Plasma gelsolin is a marker and therapeutic agent in animal sepsis*

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Objective: **Plasma gelsolin is a circulating actin-binding protein that serves a protective role against tissue injuries. Depletion of plasma gelsolin in systemic inflammation may contribute to adverse outcomes. We examined the role of plasma gelsolin in animal models of sepsis.**

Design: **Animal and laboratory experiments.**

Setting: **Academic research laboratory.**

Subjects: **Adult male mice.**

Interventions: **Mice subjected to endotoxin or cecal ligation and puncture (CLP) were treated with exogenous plasma gelsolin or placebo.**

Measurements and Main Results: **We document the depletion of plasma gelsolin (25–50% of normal) in murine models of sepsis associated with the presence of circulating actin within 6 hrs of septic challenge. Repletion of plasma gelsolin leads to solubilization of circulating actin aggregates and significantly reduces mortality in endotoxemic mice (survival rates were 88% in the gelsolin group vs. 0% in the saline group, $p < .001$) and in CLP-challenged mice (survival rates were 30% in the gelsolin group vs. 0% in the saline group, $p = .001$). Plasma gelsolin repletion also shifted the cytokine profile of endotoxemic mice toward anti-inflammatory (plasma interleukin-10 levels were 205 ± 108 pg/mL in the gelsolin group vs. 39 ± 29 pg/mL in the saline group, $p = .02$).**

Conclusions: **We propose that circulation of particulate actin is a marker for sepsis-induced cell injury, that plasma gelsolin has a crucial protective role in sepsis, and that gelsolin replacement represents a potential therapy for this common lethal condition. (Crit Care Med 2007; 35:849–855)**

KEY WORDS: septicemia; sepsis syndrome; endotoxemia; actin-binding proteins; cecal ligation and puncture; biological markers

Early in the course of sepsis, offending microorganisms and their products elicit an intense inflammatory response as evidenced by markedly increased levels of proinflammatory cytokines (1). In the absence of a response to antibiotics, organ failure and death ensue. Sepsis claims >200,000 lives in the United States annually (2). The relationship between the inflammatory response and delayed organ failure remains mysterious, as does the mechanism of that failure. Inhibition of specific individual mediators has not favorably affected the course of sepsis in clinical trials (1, 3), and histologic analyses of organs do not consistently reveal massive necrosis or apoptosis even at the time of death (4).

The lethal organ failure that complicates sepsis resonates with the general phenomenon of delayed secondary organ injury that occurs after other primary injuries such as major trauma, burns, and extensive surgery, both in humans and in experimental animal models. We and others have accumulated evidence that decreases in a circulating actin-binding protein, plasma gelsolin, to critical levels precede and therefore predict complications in these conditions (5–9).

Cytoplasmic gelsolin (cGSN) was discovered as an intracellular actin- and phosphoinositide-binding protein involved in cell motility (10). It is also an abundant secretory protein normally circulating at 190–300 μg/mL (11). The exported isoform of gelsolin, designated plasma gelsolin (pGSN), has 25 additional amino acids and originates from alternative splicing of a single gene (12). pGSN's prevalence in complex organisms, including *Drosophila* (13), is consistent with its having an important physiologic role. In humans, extensive tissue injury in trauma, acute respiratory distress syndrome, hematopoietic stem cell transplantation, acute hepatic failure, and myonecrosis lead to pGSN decrements (5, 9, 14, 15), and in critically ill surgical patients, very low pGSN levels predict poor outcomes (6).

Although decreased pGSN levels have been documented in septic patients (9), the predictive value and the significance of such reductions are unknown. The aim of this study was to determine whether pGSN depletion is associated with sepsis and if pGSN therapy favorably affects mortality in murine models of sepsis.

METHODS

Animals. Wild-type C57BL/6 male (Charles River Laboratories, Wilmington, MA), Toll-like receptor 4 mutant (C3H/HeJ) mice (Jackson Laboratory, Bar Harbor, ME) had free access to a standard feed and water, and the Harvard Medical Area Standing Committee on Animals

***See also p. 970.**

From the Pulmonary and Critical Care Division (PSL, SWC, MAP) and Translational Medicine Division (PSL, TPS), Brigham and Women's Hospital, Boston, MA; Pulmonary and Critical Care Unit, Massachusetts General Hospital, Boston, MA (ABW, KLC); and Harvard Medical School, Boston, MA (PSL, ABW, SWC, MAP, TPS).

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Since submission of this paper, Brigham and Women's Hospital has licensed issued and pending patent applications concerning diagnostic and therapeutic uses of plasma gelsolin to Critical Biologics Corporation of which Drs. Lee and Stossel are founding scientists and in which they hold stock options. None of the research reported in this paper was supported by Critical Biologics Corporation or by any other corporate entity. The remaining authors have not disclosed any potential conflicts of interest.

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approved all procedures described, according to standards as set forth in the *Guide for the Care and Use of Laboratory Animals*.

