Inactivation of Endotoxin by Human Plasma Gelsolin†

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ABSTRACT: Septic shock from bacterial endotoxin, triggered by the release of lipopolysaccharide (LPS) molecules from the outer wall of Gram-negative bacteria, is a major cause of human death for which there is no effective treatment once the complex inflammatory pathways stimulated by these small amphipathic molecules are activated. Here we report that plasma gelsolin, a highly conserved human protein, binds LPS from various bacteria with high affinity. Solid-phase binding assays, fluorescence measurements, and functional assays of actin depolymerizing effects show that gelsolin binds more tightly to LPS than it does to its other known lipid ligands, phosphatidylinositol 4,5-bisphosphate and lysophosphatidic acid. Gelsolin also competes with LPS-binding protein (LBP), a high-affinity carrier for LPS. One result of gelsolin−LPS binding is inhibition of the actin binding activity of gelsolin as well as the actin depolymerizing activity of blood serum. Simultaneously, effects of LPS on cellular functions, including cytoskeletal actin remodeling, and collagen-induced platelet activation by pathways independent of toll-like receptors (TLRs) are neutralized by gelsolin and by a peptide based on gelsolin residues 160−169 (GSN160−169) which comprise part of gelsolin's phosphoinositide binding site. Additionally, TLR-dependent NF-κB translocation in astrocytes appears to be blocked by gelsolin. These results show a strong effect of LPS on plasma gelsolin function and suggest that some effects of endotoxin in vivo may be mediated or inhibited by plasma gelsolin.

Plasma gelsolin is an abundant component of normal human plasma for which there is not yet a clearly defined function. On the basis of the actin cytoskeleton remodeling role of intracellular gelsolin, which is derived from the same gene (*1*) and is nearly identical in structure and function in vitro (*2*), the concept of an actin-scavenging system in human plasma consisting of gelsolin and Gc-globulin has been proposed to protect the microcirculation from deleterious effects of long cytoskeletal polymers released during normal or pathological cell death (*3*−*5*). Some data suggest that experimental introduction of F-actin into mammalian blood causes death when the actin levels surpass the buffering capacity of gelsolin and Gc-globulin, but evidence that these actin levels are reached in pathologic states is lacking (*6*). There is, however, a strong correlation between lowered plasma gelsolin levels in patients suffering trauma or undergoing bone-marrow transplant and their risk of succumbing to inflammatory crises leading to acute respiratory distress syndrome or multiple-organ failure (*7*−*9*). In these settings, saturation of the actin scavenging system has not been observed, and a function for gelsolin as a carrier for lysophosphatidic acid (LPA)[1] or other inflammatory mediators has been suggested (*10*).

Toll-like receptors (TLRs) are the transmembrane cellular components involved in recognizing bacterial elements. TLR-4 specifically recognizes LPS and is present in numerous cell types, including astrocytes, a cell type integral to the central nervous system's immune response (*11*, *12*). Extracellular activation of TLR-4 by LPS initiates translocation of transcription factor NF-κB to the nucleus, resulting in the transcription of various cytokines.

Gelsolin binds with high affinity and selectivity to some polyphosphoinositides, mainly PI(4,5)P_2 and PI(3,4)P_2 (*13*), and to LPA (*14*). Biochemical and mutational studies implicate a small linear sequence of basic and hydrophobic residues that bind to both the phosphomonoester and hydrophobic parts of these anionic phospholipids (*15*−*17*). Potent phosphoinositide binding activity is retained in a 10-residue peptide derived from the PIP_2-binding site of gelsolin. A rhodamine B derivative of this peptide, termed PBP10, for phosphoinositide binding peptide of 10 residues, competes strongly with intact gelsolin to bind PIP_2 (*18*).

LPS, a more complex molecule than either PIP_2 or LPA, shares the feature of having multiple negative charges due

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[1] Abbreviations: BSA, bovine serum albumin; GSN160−169, gelsolin QRLFQVKGRR peptide; HAEC, human aorta endothelial cells; LBP, LPS-binding protein; LPS, bacteria lipopolysaccharide (endotoxin); LPA, lysophosphatidic acid; NF-κB, nuclear factor κB; PBP10, phosphoinositide binding peptide of 10 residues (rhodamine B-QRLFQVKGRR); PIP_2, phosphatidylinositol 4,5-bisphosphate; PPIs, polyphosphoinositide; PS, phosphatidylserine; RT, room temperature; TNF-α, tumor necrosis factor α; TLR, toll-like receptor.

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to phosphomonoesters on a cyclic sugar, juxtaposed to a hydrophobic interface (*19*). The finding that a derivative of the 10-residue PIP_2 binding site of gelsolin has antibacterial activity similar to that of true antimicrobial peptides (*20*) motivated a study for determining whether the target of the gelsolin peptide was a bacterial lipid or LPS. These studies demonstrate a tight interaction between gelsolin and LPS that affects both gelsolin's actin binding function and some aspects of the effects of LPS on cells.

