LIPOPOLYSACCHARIDE MODULATES THE EXPRESSION OF 
\( \alpha_1 \) PROTEINASE INHIBITOR AND OTHER SERINE 
PROTEINASE INHIBITORS IN HUMAN MONOCYTES AND 
MACROPHAGES

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At sites of inflammation or tissue injury, proteolytic enzymes are released by 
neutrophils, platelets, damaged tissue, and activation products of the comple-
ment, coagulation, and fibrinolytic pathways. These proteinases must ultimately 
be inactivated to prevent incidental destruction of surrounding uninvolved tissue 
and to ensure the orderly initiation of tissue repair. It has recently been recog-
nized that protease inhibitors may be produced by macrophages as well as by 
lever and, therein, provide both local and remote mechanisms for proteinase 
inactivation at sites of tissue injury. Human monocytes and macrophages synthe-
size and secrete \( \alpha_1 \) proteinase inhibitor (PI)\(^1\) (1, 2), C1 inhibitor (3, 4), plasmino-
gen-activator inhibitor (5), \( \alpha_2 \) macroglobulin (6), and collagenase inhibitor (7).

\( \alpha_1 \) PI is a 55-kD glycoprotein that constitutes the principle serum inhibitor of 
neutrophil elastase. It is encoded by an \( \sim 10 \)-kb gene on human chromosome 14 
(8, 9). It is considered a member of a supergene family that includes antithrombin 
III, ovalbumin (10), angiotensinogen (11), \( \alpha_2 \) antiplasmin (12), \( \alpha_1 \) antichymotryp-
sin (13), protein C inhibitor (14) and C1 inhibitor (15, 16). These genes bear 
30-40% primary structural homology and have a number of residues around 
the active inhibitory region that are highly conserved. Each one of these serpins 
is characterized by a specificity for inhibition of individual serine proteinases, 
although each is also able to inhibit other serine proteinases less effectively 
(reviewed in reference 17).

There is considerable polymorphic variation of \( \alpha_1 \) PI. One variant allele, PiZ, 
is associated with a severe reduction in serum concentrations of functionally 
active \( \alpha_1 \) PI. This deficiency is often associated with premature development of 
pulmonary emphysema, and 15-20% of PiZZ individuals are affected by pro-

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1 Abbreviation used in this paper: PI, proteinase inhibitor.

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gressive liver disease (reviewed in references 18, 19). A number of studies have
directly demonstrated (2, 20–22) a selective deficit in secretion of α1 PI in PiZZ
individuals, probably affecting the transport of α1 PI from the endoplasmic
reticulum to the Golgi apparatus. The defect is expressed in liver cells and cells
of mononuclear phagocyte lineage (2).

Plasma concentrations of α1 PI increase three- to fourfold during acute
inflammation or tissue injury (23). A number of other human hepatic acute-
phase reactants have now been shown (24–25) to be regulated in human hepato-
toma cells (HepG2 and Hep3B) by the monokines IL-1 and cachectin/TNF. The
rate of synthesis of α1 PI in HepG2 and Hep3B cells is not affected by IL-1,
TNF (25), or by supernatants of LPS-stimulated peripheral blood mononuclear
cells (26). Conversely, significant increases in expression of α1 PI have been
demonstrated in human monocytes and macrophages. A product of mitogen-
stimulated T4+ lymphocytes (27) mediates a 2.0–2.5-fold increase and neutrophil
elastase (28) mediates a 3.5–8-fold increase in steady-state levels of α1 PI mRNA
and rate of synthesis of α1 PI in cultured extrahepatic mononuclear phagocytes.
In this study we examined the effect of inflammatory activation by a bacterial
product, LPS, on expression of the α1 PI gene in macrophages from normal and
homozygous PiZZ α1 PI-deficient individuals.

