.Y.), and the relative optical density of SLPI mRNA was analyzed with a Fluor-S multi-imager with a clear light filter (Bio-Rad Laboratories, Hercules, Calif.) with reference to the expression of β-actin or G3PDH.

Inhibition of protein synthesis by cycloheximide. Monolayers of primary macrophages (10^6/well) in 24-well plates were incubated with cysteine- and methionine-free RPMI medium for 1 h at 37°C, and 1 μCi of a radiolabeled [^{35}S] methionine-cysteine mixture with different concentrations of cycloheximide in triplicate was added to each well. After incubation at 37°C for 4 h, monolayers were washed three times with phosphate-buffered saline and cells were lysed with 500 μl of 1 N NaOH. Protein synthesis was determined as trichloroacetic acid-precipitable radioactivity in the lysate and expressed as a percentage of the control value (determined with cells incubated in the absence of cycloheximide).

Secretion of nitrite. Cells were plated in 96-well plates at 10^5 cells/well in 150 μl of medium and treated for 48 h with indicated concentrations of LPS, IFN-γ, or both. A volume of conditioned medium (100 μl) was mixed with an equal volume of Griess' reagent (1% sulfanilamide, 0.1% naphthylethylenediamine dihydrochloride, and 2.5% H_3PO_4). Absorbance at 550 nm was recorded with a microplate reader (MR5000; Dynatech, Chantilly, Va.) with sodium nitrite as the standard. The nitrite content of similarly incubated cell-free medium was subtracted.

Infection with *Pseudomonas aeruginosa*. Mice were infected intratracheally with a mucoid clinical isolate of *P. aeruginosa* 508 enmeshed in microbeads of agar as previously described (27). Briefly, a log-phase bacterial suspension diluted in warm Trypticase soy agar was added to heavy mineral oil, stirred vigorously, and chilled in ice to form bacterium-containing beads <200 μm in diameter. The titer of viable bacteria in the beads was determined by plating serial dilutions of homogenized bead suspension on plates containing Trypticase soy agar medium. Mice were anesthetized, and their tracheas were exposed by a ventral midline cervical incision; 50 μl of the bead suspension (5×10^4 *P. aeruginosa* organisms) followed by 50 μl of air was inoculated into the lungs through a 22-gauge intravenous catheter inserted into the trachea. After inoculation, the incision was sutured. Animals did not develop wound infections, and healing occurred within 2 to 3 days. Sham infection involved the same surgery and injection of agar microbeads prepared in the same way but without bacteria.

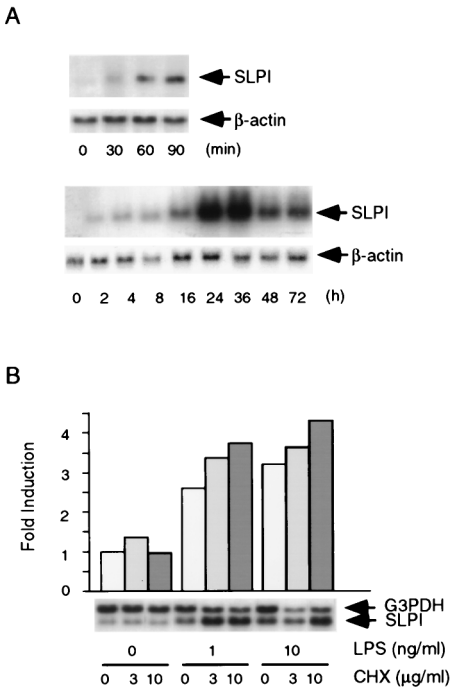

FIG. 1. Kinetics of LPS-induced expression of SLPI and the effect of cycloheximide. (A) Time course. RNA samples (25 μg/lane) were prepared from RAW 264.7 cells treated with 100 ng of LPS per ml for the indicated times, electrophoresed, and hybridized with an SLPI cDNA probe. The membrane was rehybridized with a control probe as a loading control. (B) Total RNA samples (20 μg/lane) from primary macrophages (C3H/HeN) treated with indicated concentrations of LPS and/or cycloheximide (CHX) for 4 h were prepared and hybridized as for panel A except that the control probe was derived from G3PDH. The bar graph displays the densitometric analysis of the underlying autoradiogram. The signal intensity ratio of SLPI to G3PDH for cells not treated in vitro (first column in autoradiogram) was set at 1 and is termed the control ratio. Signal intensity ratios of SLPI to G3PDH for all other sets are expressed relative to the control ratio. The same principle was used for comparison of results of densitometric analyses in all figures.