Murine Peritonitis by Cecal Ligation and Puncture (CLP). Male C57BL/6 mice, 8–10 wks old, were anesthetized by intraperitoneal injection of 0.015–0.017 mg/g Avertin (Fluka Chemie, Buchs, Switzerland). The cecum of each anesthetized animal was exposed through a small incision in the lower anterior abdomen and punctured once by an 18.5-gauge needle. A small amount of intestinal content was extruded, and the cecum was ligated without obstructing the intestinal tract with a 6-0 silk suture. The abdomen was closed with a 4-0 silk suture. In one experiment, immediately after surgery, ten mice received subcutaneously 1 mL of 150 mM NaCl (saline) and ten other mice received subcutaneously 1/mL of 8 mg/mL recombinant human pGSN with 0.4 mM Ca in saline. This protein was produced in *Escherichia coli*, refolded with oxidized glutathione (16), tested for endotoxin, formulated as described, and stored at −70°C by Biogen (Cambridge, MA). The protein has subsequently been transferred to and is currently stored in our laboratory and is the material used previously in animal models (8, 17). Five mice that did not undergo CLP served as controls. The animals were allowed to recover with free access to food and water. At 6 hrs and 24 hrs after CLP, five mice from each treatment group (one saline-treated mouse died before the 24-hr collection) were anesthetized with Avertin, and blood was collected before kill by retro-orbital bleeding into 0.1 volume of Aster-Jandl anticoagulant solution (18) and centrifuged at 2000 × *g* for 10 mins to generate plasma. Plasma was frozen in liquid nitrogen and stored at −80°C. In a separate experiment, 20 animals were subjected to CLP; ten animals received saline and ten received recombinant human pGSN immediately as described and repeated at 24 hrs after CLP. Survival was recorded daily for 7 days, and surviving animals were killed.

Murine Endotoxemia. Male C57BL/6 mice, 6–8 wks old, each weighing 18–20 g, were injected intraperitoneally with 25 mg/kg lipopolysaccharide (LPS; *E. coli* O55:B5, Sigma, St. Louis, MO) and immediately given 400-μL subcutaneous injections of sterile saline alone (nine animals) or 20 mg/mL recombinant human pGSN with 0.4 mM Ca in saline (nine animals).