Materials and Methods

DME and DME lacking methionine were purchased from Gibco Laboratories, Grand
Island, NY. HBSS and Medium 199 (M199) were purchased from Microbiological Asso-
ciates, Walkersville, MD. FCS, L-glutamine, and penicillin-streptomycin were from Flow
Laboratories, Inc., McLean, VA. [35S]Methionine (specific radioactivity ~1,000 Ci/mmol)
and [32P]deoxycytidine triphosphate (specific radioactivity ~5,000 Ci/mmol) were ob-
tained from New England Nuclear, Boston, MA, and [3H]methylated protein standards
were from Amersham Corp., Arlington Heights, IL. Other reagents included IgG-Sorb
from Enzyme Center, Cambridge, MA; cesium chloride from Bethesda Research Labo-
ratories, Gaithersburg, MD; guanidine isothiocyanate from Fluka AG, Buchs, Switzerland;
and sodium-N-laurylsarcosinate from ICN Pharmaceuticals, Inc., Irvine, CA. Goat anti-
human factor B, goat anti–human C3, goat anti–human C1 inhibitor were purchased
from Atlantic Antibodies, Scarborough, ME; rabbit anti–human α1 PI and rabbit anti-
human α1 macroglobulin were from Dako Corp., Santa Barbara, CA; and sheep anti-
human C2 came from Miles Laboratories Inc., Naperville, IL. Goat anti–human neutro-
phil elastase was provided by James Travis, University of Georgia, Athens, GA. LPS
preparations extracted from two different strains of Escherichia coli (serotypes 0111:B4
and 026:B6), each extracted by two different procedures (TCA precipitation and Westphal
phenolic extraction) were purchased from Sigma Chemical Co. E. coli 0113 LPS was also
purchased from Associates of Cape Cod, Inc., Woods Hole, MA. mAb to the lipid A moiety
of LPS (BA1 [29]) was kindly provided by Dr. Warren C. Bogard, Centocor
Malvern, PA.

Cell Culture. Confluent monolayers of human peripheral blood monocytes from 24
normal PiMM and 3 symptomatic PiZZ individuals (as defined by IEF, serum levels,
and family studies) were established by adherence of dextran-purified leukocytes on siliconized
glass as previously described (30). Bronchoalveolar macrophages were obtained from
sterile saline bronchial lavage. After centrifugation and washing, cells were allowed to
adhere to siliconized glass cover slips (31). HepG2 and Hep3B cells were maintained in
culture as previously described (32). Murine L cells transfected with the cloned human α1
PI gene (TfAT2) were maintained in selective medium. The TfAT2 cell line was derived by
cotransfection of murine Ltk+ cells with HSV thymidine kinase DNA and a genomic
DNA clone bearing the entire human α1 PI gene and 5–6 kb of 5' and 3' flanking regions
(AT73 [33], kindly provided by Professor R. Cortese, Heidelberg, Federal Republic of Germany) by calcium phosphate precipitation (34). Synthesis and secretion of α1 PI was demonstrated in this cell line, but not in the parent untransfected Ltk- cell line (28).

LPS contamination was detected and quantified by limulus amebocyte lysate assay (Associates of Cape Cod, Inc.). The LPS concentration of the cell culture before the addition of exogenous LPS was <10 pg/ml, when detectable.

**Biosynthetic Labeling.** Confluent monolayers were rinsed and incubated at 37°C in the presence of methionine-free medium containing [35S]methionine, 500 μCi/ml (pulse period). To determine the rate of synthesis of α1 PI or control secretory proteins, cells were subjected to a short pulse interval (30 min), and radiolabeled proteins were detected in the cell lysate alone. To determine the rate of secretion of α1 PI, cells were subjected to a pulse period of 30 min, rinsed, and incubated in serum-free medium containing an excess of unlabeled methionine (chase period). Radiolabeled α1 PI was detected in cell culture fluid and cell lysates at specified intervals of the chase period. To determine the accumulation of α1 PI in the cell culture fluid, cells were subjected to a long pulse interval (3 h) and radiolabeled α1 PI was identified in cell culture fluid alone. Methods for solubilization of cells and clarification of cell lysates after labeling have been described (1). Total protein synthesis was estimated by TCA precipitation of aliquots of cell lysates and culture fluid (35).

**Immunoprecipitation and SDS-PAGE.** Aliquots of cell lysate or medium were incubated overnight at 4°C in 1% Triton X-100/1.0% SDS/0.5% deoxycholic acid, with excess antibody. Immune complexes were precipitated with excess formalin-fixed staphylococci-bearing protein A, washed, released by boiling in sample buffer, and applied to 9.0% SDS-PAGE under reducing conditions as described by Laemmli (36). 14C-methylated molecular size markers (200,000; 92,500; 68,000; 46,000; 30,000; and 17,000 mol wt) were included on all gels. After electrophoresis, gels were stained in Coomassie Brilliant Blue, destained, impregnated with 2,5-diphenyloxazole (EN3HANCE, New England Nuclear), and dried for fluorography on XAR x-ray film (Eastman Kodak Co., Rochester, NY). LKB Instruments, Inc. Houston, TX laser densitometer 2222 ultrascan XL was used for scanning of fluorograms.

