Effects of Methylprednisolone on Intracellular Bacterial Growth

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Clinical studies have shown positive associations among sustained and intense inflammatory responses and the incidence of bacterial infections. Patients presenting with acute respiratory distress syndrome (ARDS) and high levels of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), and IL-6, have increased risk for developing nosocomial infections attributable to organisms such as Staphylococcus aureus, Pseudomonas aeruginosa, and Acinetobacter spp., compared to those patients with lower levels. Our previous in vitro studies have demonstrated that these bacterial strains exhibit enhanced growth extracellularly when supplemented with high concentrations of pure recombinant TNF-α, IL-1β, or IL-6. In addition, we have shown that the intracellular milieu of phagocytic cells that are exposed to supraoptimal concentrations of TNF-α, IL-1β, and IL-6 or lipopolysaccharide (LPS) favors survival and replication of ingested bacteria. Therefore, we hypothesized that under conditions of intense inflammation the host’s micromilieu favors bacterial infections by exposing phagocytic cells to protracted high levels of inflammatory cytokines. Our clinical studies have shown that methylprednisolone is capable of reducing the levels of TNF-α, IL-1β, and IL-6 in ARDS patients. Hence, we designed a series of in vitro experiments to test whether human monocytic cells (U937 cells) that are activated with high concentrations of LPS, which upregulate the release of proinflammatory cytokines from these phagocytic cells, would effectively kill or restrict bacterial survival and replication after exposure to methylprednisolone. Fresh isolates of S. aureus, P. aeruginosa, and Acinetobacter were used in our studies. Our results indicate that, compared with the control, stimulation of U937 cells with 100-ng/ml, 1.0-µg/ml, 5.0-µg/ml, or 10.0-µg/ml concentrations of LPS enhanced the intracellular survival and replication of all three species of bacteria significantly (for all, \( P = 0.0001 \)). Stimulation with \( \approx 10.0 \) µg of LPS generally resulted in efficient killing of the ingested bacteria. Interestingly, when exposed to graded concentrations of methylprednisolone, U937 cells that had been stimulated with 10.0 µg of LPS were able to suppress bacterial replication efficiently in a concentration-dependent manner. Significant reduction in numbers of CFU was observed at \( \approx 150 \) µg of methylprednisolone per ml (\( P \) values were 0.032, 0.008, and 0.009 for S. aureus, P. aeruginosa, and Acinetobacter, respectively). We have also shown that steady-state mRNA levels of TNF-α, IL-1β, and IL-6 in LPS-activated cells were reduced by treatment of such cells with methylprednisolone, in a concentration-dependent manner. The effective dose of methylprednisolone was 175 mg, a value that appeared to be independent of priming level of LPS and type of mRNA. We therefore postulate that a U-shaped relationship exists between the level of expression of TNF-α, IL-1β, and IL-6 within the phagocytic cells and their abilities to suppress active survival and replication of phytophagized cytobacteria.

The inflammatory process is part of the innate immune response of the host to an infectious or noninfectious assault. The most proximal expression of such a response is the elaboration of the proinflammatory cytokines tumor necrosis factor alpha (TNF-α), interleukin 1β (IL-1β), and IL-6. When present at optimal concentrations, these biologically active molecules recruit both specific and nonspecific immune cells—nonlymphoid leukocytes and lymphocytes—to the site of assault and activate them, thereby helping to eradicate the assault and to restore homeostasis. Whereas optimal levels of these peptides are important for a successful defense, at progressively higher concentrations they mediate proportionately stronger local and finally systemic responses (systemic inflammation), with predominantly destructive rather than protective effects on the host (4). Reduction in the effective concentration of proinflammatory mediators is an important component in the resolution of inflammation (27).