EXPERIMENTAL PROCEDURES

Peptide Synthesis and Labeling. QRLFQVKGRR (gelsolin residues 160−169), FRVKLKQGQR (scrambled peptide from gelsolin residues 160−169), and QRL peptides were prepared by solid-phase peptide synthesis and fluorescently labeled at their N-termini by reaction with succinimidyl esters of rhodamine B or pyrene as previously described (*18*). All peptides were purified by reverse-phase HPLC on a silica C-18 column using a 20 to 60% acetonitrile gradient in 0.1% trifluoroacetic acid and dried.

Interaction of LPS with Gelsolin and Fluorescence Peptides Derived from Gelsolin's PIP_2 Binding Site. The fluorescence of rhodamine B- or pyrene-labeled QRLFQVKGRR ($\lambda_{em} = 590$ nm and $\lambda_{ex} = 560$ nm or $\lambda_{em} = 365-550$ nm and $\lambda_{ex} = 343$ nm, respectively) was measured 15 min after addition of various concentrations of LPS (*Escherichia coli* serotype O26:B6, Sigma), PIP_2, LPA, or phosphatidylserine (PS) to 2 μM peptide solutions in buffer A [10 mM Tris and 10 mM MES (pH 7.0)]. The expectation was that if peptides bound to lipids, their surface concentration would become much higher than their bulk concentration, thereby resulting in either changes in rhodamine B fluorescence or formation of pyrene excimers with a shift of fluorescence emission from the monomer wavelength at 378 nm to the excimer emission at 473 nm. To determine the potential effects of lipids on the structure of gelsolin, the optical density at 280 nm was measured in solutions containing different amounts of LPS, LPA, or PS added to 0.1 mg/mL human plasma gelsolin in PBS. A decrease in tyrosine and tryptophan fluorescence, due to the decreased absorbance, has been documented as an assay for binding of PIP_2 to gelsolin (*21*).

Radiolabeled LPS and LBP. Tritium labeling of LPS was performed by modifying a procedure of Watson and Riblet (*23*). A sample (2 mg) of LPS from *Salmonella minnesota* Re595 was oxidized (150 min, 20 °C) with sodium periodate (30 mM). After destruction of the oxidant with 1 M ethylene glycol, aldehyde groups were reduced (18 h at 4 °C) with an ice-cold solution of $NaB[^3H]_4$ (0.46 GBq, 481 GBq/mmol) in 200 μL of ice-cold borate buffer (0.05 M, pH 9.5). Excess sodium borohydride was destroyed with 5 μL of acetic acid. After two washings (centrifugations at 100000*g* for 15 min) in 400 μL of an ice-cold water/ethanol mixture (1:1 by volume), the radiolabeled [^{3}H]LPS (9×10^5 cpm/μg; 2×10^3 cpm/pmol) was collected and stored at −20 °C until it was used. Mouse recombinant LBP (Biometec, Greifswald, Germany) was labeled with ^{125}I by the method of Greenwood et al. (*22*). A freshly prepared solution (60 μL) of 1,3,4,6-tetrachloro-3a,6b-diphenylglycouril (Iodogen; 1 mg/mL in chloroform) was evaporated under vacuum in a glass tube. NaCl (0.15 M, 30 μL), LBP (1 mg/mL in 0.15 M NaCl, 5 μL), and $Na^{125}I$ (3.9 MBq, 5 μL) were sequentially added to the Iodogen-coated tube. After incubation for 10 min at room temperature, the solution was transferred into a polypropylene tube containing dithiothreitol (2 mg/mL in 0.15 M NaCl, 7.5 μL). Radioiodinated LBP was separated from the unreacted $Na^{125}I$ by dialysis. The specific activity of [^{125}I]-LBP was 6×10^6 cpm/μg, and its purity was assessed by SDS−PAGE analysis. Its bioactivity was demonstrated by the observation that the binding of [^{125}I]LBP to LPS-coated plates was markedly inhibited (92 ± 4%) by addition of 20 μg/mL LPS.

[^{3}H]LPS Binding Assay. Polystyrene ELISA-type microplates were coated with a 10-residue phosphoinositide-binding peptide (PBP10), a scrambled peptide, or RhB-QRL by incubation for 18 h at 4 °C with a solution (100 μL, 500 pmol/well) in saline. After being washed three times with 100 μL of saline, the wells were incubated at room temperature with [^{3}H]LPS (360 000 cpm), with or without unlabeled LPS (20 μg/well), in a binding medium (100 μL) containing bovine serum albumin (50 μg) in saline. After being incubated for 3 h, the plates were washed three times with 100 μL of saline, and the remaining bound radioactivity was measured.

