Effect of CD14 Blockade in Rabbits with Escherichia coli Pneumonia and Sepsis


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**Effect of CD14 Blockade in Rabbits with *Escherichia coli* Pneumonia and Sepsis**


CD14, a pattern recognition receptor found on myeloid cells, is a critical component of the innate immune system that mediates local and systemic host responses to Gram-negative and Gram-positive bacterial products. Previous studies in normal animals have tested the effect of CD14 blockade on the systemic response to i.v. LPS. The goals of the study were to determine whether CD14 blockade protected against the deleterious systemic response associated with *Escherichia coli* pneumonia and to determine whether this strategy affected the pulmonary response to tissue infection. Rabbits were pretreated with either anti-CD14 mAb or isotype control mAb at 2.5 mg/kg. *E. coli* (1 × 10^9 CFU) was inoculated into the lungs, and the animals were observed for either 4 or 24 h. The blockade of CD14 improved the mean arterial blood pressure (\( p = 0.001 \)) and decreased the i.v. fluid requirements (\( p = 0.01 \)). Although this therapy protected the vascular compartment, rabbits treated with anti-CD14 mAb had increased bacterial burdens in the bronchoalveolar lavage fluid recovered from the instilled lung (\( p = 0.005 \)) and widened alveolar-arterial oxygen difference. Blockade of CD14 prevents the deleterious systemic responses that occur in sepsis; however, other measures are necessary to control bacterial proliferation at the primary site of infection. *The Journal of Immunology*, 2000, 164: 5439–5445.

Proinflammatory mediators released by cells of the innate immune system are important in the pathophysiology of sepsis. The innate immune system has evolved a set of germline-encoded receptors that recognize common motifs on microbial pathogens. These receptors, commonly referred to as pattern recognition receptors, include the mannose receptor, scavenger receptors, and CD14 (1–3). CD14 is an important recognition and signaling receptor for LPS and Gram-negative bacteria. The recognition and signaling through CD14-dependent pathways requires three proteins, LPS binding protein, CD14, and Toll-like receptors (TLR)\(^3\) (4, 5). LPS-binding protein, a plasma lipid transfer protein, acts on LPS aggregates or bacterial membranes and presents LPS monomers to binding sites on CD14 (6). CD14, a glycosylphosphatidylinositol-linked protein found on the surface of myeloid cells, binds to LPS, resulting in cellular activation, production of proinflammatory cytokines (e.g., TNF-\(\alpha\), IL-6), and chemokines (e.g., IL-8), and activation of inducible nitric oxide synthase (7, 8). Although LPS recognition is mediated by CD14, this receptor requires another receptor for intracellular signaling (9). Toll-like receptor-4 is a likely candidate for CD14-dependent signal transduction because TLR-4 initiates NF-\(\kappa\)B activation and LPS-resistant mice have a critical mutation in the intracellular portion of TLR-4 (10–12). Because CD14-LPS interactions result in cellular signaling and production of proinflammatory cytokines, blockade of this early step in the acute inflammatory cascade represents a potential therapeutic strategy.

To evaluate the effects of blocking CD14-LPS and CD14-bacterial interactions, several animal models have been studied. CD14-deficient mice are resistant to i.v. LPS and have reduced bacteremia after the i.p. instillation of *Escherichia coli* (13). In a nonhuman primate model of endotoxin-induced shock, anti-CD14 mAbs prevented hypotension and reduced plasma cytokine levels and lung epithelial permeability (14). In a rabbit model of the generalized Schwartzman reaction after LPS treatment, an anti-CD14 mAb protected against organ injury and death even when the Ab was administered 4 h after the endotoxin injection (15). Although these studies support the concept that CD14 blockade is beneficial, there is no information about the effect of blocking CD14-bacterial interactions on host defenses in a model of tissue infection associated with severe systemic responses that mimics clinical events in humans.

The goal of this study was to determine the effects of CD14 blockade on systemic and pulmonary responses of rabbits with Gram-negative pneumonia. We hypothesized that blockade of CD14 would protect against the deleterious systemic responses that occur in rabbits with bacterial pneumonia (16). Because CD14 has a critical role in bacterial recognition, we also monitored pulmonary physiological and inflammatory responses to determine whether the blockade of CD14 affected lung function or local intrapulmonary host defenses.