Acute respiratory distress syndrome (ARDS) is a frequent form of hypoxic respiratory failure caused by excessive systemic inflammation, with an associated mortality of 40 to 60% (15). We have previously investigated the longitudinal relationship between pulmonary and circulatory proinflammatory TNF-α, IL-1β, and IL-6 levels, infections, and outcome in patients with (sepsis-induced) ARDS (9, 19, 22). We reported that at the onset of ARDS and over time, nonsurvivors (\( n = 17 \)) had significantly (\( P < 0.001 \)) higher plasma TNF-α, IL-1β, and IL-6 levels than survivors (\( n = 17 \)) did (19). The rate of nosocomial infection per day of mechanical ventilation was 1% in survivors and 8% in nonsurvivors (9). Moreover, none of the proven (\( n = 36 \)) or suspected (\( n = 55 \)) nosocomial infections caused either a transient or a sustained increase in plasma TNF-α, IL-1β, IL-6, and IL-8 levels above preinfection values (9). The findings of these studies (9, 19, 22) suggested that final outcome in patients with ARDS is related to the magnitude
and duration of the host inflammatory response and that nosocomial infections might be an epiphenomenon of prolonged intense inflammation.

Until recently, very little was known of the ability of bacteria to interfere with or to utilize extracellular cytokines secreted by the host cells or intracellular cytokines within phagocytic cells. Recent reports have shown that certain bacteria have receptors for the cytokines IL-1β and TNF-α and that exposure of bacteria to these cytokines enhanced their growth (12, 25, 29) and virulence (16). Furthermore, studies have reported enhancement of bacterial growth in the presence of cytokines for Escherichia coli (IL-1β [25] gamma interferon [10], IL-2, and granulocyte-macrophage colony-stimulating factor [7]), Staphylococcus aureus (IL-4 [11]), Legionella pneumophila (IL-10 [24]), and Mycobacterium avium (IL-3, granulocyte-macrophage colony-stimulating factor [6, 28], and IL-6 [6, 28]).

In the context of our clinical observations (9) and the experimental work described above, we hypothesized that cytokines secreted by the host during ARDS may indeed favor the growth of bacteria and explain the association between exaggerated and protracted systemic inflammation and the frequent development of nosocomial infections. To test this hypothesis, we conducted in vitro studies evaluating the extracellular and intracellular growth response of three clinically relevant bacteria—S. aureus, Pseudomonas aeruginosa, and Acinetobacter—in response to graded concentrations of proinflammatory cytokines TNF-α, IL-1β, and IL-6 (13, 21). In these studies, we identified a U-shaped response of bacterial growth to proinflammatory cytokines. When the tested bacteria were exposed in vitro to a lower concentration of TNF-α, IL-1β, or IL-6, similar to the values in plasma of ARDS survivors (19), extracellular and intracellular bacterial growth was not promoted and human monocytic cells were efficient in killing the ingested bacteria (13, 21). In contrast, when bacteria were exposed to higher concentrations of these proinflammatory cytokines, similar to the values in plasma of ARDS nonsurvivors (19), intracellular and extracellular bacterial growth was enhanced in a dose-dependent manner (13, 21).

Recently, we completed a randomized, double-blind, placebo-controlled trial showing significant physiological and survival benefit when prolonged methylprednisolone treatment was administered to ARDS patients failing to improve after 1 week of mechanical ventilation (18). Similar to results of a previous uncontrolled study (20), improvement during methylprednisolone administration was associated with a significant reduction in plasma TNF-α, IL-1β, and IL-6 levels (23).

In the present study, we tested the hypothesis that methylprednisolone can decrease cytokine-mediated enhancement of in vitro bacterial growth and that LPS-stimulated monocytic cells that are impaired in their abilities to kill ingested bacteria would regain their abilities to suppress the survival and/or replication of internalized bacteria when such cells are exposed to adequate concentrations of methylprednisolone.

**MATERIALS AND METHODS**

**Bacteria.** Fresh clinical isolates of *S. aureus*, *P. aeruginosa*, and *Acinetobacter* were obtained from bronchoalveolar lavage fluid or peripheral blood of patients admitted to the University of Tennessee Bowld Hospital, Memphis. All the bacterial isolates were tested for susceptibility to a gentamicin (100 μg/ml), streptomycin (100 μg/ml), and penicillin (100 U/ml) combination using standard in vitro antibiotic susceptibility testing techniques. These fresh isolates of bacteria were grown in 3 ml of RPMI medium with fetal calf serum and antibiotics (Life Technologies, Bethesda, Md.) at 37°C for 8 h. The bacterial cultures were washed and resuspended in 1 ml of RPMI medium without antibiotics to a concentration of 10^5 CFU/ml.