Competition between Gelsolin and LPS-Binding Proteins. LPS-coated wells (1 nmol/well of LPS Re-595 from *S. minnesota*) were saturated by incubation (2 h at 20 °C) with gelatin (2 mg/mL in RPMI), washed, and preincubated with different amounts of gelsolin (0−2 ng/well) in gelatin (0.5 mg/mL) containing RPMI. Then 10 μL of [^{125}I]LBP (6×10^4 cpm) was added. After incubation for 90 min at 37 °C, the plate was washed six times with RPMI and bound radioactivity was measured. Similarly, gelsolin-coated wells were used to determine if gelsolin can directly interact with [^{125}I]LBP.

F-Actin Preparation and Severing Activity of Gelsolin. Monomeric G-actin was prepared from rabbit skeletal muscle (*23*) and labeled with pyrene-iodoacetamide (*24*). The nonpolymerizing solution contained 2 mM Tris, 0.2 mM $CaCl_2$, 0.5 mM ATP, and 0.2 mM DTT (pH 7.4). Actin was polymerized by adding 150 mM KCl and 2 mM $MgCl_2$ to G-actin and incubating the mixture for 1 h at RT. Recombinant human gelsolin and blood serum severing activity was measured in 0.4 μM pyrene-labeled F-actin samples after adding gelsolin, serum, or their combination with LPS (from different species), PIP_2, LPA, Lipid A, or PS. The fluorescence intensity of F-pyrene-actin was monitored for 5 min, and calculation of severing activity based on the rate of fluorescence decrease was performed as described in ref *25*.

Cell Culture Study. Rat primary astrocytes were obtained from prenatal rats and maintained for 14 days in culture before being used as previously described (*26*). Human aorta endothelial cells (HAEC) and EBM-2 medium were obtained from Cambrex Bio Science (Walkersville, MD). In all experiments, the medium was changed to serum-free medium 6−12 h prior to addition of LPS, TNF-α, or gelsolin. For F-actin cytoskeleton disruption by LPS, HAEC and astrocyte cultures were incubated for 10 min in medium containing 10 μg/mL LPS alone (*E. coli* serotype O26:B6, Sigma), or LPS that had been preincubated with 0.16 mg/mL human gelsolin. Cultures were fixed with 4% paraformaldehyde and stained with FITC-labeled phalloidin (Molecular Probes). In astrocyte cultures, NF-κB translocation was manipulated by