**Materials and Methods**

**Reagents**

The rabbit-specific anti-CD14 mAb, designated 1116 1a6, and the control Ab, designated W6/32 (both murine IgG2a), were provided by ICOS (Bothell, WA). The ELISAs for TNF-\(\alpha\), IL-8, melanoma/growth-related protein (GRO), and monocyte chemotactic peptide-1 (MCP-1) are specific.
CD14 BLOCKADE IN RABBITS WITH E. coli PNEUMONIA AND SEPSIS

for the rabbit (17, 18). E. coli serotype K-1 was a clinical isolate obtained from a patient with bacteremia due to biliary sepsis.

Preparation of bacteria

The bacteria were passaged by inoculation into the peritoneal cavity of a specific pathogen-free New Zealand white rabbit. Twenty-four hour after inoculation, the spleen was aseptically removed, homogenized in 0.9% NaCl, diluted to 30% glycerol, and stored in aliquots at −70°C. After each passage, the bacteria were identified using standard microbiological techniques. On the day before an experiment, a frozen aliquot of E. coli was thawed, inoculated into 50 ml Lennox-B broth, and incubated overnight at 37°C in a shaking incubator. The bacteria were recovered by centrifugation, washed once in PBS, and resuspended in sterile water to 10^9 CFU/ml. Bacterial concentrations were confirmed by quantitative culture using the pour plate method. The anti-CD14 mAb and the isotype control mAb were diluted into 0.9% NaCl at a concentration of 2.5 mg/ml.

Animal protocols

The Animal Research Committee of the Veterans Affairs Puget Sound Health Care System approved all experiments. Female NZW rabbits, specific pathogen free, weighing 3.0–3.5 kg, were purchased from Western Oregon Rabbit (Philomath, OR) and were housed in the animal facility until the day of the experiment.

Rabbit model of bacterial pneumonia

Rabbits were anesthetized with a combination of ketamine (10 mg/kg) and xylazine (3 mg/kg) i.v. and allowed to breathe spontaneously through an endotracheal tube (Kendall/Sheridan, Argyle, NY). For the 24-h studies, cerebral venous and arterial catheters were placed via a cutaneous incision. For 4-h studies, cerebral venous and arterial catheters were not used.

Induction of Gram-negative bacterial pneumonia. To induce pneumonia, rabbits were placed in a right lateral recumbent position on a 20-degree incline with the head elevated. Then 1.0 ml of the E. coli suspension (1 × 10^8 CFU/ml) was instilled in the right lower lobe bronchus using a 5 French catheter (AccuMark, Keene, NH) advanced through the endotracheal tube. Bacteria were mixed with 1% colloidal carbon (Pelikan, Hanover, Germany) to aid in identifying the instilled areas at necropsy (19). The rabbits were placed on a heated water blanket (Gaymar Industries, Orchard Park, NY) for the first 2 h after the instillation to maintain body temperature after anesthesia. Although the intent was to instill the right lower lobe, on 2 of the 23 installations the left lung was instilled. Due to the instillation of the left lung in these two rabbits, the lung that received the bacterial inoculation is described as the “instilled lung,” and the contralateral lung is designated as the “uninstilled lung.” The experiments were not designed to study the inflammatory response in the uninstilled lungs due to the potential for a low level contamination of the contralateral lung with E. coli.

In the 24-h studies, arterial blood pressure and central venous pressures (CVP) were measured continuously using an endotracheal tube (Kendall/Sheridan, Argyle, NY). For the 24-h studies, arterial blood pressure and central venous pressures were monitored every 10 min for ABP between 55 and 64 mm Hg, and every 5 min for ABP below 55 mm Hg.

Bronchoalveolar lavage. The animals were euthanized with pentobarbital (120 mg/kg) and then exsanguinated by direct cardiac puncture. The trachea was isolated and cross-clamped, and the trachea, lungs, and heart were removed en bloc. The trachea and lungs were dissected free from the heart and surrounding tissue, and a sterile plastic catheter was inserted into the middle portion of the trachea and secured with silk suture. Each lung was lavaged separately. First, the right mainstem bronchus was cross-clamped, and the left lung was lavaged with five separate 15-ml aliquots of 0.9% NaCl containing 0.6 mM EDTA at 37°C. The left mainstem bronchus was then cross-clamped, and the right lung was lavaged using the same protocol. After bronchoalveolar lavage (BAL), a 1.0-ml aliquot of BAL fluid was removed and processed for leukocyte counts and quantitative cultures. The remaining BAL fluid was spun at 200 × g to pellet cells, and aliquots of the cell-free supernatant fluid were stored at −70°C.