**Detection of RNA by RNA Blot Analysis.** Total cellular RNA was isolated from adherent monolayers of monocytes and macrophages by guanidine isothiocyanate extraction and ethanol precipitation (37). RNA was quantified by absorbance at 260 nm and solubilized for agarose-formaldehyde gel electrophoresis and transfer to nitrocellulose filters (38). Filters were then hybridized with 32P-labeled cDNA specific for human α1 PI (39), human factor B (40), human C3 (41), and human α2 macroglobulin (kindly provided by Dr. B. F. Tack, Scripps Clinic and Research Institute, La Jolla, CA).

**Results**

**LPS Mediates an Increase in the Rate of Synthesis of α1 PI in Human Blood Monocytes and Bronchoalveolar Macrophages.** Monolayers of peripheral blood monocytes after 24 h in culture were incubated for an additional 24 h in control serum-free medium or medium supplemented with LPS (Fig. 1, left). LPS mediates a concentration-dependent increase in the rate of synthesis of α1 PI. The effect of LPS is evident at concentrations of LPS as low as 1 ng/ml. At the highest concentration tested (1 μg/ml), the rate of α1 PI synthesis increased 8.7-fold. LPS from three different strains of E. coli, each prepared by two different extraction procedures, have the same effect on synthesis of α1 PI (data not shown). Accumulation of α1 PI in the intracellular contents and cell culture fluid after a long interval of pulse radiolabeling increased 5.0- and 4.6-fold, respectively (Fig. 1, right), suggesting that LPS does not have a significant effect on the rate of posttranslational processing or secretion of newly synthesized α1 PI. Pulse-chase experiments, described below, also demonstrate that LPS affects the rate
of synthesis of \( \alpha_1 \) PI but not the rate of its secretion. The effect of LPS results in an increase of similar magnitude in the accumulation of native 55-kD \( \alpha_1 \) PI and a 75-kD form of \( \alpha_1 \) PI in complex with an endogenous monocyte serine elastase (1, 42, 43) (Fig. 1, right). Activation of monocytes by LPS, therefore, results in an increase in functional activity of \( \alpha_1 \) PI, as defined by the capacity to form a stable complex with serine elastase, as well as an increase in rate of synthesis of \( \alpha_1 \) PI (Fig. 1, left). Moreover, there is an increase in functional activity of \( \alpha_1 \) PI despite the potential for inactivation by oxygen radicals (44), cysteine proteases (45), or metalloenzymes (46), which may also be released by LPS-activated monocytes. Conversely, the rate of synthesis of elastase in monocytes is not affected by LPS (Fig. 2).

The effect of LPS is time dependent (Fig. 3). Rate of synthesis of \( \alpha_1 \) PI increases within 8 h. The effect of LPS is still evident after 24 h.

LPS has a similar effect on \( \alpha_1 \) PI expression in blood monocytes and tissue macrophages. The rate of synthesis of \( \alpha_1 \) PI increases 4.8–7.2-fold in monocytes incubated with LPS after 1, 5, and 10 d in culture (Fig. 4), despite the specific decrease in expression of \( \alpha_1 \) PI that ordinarily accompanies in vitro maturation of monocytes (1, 27). The rate of synthesis of \( \alpha_1 \) PI increases 4.5-fold in bronchoalveolar macrophages incubated with LPS after 1 d in culture (Fig. 5).

LPS also regulates the expression of other serine protease inhibitors in monocytes and macrophages. The rate of synthesis of Cl inhibitor increases 5-fold in LPS-treated alveolar macrophages, while that of \( \alpha_2 \) macroglobulin decreases 3.7-fold (Fig. 5). These effects are specific in that other products of the macrophage increase to a different extent, including complement components C3 (3-fold) and factor B (4.2-fold), or are not affected, as shown by C2 (Fig. 5).
The effect of LPS on expression of α1 PI in mononuclear phagocytes was neutralized by preincubation with mAb to E. coli lipid A (Fig. 6). LPS had no effect on the rate of synthesis of α1 PI in human hepatoma cells HepG2 or Hep3B or in a stable transfected cell line, mouse fibroblast L cells transfected with the human α1 PI gene (data not shown).