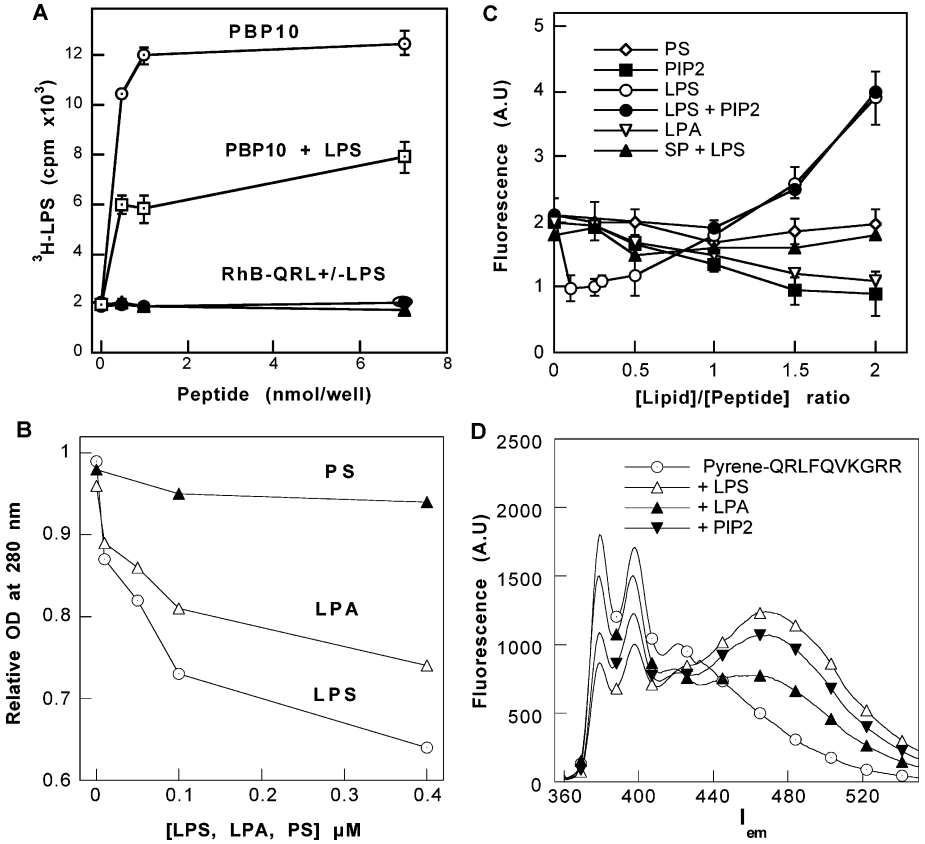

FIGURE 1: Interaction of gelsolin and rhodamine B- and pyrene-labeled QRLFQVKGRR with LPS. (A) Binding of ^{3}H-labeled LPS to PBP10 peptides with or without cold LPS. (B) Optical density (OD) of gelsolin in solutions containing varying amounts of LPS, LPA, or PS. (C) Rhodamine B fluorescence changes of RhB-QRLFQVKGRR (PBP10) and RhB-FRVKLKQGQR (SP) peptides in the presence of different lipids. (D) Fluorescence emission spectra of pyrene-QRLFQVKGRR (2 μM) in the presence of LPS from *E. coli* (1.2 μM), PIP_2 (0.82 μM), and LPA (0.82 μM). Data in panels A−C are means ± the standard deviation of two to four experiments; data in panel D are representative of three independent experiments.

a 2 h incubation in serum-free medium containing either 10 ng/mL TNF-α, 0.1−10 μg/mL LPS alone, or LPS that had been preincubated with 0.16 mg/mL human gelsolin. Localization of NF-κB was observed using a monoclonal antibody to NF-κB (Molecular Probes), and cell nuclei were detected by counterstaining with 4′,6-diamidino-2-phenylindole dihydrochloride (Sigma). Individual cells were counted to assess NF-κB localization (200−600 cells per treatment) as nuclear if the two stains colocalized or as cytoplasmic if they did not.

Isolation of Human Platelets, Platelet Aggregation, and Secretion. Blood from healthy volunteers was collected in acid-citrate dextrose, and gel-filtered platelets (GFP) were prepared as previously described (*27*). Platelet aggregation was initiated by adding collagen (10 μg/mL) and monitored using a Chronolog (Havertown, PA) Lumi-Aggregometer. Platelet secretion was monitored using a Luciferin-Luciferase reagent (Chrono-lum #395 from Chrono-Log) according to the manufacturer's recommendation. When required, before activation the platelet suspension was treated with LPS (*E. coli* serotype O26:B6, Sigma) or LPS preincubated with the QRLFQVKGRR peptide.

RESULTS

Interactions of LPS with Gelsolin and Its PPI Binding Sequence. Solid-phase binding, fluorescence, and absorbance measurements all show an interaction between LPS and gelsolin. In a solid-phase binding assay, 4 μg/mL [^{3}H]LPS bound in a saturable manner to surfaces that were coated with the PIP_2-binding peptide rhodamine B-QRLFQVKGRR (PBP10) but not to surfaces coated with rhodamine B-QRL, which does not bind PIP_2. Addition of a 5-fold excess of unlabeled LPS reduced the level of binding to PBP10 by approximately 50%, but had no effect on the small amount of nonspecific binding to the control peptide-treated surface (Figure 1A). Binding of LPS to intact gelsolin was evident by a change in UV absorbance shown in Figure 1B. Figure 1B shows that LPS decreases the absorbance of gelsolin, with a maximal decrease of approximately 35%, similar to that seen with PIP_2 (data not shown) and somewhat greater than that caused by LPA. Other acidic lipids such as PS have no significant effect on gelsolin absorbance. The fluorescence of rhodamine B-QRLFQVKGRR is also changed by LPS as shown in Figure 1C. After an initial decrease in fluorescence at low LPS:peptide ratios, the peptide fluorescence increased strongly, which is suggestive of insertion of the peptide-bound rhodamine B into a more hydrophobic environment. At these molar ratios, only the first stage of lowered fluorescence was seen with LPA or PIP_2, as reported earlier, and no change was seen after addition of nonspecific anionic lipids such as PS (*16*). There was also no significant fluorescence change after addition of LPS to a scrambled peptide (RhB-FRVKLKQGQ) or control peptide with the sequence rhodamine B-QRL (RhB-QRL, data not shown). Figure 1D confirms these results using a pyrene-derivatized gelsolin peptide in which binding to LPS induced clustering