**Maintenance of U937 cells.** Human monocytic cell line U937 was obtained from the American Type Culture Collection (ATCC; Rockville, Md.). These cells were maintained in RPMI medium with 10% fetal calf serum, 100 U of penicillin per ml and 100 μg of streptomycin (Life Technologies) per ml. Prior to each experiment, cells were centrifuged, resuspended in RPMI containing 2% fetal bovine serum without antibiotics, and seeded into 12-well tissue culture plates (Costar, Cambridge, Mass.) to a concentration of 2 × 10^6 cells/ml. RPMI medium lacks complex organic materials that may be present in a conventional bacteriological medium and does not interfere with the biological activities of the tested cytokines.

**Priming of U937 cells with graded concentrations of LPS.** Replicates of U937 cells (2 × 10^5 cells/ml) were exposed to various amounts (0, 10, and 100 ng and 1.0, 5.0, and 10.0 ng) of lipopolysaccharide (LPS) purified from *E. coli* strain K235 (Sigma Chemicals, St. Louis, Mo.). Cells were then incubated for 6 h at 37°C in a humid atmosphere of 5% CO₂.

**Exposure of LPS-primed U937 cells to graded concentrations of methylprednisolone.** U937 cells (2 × 10^5 cells/ml) were exposed to various amounts (0, 25, 50, 75, 100, and 250 ng) of methylprednisolone sodium succinate (Pharmacia-Upjohn Company, Kalama-zoo, Mich.) and incubated at 37°C in a humidified atmosphere of 5% CO₂ for 4 to 6 h.

**Cell viability as assessed by trypan blue dye exclusion.** The viability of cells was assessed by suspending the isolated cells in 0.01% trypan blue and counting the viable cells under a light microscope. Viabilities of the cells were 70 to 80% in both control and experimental samples (microscopic data not shown; no photographs taken).

**Bacterial infection of LPS-primed U937 cells.** Intracellular bacterial survival and replication of the tested bacteria were investigated under two conditions: (i) U937 cells primed with graded concentrations of LPS and (ii) LPS-primed U937 cells exposed to graded concentrations of methylprednisolone. Before these experiments were started, the maintenance or growth culture media were removed from the cell sheets and fresh media without fetal bovine serum or antibiotics were added. These cells (2 × 10^6 cells/ml) were mixed with 4 × 10^6 CFU of the above-mentioned bacterial species (*S. aureus*, *P. aeruginosa*, and *Acinetobacter* spp.) per ml and incubated at 37°C in a humidified atmosphere of 5% CO₂ for 18 h. The bacterial colonies were counted, and the results were expressed as CFU per milliliter of lysate.

**Total cellular RNA** was isolated from U937 cells stimulated with various amounts of LPS (100 ng, 1.0 μg, 5.0 μg, and 10.0 μg) and treated with various amounts (0, 25, 50, 75, 100, 150, and 250 ng) of methylprednisolone, using a modified procedure previously described (14). Briefly, the cells were harvested, washed in sterile normal phosphate-buffered saline, and lysed using Trizol reagent (Life Technologies). Total cellular RNA was extracted from the cell lysates with chloroform (Sigma Chemicals) followed by ethanol (Sigma Chemicals) precipitation. The RNA was stored as dry pellets or as aliquots of aqueous solutions at −80°C until used.