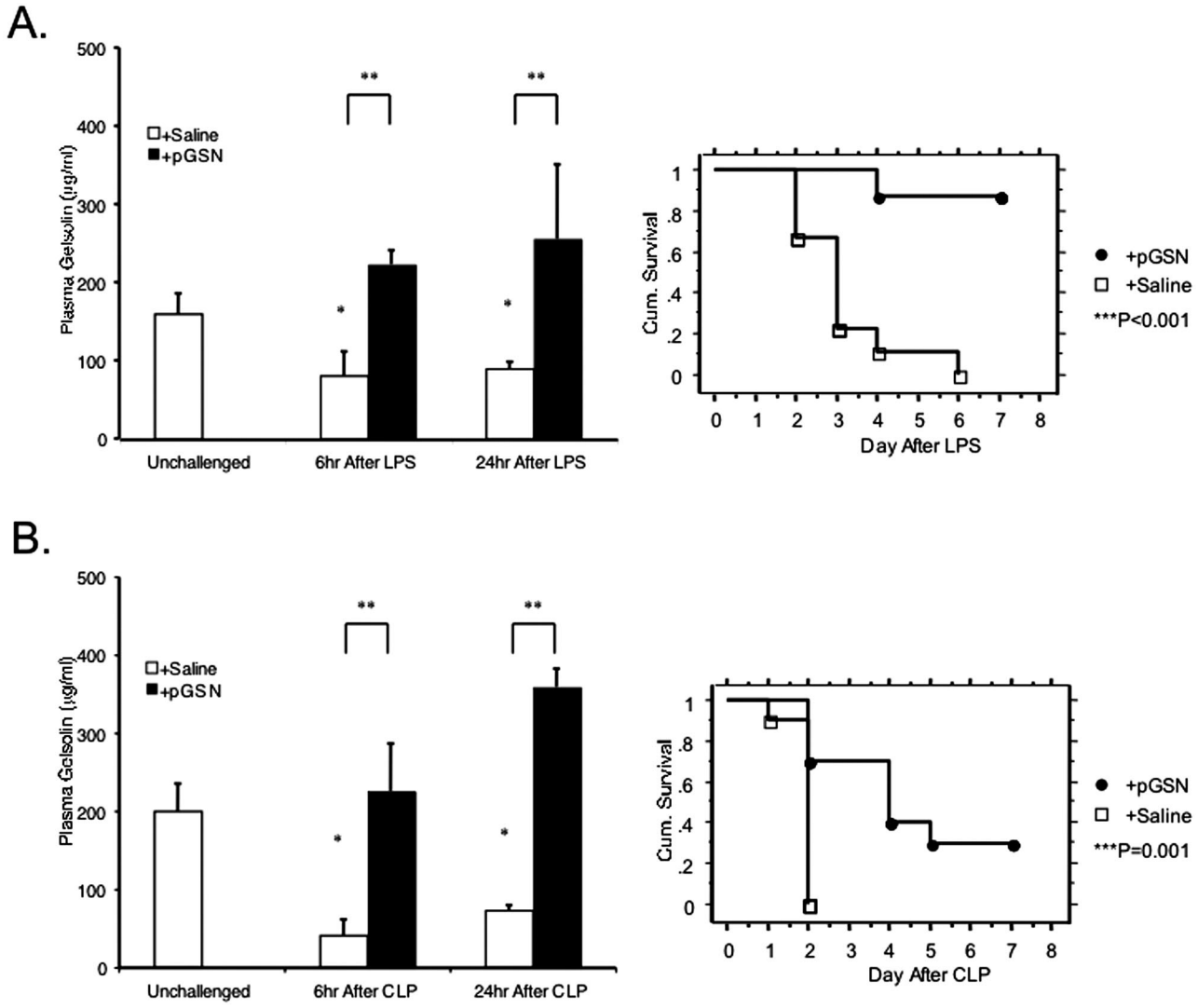

Figure 1. Plasma gelsolin (*pGSN*) levels and mortality plots in lipopolysaccharide (LPS)- or cecal ligation and puncture (CLP)-challenged mice treated with or without exogenous pGSN. Open bars denote mice that received saline treatment (+*saline*), and filled bars denote mice that received exogenous pGSN treatment (+*pGSN*). Left panels show that endogenous pGSN levels dropped to near 50% of normal within 6 hrs of LPS challenge or CLP challenge and persisted for ≥24 hrs (*$p < .02$, compared with unchallenged mice). Administration of exogenous pGSN at the time of LPS or CLP challenge successfully raised pGSN levels (**$p < .03$, comparing pGSN treated and untreated mice within the same group). Right panels show that exogenous pGSN improves survival of septic mice. In mice challenged with lethal LPS, those treated with pGSN had a significantly better survival compared with saline-treated mice (***$p < .001$). Mice subjected to CLP had a similar favorable response to pGSN with much better survival (***$p = .001$).

The mice were anesthetized for plasma collections and then killed at 6 hrs (five mice per treatment group) or 24 hrs (four mice per treatment group) after LPS challenge. In addition, control mice without LPS challenge were given only subcutaneous saline (five mice) or pGSN (three mice) 24 hrs before being killed for plasma collection. In a separate experiment, mice received the same LPS challenge and were allocated to receive saline (nine mice) or pGSN (eight mice) treatment immediately as described and again at 24, 48, and 72 hrs after LPS injections. The animals were monitored frequently, and survival was recorded for 7 days. Surviving mice were killed.

pGSN Concentrations in LPS-Resistant Mice. Male C3H/Hej mice, 8–10 wks old, were injected intraperitoneally with 25 mg/kg *E. coli* LPS (five mice) or subjected to CLP as described (four mice). Unchallenged mice served as controls (four mice). At 24 hrs after LPS challenge, plasma samples were collected from anesthetized mice as described previously.

Mouse Cytokine Measurements. Plasma granulocyte-macrophage colony-stimulating factor, interferon-γ, interleukin (IL)-1β, IL-6, IL-10, and tumor necrosis factor (TNF)-α cytokines were measured using enzyme-linked immunosorbent assays (LINCO Research, St. Charles, MO). The lower range of the assay is <3.2 pg/mL for each cytokine, and levels <3.2 pg/mL were assigned a value of zero.

Gelsolin and Albumin Measurements. Plasma gelsolin was measured in duplicate by its ability to stimulate actin nucleation as previously described in detail (6, 19). Gelsolin quantification by the actin nucleation assay correlates well with levels obtained from Western blotting measurements (7). The assay is highly specific, as evidenced by virtually zero activity in plasma of LPS-treated gelsolin-null mice (20); however, the assay does not discriminate between cGSN and pGSN. It is also not species-specific and is thus able to approximate total gelsolin levels in mice treated with recombinant human pGSN. Actin or lipids complexing to pGSN do not affect pGSN's actin nucleation activity (21, 22).

Albumin levels were measured colorimetrically using a commercial kit (Stanbio, Boerne, TX) according to the manufacturer's instruction.

Preparation of Plasma for Western Blotting. Mouse plasma was subjected to centrifugation at 2000 × *g* for 10 mins to clear any remaining cell debris. Then 100 μL of the precleared plasma was centrifuged at 250,000 × *g* at 4°C for 30 mins; 90 μL of the supernatant fraction was carefully removed and designated as the supernatant. The remaining solution was subjected to vortexing to resuspend sediments and was designated as the pellet fraction. Each plasma sample was diluted 1:200 in phosphate-buffered saline and analyzed by Western blotting. Passing plasma samples through a 0.22-μm filter (Sigma, St. Louis, MO) did not affect the results, indicating that plasma samples did not contain large cell fragments.