Rabbit whole blood experiments. To evaluate the effectiveness of the treatment protocols in blocking CD14 in vivo, rabbits were treated with either 2.5 or 5.0 mg/kg anti-CD14 mAb i.v. Heparinized venous blood samples were collected before treatment and then at 1-h intervals for a total of 4 h. The whole blood samples were diluted with an equal volume of RPMI 1640 containing penicillin (100 U/ml), streptomycin (100 µg/ml), HEPES (10 mM), and L-glutamine (2 mM) (complete RPMI 1640), and 150-µl aliquots were placed in 96-well tissue culture wells (Costar, Cambridge, MA). The blood samples were washed once in PBS, and resuspended in sterile water to 10^9 bacterial cells/ml. The samples were gently mixed for 30 s and then incubated for 1 h at 37°C in 5% CO2, and then LPS (Re595; List Laboratories, Campbell, CA) was added to the appropriate wells at final concentrations of 0, 1, or 10 ng/ml. The plates were gently mixed for 30 s and then incubated for 18 h at 37°C in 5% CO2. After incubation, the samples were spun at 400 × g for 5 min. The supernatants were removed and stored frozen at −70°C. The concentration of IL-8 in each plasma sample was measured using an immunoassay specific for rabbit IL-8 (17).

Measurements performed on biological fluids

Total and differential cell counts. Total and differential cell counts were performed on whole blood and BAL fluid. The samples were diluted with trypan blue to determine viability and with crystal violet containing citric acid to measure total leukocyte concentrations using a hemocytometer. Differential cell counts for the BAL fluids were performed on cyto-centrifuge preparation stained with Diff-Quik (American Scientific Products, McGaw Park, IL.).

Measurement of nitric oxide metabolites. Plasma nitrate (NO3−) and nitrite (NO2−) levels were measured using a nitrate/nitrite colorimetric assay kit (Cayman Chemical Company, Ann Arbor, MI). Plasma aliquots (80 µl) were filtered by centrifugation for 12 min at 14,000 × g at 25°C using 30,000 MW cutoff centrifuge tubes (Amicon, Beverly, MA). A standard curve for nitrite was prepared, and then 200 µl of the assay buffer were placed in a blank well. Plasma samples (20 µl) and assay buffer (60 µl) were added to individual wells. Then 10 µl of the enzyme cofactor and 10 µl of nitrate reductase were added to each well. The wells were covered, and the plate was incubated for 3 h at room temperature. To each of the wells 50 µl of Griess component 1 (R1) and then 50 µl of Griess component 2 (R2) were added and then incubated for 10 min at room temperature. The plate was read at OD540 nm, and the absorbance from the blank well was subtracted from all other wells. Nitrite and nitrate levels were calculated according to the equation, nitrate + nitrite (µM) = (OD540 − Y intercept/slope)(200 µl/volume of sample) × dilution.

Bacterial cultures. Quantitative bacterial counts were performed by the serial dilution and pour plate method. To detect low numbers of bacteria, 5 µl of each sample were also inoculated into 5 ml of trypticoye broth, incubated overnight at 37°C, and then subcultured on sheep’s blood agar and McConkey agar plates. Bacterial colonies were identified by standard microbiological methods.

Measurement of total proteins, rabbit cytokines, and chemokines. Total protein in the BAL fluid was measured using a bicinchoninic acid assay method (Pierce, Rockford, IL.). The rabbit-specific cytokines MCP-1, and TNF-α were all measured with rabbit-specific immunoassays (17, 18). The assay sensitivities were GRO and MCP-1, 0.1 ng/ml; IL-8, 0.03 ng/ml; and TNF-α, 0.75 ng/ml.