**Reverse transcription (RT) reactions** were performed in accordance with a procedure described by Kanangat et al. (14). Five micrograms of total cellular RNA were reverse transcribed using avian myeloblastosis virus reverse transcriptase and oligo(dT)₃₄ primer (Promega Corporation, Madison, Wis.). In addition to avian myeloblastosis virus reverse transcriptase and oligo(dT)₃₄ primer, the reaction mixture consisted of 5 mM MgCl₂, 50 mM KCl, 0.1% Triton X-100, 2 mM deoxynucleoside triphosphate, and 40 U of ribonuclease inhibitor (Pro-
FIG. 1. Intracellular bacterial survival/replication of *S. aureus*, *P. aeruginosa*, and *Acinetobacter* in U937 cells primed with graded concentrations of LPS. Concentration-dependent responses were observed, with reductions in intracellular bacterial survival and replication at lower concentrations of LPS (1.0 and 10.0 ng/ml, except for *S. aureus*) and enhancements in intracellular bacterial survival and replication at higher concentrations (≥100 ng/ml, except for *S. aureus* at ≥10.0 ng/ml). Standard errors were estimated by the square root of the mean square error from ANOVA divided by the square root of 3. Each P value is probability that the difference between the mean intracellular survival and replication for cells primed with a particular concentration of LPS and the mean of the control cells (i.e., no LPS) is zero, given that the null hypothesis is true. *, P = 0.0001;

PCR. PCR was done using a previously described method (14). Five microliters of the RT mixture (cDNA) was used in a 25.0-μl PCR mixture for qualitative detection of β-actin (used as a control for RNA isolation and RT efficiency) and mRNA of TNF-α, IL-1β, and IL-6. All these reactions were done in separate tubes to avoid possible competition among different target and primer pairs. The reaction mixture consisted of 1.5 to 2.5 mM MgCl₂, 0.1% Triton X-100, 125 μM (each) dATP, dCTP, dGTP, and dTTP; 50 mM Tris HCl (pH 8.3), and 1.0 U of Taq DNA polymerase (Life Technologies). The conditions for PCR consisted of denaturing at 94°C for 90 s and annealing at 55°C for 60 s followed by extension at 72°C for 120 s. These cycles were repeated 35 times for each message mentioned above. The primers were used at a concentration of 15 pmol per reaction. PCR products were analyzed on a 2.5% gel (Life Technologies), stained with ethidium bromide (Sigma Chemicals), and photographed. The intensities of the bands were measured using the Alpha Imager 2000 documentation and analysis system (Alpha Inotech Corporation, San Leandro, Calif.).

Repetition of experiments. The experiments evaluating intracellular survival and replication of bacteria within U937 cells primed with graded concentrations of LPS were run in triplicate. The experiments assessing the intracellular survival and replication of bacteria in LPS-primed U937 cells exposed to graded concentrations of methylprednisolone were run in duplicate. The experiments with U937 cells exposed to 10.0 μg of LPS and 250 μg of methylprednisolone were done in triplicate. The quantification of steady-state mRNA levels of TNF-α, IL-1β, and IL-6 were done in duplicate.

Statistical analysis. For U937 cells primed with graded concentrations of LPS, intracellular survival and replication (10⁵ CFU/ml) were analyzed with two-way ANOVA for linear regression, such values are assumed to be invariate. For each cytokine, levels of methylprednisolone, mRNA expression of TNF-α, IL-1β, and IL-6 within each level of LPS was analyzed with simple linear regression with expression of TNF-α (units of intensity) as the dependent variable and methylprednisolone as the independent variable. For each regression model (i.e., for each type of mRNA expression at each level of LPS), the effective dose of methylprednisolone was defined as the level of methylprednisolone associated with a 50% reduction in mRNA expression. The value for mRNA expression that corresponded to a 50% reduction was empirically derived according to the following formula: median maximum expression/2. Then, each value was substituted in the appropriate regression equation and the effective dose determined by solving for the unknown level of methylprednisolone. Because of the assumptions necessary for linear regression, such values are assumed to be invariate. For each cytokine, effective doses were averaged over levels of LPS. Because the average effective doses were remarkably similar, the effective dose was estimated by averaging over both levels of LPS and type of cytokine.

RESULTS

Bacterial survival and replication in U937 cells primed with graded concentrations of LPS. Figure 1 shows the intracellular survival and replication of *S. aureus*, *P. aeruginosa*, and *Acinetobacter* spp. within U937 cells exposed to graded concentrations of LPS. For all three species of bacteria, concentration-dependent responses were observed, with reductions in intracellular survival and replication at lower concentrations of LPS and enhancements at higher concentrations (≥100 ng of LPS per ml) except *S. aureus*, for which the lowest enhancing concentration was 10 ng of LPS per ml. However, at priming concentration of 1 ng of LPS per ml, reductions in intracellular...
survival and replication in comparison to the control (i.e., no LPS) were observed for all three bacterial species (P values, 0.0001, 0.02, and 0.0001, respectively).