Western Blot Analysis. Each sample was heated at 95°C for 3 mins in sodium dodecyl sulfate-sample buffer (Boston Bioproducts) and then analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis using 12% Tris-glycine gel (Invitrogen, Carlsbad, CA) and transferred to polyvinylidene fluoride membranes (Millipore, Bedford, MA). After blocking the membrane overnight in 5% nonfat dry milk in Tris-buffered saline with 0.05% Tween 20, primary antibodies were added and incubated at room temperature for 1 hr. To assay for plasma actin, a rabbit polyclonal anti-actin antibody (A2103, Sigma, St. Louis, MO) was used at a 1:2000 dilution. Bound primary antibodies were probed with horseradish peroxidase-linked anti-rabbit immunoglobulin G (Cell Signaling, Beverly, MA) at 1:2000 dilution. Chemiluminescence of horseradish peroxidase was developed with LumiGLO (Cell Signaling, Beverly, MA). Exposed and developed photofilm was scanned (Hewlett-Packard ScanJet, Palo Alto, CA) and immunoreactive signals were quantified by Adobe Photoshop (San Jose, CA).

Gelsolin-LPS Binding. Each well of a Microlite 2, white, 96-well, flat-bottom plate (Dynex Technologies, Chantilly, VA) was coated with various amounts of recombinant human pGSN or bovine serum albumin and incubated at 4°C overnight. After four washes with platelet buffer (PB) (145 mM NaCl, 5 mM KCl, 2 mM MgCl2, 3.5 mM NaH_2PO_4, 10 mM glucose, 10 mM HEPES, 3 mg/mL bovine serum albumin, 1 mM CaCl2, pH 7.4), 2 μg of Alexa488-labeled LPS (*E. coli* serotype 055:B5,