Fluid therapy. To maintain the patency of the catheters and to provide maintenance fluids, 0.9% NaCl was infused through the venous and arterial catheters at 4 ml/h. All animals were treated with 10 ml 0.9% NaCl immediately after surgery. During the observation period, fluid boluses of 10 ml 0.9% NaCl were administered if the animals met any of the following criteria: 1) arterial blood pressure (ABP) < 75 mm Hg, or a decrease in ABP from baseline >15%; 2) central venous pressure (CVP) < −10 cm H2O; or 3) arterial pH < 7.30. The frequency of fluid bolus administration depended on the ABP: every 15 min for ABP between 65 and 75 mm Hg; every 10 min for ABP between 55 and 64 mm Hg, and every 5 min for ABP below 55 mm Hg.
Statistical analysis

Paired comparisons between groups were performed with the Wilcoxon signed rank test. Unpaired comparisons were performed with the Mann-Whitney U test. Changes over time were tested using repeated measures ANOVA. A p value of ≤ 0.05 was considered significant. Values are means ± SEM unless otherwise specified.

Results

To verify that the dose of anti-CD14 mAb used inhibited LPS responses in vivo, blood was collected (i.e., 0 h), and then rabbits were treated i.v. with either 2.5 mg/kg (A) or 5 mg/kg (B) of anti-CD14 mAb, and blood samples were collected at the times specified (x-axis). The whole blood was then incubated with the indicated concentrations of LPS ex vivo at 37°C for 18 h, and the amount of IL-8 produced was measured by ELISA. Both doses of anti-CD14 mAb were effective at blocking IL-8 production in response to LPS ex vivo.

Systemic physiological responses

Blockade of CD14 significantly improved the systemic physiological responses in rabbits with E. coli pneumonia. Animals treated with the anti-CD14 mAb had a higher MABP than the control animals (p < 0.001) (Fig. 2). This effect was detectable as early as 2 h after the induction of pneumonia and was sustained throughout the 24-h observation period. In addition, animals treated with anti-CD14 mAb required significantly less fluid to maintain a MABP above 75 mm Hg (p = 0.012) (Fig. 3). There were no significant differences in CVP, heart rate, or respiratory rate between rabbits treated with anti-CD14 mAb and rabbits treated with control mAb (data not shown).

Systemic inflammatory responses

To evaluate the systemic inflammatory response, we measured the number of white blood cells and neutrophils (PMNs) and the concentrations of TNF-α, IL-8, GRO, and MCP-1 in blood collected at 4 and 24 h (Table I). There were no differences in the total number of circulating white blood cells or PMNs at 4 or 24 h. Although GRO and MCP-1 were detected in plasma, there were no differences between the anti-CD14 and the control groups. TNF-α was not detectable in the plasma at either 4 or 24 h. IL-8 was detected in the plasma of all rabbits at 4 h, but the concentration was much lower than that detected for GRO, another CXC chemokine. There was no difference in the amount of IL-8 in the plasma of the two groups at either 4 or 24 h. None of the animals was bacteremic at any time.

To determine whether anti-CD14 mAb affected the amount of nitric oxide metabolites in the systemic circulation, nitrate and nitrite were measured in blood samples using a modification of the Greiss reaction (Fig. 4). There was a significant increase in the plasma concentrations of nitrate and nitrite over time in the control animals (p = 0.02). In contrast, nitrate and nitrite did not increase...
significantly in the plasma of rabbits treated with the anti-CD14 mAb. The total accumulation of nitrate and nitrite in plasma was significantly less in the anti-CD14 mAb-treated rabbits than in the control rabbits at 24 h (mean, 37 ± 14 µM vs 99 ± 25 µM, respectively, \( p = 0.05 \)).

**Bacterial clearance from the lungs**

Culture of the bacterial suspension instilled into the right lungs of the rabbits showed that both groups received equivalent bacterial inocula (control, 0.98 ± 0.18 \( \times 10^6 \) CFU; anti-CD14, 1.13 ± 0.21 \( \times 10^6 \) CFU). The clearance of bacteria from the lungs of anti-CD14-treated animals was significantly delayed (Fig. 5).

There was a significantly greater bacterial burden at 24 h in the anti-CD14-treated animals was significantly delayed (Fig. 5).