**Bacterial survival and replication in LPS-primed U937 cells treated with graded concentrations of methylprednisolone.**

Figure 2 shows the intracellular survival and replication (10^6 CFU/ml) of *S. aureus*, *P. aeruginosa*, and *Acinetobacter* in U937 cells primed with 10 µg of LPS per ml and then exposed to graded concentrations of methylprednisolone. (These experiments were done in duplicate.) Gray bars, U937 monocytic cells primed with LPS alone; hatched bars, U937 cells primed with LPS and then exposed to methylprednisolone (0, 25, 50, 75, 100, 150, and 250 µg per ml). Standard errors for each bacterial species were estimated from the square root of the mean square error from ANOVA divided by the square root of 2, and the P values reflect the probability that the mean intracellular bacterial survival and replication of primed U937 cells with exposure to methylprednisolone is equal to the mean of primed U937 cells without exposure *, P = 0.0001.

FIG. 2. Intracellular bacterial survival and replication of *S. aureus*, *P. aeruginosa*, and *Acinetobacter* in U937 cells primed with 10 µg of LPS per ml and then exposed to graded concentrations of methylprednisolone. (These experiments were done in duplicate.) Gray bars, U937 monocytic cells primed with LPS alone; hatched bars, U937 cells primed with LPS and then exposed to methylprednisolone (0, 25, 50, 75, 100, 150, and 250 µg per ml).
In the present study, we found that the intracellular survival and replication of *S. aureus*, *P. aeruginosa*, and *Acinetobacter* in LPS-primed U937 monocyteic cells was affected by the degree of cell activation obtained with exposure to increasing concentrations of LPS. Activation of phagocytic cells with high concentrations of LPS (∼100 ng/ml) significantly enhanced intracellular bacterial survival and replication, while stimulation with lower concentrations of LPS (1.0 or 10.0 ng/ml) effectively suppressed bacterial survival and replication (Fig. 1), depending on the type of bacteria. The impairment in intracellular bacterial killing coincided with an increased expression of proinflammatory cytokines (Fig. 4A). Exposure of LPS-stimulated cells (10.0 μg/ml) to graded concentrations of methylprednisolone resulted in appreciable dose-dependent reductions in the survival and replication of internalized bacteria (Fig. 2 and 3 and Table 1). Replication of all three bacterial species was significantly reduced in LPS-activated cells treated with 250 μg of methylprednisolone per ml (Table 1). Methylprednisolone treatment of LPS-activated U937 cells resulted in appreciable reductions in the steady state levels of mRNA for TNF-α, IL-1β, and IL-6 in a manner independent of the priming conditions because our design provided appropriate controls.

Our investigation utilized in vitro studies wherein potentially phagocytic cells were tested in microenvironments that mimic selected aspects of in vivo inflammation. Certain technical aspects of the protocol require clarification. We have not quantified the phagocytic indices under the different experimental conditions because our design provided appropriate controls. We eliminated the extracellular bacteria with appropriate an-

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**DISCUSSION**

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<table>
<thead>
<tr>
<th>Organism</th>
<th>10^6 CFU/ml (mean ± SD)*</th>
<th>P b</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Without methylprednisolone</td>
<td>With methylprednisolone</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>275 ± 62</td>
<td>82 ± 7</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>381 ± 53</td>
<td>104 ± 11</td>
</tr>
<tr>
<td><em>Acinetobacter</em></td>
<td>375 ± 71</td>
<td>127 ± 30</td>
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</tbody>
</table>

* Values are means for three experiments.

b Each P value is the probability that the mean of the group treated with methylprednisolone is equal to the mean of the control group, given that the null hypothesis is true. Independent sample t tests for equal or unequal variants were used.