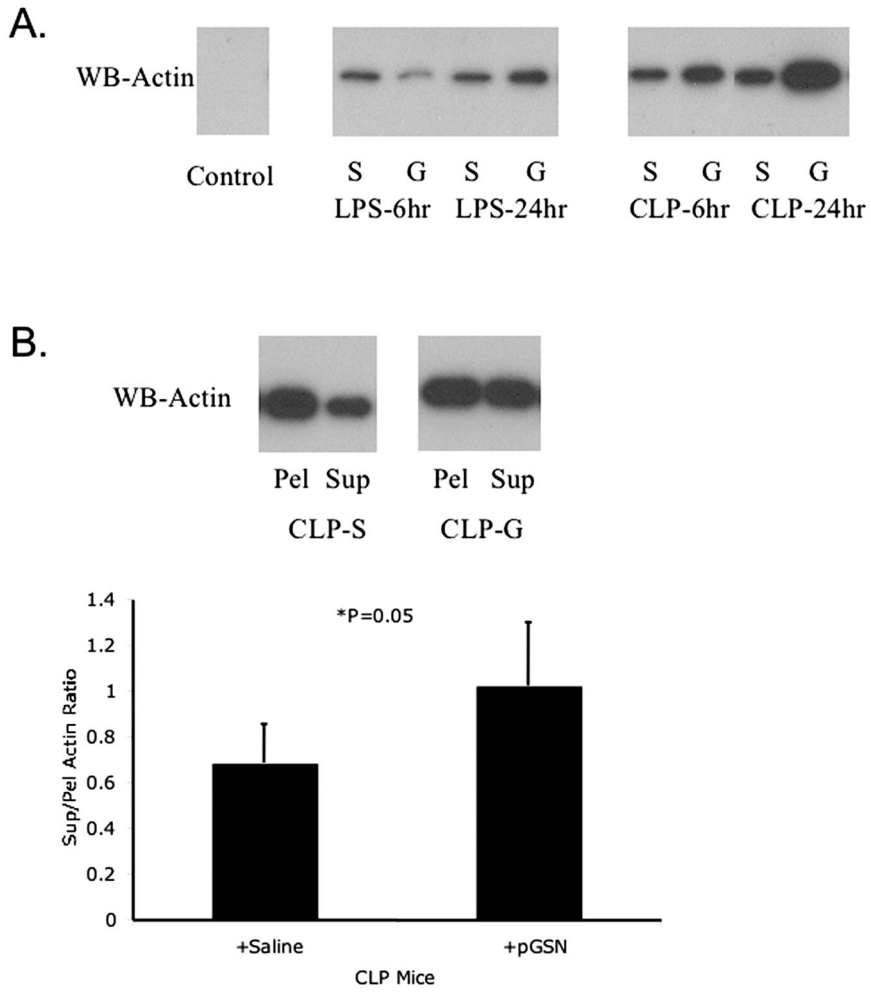

Figure 2. Representative Western blot (*WB*) of mouse plasma staining for actin. *A*, within 6 hrs of lipopolysaccharide (*LPS*) or cecal ligation and puncture (*CLP*) challenge, circulating actin was detected in the plasma of saline-treated (*S*) and pGSN-treated (*G*) mice. The presence of actin persisted for ≥24 hrs. pGSN treatment did not decrease the total circulating actin when compared with saline treatment. *B*, after ultracentrifugation, the pellet fraction (*Pel*) of CLP-challenged mice treated with saline (*CLP-S*) showed higher amount of actin compared with the supernatant fraction (*Sup*). pGSN-treated mice (*CLP-G*) show the same amount of actin in pellet and supernatant fractions of plasma. Upper panel shows the representative Western blot of Pel and Sup fractions of CLP mice plasma, and lower panel shows the graph of quantifying actin by densitometry expressed as ratio of Sup/Pel, representing relative amount of soluble/filamentous actins.

Molecular Probes, Eugene, OR) was added to each well with 100 μL of PB buffer and incubated at room temperature for 1 hr. After 4 washes with PB buffer, the fluorescence of each well was analyzed in a spectrofluorometer at excitation and emission wavelengths of 488 and 520 nm, respectively. The amount of Alexa488-LPS bound was estimated by extrapolating from a standard curve generated by seeding various amounts of Alexa488-LPS in PB buffer. All studies were done in duplicate.

LPS Stimulation of Monocytic Cells. The human monocytic cell line THP-1 was purchased from the American Type Culture Collection (Manassas, VA). The cells were maintained in RPMI (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum and 2% penicillin-streptomycin (GIBCO) at 37°C. Next 50,000 cells were seeded into each well of a 24-well plate and stimulated with or without 100 ng of *E. coli* LPS and treated with 200 μg/mL human recombinant pGSN or bovine serum albumin. Two hours after LPS addition, 200 μL of media was collected and cells were removed by centrifugation at $1000 \times g$ for 10 mins. TNF-α levels of cell-free media were determined by an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN). Studies were done in triplicate.

Statistics. Values are presented as mean ± SD. The Mann-Whitney U test was used to evaluate differences between cytokine, actin, and pGSN levels. Animal mortality is presented as Kaplan-Meier curves, and the log-rank test was used to analyze treatment impact on animal mortality. A p value $\leq .05$ was considered significant.

RESULTS

pGSN Levels Decrease in Mice Subjected to LPS or CLP. Mice subjected to lethal challenges of LPS or CLP had decreased pGSN levels as early as 6 hrs after challenge. LPS administration reduced pGSN levels to about 50% of controls (pGSN levels were 80 ± 31 μg/mL in endotoxemic mice vs. 160 ± 26 μg/mL in controls, $p < .02$), whereas CLP, a more traumatic insult, diminished them further, to about 25% of the basal value (pGSN levels were 41 ± 20 μg/mL in CLP-challenged mice vs. 200 ± 35 μg/mL in controls, $p < .02$). The pGSN concentrations of injured mice remained low for ≥24 hrs after the insults (Fig. 1, *left panels*), whereas albumin levels did not significantly decrease at 24 hrs after LPS or CLP challenge (data not shown). Administration of exogenous recombinant human pGSN according to the dosage and schedule employed maintained the pGSN levels of LPS- or CLP-treated mice at normal or above normal levels.

Repletion of pGSN Improves Survival in Septic Mice. Mice challenged with LPS at a dose of 25 mg/kg intraperitoneally all died within 7 days of injection. In contrast, treatment with exogenous pGSN at the time of LPS challenge significantly improved survival (88% survival rate, $p < .001$) in endotoxemic mice (Fig. 1*A*, *right panel*). Mice treated with saline after CLP died within 48 hrs of injury, whereas those mice treated with exogenous pGSN after CLP had significantly enhanced survival (30% survival rate, $p = .001$, Fig. 1*B*, *right panel*).

Appearance of Circulating Actin After LPS and CLP and the Effect of pGSN Treatment on Its Sedimentability. As early as 6 hrs after LPS or CLP challenge, actin was detectable in the plasma of septic mice. Total actin appeared to increase with time after injury in both LPS- and CLP-challenged mice to about 250–500 μg/mL estimated from immunoblots using purified rabbit muscle actin as reference. Surprisingly, treating injured mice with pGSN did not decrease the levels of circulating actin compared with mice treated with saline; in contrast, pGSN-treated mice even appeared to have slightly higher level of circulating actin, especially in CLP-challenged mice (Fig. 2*A*). Subjecting plasma to ultracentrifugation and separating plasma into pellet and supernatant fractions determined the aggregation state of the actin protein (Fig. 2*B*, *upper panel*). Approximately 40% of the circulating actin in plasmas of saline-treated CLP-challenged mice was sedimentable. In contrast, pGSN-treated CLP-challenged mice had similar amounts of soluble and total plasma actin, indicating that pGSN-treated mice had mostly soluble actin in the plasma (Fig. 2*B*, *lower panel*).

pGSN Binds LPS But Does Not Inhibit LPS Activation of Monocytes. Since a recent study reported that a peptide derived from pGSN binds LPS and that pGSN may neutralize some cellular effects of LPS (23), we explored the possibility that pGSN directly binds and neutralizes inflammatory actions of LPS. We confirmed that pGSN specifically bound to fluorescently labeled LPS (Fig. 3*A*). However, pGSN did not interfere with the ability of LPS to elicit TNF-α secretion from human monocytes *in vitro* (Fig. 3*B*).