RESULTS

SLPI is encoded by an LPS-induced immediate-early gene, yet its full expression is delayed and prolonged. LPS induces the production of SLPI in both primary macrophages and the RAW 264.7 macrophage cell line (32). Here, we examined the kinetics of this process. After incubation with 100 ng of LPS per ml, an increase in SLPI mRNA in RAW 264.7 cells was detected as early as 30 min, the first time point examined. However, expression did not peak until 24 h and was still high at 72 h (Fig. 1A). The early onset of expression suggested that transcription of the SLPI gene might be induced by LPS without the mediation of a newly synthesized protein. To address this, we examined the effect of cycloheximide. At protein synthesis-blocking concentrations, cycloheximide was toxic to RAW 264.7 cells but not to primary macrophages. In the latter cells, cycloheximide inhibited protein synthesis by 84 or 95% (at concentrations of 3 and 10 μg/ml, respectively), but the increased expression of SLPI by LPS was unaffected (Fig. 1B).

Induction of SLPI expression by IL-10 and IL-6. Even though SLPI appeared to be an early response gene, its gradually increasing and prolonged expression suggested that the production of other proteins might contribute to the later

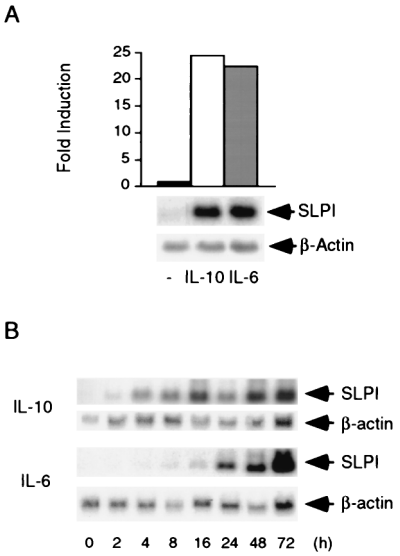

FIG. 2. Induction of SLPI by IL-10 and by IL-6 plus IL-6R. (A) Primary macrophages. Macrophages (C3H/HeN) were incubated alone or with IL-10 (100 ng/ml) or crude human IL-6 together with IL-6R (with a bioactivity equivalent to 25 U/ml) for 24 h. SLPI mRNA levels were analyzed with Northern blots, controlled, and expressed as in Fig. 1. (B) RAW 264.7 cells: kinetics of the response to IL-10 or IL-6. RNA samples (25 μg/lane) were prepared from RAW 264.7 cells treated with IL-10 or with IL-6 plus IL-6R as for panel A for the indicated hours. Northern blots were prepared and analyzed as described for panel A.

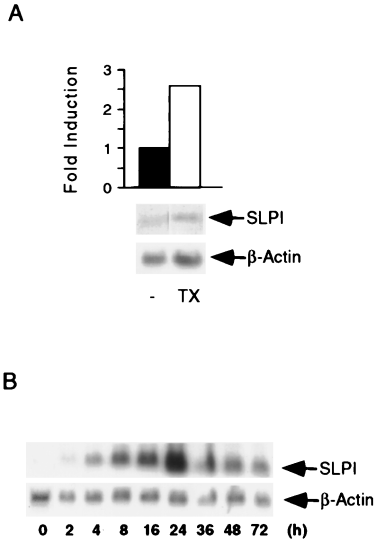

FIG. 3. Induction of SLPI by taxol. (A) Primary macrophages. Macrophages (C3H/HeN) were incubated with 10 μM taxol (TX) for 24 h. SLPI mRNA levels were analyzed with Northern blots, controlled, and expressed as in Fig. 1. (B) RAW 264.7 cells: kinetics of the response to taxol. RNA samples (25 μg/lane) were prepared from RAW 264.7 cells treated with taxol (10 μM) for the indicated times, and the expression of SLPI mRNA was assessed by Northern blot analysis as for Fig. 1.