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**TABLE 1. Reduced survival and replication of bacteria in LPS-activated U937 cells exposed to methylprednisolone at 250 μg/ml compared to controls**

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FIG. 3. Intracellular survival and replication of *S. aureus* in U937 cells primed with 10 μg of LPS per ml and then exposed to methylprednisolone at 0, 150, and 250 μg per ml. Note the reductions in intracellular survival and replication with methylprednisolone.
tibiotic combinations. However, we did not add antibiotics to the final step of the culture process. Our intent was to quantify the survival and replication of the bacteria engulfed by the monocytic cells. We recognize that some cells may have died during the process and released bacteria to the external medium. The external medium was likely rich in cytokines and might have favored the survival and replication of bacteria. Because antibiotics in the extracellular medium would have affected survival of the released bacteria, at this step we avoided any inclusion of antibiotics in the extracellular medium.

The results of our in vitro experiments indicate that methylprednisolone does not impair the ability of activated monocytes to phagocytize and kill ingested bacteria. To the contrary, we found that methylprednisolone restored the intracellular killing capacity of phagocytic cells primed with high concentrations of LPS. In methylprednisolone-treated cells, the reduction in intracellular bacterial survival and replication in these cells coincided (Table 1) with a reduction in the expression of TNF-α, IL-1β, and IL-6 (Fig. 4; Table 2). Hence we presume that bacterial survival and replication within phagocytic cells is in part associated with the cytokines expressed by such cells. How exactly the cells lose their killing functions without any apparent impairment in the phagocytic abilities is not known. It is likely that once bacteria are internalized and are under selective pressure they adapt to an otherwise hostile microenvironment by switching on novel genes’ expression, which enables them to utilize cytokines as growth factors. It has previously been shown that the ability of fresh bacterial isolates (obtained from infected patients) to respond to proinflammatory cytokines is lost after several in vitro passages on a bacteriological medium without added cytokines (21). LPS activation of monocytes may also induce the expression of a variety of small mRNA molecules, which are thought to be converted into biologically active low-molecular-weight proteins (26). The low-molecular-weight mRNA molecules may code for proteins or small polypeptides that become growth factors for certain bacteria, or bacteria may acquire the ability to utilize those peptides under selective pressure.

It is unclear how bacteria may use cytokines for their growth, since bacteria are prokaryotes without a defined nucleus and cytokines are intended to work on well-defined eukaryotic cells with consequent signal transduction events. However, in a host milieu, bacteria may adapt to eukaryotic cellular processes (8). Although the subsequent sequence of intracellular events has not been delineated, it is possible that bacteria might use cytokines through receptor-mediated, signal transduction-induced activities that would require the presence of biochemical processes akin to those seen in eukaryotic cells; cytokines may act on bacteria through a signaling process similar to that of eukaryotes but involving different biochemical pathways; or bacteria may break down cytokines into biologically active fragments that are transported across the bacterial cell membranes and act on specific gene transcription and translation. In-depth studies of LPS activation and bacterial infections at the cellular and molecular levels are necessary to investigate the foregoing speculations.

Our results suggest that glucocorticoids may have the capacity to restore the intracellular killing functions of phagocytic cells impaired by excessive activation. By showing that meth-
ship between excessive inflammation and bacterial growth. In vitro experimental data suggesting a cause-and-effect relationship, we provide in the present study, we have assumed that the effects of methylprednisolone can reduce, in a dose-dependent manner, the cytokine/β-actin ratio of the tested bacteria, we did not observe any significant change in the number of CFU of bacteria from the cell culture (unpublished data).