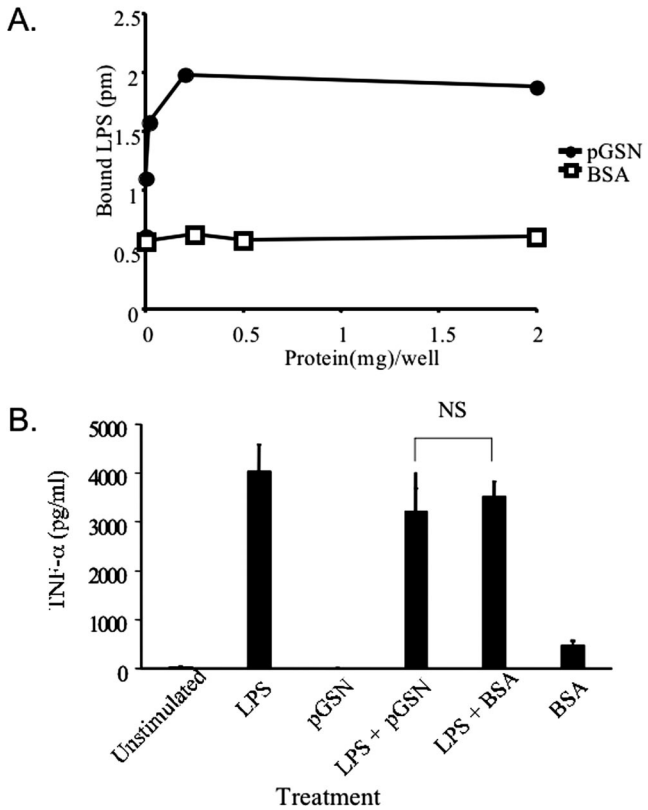

Figure 3. *A*, binding study of plasma gelsolin (*pGSN*) and fluorescent lipopolysaccharide (*LPS*) showing a binding curve of fluorescent LPS plateauing at 250 μg/well pGSN. Bovine serum albumin (*BSA*) had minimal affinity to LPS. *B*, tumor necrosis factor (*TNF*)-α levels of media from THP-1 cells treated without LPS (unstimulated), LPS only, pGSN only, LPS and pGSN, LPS and BSA, and BSA only. LPS stimulated THP-1 cells treated with pGSN or BSA had similar levels of TNF-α (*NS*, not significant).

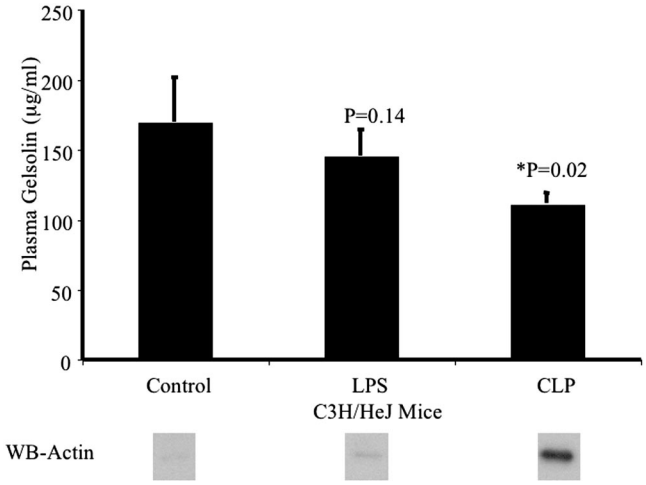

Figure 4. Effect of lipopolysaccharide (*LPS*) and cecal ligation and puncture (*CLP*) challenge in Toll-like receptor 4 mutants. LPS had no effect on plasma gelsolin (pGSN) levels of Toll-like receptor 4 mutants, whereas CLP induced pGSN depression in these mice. Representative Western blot (*WB*) of plasma staining for actin shows that increased circulating actin only occurred in CLP-challenged mice, corresponding with pGSN depletion.