phases of its expression. Accordingly, we tested the effects of five LPS-induced macrophage proteins—TNF-α, IL-1, IL-6, IL-10, and TGF-β—as well as dexamethasone, one of the glucocorticoids, which are LPS-induced, anti-inflammatory autacoids that induce macrophage inhibition factor, another macrophage-derived protein active on macrophages (17). Of all these, only IL-10 and IL-6 stimulated SLPI expression (Fig. 2A and data not shown). The IL-10-stimulated increase in SLPI expression was strong at 16 h. The SLPI level appeared to decrease at 24 h but bounced back at 48 h and remained high throughout the study (until 72 h). The IL-6-induced increase in SLPI expression was not obvious until 24 h, and the SLPI level was still rising at 72 h (Fig. 2B). This is in contrast to the decrease in the level of SLPI induced by LPS at late time points (Fig. 1A). Lack of induction of SLPI in macrophages by dexamethasone (10^{-14} to 10^{-6} M), TNF (0.1 to 10 ng/ml), IL-1 (0.1 to 100 ng/ml), or TGF-β (0.1 to 100 ng/ml) is in contrast to the case in human epithelial cells (1, 2, 37, 45).

Induction of SLPI by taxol. The actions on mammalian cells of taxol, an antitumor drug derived from the stem bark of the western yew, *Taxus brevifolia*, were originally believed to be confined to promotion of the abnormal assembly of microtubules and mitotic spindles. More recently, however, it has been extensively demonstrated that taxol mimics numerous actions of LPS in macrophages in a manner under the control of the *Lps* gene (12, 22, 23, 31) but apparently independent of CD14 (34), a receptor responsible for mediating responses to pathophysiologically relevant concentrations of LPS (53, 54). Consistent with this, LPS-free taxol induced SLPI. In primary macrophages, the induction by taxol was less than the induction by LPS (Fig. 3A), while in RAW 264.7 cells, the effect of taxol on SLPI expression (Fig. 3B) matched that of LPS in both extent and kinetics.

Induction of SLPI by LTA. Next, we checked whether LTA, the counterpart of LPS in gram-positive bacteria, affects SLPI expression. LTA shares the ability of LPS to bind CD14 (52), and both trigger macrophages to secrete similar spectrums of inflammatory mediators (10, 15, 18, 33, 38, 48). When primary macrophages were incubated for 8 h with 100 ng of LTA per ml, SLPI expression increased more than 20-fold (Fig. 4A). However, the kinetics of response to LTA differed from the kinetics of response to LPS. The level of SLPI induced by LTA in RAW 264.7 cells reached its peak at 8 h and rapidly declined thereafter (Fig. 4B). This difference in kinetics ruled out the

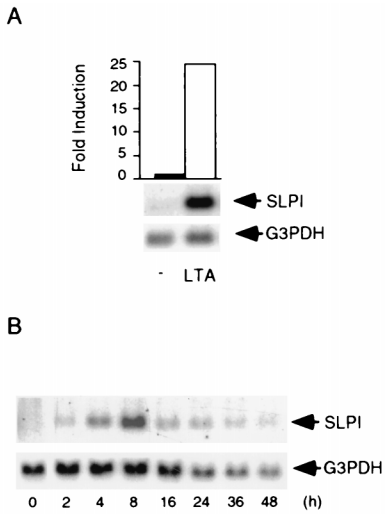

FIG. 4. Induction of SLPI by LTA. (A) Primary macrophages. Macrophages (C3H/HeN) were incubated with or without 100 μg of LTA per ml for 8 h. SLPI mRNA levels were analyzed with Northern blots, controlled, and expressed as in Fig. 1. (B) RAW 264.7 cells: kinetics of the response to LTA. RNA samples (25 μg/lane) were prepared from RAW 264.7 cells treated with LTA (100 μg/ml) for the indicated times, and the expression of SLPI mRNA was assessed by Northern blot analysis as for Fig. 1.