The Effect of LPS on Expression of α1 PI in Mononuclear Phagocytes Involves Both
Pretranslational and Translational Mechanisms. Total cellular RNA was isolated from confluent monolayers of control or LPS-treated monocytes or macrophages and subjected to RNA blot analysis. In contrast to its effect on the rate of synthesis of $\alpha_1$ PI, the effect of LPS on steady-state levels of $\alpha_1$ PI mRNA is variable. In some experiments, accumulation of $\alpha_1$ PI mRNA increases less than 2-fold in the presence of LPS (Fig. 7). In other experiments, steady-state levels of $\alpha_1$ PI increase 2.5-fold (Fig. 8). The difference in the magnitude of the LPS effect on steady-state levels of $\alpha_1$ PI mRNA (1.5–2.5-fold increase) and rate of
FIGURE 8. Time-dependent changes in $\alpha_1$ PI mRNA in LPS-activated monocytes and macrophages. After 24 h in culture, monolayers of monocytes were incubated for 24 h in serum-free medium alone (0) or for the specified time intervals in serum-free medium supplemented with LPS, 100 ng/ml. RNA was then subjected to RNA blot analysis with radiolabeled $\alpha_1$ PI, $\alpha_2$ macroglobulin, or factor B cDNA as probe. Similar amounts of ethidium bromide-stained 18S and 28S RNA were visualized in each lane. 18S and 28S ribosomal RNA is indicated in the right margin.

The Effect of LPS on Expression of $\alpha_1$ PI in Monocytes From Deficient Individuals Exaggerates the Intrinsic Cellular Defect. The cellular defect in homozygous PiZZ $\alpha_1$ PI deficiency, a selective decrease in the rate of secretion of $\alpha_1$ PI, is expressed in monocytes from PiZZ individuals (2). Therefore, it is possible to examine the effect of enhanced translation of $\alpha_1$ PI, as mediated by LPS, on a cell in which there is a defect in posttranslational processing/transport. Monocytes from PiMM and PiZZ individuals were separately incubated in control medium or medium supplemented with LPS and then subjected to pulse-chase radiolabeling (Fig. 9). The rate of synthesis of $\alpha_1$ PI increases to a similar extent in PiMM and PiZZ monocytes (compare IC time 0 left top with right top and IC time 0 left bottom to IC time 0 right bottom). Although $\alpha_1$ PI is already disappearing from the intracellular contents and appearing in the extracellular fluid by 30 min of the chase period in PiMM monocytes, it accumulates in intracellular contents over the entire chase period in PiZZ monocytes. These results indicate that upregulation of the expression of $\alpha_1$ PI by a pretranslational mechanism, as mediated by a product of T4+ lymphocytes (27) or by serine elastase (28), or by a translational mechanism, as mediated by LPS, results in a greater intracellular accumulation of $\alpha_1$ PI in PiZZ monocytes.
FIGURE 9. Effect of LPS on the kinetics of secretion of $\alpha_1$ PI in PiMM and PiZZ monocytes. After 24 h in culture monocytes from PiMM (top panels) and PiZZ individuals (bottom panels) were incubated for 24 h in medium alone (control) or medium supplemented with LPS 100 ng/ml (LPS). Monolayers were then subjected to pulse-chase radiolabeling. M, markers are indicated.

Discussion

These experiments demonstrate tissue-specific, predominantly translational regulation of the expression of $\alpha_1$ PI in human mononuclear phagocytes by LPS. The increase in $\alpha_1$ PI expression in blood monocytes and bronchoalveolar macrophages is part of a program of specific changes in expression of serine protease inhibitors that accompany activation by LPS. There is an increase in expression of $C_1$ inhibitor and a decrease in expression of $\alpha_2$ macroglobulin.