**Pulmonary physiologic responses**

To evaluate pulmonary gas exchange in infected rabbits, arterial blood gases were measured, and the alveolar-arterial oxygen difference (\( D(\text{A} - \text{a})\text{O}_2 \)) was calculated. The PaO\(_2\) in animals treated with anti-CD14 was significantly lower than in the control animals. This difference was greatest at 4 h (\( p = 0.02 \)) (Fig. 6A). The \( D(\text{A} - \text{a})\text{O}_2 \) was significantly higher in the anti-CD14-treated animals (\( p = 0.05 \)) (Fig. 6B).

There was a trend for increased total protein in BAL fluid of rabbits treated with the anti-CD14 mAb, but this was not statistically significant because of the variability in the control group (Fig. 7). The highest BAL protein concentrations occurred in the anti-CD14-treated rabbits.

**Pulmonary inflammatory responses**

To evaluate the pulmonary inflammatory response, the total and differential cell counts and the amount of TNF-\( \alpha \), IL-8, GRO, and MCP-1 in BAL fluid were determined at 4 and 24 h (Table II). There was no difference in the number of BAL leukocytes at either 4 or 24 h in the treatment and control groups (Table II). No significant differences were observed in any of the cytokines in the BAL fluid at 4 or 24 h in one of the rabbits treated with anti-CD14 Ab and followed for 24 h, the amount of MCP-1 in the BAL was 118 ng/ml. This value influenced the mean concentration of MCP-1 in the BAL fluid of the 24-h anti-CD14-treated rabbits.

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### Table I. White blood cells and cytokines in blood

<table>
<thead>
<tr>
<th></th>
<th>Control mAb</th>
<th>Anti-CD14 mAb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
<td>24 h</td>
</tr>
<tr>
<td>WBCs (cells/ml)</td>
<td>5.37 ± 0.8 ( \times 10^6 )</td>
<td>10.8 ± 1.0 ( \times 10^6 )</td>
</tr>
<tr>
<td>PMN (%)</td>
<td>35.42 ± 3.9</td>
<td>39.20 ± 4.7</td>
</tr>
<tr>
<td>PMN (cells/ml)</td>
<td>2.09 ± 0.5 ( \times 10^6 )</td>
<td>4.39 ± 0.9 ( \times 10^6 )</td>
</tr>
<tr>
<td>TNF-( \alpha ) (ng/ml)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>IL-8 (ng/ml)</td>
<td>0.03 ± 0.01</td>
<td>0.11 ± 0.07</td>
</tr>
<tr>
<td>GRO (ng/ml)</td>
<td>5.42 ± 1.19</td>
<td>0.41 ± 0.32</td>
</tr>
<tr>
<td>MCP-1 (ng/ml)</td>
<td>5.08 ± 1.30</td>
<td>0.64 ± 0.55</td>
</tr>
</tbody>
</table>

* WBC, white blood cells; ND, not detectable. Data are the mean ± SEM for each parameter. Data for TNF-\( \alpha \), IL-8, GRO, and MCP-1 are also presented as the number of rabbits with detectable amounts divided by the number of rabbits tested.
Discussion

The goal of this study was to determine the effect of CD14 blockade on the systemic and pulmonary responses in rabbits with Gram-negative bacterial pneumonia. We hypothesized that the blockade of CD14 would protect against the deleterious systemic responses that occur in rabbits with bacterial pneumonia. The pulmonary physiological and inflammatory responses were monitored to determine whether the blockade of CD14 affected lung function or pulmonary host defenses.

In human patients with Gram-negative sepsis, the deleterious systemic inflammatory response is the result of tissue infection rather than blood stream infection. In two previous studies, CD14 blockade was tested in animal models in which the systemic inflammatory response was the result of direct activation of systemic responses with i.v. LPS (14, 15). The present study addresses the role of CD14 blockade in an animal model of tissue infection that is similar to the events that occur in humans with Gram-negative sepsis associated with pneumonia. This model allowed us to evaluate the systemic effects of CD14 blockade and also to determine the local effects on gas exchange and host defenses at the site of infection in the lungs.

The data show that pretreatment with anti-CD14 mAb protected rabbits against sustained hypotension and reduced the i.v. fluid requirements of rabbits with *E. coli* pneumonia. In addition, the anti-CD14 mAb reduced the concentrations of nitrate and nitrite in the blood. This suggests that the mechanism of improvement in the hemodynamic responses involves blockade of NO production in the vasculature. Although the blockade of CD14 protected the systemic compartment, rabbits treated with anti-CD14 mAb had significantly worse hypoxemia, delayed intrapulmonary bacterial clearance, and a trend toward higher total protein in BAL fluid recovered from the instilled lung at 24 h.