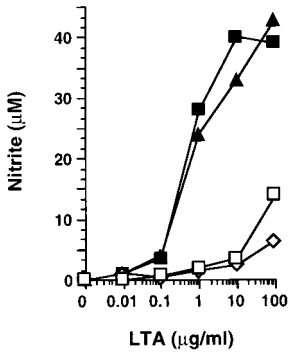

FIG. 5. Inhibition of LTA-induced nitrite production in SLPI-expressing macrophages. HeNC2 macrophages (■), HeNC2 cells transfected with vector alone (▲), and two independently selected clones of HeNC2 cells transfected with SLPI (□ and ◇) were cultured for 48 h at 10^5 cells per well with the indicated concentrations of LTA, and nitrite accumulation was measured in the conditioned media. Results are means of triplicates from one of four similar experiments. Standard errors of the means fall within the range covered by the symbols.

FIG. 6. Induction of SLPI expression in the lung by infection with *P. aeruginosa*. RNA was prepared from lungs of mice that were untreated (0 h) or injected intratracheally with *P. aeruginosa* embedded in agar beads as described in Materials and Methods (top) or injected in the same manner but without bacteria (bottom) after the indicated times postinfection. Northern blots were performed as described for Fig. 1 except that the membrane was exposed for 4 days. d, days.

possibility that the effect of LTA could be attributed to its contamination with LPS, which in any event was miniscule (see Materials and Methods).

Hyporesponsiveness to LTA in SLPI-expressing macrophages. Forced expression of SLPI in macrophage cell lines induced an LPS-hyporesponsive state (32). Here, we tested the LTA responsiveness of the same two independently transfected clones of HeNC2 cells expressing recombinant SLPI, along with the parental cells and one clone transfected with the p463-neo vector alone. The parental cells and the vector control transfectant expressed no detectable SLPI, while clones HeNC2-pSLPIa and HeNC2-pSLPIb expressed SLPI mRNA and protein at levels expressed by LPS-stimulated primary macrophages (32). As judged by nitrite release, a measure of nitric oxide production, LTA responsiveness was preserved in HeNC2 vector control cells, just as in the parental HeNC2 cells; both responded to as little as 1 μg of LTA per ml. In contrast, the two SLPI transfectants became markedly LTA hyporesponsive, requiring more than 100-fold-greater concentrations of LTA to produce the same amounts of nitrite (Fig. 5).

Induction of SLPI expression in vivo by *P. aeruginosa* infection. The results reported so far were from in vitro studies. To see whether SLPI could be induced by bacteria in vivo, we employed an established mouse model in which pneumonitis leading to emphysema is established with live *P. aeruginosa* by delivering the bacteria intratracheally within agar microbeads (27). Controls were injected in the same way with microbeads containing no bacteria. SLPI mRNA levels in lungs from infected mice peaked at day 3 and returned to basal levels by day 14 (Fig. 6, top). Injection with control beads induced little SLPI expression (Fig. 6, bottom).

DISCUSSION

The identification of new sources (macrophages and neutrophils) and a new function (inhibition of LPS responses) (32) for SLPI prompted us to search for regulators of SLPI expression themselves induced by or similar to LPS. The new inducers of SLPI (IL-10, IL-6, and LTA) and the kinetics of its induction (both direct and indirect, slow to rise and then prolonged) are both consistent with the hypothesis that SLPI may act in autocrine fashion as a brake on the response of macrophages to microbial inflammation. In addition, induction of SLPI by taxol extends the list of ways in which this compound duplicates actions of LPS (12, 22, 23, 31, 35).

Human SLPI is a potent inhibitor of the serine proteases trypsin, chymotrypsin, elastase, cathepsin G, chymase, and tryptase. Its only known function has been the protection of mucosal surfaces from degradation by proteases during inflammation (24, 43, 51), although human SLPI also displays broad-spectrum bactericidal activity (29). Mouse SLPI shares considerable structural homology with human SLPI but bears a variant residue at the active site (32, 56). Although mouse SLPI is indeed a poor inhibitor of bovine trypsin, it is nonetheless a potent inhibitor of human neutrophil elastase, cathepsin G, and bovine chymotrypsin (56). The actions of mouse SLPI on mouse proteases remain to be characterized. The LPS-antagonizing ability of mouse SLPI (32) was confirmed by Zhang et al. (55) with recombinant human SLPI in tests of monocyte production of cyclooxygenase 2, prostaglandin E_2, and matrix metalloproteinase and was extended to inhibition of monocytes' concanavalin A-induced responses as well. McNeely and colleagues have also identified a fourth function of human SLPI: inhibition of monocyte susceptibility to infection by HIV (39). In both these studies, the novel biological actions of SLPI were independent of its ability to inhibit serine proteases (40, 55). Thus, both SLPI's range of anti-inflammatory and antiinfectious effects and its mechanisms of action are more diverse than previously understood and deserve to be further explored.