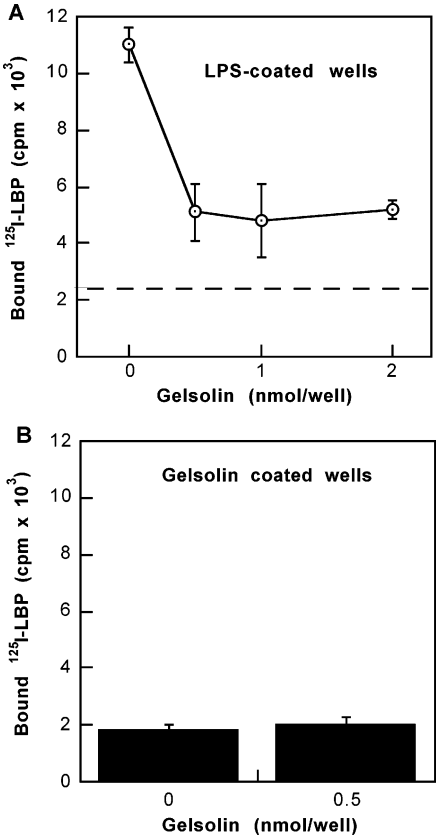

FIGURE 2: Gelsolin inhibits LBP−LPS binding without interaction with LBP. Polypropylene plates were coated with LPS (1 nmol/well) (A) or gelsolin (0.5 nmol/well) (B) and saturated by incubation (2 h at 20 °C) with gelatin (2 mg/mL). After preincubation (30 min at 37 °C) with a solution (90 μL) of gelsolin in 0.5 mg/mL gelatin (A) or with gelatin alone (B), 10 μL of [^{125}I]LBP (6×10^4 cpm, 10 ng) was added. After 90 min at 37 °C, the plates were washed and bound radioactivity was measured. The dashed horizontal line (A) represents the nonspecific level of binding of [^{125}I]LBP to uncoated wells. Data are means ± the standard deviation of four experiments.

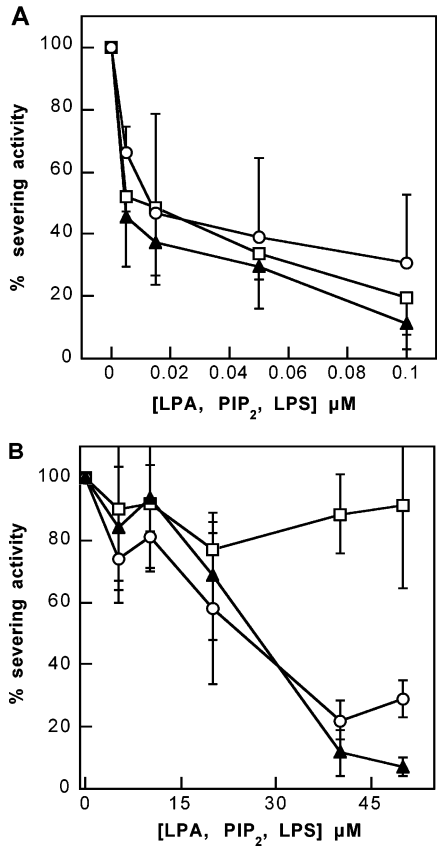

FIGURE 3: Effect of LPA (□), PIP_2 (○), and LPS (▲) on the actin filament severing activity of recombinant human gelsolin (A) or human blood plasma (B). Severing activity was determined from rates of fluorescence decrease during depolymerization after addition of 16 nM gelsolin or 2.5 μL of blood plasma to an F-actin solution (0.4 μM) polymerized with 50% pyrene-labeled G-actin. Data are means ± the standard deviation of four to six experiments.

of the peptide. Peptide clustering brings the aromatic rings of the pyrene groups sufficiently close that the molecular orbitals that determine fluorescence are perturbed and exhibit excimer fluorescence of the pyrene group, which is evident as a large increase in fluorescence emission at 480 nm. LPS, LPA, and PIP_2 had similar abilities to induce excimer formation, whereas PS had no effect (data not shown and ref *16*).

Gelsolin Competes with LPS-Binding Protein (LBP) for LPS. Using a solid-phase binding assay, we found that nanomolar concentrations of gelsolin inhibit binding of [^{125}I]-LBP (LPS-binding protein) to LPS-coated wells by 69 ± 6% (Figure 2A). In contrast, the scrambled peptide RhB-FRVKLKQGQR (2 nmol/well) induced a much lower and statistically nonsignificant inhibition (26 ± 24%). Inhibition of LBP−LPS binding is not due to direct binding of LBP to gelsolin since [^{125}I]LBP does not bind to gelsolin-coated surfaces (Figure 2B). Since plasma contains higher levels of gelsolin than LBP, 150−300 and 3−10 μg/mL, respectively (*3*, *28*), these data, showing comparable affinity of gelsolin and LBP for LPS [the affinity of LBP for LPS (K_d ∼ 1 nM) (*29*) is much higher than that of soluble CD14 (K_d ∼ 74 nM) (*28*)], suggest that gelsolin may play a role in LPS buffering or presentation.