![FIGURE 6. Arterial PaO₂ (A) and alveolar-arterial oxygen difference (B) in rabbits with *E. coli* pneumonia. In rabbits treated with anti-CD14 mAb, the PaO₂ was lower and the D(A – a)O₂ was higher, indicating worse gas exchange in this group. Values are means ± SEM; * is = 6 rabbits/group. * p < 0.02; ** p < 0.05.](image)

**FIGURE 7.** Total protein in the BAL fluid recovered from the instilled lung at 24 h after the intratracheal instillation of *E. coli* into the lungs of rabbits. Values are the individual data points; *n* = 6 rabbits/group. Bars, median values.

### Table II. Cells and cytokines in BAL fluid collected from the instilled lung

<table>
<thead>
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<th></th>
<th>Control mAb</th>
<th>Anti-CD14 mAb</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>4 h</td>
<td>24 h</td>
</tr>
<tr>
<td>PMN (%)</td>
<td>37.0 ± 10.9</td>
<td>54.0 ± 7.5</td>
</tr>
<tr>
<td>PMN (total cells)</td>
<td>1.68 ± 0.63 × 10⁷</td>
<td>2.83 ± 0.72 × 10⁷</td>
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<tr>
<td>AM (%)</td>
<td>62 ± 10.1</td>
<td>45.3 ± 7.5</td>
</tr>
<tr>
<td>AM (total cells)</td>
<td>2.2 ± 0.3 × 10⁷</td>
<td>1.95 ± 0.28 × 10⁷</td>
</tr>
<tr>
<td>TNF-α (ng/ml)</td>
<td>44.06 ± 8.58</td>
<td>3.03 ± 0.79</td>
</tr>
<tr>
<td></td>
<td>6/6</td>
<td>2/6</td>
</tr>
<tr>
<td>IL-8 (ng/ml)</td>
<td>4.33 ± 0.96</td>
<td>0.25 ± 0.19</td>
</tr>
<tr>
<td></td>
<td>6/6</td>
<td>2/6</td>
</tr>
<tr>
<td>GRO (ng/ml)</td>
<td>1.92 ± 0.24</td>
<td>0.48 ± 0.25</td>
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<tr>
<td></td>
<td>6/6</td>
<td>3/6</td>
</tr>
<tr>
<td>MCP-1 (ng/ml)</td>
<td>13.15 ± 2.79</td>
<td>15.88 ± 7.46</td>
</tr>
<tr>
<td></td>
<td>6/6</td>
<td>5/5</td>
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</table>

*ND, not detectable; AM, alveolar macrophage. Data are the mean ± SEM for each parameter. The data for TNF-α, IL-8, GRO, and MCP-1 are also presented as the number of rabbits with detectable amounts divided by the number of rabbits tested.
The deleterious systemic responses to Gram-negative pneumonia are a consequence of the activation of the innate host responses and amplification of critical inflammatory pathways. Pretreatment with anti-CD14 mAb was very effective at preventing the hypotension that occurred in rabbits after the intratracheal inoculation of E. coli. The effect of CD14 blockade on MABP was even more dramatic when fluid administration is considered. Not only did the rabbits treated with anti-CD14 have a significant increase in their MABP, they maintained this increase in MABP with less fluid replacement than rabbits treated with the control Ab. The ability of CD14 blockade to protect against systemic hypotension has been reported in nonhuman primates treated with a continuous infusion of LPS (14), and rabbits after intermittent doses of LPS (15). Herein we provide the first evidence that CD14 blockade protects against systemic consequences of localized tissue infection.

To determine the mechanisms responsible for the improved hemodynamic responses, we first evaluated the systemic inflammatory response (Table I). No differences in systemic cytokine responses occurred in the rabbits treated with anti-CD14 mAb. This is in contrast to a previous study in which primates challenged with i.v. LPS and treated with a blocking anti-CD14 mAb showed improved hemodynamics and decreased systemic production of proinflammatory cytokines such as TNF-α, IL-6, and IL-8 (14). Our findings suggest that in this model, mechanisms other than decreased systemic production of proinflammatory cytokines are responsible for the improved MABP.