That the regulation of SLPI described here differs from that in previous reports probably reflects the cell types studied and the concentrations of stimuli used; species differences may also have played a role. Thus, in human epithelial cells, SLPI expression was induced by TNF and LPS at concentrations that could be considered supraphysiologic (10 μg/ml) (37, 45). In mouse macrophages, SLPI was induced by LPS at concentrations in the nanogram-per-milliliter range but was not induced by TNF (100 ng/ml) or IL-1 (100 ng/ml). However, IL-1 and TNF can induce IL-6 (42), and LPS can induce both IL-6 (28, 46) and IL-10 (25) in macrophages. Thus, it was of interest that IL-6 and IL-10 triggered macrophages to express SLPI. IL-6 can be considered both pro- and anti-inflammatory, sharing and enhancing some biological properties of IL-1 and TNF (19, 20) while suppressing their production in response to LPS (4, 47). Induction of a wide spectrum of acute-phase proteins

is a special property of IL-6 (6); SLPI can now be added to the list of these presumably protective proteins.

LPS tolerance, an LPS-refractory state induced by prior treatment with a subeffective dose of LPS (7), has been attributed to LPS-induced production of IL-10, TGF-β, and corticosteroids (8, 14, 21, 30, 44). In our studies, neither dexamethasone nor TGF-β had any effect on SLPI mRNA expression in macrophages. However, IL-10 was a potent inducer of SLPI. The possibility that SLPI may mediate some of the effects of IL-10, such as contributing to LPS tolerance, should be considered.

Septic shock is caused not only by gram-negative bacteria (~40% of culture-positive cases) but also by gram-positive bacteria (~55% of culture-positive cases) (49). Phagocytic leukocytes generate a similar spectrum of biological activities in response to LPS and LTA (10, 15, 18, 33, 38, 48). Both bacterial cell wall constituents bind CD14 (18, 52). That SLPI expression inhibited both LPS- (32) and LTA-induced nitric oxide production (Fig. 5) suggests that SLPI may bind CD14 in a manner that interferes with the binding of both LPS and LTA or their subsequent interactions with coreceptors. However, given that SLPI also inhibits concanavalin A-induced responses (55), it seems more likely to block signal transduction by interacting with another membrane protein. Indeed, human SLPI blocks infectivity of HIV in monocytes by interacting specifically with cell surface proteins (39, 40).

Taken together with previous findings, the present work suggests a multifaceted role for SLPI during infection: exerting antimicrobial activity (29), inhibiting leukocyte-derived proteases, and suppressing the ongoing secretion of inflammatory mediators.

ACKNOWLEDGMENTS

We thank JianXun Li and Selina Cheng-Kiang for providing us with reagents.

This work was supported by NIH grants AI30165 and GM53921.