LPS Inhibits the Actin Filament Severing Activity of Gelsolin and in Blood Serum. When added to purified human gelsolin, 0.02 μM LPS (100 ng/mL) inhibits 50% of the severing activity of 0.016 μM gelsolin, and severing is 90% inhibited by 0.1 μM LPS (Figure 3). On a molar basis, LPS was a more potent inhibitor than either LPA or PIP_2. Lipid A, the LPS derivative-lacking polysaccharide, had a weaker but still significant inhibitory effect, and nonspecific anionic lipids such as PS had no effect (data not shown). When added to whole human serum, LPS and PIP_2 were also able to inhibit gelsolin activity, although larger amounts were needed compared to inhibition of pure gelsolin in aqueous solution. Measurements of the effects on blood serum severing function showed that the inhibitory efficiencies of different LPS species that were tested were in the following order: *Klebsiella pneumoniae* (strain ATCC15380, Sigma) < *Salmonella enteriditis* (strain ATCC 13076, Sigma) < *E. coli* (data not shown). LPA appeared to lack its effect in serum compared to that in pure solution, possibly because the greater lability of the lysolipid allows its more rapid partitioning into lipoprotein particles or Ca^{2+}-mediated aggregates that are incapable of binding gelsolin. Inhibition by LPS of actin depolymerizing activity in serum is due to effects on gelsolin because the actin sequestering activity of Gc-globulin (vitamin D-binding protein) which is the other component of the plasma actin scavenger system was

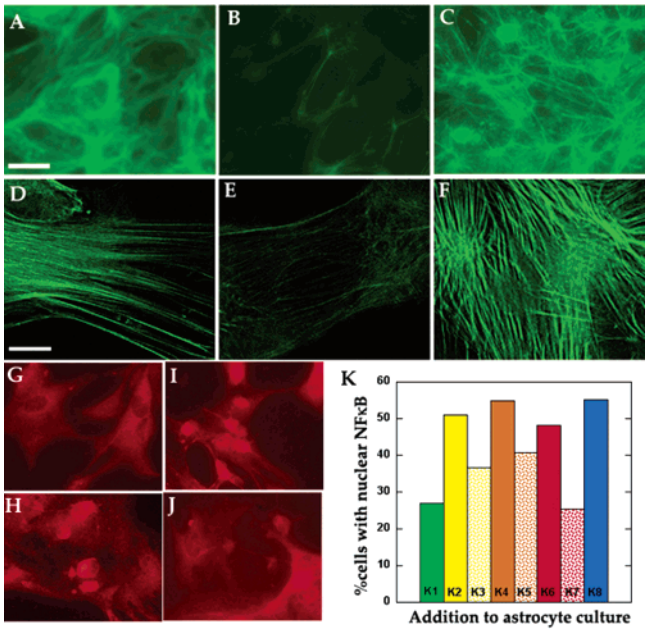

FIGURE 4: LPS-induced disruption of cellular responses is inhibited by preincubating LPS with plasma gelsolin. Staining for F-actin with phalloidin (A−F) was used to demonstrate the prevention by plasma gelsolin of LPS-induced actin cytoskeleton disassembly. Endothelial cells (A−C) and astrocytes (D−F) were treated with 2 μM LPS (B and E) or 2 μM LPS and 1.8 μM gelsolin (C and F). Control cells are also shown (A and D). LPS-induced translocation of NF-κB to the nucleus was blocked by plasma gelsolin as shown by immunostaining with an antibody against NF-κB (G−J). In astrocytes, addition of 10 ng/mL TNF-α (H) or 2 μM LPS (I) caused translocation of NF-κB to the nucleus when compared with control cells (G). Preincubation with 1.8 μM gelsolin blocked this translocation (J). In panel K, quantification of NF-κB translocation after K1 (no treatment), K2 (10 ng/mL LPS), K3 (10 ng/mL LPS and gelsolin), K4 (100 ng/mL LPS), K5 (100 ng/mL LPS and gelsolin), K6 (10 μg/mL), K7 (10 μg/mL LPS and gelsolin), and K8 (10 ng/mL TNF-α). This experiment was repeated three times, and data from one representative experiment are shown.

unaffected by LPS at concentrations at least up to 100 μM (data not shown).

Gelsolin Prevents LPS-Induced Cellular Effects. Binding of gelsolin to LPS also influenced in vitro cellular responses to LPS. When added to either cultured human aortic endothelial cells (HAEC) (Figure 4A−C) or primary rat astrocytes (Figure 4D−F), LPS at relatively high concentrations rapidly induced disassembly of the actin cytoskeleton as seen in the disrupted and diffuse phalloidin staining in panels B and E of Figure 4, compared to the abundant actin bundles seen in controls (panels A and D of Figure 4). However, when first incubated with an excess of plasma gelsolin, LPS had no discernible effect on the actin cytoskeletons of either HAEC or astrocytes.