Changes in expression of $\alpha_1$ PI and other serine protease inhibitors accompany many other specific changes in gene expression in macrophages during activation by LPS. Production of tumoricidal activity (47), superoxide anions (44), monokines (48, 49), prostaglandin metabolites (50), neutral proteases (51), proteases of the complement (40, 52, 53), coagulation (54), and fibrinolytic pathways (55), and proto-oncogenes (56) in human mononuclear phagocytes are regulated during LPS activation. Recent studies (49, 57, 58) of endotoxin-resistant states have suggested that regulation of macrophage gene expression by LPS involves
distinct transcriptional and translational control mechanisms. In cord blood monocytes, endotoxin mediates an increase in steady-state levels of C3 and factor B mRNA (58), but there is no accompanying change in the synthesis or secretion of C3 or factor B (57). In macrophages of the endotoxin-resistant C3H/HeJ mouse or in dexamethasone-treated macrophages of the endotoxin-sensitive C3H/HeN mouse there are increases in cachectin/TNF mRNA levels in response to endotoxin but no changes in the accumulation of cachectin in the extracellular fluid (49). A conserved sequence of 33 nucleotides composed entirely of A and T residues has been noted in the 3'-untranslated region of genes encoding several cytokines that are regulated by LPS, including cachectin/TNF, lymphotoxin, IL-1, granulocyte/macrophage colony-stimulating factor, and the IFNs (59). This sequence is thought to be a recognition signal for degradation of specific mRNAs (60) and to be involved in posttranscriptional stabilization of these mRNAs in response to LPS (61). This sequence is not found in the 3'-untranslated region of the human α1 PI gene (62). Moreover, there is a variable and less significant increase in steady-state levels of α1 PI mRNA levels in response to endotoxin, indicating that a predominantly translational mechanism is involved in this particular regulatory effect of endotoxin.

The results of these experiments also extend our understanding of the factors that affect the net biological activity of macrophage-derived α1 PI in tissues. During homeostasis, α1 PI activity may be affected by the state of differentiation of the mononuclear phagocyte, by the local microenvironment, by interaction with serine elastase (1), by interaction with metallo elastases (46), as well as with cysteine proteases (45). During inflammation or tissue injury, expression of α1 PI in cells of mononuclear phagocyte lineage may be directly regulated by products of activated lymphocytes (27), by proteolytic enzymes such as elastase (28), by products of bacteria such as LPS, and probably by products of oxidative metabolism (63). In contrast, expression of α1 PI in the only well-characterized human liver cell culture systems, human hepatoma cell lines HepG2 and Hep3B, is regulated to only a limited extent. The rate of synthesis of α1 PI may be increased by twofold in HepG2 cells incubated with crude supernatants of alloantigen-stimulated T4+ clones (27, 28), but is not affected by IL-1, TNF (25), IFN-γ or crude supernatants of LPS-stimulated peripheral blood mononuclear cells (26). Regulation of other hepatic acute-phase genes in these cell lines is mediated by each of these cytokines or cytokine preparations (25, 26, 64). These observations raise the possibility that in humans, expression of α1 PI by hepatocytes is largely constitutive while that of macrophages is regulated.

Expression of α1 PI in macrophages from PiZZ individuals is also regulated by LPS. In this instance, an increase in the efficiency of translation of α1 PI mRNA may further compromise the deficient host. Rate of synthesis of α1 PI increases but rate of secretion is not affected. The result is greater intracellular accumulation of the inhibitor. An increase in steady-state levels of α1 PI mRNA as mediated by crude lymphokine supernatants (27) or by serine elastase (28) also results in greater intracellular accumulation of α1 PI in deficient macrophages. Nevertheless, the effect of LPS in the deficient individual may be especially profound since it also directly downregulates the expression of α2 macroglobulin,
another elastase inhibitor expressed in macrophages at local tissue sites of inflammation or tissue injury.

Summary

α1 Proteinase inhibitor (PI) is the principle inhibitor of neutrophil elastase, an enzyme that degrades many components of the extracellular matrix. Expression and regulation of α1 PI, therefore, affects the delicate balance of elastase and antielastase, which is critical to turnover of connective tissue during homeostasis, tissue injury, and repair. In this study we show that expression of α1 PI in human monocytes and macrophages is regulated during activation by LPS. LPS mediates a concentration- and time-dependent increase in the rate of synthesis of α1 PI in mononuclear phagocytes. There is a 4.5–8.7-fold increase in functionally active inhibitor delivered to the cell culture fluid of monocytes. The effect of LPS is specific in that it is neutralized by an mAb to the lipid A moiety. The increase in expression of α1 PI mediated by LPS occurs in the context of other specific changes in the expression of serine proteinase inhibitor genes in mononuclear phagocytes. There is an increase in the rate of synthesis of C1 inhibitor and a decrease in synthesis of α2 macroglobulin. Regulation of α1 PI by LPS is distinctive in that it is largely determined by a change in the efficiency of translation of α1 PI mRNA. LPS has no effect on the rate of posttranslational processing and/or secretion of α1 PI and, therein, causes greater intracellular accumulation of α1 PI in mononuclear phagocytes from individuals with homozygous PiZZ α1 PI deficiency.

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ENDOTOXIN REGULATES $\alpha_1$ PROTEINASE INHIBITOR EXPRESSION

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