REFERENCES

1. **Abbinante-Nissen, J. M., L. G. Simpson, and G. D. Leikauf.** 1995. Corticosteroids increase secretory leukocyte protease inhibitor transcript levels in airway epithelial cells. Am. J. Physiol. **268:**L601–L606.
2. **Abbinante-Nissen, J. M., L. G. Simpson, and G. D. Leikauf.** 1993. Neutrophil elastase increases secretory leukocyte protease inhibitor transcript levels in airway epithelial cells. Am. J. Physiol. **265:**L286–L292.
3. **Abe, T., N. Kobayashi, K. Yoshimura, B. C. Trapnell, H. Kim, R. C. Hubbard, M. T. Brewer, R. C. Thompson, and R. G. Crystal.** 1991. Expression of the secretory leukoprotease inhibitor gene in epithelial cells. J. Clin. Invest. **87:**2207–2215.
4. **Aderka, D., J. M. Le, and J. Vilcek.** 1989. IL-6 inhibits lipopolysaccharide-induced tumor necrosis factor production in cultured human monocytes, U937 cells, and in mice. J. Immunol. **143:**3517–3523.
5. **Akagawa, K. S., K. Kamoshita, S. Onodera, and T. Tokunaga.** 1987. Restoration of lipopolysaccharide-mediated cytotoxic macrophage induction in C3H/HeJ mice by interferon-gamma or a calcium ionophore. Jpn. J. Cancer Res. **78:**279–287.
6. **Akira, S., T. Taga, and T. Kishimoto.** 1993. Interleukin-6 in biology and medicine. Adv. Immunol. **54:**1–78.
7. **Beeson, P. B.** 1947. Tolerance to bacterial pyrogens. J. Exp. Med. **86:**29–44.
8. **Beutler, B., N. Krochin, I. W. Milsark, C. Luedke, and A. Cerami.** 1986. Control of cachetin (tumor necrosis factor) synthesis: mechanisms of endotoxin resistance. Science **232:**977–980.
9. **Beutler, B., V. Tkacenko, I. Milsark, N. Krochin, and A. Cerami.** 1986. Effect of gamma interferon on cachectin expression by mononuclear phagocytes. Reversal of the *lps*^{d} (endotoxin resistance) phenotype. J. Exp. Med. **164:**1791–1796.
10. **Bhakdi, S., T. Klonisch, P. Nuber, and W. Fischer.** 1991. Stimulation of monokine production by lipoteichoic acids. Infect. Immun. **59:**4614–4620.
11. **Blasi, E., D. Radzioch, S. K. Durum, and L. Varesio.** 1987. A murine macrophage cell line, immortalized by v-raf and v-myc oncogenes, exhibits normal macrophage functions. Eur. J. Immunol. **17:**1491–1498.
12. **Bogdan, C., and A. Ding.** 1992. Taxol, a microtubule-stabilizing antineoplastic agent, induces expression of tumor necrosis factor alpha and interleukin-1 in macrophages. J. Leukocyte Biol. **52:**119–121.
13. **Bogdan, C., and C. Nathan.** 1993. Modulation of macrophage function by transforming growth factor beta, interleukin-4, and interleukin-10. Ann. N. Y. Acad. Sci. **685:**713–739.
14. **Bogdan, C., Y. Vodovotz, and C. Nathan.** 1991. Macrophage deactivation by interleukin 10. J. Exp. Med. **174:**1549–1555.
15. **Bone, R. C.** 1994. Gram-positive organisms and sepsis. Arch. Intern. Med. **154:**26–34.
16. **Bone, R. C.** 1991. The pathogenesis of sepsis. Ann. Intern. Med. **115:**457–469.
17. **Calandra, T., J. Bernhagen, C. N. Metz, L. A. Spiegel, M. Bacher, T. Donnelly, A. Cerami, and R. Bucala.** 1995. MIF as a glucocorticoid-induced modulator of cytokine production. Nature **377:**68–71.
18. **Cleveland, M. G., J. D. Gorham, T. L. Murphy, E. Tuomanen, and K. M. Murphy.** 1996. Lipoteichoic acid preparations of gram-positive bacteria induce interleukin-12 through a CD14-dependent pathway. Infect. Immun. **64:**1906–1912.
19. **Crowl, R. M., T. J. Stoller, R. R. Conroy, and C. R. Stoner.** 1991. Induction of phospholipase A2 gene expression in human hepatoma cells by mediators of the acute phase response. J. Biol. Chem. **266:**2647–2651.
20. **Dinarello, C. A.** 1996. Cytokines as mediators in the pathogenesis of septic shock. Curr. Top. Microbiol. Immunol. **216:**133–165.