Effects of LPS on Astrocyte NF-κB Localization. In untreated primary astrocytes, NF-κB was located in the cytoplasm (Figure 4G) and activation by LPS resulted in its translocation to the nucleoplasm (Figure 4I), like the localization induced by TNF-α (Figure 4H). LPS-induced translocation of NF-κB was blocked by preincubation with gelsolin (Figure 4J). Quantification of NF-κB translocation is shown in Figure 4K. This effect was not observed when LPS-induced translocation of NF-κB was evaluated in NIH-3T3 fibroblasts (data not shown). This may indicate that gelsolin prevents LPS-induced pathways in specific cell types, perhaps in association with the expression of TLRs after LPS activation.

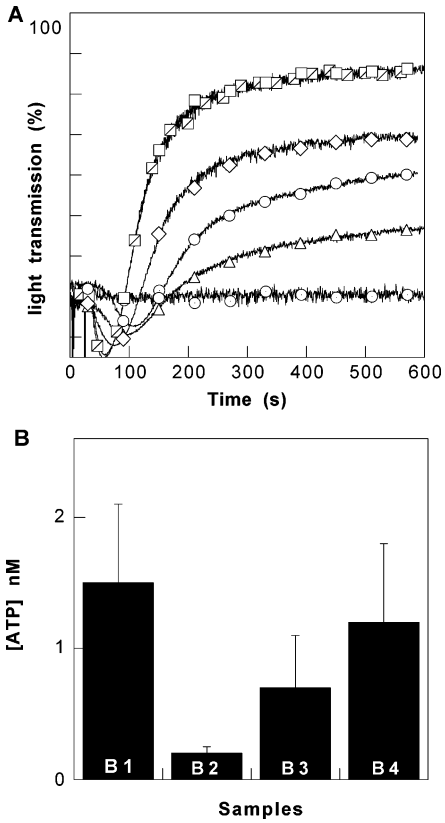

FIGURE 5: Effect of LPS on collagen (10 μg/mL)-activated platelets. Inhibition of platelet aggregation (A) and secretion (B) by LPS and reversal of the inhibition by the QRLFQVKGRR peptide. In panel A, marked and empty squares represent platelet aggregation induced with collagen, with and without 25 μM GSN160−169, respectively. Triangles represent platelet aggregation when collagen was added to platelet suspensions preincubated with 80 μM LPS. The inhibitory effects of LPS on collagen-induced platelet aggregation reversed by preincubation of LPS with 12.5 and 25 μM GSN160−169 are represented by circles and diamonds, respectively. The lack of a direct effect of GSN160−169 peptide on resting platelets is shown with dotted circles. In panel B, ATP secretion after B1 (collagen), B2 (LPS), B3 (LPS and collagen), and B4 (LPS, GSN160−169, and collagen). Data in panel A are representative of three independent experiments; data in panel B are means ± the standard deviation of three experiments.

Effects of LPS on Platelet Function. Generalized coagulation dysfunction is a common complication of LPS-induced septic shock syndrome. The effect of LPS on platelets depends on the LPS concentration. It has been found that LPS at low concentrations caused priming of platelet activation (*30*), but LPS at 100−300 μg/mL inhibits platelet aggregation stimulated by collagen (*31*). Figure 5 shows a protective effect of the gelsolin peptide QRLFQVKGRR (GSN160−169) on LPS-mediated alteration of platelet function. When added alone, LPS strongly inhibited the aggregation and secretion of platelets induced by collagen. Addition of GSN160−169 to LPS before its addition to platelets restored, in a peptide concentration-dependent manner, the normal collagen-induced platelet activation. GSN160−169 by itself had no effect on collagen-mediated platelet functions.

DISCUSSION

Toll-like receptors (TLRs) appear to be the primary or sole transmembrane proteins in cells that activate in response to

LPS, but the delivery of LPS from external fluids to the cell membrane and ultimately to the TLR is complex and involves a number of external proteins, including CD14, LBP, MD-2, and other factors (*19*). The structural diversity of LPS variants from different bacterial species with similar pathologic effects may explain why a unique, highly specific pharmacologic treatment for this toxin has not emerged, and many LPS antagonists or ligands share a mainly cationic and hydrophobic character (*32*, *33*). A critical factor in LPS toxicity is the aggregation state of this amphipathic molecule. A number of studies demonstrate that when LPS is packed into lamellar phases toxicity is low, while nonlamellar phases such as cubic or hexagonal phases characteristic of cone-shaped amphiphiles promote the toxicity of LPS (*34*). The fact that the aggregation state of the lipopolysaccharide determines its toxicity suggests that complexation of LPS to prevent formation of toxic structures may be achieved by LPS-binding proteins or lipids. Alternatively, binding of LPS to proteins may be necessary to extract LPS from nontoxic aggregates or to transfer it to or from lipoprotein particles as part of the pathway from the bacterial membrane to the TLR.