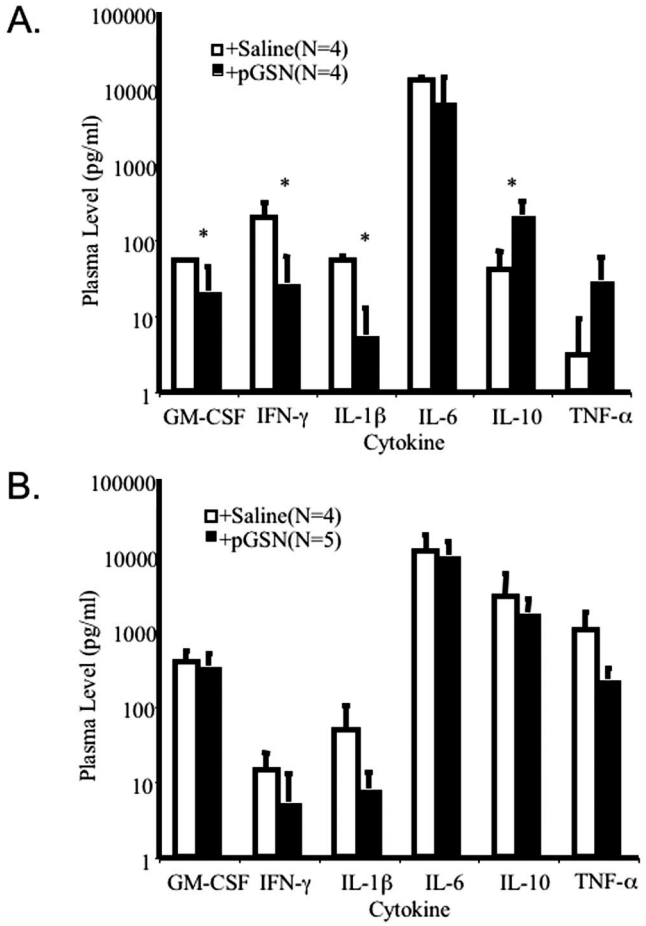

Figure 5. Cytokine profiles of endotoxemic mice treated with (*filled bars*) or without (*open bars*) plasma gelsolin (*pGSN*) at 24 hrs after lipopolysaccharide (*A*) or cecal ligation and puncture (CLP; *B*) challenge. The y-axis is in log scale. *A*, 24 hrs after LPS, saline-treated mice had with significantly higher levels of granulocyte-macrophage colony-stimulating factor (*GM-CSF*), interferon (*IFN*)-γ, and interleukin (*IL*)-1β ($p < .03$ for all) by as much as ten-fold compared with pGSN-treated mice. In contrast, IL-10 level was significantly higher in pGSN-treated mice ($p < .03$). *B*, cytokine profiles did not significantly differ between pGSN-treated and untreated mice 24 hrs after CLP challenge. *TNF*, tumor necrosis factor.

LPS Without Concurrent Cellular Response Does Not Deplete pGSN. To examine if LPS binding to pGSN alone can cause pGSN depletion, we studied pGSN levels in C3H/HeJ mice, a strain expressing mutated Toll-like receptor 4 that renders the mice resistant to LPS-induced inflammation (24). We found that pGSN levels and circulating actin did not differ significantly between LPS-challenged and unchallenged C3H/HeJ mice (Fig. 4). Consistent with their known resistance to LPS, C3H/HeJ mice also appeared unaffected by LPS challenge. However, CLP-challenged C3H/HeJ mice had significantly subnormal pGSN levels and increased levels of circulating actin, suggesting that the release of actin associated with cell injury was the main cause of pGSN depletion in septic mice (Fig. 4).

pGSN Repletion Altered the Cytokine Profiles of Endotoxemic Mice. Having shown that pGSN depletion precedes death and exogenous pGSN repletion improves survival, we evaluated whether administration of pGSN alters the cytokine profile of septic mice. Plasma cytokine profiles between pGSN-treated and saline-treated endotoxemic mice 6 hrs after LPS were similar (data not shown). However, 24 hrs after LPS challenge, pGSN-treated mice had significantly lower levels of several proinflammatory cytokines (granulocyte-macrophage colony-stimulating factor, interferon-γ, and IL-1β; $p < .03$), although IL-6 and TNF-α levels were not detected to be different (Fig. 5*A*). In addition, pGSN treatment resulted in a significantly higher IL-10 level (plasma IL-10 levels were 205 ± 108 pg/mL in gelsolin group vs. 39 ± 29 pg/mL in saline group, $p = .02$). pGSN does not directly stimulate IL-10 secretion in the absence of LPS as IL-10 was not increased in pGSN-treated unchallenged mice (data not shown). In contrast to LPS-challenged mice, pGSN repletion in CLP-challenged mice did not significantly affect the cytokine profile 24 hrs after injury (Fig. 5*B*). Although plasma levels of IL-1β and TNF-α were moderately lower in pGSN-treated mice, the differences did not reach statistical significance. This may be due to an insufficient number of experimental observations.