21. **Ding, A., C. F. Nathan, J. Graycar, R. Derynck, D. J. Stuehr, and S. Srimal.** 1990. Macrophage deactivating factor and transforming growth factors-beta 1, -beta 2 and -beta 3 inhibit induction of macrophage nitrogen oxide synthesis by IFN-gamma. J. Immunol. **145:**940–944.
22. **Ding, A., E. Sanchez, and C. F. Nathan.** 1993. Taxol shares the ability of bacterial lipopolysaccharide to induce tyrosine phosphorylation of microtubule-associated protein kinase. J. Immunol. **151:**5596–5602.
23. **Ding, A. H., F. Porteu, E. Sanchez, and C. F. Nathan.** 1990. Shared actions of endotoxin and taxol on TNF receptors and TNF release. Science **248:**370–372.
24. **Fink, E., R. Nettelbeck, and H. Fritz.** 1986. Inhibition of mast cell chymase by eglin c and antileukoprotease (HUSI-I). Indications for potential biological functions of these inhibitors. Biol. Chem. Hoppe-Seyler **367:**567–571.
25. **Fiorentino, D. F., A. Zlotnik, T. R. Mosmann, M. Howard, and A. O'Garra.** 1991. IL-10 inhibits cytokine production by activated macrophages. J. Immunol. **147:**3815–3822.
26. **Fritz, H.** 1988. Human mucus proteinase inhibitor (human MPI). Human seminal inhibitor I (HUSI-I), antileukoprotease (ALP), secretory leukocyte protease inhibitor (SLPI). Biol. Chem. Hoppe-Seyler **369:**79–82.
27. **Gosselin, D., J. DeSanctis, M. Boulé, E. Skamene, C. Matouk, and D. Radzioch.** 1995. Role of tumor necrosis factor alpha in innate resistance to mouse pulmonary infection with *Pseudomonas aeruginosa*. Infect. Immun. **63:**3272–3278.
28. **Helfgott, D. C., L. T. May, Z. Sthoeger, I. Tamm, and P. B. Sehgal.** 1987. Bacterial lipopolysaccharide (endotoxin) enhances expression and secretion of beta 2 interferon by human fibroblasts. J. Exp. Med. **166:**1300–1309.
29. **Hiemstra, P. S., R. J. Maassen, J. Stolk, R. Heinzel-Wieland, G. J. Steffens, and J. H. Dijkman.** 1996. Antibacterial activity of antileukoprotease. Infect. Immun. **64:**4520–4524.
30. **Hogan, M. M., and S. N. Vogel.** 1988. Inhibition of macrophage tumoricidal activity by glucocorticoids. J. Immunol. **140:**513–519.
31. **Hwang, S., and A. Ding.** 1995. Activation of NF-kappa B in murine macrophages by taxol. Cancer Biochem. Biophys. **14:**265–272.
32. **Jin, F. Y., C. Nathan, D. Radzioch, and A. Ding.** 1997. Secretory leukocyte protease inhibitor: a macrophage product induced by and antagonistic to bacterial lipopolysaccharide. Cell **88:**417–426.
33. **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.
34. **Kirikae, F., T. Kirikae, N. Qureshi, K. Takayama, D. C. Morrison, and M. Nakano.** 1995. CD14 is not involved in *Rhodobacter sphaeroides* diphosphoryl lipid A inhibition of tumor necrosis factor alpha and nitric oxide induction by taxol in murine macrophages. Infect. Immun. **63:**486–497.
35. **Manthey, C. L., N. Qureshi, P. L. Stutz, and S. N. Vogel.** 1993. Lipopolysaccharide antagonists block taxol-induced signaling in murine macrophages. J. Exp. Med. **178:**695–702.
36. **Marino, M. W., A. Dunn, D. Grail, M. Inglese, Y. Noguchi, E. Richards, A. Jungbluth, H. Wada, M. Moore, B. Williamson, S. Basu, and L. J. Old.** 1997. Characterization of tumor necrosis factor-deficient mice. Proc. Natl. Acad. Sci. USA **94:**8093–8098.
37. **Maruyama, M., J. G. Hay, K. Yoshimura, C. S. Chu, and R. G. Crystal.** 1994. Modulation of secretory leukoprotease inhibitor gene expression in human bronchial epithelial cells by phorbol ester. J. Clin. Invest. **94:**368–375.
38. **Mattsson, E., L. Verhage, J. Rollof, A. Fleer, J. Verhoef, and H. van Dijk.** 1993. Peptidoglycan and teichoic acid from Staphylococcus epidermidis stimulate human monocytes to release tumour necrosis factor-alpha, interleukin-1 beta and interleukin-6. FEMS Immunol. Med. Microbiol. **7:**281–287.
39. **McNeely, T. B., M. Dealy, D. J. Dripps, J. M. Orenstein, S. P. Eisenberg, and S. M. Wahl.** 1995. Secretory leukocyte protease inhibitor: a human saliva