The tight and selective binding of gelsolin to LPS has several implications for the function of plasma gelsolin and may suggest methods for counteracting the toxic effects of LPS. The strong clinical correlation between lowered gelsolin levels and susceptibility to inflammatory shock has thus far not been explained by the interaction of gelsolin with its only known ligands, actin and anionic phospholipids. The current findings suggest that LPS, in addition to LPA and actin, may be the harmful agent responsible for the depletion of gelsolin following trauma or burns (*5*, *7*, *35*).

The possible role of gelsolin in determining the fate of LPS released into blood may be different from those of LBP, MD-2, or soluble CD14. In normal blood, gelsolin is present at much higher concentrations (2 μM) than the other high-affinity LPS ligands, unlike LBP and CD14 (*36*, *37*) it is not an acute-phase protein, and there is no reported condition during which plasma gelsolin levels are upregulated. By analogy with the actin scavenger hypothesis for plasma gelsolin function (*38*), it seems plausible to suggest that gelsolin may have a buffering effect on the availability of LPS for other targets, but other possibilities such as changing the aggregation state of LPS complexes or facilitating transfer of LPS cannot be ruled out.

The activity of gelsolin to weaken effects of LPS on astrocytes in vitro is observed at concentrations of LPS as low as 10 ng/mL, near the range of 1−3 ng/mL circulating LPS detected in blood plasma (*39*) or cerebrospinal fluid (*40*) in patient samples. Locally, LPS levels may be higher since a majority of LPS is reported to be lost on the surface of platelets and other cells removed during preparation of plasma (*41*), and lethal doses of LPS in animals injected intraperitoneally (*42*) or intravenously (*43*) are orders of magnitude higher than those detected as circulating in blood plasma during sepsis.

The inhibition of LPS effects reported here may represent only one aspect of the gelsolin−LPS interaction. In other settings, gelsolin−LPS complexes may also activate cell receptors, as has been shown for the soluble form of the LPS CD14 receptor that can either inhibit or enhance the cellular response to LPS (*44*). LBP can also either enhance or inhibit the cellular response to LPS, depending in part on the presence of other LPS-binding molecules to which LBP may facilitate transfer (*45*). The finding that gelsolin competes with LBP for binding LPS suggests that in plasma, the delivery of LPS to cellular, lipoprotein, or other targets mediated by LBP would be perturbed when plasma gelsolin levels drop. LBP is one of the first plasma components interacting with the lipid A moiety of LPS (*46*), influencing interactions of LPS with lipoprotein and cellular targets and serving as an acute-phase reactant, its level rising during trauma or infection (*28*, *37*). The dual role of LBP and CD14 in innate immunity has recently been established (*47*), and the potential that gelsolin may also participate in the interactions of these proteins with LPS deserves future investigation. MD-2 also interacts strongly with LPS, and further studies are required to determine if gelsolin can also alter this interaction.

Since gelsolin is expressed as both a plasma and a cytosolic/cytoskeletal protein, there may also be significance to an intracellular gelsolin−LPS interaction for Gram-negative bacteria such as *Shigella flexneri* that invade the host cytoplasm and trigger actin assembly to move within the cell and from one cell to another. Gelsolin has profound effects on the motility and actin remodeling induced by the Gram-positive bacterium *Listeria monocytogenes* (*48*), and the mechanism by which Gram-negative bacteria remodel actin is similar (*49*). The data in Figure 1 show that binding of LPS to gelsolin occurs at the same site to which PIP_2 binds. Several cytoskeletal proteins have PIP_2-regulated sites similar to those in cytoplasmic gelsolin (*50*), suggesting that they may also be altered by LPS. The possibility that LPS may also alter the activity of proteins such as N-WASP, profilin, capping protein, and cofilin would be relevant in explaining the finding that mutations in *Shigella* that change LPS structure but not the protein IcsA which engages the actin polymerization system of the host cell have a strong effect on actin-dependent bacterial motility (*51*).

In summary, the biochemical and biophysical data presented here demonstrate a strong interaction between plasma gelsolin and bacterial lipopolysaccharide that inactivates the actin severing function of gelsolin and that competes with the binding of LPS to LBP, a plasma protein that is crucial to the trafficking of LPS. Studies of effects of LPS on endothelial cells, astrocytes, and platelets show a partial inhibition of LPS-dependent stimulation, suggesting that gelsolin may be part of the complex system of soluble plasma proteins that determine how LPS is delivered to cellular receptors and other targets.

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