Additionally, when we removed methylprednisolone from the culture medium of LPS-activated cells before and after adding methylprednisolone (0, 25, 50, 75, 100, 150, and 250 μg/ml) with LPS at 10,000 ng/ml, a value that is 2.5 times the 50% reduction in levels of methylprednisolone (MP). U937 cells (2 × 10^6/ml) were primed with 10 μg of LPS and exposed to graded concentrations of methylprednisolone. U937 cells (2 × 10^6/ml) were primed with 10 μg of LPS and exposed to graded concentrations of methylprednisolone (0, 25, 50, 75, 100, 150, and 250 μg per ml [bands 1 through 7, respectively]) for 4 to 6 h. The cells were then harvested, and the total cellular RNA was extracted. The cellular mRNAs were then reverse transcribed using oligo(dT)18 primers, and the steady-state level of TNF-α, IL-1β, and IL-6 were measured by semiquantitative PCR, taking β-actin mRNA levels as the internal control for total mRNA extraction and general efficiency of the reverse transcription. The three-dimensional measurement of intensities of the bands (visualized by ethidium bromide staining) was obtained using the Alpha Inotech 2000 imaging system. The values related to the levels of β-actin do not differ appreciably from experiment to experiment and are presented above the gel.

Overall, our findings indicate that excessive systemic inflammation not only leads to inappropriate tissue damage and maladaptive tissue repair (17) but may also create an “acquired” host environment that favors the growth of bacteria. We have provided in vitro experimental evidence to suggest a possible cause-and-effect relationship between excessive inflammation (TNF-α, IL-1β, and IL-6) and enhanced bacterial survival. We and others have previously shown that in patients with life-threatening systemic inflammation, prolonged glucocorticoid treatment has to be sustained to decrease circulat-

### TABLE 2. Parameter estimates from regression of ratios of mRNA for TNF-α, IL-1β, and IL-6 to mRNA for β-actin obtained from LPS-stimulated U937 cells on level of methylprednisolone (MP)

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>LPS concn (ng/ml)</th>
<th>Cytokine/β-actin ratio</th>
<th>Regression equation</th>
<th>Effective dose of MP (μg/ml)</th>
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<tbody>
<tr>
<td></td>
<td>Minimum&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Maximum&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50% reduction&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
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<tr>
<td>TNF-α</td>
<td>100</td>
<td>77.0</td>
<td>185.0</td>
<td>92.50</td>
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<tr>
<td></td>
<td>1,000</td>
<td>99.5</td>
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<td>10,000</td>
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<tr>
<td>Mean</td>
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<tr>
<td>IL-1β</td>
<td>100</td>
<td>96.0</td>
<td>272.5</td>
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<td>10,000</td>
<td>189.5</td>
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<td>Mean</td>
<td></td>
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<tr>
<td>IL-6</td>
<td>100</td>
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<td>348.0</td>
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<td>Overall mean</td>
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<td>Overall median</td>
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<sup>a</sup> Median of duplicate measurements.<br>
<sup>b</sup> 50% reduction = (median maximum expression)/2.<br>
<sup>c</sup> $Y = \hat{a} - \hat{b}$ (level of MP), where Y is the ratio of mRNA for a particular cytokine, $\hat{a}$ is the intercept, and $\hat{b}$ is the regression coefficient.<br>
<sup>d</sup> After the value of a 50% reduction was substituted for $Y$, the effective dose was obtained by solving for the unknown level of MP.

**FIG. 5.** Steady-state mRNA levels of TNF-α, IL-1β, and IL-6 in U937 cells primed with 10 μg of LPS and exposed to graded concentrations of methylprednisolone. U937 cells (2 × 10^6/ml) were primed with LPS at 10 μg and exposed to graded concentrations of methylprednisolone (0, 25, 50, 75, 100, 150, and 250 μg per ml [bands 1 through 7, respectively]) for 4 to 6 h. The cells were then harvested, and the total cellular RNA was extracted. The cellular mRNAs were then reverse transcribed using oligo(dT)18 primers, and the steady-state level of TNF-α, IL-1β, and IL-6 mRNAs were measured by semiquantitative PCR, taking β-actin mRNA levels as the internal control for total mRNA extraction and general efficiency of the reverse transcription. The three-dimensional measurement of intensities of the bands (visualized by ethidium bromide staining) was obtained using the Alpha Inotech 2000 imaging system. The values related to the levels of β-actin do not differ appreciably from experiment to experiment and are presented above the gel.
ing levels of inflammatory mediators (3, 20) and to improve organ function (1, 2, 5, 18). This experimental work suggests that under such conditions, administration of exogenous glucocorticoids may also restore the host’s ability to counteract infections.

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