To investigate an alternative mechanism for the improved hemodynamic responses, we measured the NO-derived products, nitrate and nitrite, in plasma. The anti-CD14-treated rabbits had less accumulation of NO-derived products in plasma at 24 h (Fig. 4). Nitric oxide is an important mediator of the hypotension that occurs in endotoxemic animals and patients with sepsis and septic shock (reviewed in Refs. 20 and 21). The expression of the inducible form of nitric oxide synthase, the most likely source of increased nitric oxide production during endotoxemia and sepsis, is regulated in part through CD14 (8, 22). Although more than one mechanism is likely to be responsible for the improved MABP seen in rabbits treated with anti-CD14 mAb, the data support the interpretation that a reduction in either the induction or the activity of inducible NO synthases accounts for a substantial part of the improved hemodynamics when CD14-dependent pathways are blocked.

Whereas the blockade of CD14 improved hemodynamic responses in the systemic compartment, it was associated with impaired pulmonary responses to the tissue infection in the lungs. An increased bacterial burden in the lungs of rabbits treated with anti-CD14 mAb suggests that CD14 blockade impairs pulmonary host defenses and impairs local bacterial clearance (Fig. 5). To evaluate whether the anti-CD14 mAb affected the pulmonary inflammatory response, we compared the recovery of PMNs as well as the cytokines TNF-α, IL-8, GRO, and MCP-1 in BAL fluid recovered from the instilled lung at 4 and 24 h (Table II). There were no significant differences in either the pulmonary recruitment of PMNs or the production of the inflammatory cytokines. This suggests that the defect in pulmonary host defenses after CD14 blockade was not the result of impaired production of these cytokines or a defect in the pulmonary recruitment of PMNs. It is possible that anti-CD14 mAb affects the interactions between bacteria and leukocytes, given that phagocytosis of Gram-negative bacteria occurs via a CD14-dependent mechanism in vitro (23).

The treatment of rabbits with CD14 Ab was also associated with decreased pulmonary gas exchange (Fig. 6) and a trend toward higher total protein in BAL fluid recovered from the instilled lung (Fig. 7). The impaired pulmonary function probably reflects the inability of pulmonary host defenses to efficiently clear bacteria from the lungs after CD14 blockade. These findings contrast with previous studies in which CD14 blockade was shown to protect against organ injury following LPS treatment in primates and rabbits (14, 15). The different outcome in these studies shows the importance of evaluating novel therapeutic strategies for sepsis and septic shock in animal models of tissue infection.

Another important finding in this study is that the effect of CD14 blockade is compartmentalized. In the systemic compartment, CD14 blockade is beneficial, resulting in improved hemodynamics and a decreased requirement for i.v. fluid support. In contrast, in the primary compartment where tissue infection occurs (i.e., lungs), CD14 blockade is detrimental and impairs bacterial clearance. This observation is important and suggests that therapeutic strategies for sepsis and septic shock should involve a combinatorial approach to treat both the primary and systemic compartments. Such an approach would require strategies to improve host defense at the site of tissue infection to effectively eliminate the primary source of infection. At the same time, systemic treatment strategies are needed that down-regulate the inflammatory responses in the systemic compartment to minimize the deleterious consequences of tissue infection.

A limitation to this study is that only one pathogen (i.e., E. coli) was evaluated. This organism was isolated from a patient with biliary sepsis and causes pneumonia in rabbits (16). Although the results do not bear directly on other Gram-negative and Gram-positive pathogens, the CD14 pathway is involved in the host response to Gram-negative, Gram-positive, and fungal organisms (1), probably via interactions with specific Toll-like receptors (24). This suggests that the anti-CD14 strategy will be relevant for other pathogens in addition to E. coli but further studies are needed.

In conclusion, the data show that systemic CD14 blockade limits the systemic consequences of Gram-negative bacterial pneumonia. The data also show the importance of CD14-dependent pathways in the clearance of bacteria at the primary site of infection. An anti-CD14 strategy may be useful to limit systemic effects of local infections, but other measures are needed to enhance bacterial elimination at the primary site of infection.

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References