protein exhibiting anti-human immunodeficiency virus 1 activity in vitro. J. Clin. Invest. **96:**456–464.
40. **McNeely, T. B., D. C. Shugars, M. Rosendahl, C. Tucker, S. P. Eisenberg, and S. M. Wahl.** 1997. Inhibition of human immunodeficiency virus type 1 infectivity by secretory leukocyte protease inhibitor occurs prior to viral reverse transcription. Blood **90:**1141–1149.
41. **Nathan, C. F., H. W. Murray, M. E. Wiebe, and B. Y. Rubin.** 1983. Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J. Exp. Med. **158:** 670–689.
42. **Neta, R., S. N. Vogel, J. D. Sipe, G. G. Wong, and R. P. Nordan.** 1988. Comparison of in vivo effects of human recombinant IL 1 and human recombinant IL 6 in mice. Lymphokine Res. **7:**403–412.
43. **Ohlsson, K., M. Bergenfeldt, and P. Bjork.** 1988. Functional studies of human secretory leukocyte protease inhibitor. Adv. Exp. Med. Biol. **240:** 123–131.
44. **Randow, F., U. Syrbe, C. Meisel, D. Krausch, H. Zuckermann, C. Platzer, and H. D. Volk.** 1995. Mechanism of endotoxin desensitization: involvement of interleukin 10 and transforming growth factor beta. J. Exp. Med. **181:** 1887–1892.
45. **Sallenave, J. M., J. Shulmann, J. Crossley, M. Jordana, and J. Gauldie.** 1994. Regulation of secretory leukocyte proteinase inhibitor (SLPI) and elastase-specific inhibitor (ESI/elafin) in human airway epithelial cells by cytokines and neutrophilic enzymes. Am. J. Respir. Cell Mol. Biol. **11:**733–741.
46. **Sanceau, J., F. Beranger, C. Gaudelet, and J. Wietzerbin.** 1989. IFN-gamma is an essential cosignal for triggering IFN-beta 2/BSF-2/IL-6 gene expression in human monocytic cell lines. Ann. N. Y. Acad. Sci. **557:**130–141.
47. **Schindler, R., J. Mancilla, S. Endres, R. Ghorbani, S. C. Clark, and C. A. Dinarello.** 1990. Correlations and interactions in the production of interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF) in human blood mononuclear cells: IL-6 suppresses IL-1 and TNF. Blood **75:**40–47.
48. **Standiford, T. J., D. A. Arenberg, J. M. Danforth, S. L. Kunkel, G. M. VanOtteren, and R. M. Strieter.** 1994. Lipoteichoic acid induces secretion of interleukin-8 from human blood monocytes: a cellular and molecular analysis. Infect. Immun. **62:**119–125.
49. **Stone, R.** 1994. Search for sepsis drugs goes on despite past failures. Science **264:**365–367.
50. **Sweet, M. J., and D. A. Hume.** 1996. Endotoxin signal transduction in macrophages. J. Leukoc. Biol. **60:**8–26.
51. **Thompson, R. C., and K. Ohlsson.** 1986. Isolation, properties, and complete amino acid sequence of human secretory leukocyte protease inhibitor, a potent inhibitor of leukocyte elastase. Proc. Natl. Acad. Sci. USA **83:**6692–6696.
52. **Ulevitch, R. J., and P. S. Tobias.** 1995. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. Annu. Rev. Immunol. **13:**437–457.
53. **Viriyakosol, S., and T. N. Kirkland.** 1995. A region of human CD14 required for lipopolysaccharide binding. J. Biol. Chem. **270:**361–368.
54. **Wright, S. D., R. A. Ramos, P. S. Tobias, R. J. Ulevitch, and J. C. Mathison.** 1990. CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. Science **249:**1431–1433.
55. **Zhang, Y., D. L. DeWitt, T. B. McNeely, S. M. Wahl, and L. M. Wahl.** 1997. Secretory leukocyte protease inhibitor suppresses the production of monocyte prostaglandin H synthase-2, prostaglandin E2 and matrix metalloproteinases. J. Clin. Invest. **99:**894–900.
56. **Zitnik, R. J., J. Zhang, A. A. Kashem, T. Kohno, D. E. Lyons, C. D. Wright, E. Rosen, I. Goldberg, and A. C. Hayday.** 1997. The cloning and characterization of a murine secretory leukocyte protease inhibitor cDNA. Biochem. Biophys. Res. Commun. **232:**687–697.

Editor: R. N. Moore