DISCUSSION

We report that in animal models of sepsis, critical declines in pGSN levels precede adverse outcomes and repletion of pGSN can have a rescuing effect. These

findings represent the first example in which pGSN therapy has a positive impact on mortality. Subcutaneous dosing in the experiments documented here increased pGSN concentrations sufficiently rapidly to offset pGSN clearance induced by LPS or CLP, leading to normal or greater pGSN values, thereby permitting a correlation between maintaining these levels and a favorable therapeutic effect. Since pGSN is a major blood constituent of healthy individuals, pGSN therapy is unlikely to have toxic side effects. Therefore, an evaluation of a possible therapeutic impact of pGSN replacement appears warranted.

The beneficial basis of pGSN's effect is unclear. Since pGSN is an actin-binding protein, its depletion from the blood is presumably a result of actin exposure in response to cellular injury. Although pGSN and another circulating actin-binding protein, Gc globulin (plasma vitamin D-binding protein), have been designated as an "actin scavenger system," proposed to clear actin from the circulation where it presumably has toxic effects (25), the evidence that the primary role of these proteins is for actin scavenging is not established.

The experimental basis for this scavenger hypothesis consists of two sets of results. In one, large amounts of monomeric actin infused into rats led to actin aggregate formation in the circulation, pulmonary microvascular thrombosis, and death of the animals; concomitant administration of Gc globulin blunted this effect (26). In the other, plasma samples from patients with acute respiratory distress syndrome reportedly contain actin (15), and, in one study, this plasma actin was filamentous and toxic to cultured sheep endothelial cells, whereas exogenous gelsolin lessens the toxicity (27). Since pGSN is the only known circulating protein that can sever and depolymerize actin filaments (20, 28), pGSN depletion in the setting of significant cell injury may cause actin aggregation, leading to further tissue injury. Our findings that pGSN replacement in septic mice depolymerizes circulating actin aggregates and enhances survival lend support to this hypothesis.

Previous research has indicated that Gc globulin and not pGSN is responsible for the clearance of actin injected into the circulation of experimental animals (28, 29). The observation that pGSN administration did not diminish the plasma concentration of actin in LPS- or CLP-treated animals supports that conclusion. However, our documentation that a significant fraction of circulating actin in LPS- or CLP-treated mice was particulate and that pGSN therapy solubilized the circulating actin implies that the pGSN in LPS- or CLP-treated animals was either quantitatively insufficient or qualitatively incompetent for disaggregating the plasma actin. Since pGSN binding by actin and by lipid mediators such as lysophosphatidic acid and LPS inhibits the ability of pGSN to sever actin filaments (23, 30), such binding may explain this incompetence. The ability of administered pGSN to solubilize circulating actin in injured mice suggests that this exogenous protein was present in quantitative excess of the inhibitory mediators, thereby accounting for its therapeutic effect.

An alternative mechanism for pGSN's protective effect is that although actin exposure is indeed responsible for pGSN depletion following injury, the pathologic consequence of that depletion is the loss of an inhibitor of diverse inflammatory mediators including lysophosphatidic acid (30, 31), aβ peptide (32), platelet-activating factor (33), and possibly others. The mediator onslaught evoked by primary injury and unopposed by pGSN buffering then results in secondary injury including death. Our observation that pGSN therapy had no effect on the inflammatory cytokines released early following LPS injury but favorably influenced the cytokine profile subsequently suggests that pGSN modifies factors acting late in the course of sepsis.

Although conditions such as trauma, major surgery, burns, and acute respiratory distress syndrome create major tissue injury likely to expose sufficient actin locally to entrap considerable pGSN, such injury is not obvious in sepsis in general or in the animal sepsis models. Our finding that large amounts of actin circulate in the blood in these conditions explains the basis of the pGSN depletion in response to LPS and CLP challenges and indicates that significant cell damage occurs very early in sepsis. The source of such early cell damage may be circulating blood cells, since these cells are the first to encounter inflammatory stimuli and have been demonstrated to release microparticles in respond to endotoxin (34). This loss of actin-rich cell surface material presumably containing receptors, signaling intermediates, and other essential cell components might account for organ dysfunction associated with sepsis syndromes, and measurements of actin released into the circulation under these circumstances might provide an objective metric for major cellular damage in the sepsis setting. The results are consistent with an earlier report documenting complexed Gc globulin in plasma of septic patients (35) and suggest that acute respiratory distress syndrome patients providing plasmas containing actin-pGSN complexes may have been septic (15, 27).

Questions remain that are not answered by our study. We do not know if an optimal level of pGSN mediates surviving sepsis or the time course of pGSN changes in septic patients. Perhaps clinical improvement occurs only after pGSN recovery. Nevertheless, our study is the first to demonstrate that pGSN plays a critical role in the response to a septic insult and can be a biomarker of sepsis severity. Repletion of pGSN may represent a novel treatment for sepsis. We recognize that such a proposal must be cautious since many therapies directed against sepsis-related inflammatory mediators with effects in animal models have failed in human clinical trials (1, 36). Gelsolin replacement, however, would differ from these precedents in being driven by depletion of gelsolin rather than clinical indication and in potentially targeting a spectrum of mediators.

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