IC14, an Anti-CD14 Antibody, Inhibits Endotoxin-Mediated Symptoms and Inflammatory Responses in Humans


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IC14, an Anti-CD14 Antibody, Inhibits Endotoxin-Mediated Symptoms and Inflammatory Responses in Humans


CD14 is a receptor for cell wall components of Gram-negative and Gram-positive bacteria that has been implicated in the initiation of the inflammatory response to sepsis. To determine the role of CD14 in LPS-induced effects in humans, 16 healthy subjects received an i.v. injection of LPS (4 ng/kg) preceded (~2 h) by i.v. IC14, a recombinant chimeric mAb against human CD14, at a dose of 1 mg/kg over 1 h, or placebo. In subjects receiving IC14, saturation of CD14 on circulating monocytes and granulocytes was >90% at the time of LPS injection. IC14 attenuated LPS-induced clinical symptoms and strongly inhibited LPS-induced proinflammatory cytokine release, while only delaying the release of the anti-inflammatory cytokines soluble TNF receptor type I and IL-1 receptor antagonist. IC14 also inhibited leukocyte activation, but more modestly reduced endothelial cell activation and the acute phase protein response. The capacity of circulating monocytes and granulocytes to phagocyte Escherichia coli was only marginally reduced after infusion of IC14. These data provide the first proof of principle that blockade of CD14 is associated with reduced LPS responsiveness in humans in vivo. The Journal of Immunology, 2001, 166: 3599–3605.

Materials and Methods

Study design

Sixteen healthy, male volunteers (mean age 23, range 20–33 years) were enrolled in this double-blind, randomized placebo-controlled trial. Written informed consent was obtained from all volunteers, and the study was approved by the institutional scientific and ethics committees. Medical history, physical examination, routine laboratory examination, and electrocardiogram were all normal. Tests for HIV infection and hepatitis B and C were negative. The participants did not smoke, use any medication, have any febrile illness in the month preceding the study, and never received mAb therapy before. The subjects fasted overnight before LPS administration. On the study day, blood was collected from i.v. cannulas, and LPS, IC14, or placebo was administered i.v. Eight of the volunteers received IC14, and eight were given placebo. All participants were challenged with LPS 1 h after the end of the IC14 or placebo infusion.

The study drug, IC14, was supplied by ICOS (Bothell, WA). IC14 is a recombinant chimeric (murine/human) mAb recognizing human CD14. The murine parent is an Ab designated 28C5 (10, 11). It is secreted from the Chinese hamster ovary cells as an L2 H2 molgus monkey. Pretreatment with anti-CD14 Abs derived from the same murine component 28C5 (10) as IC14 prevented LPS-induced hypotension and reduced plasma cytokine levels (11). However, at present, evidence that CD14 mediates LPS effects in humans in vivo is lacking. Therefore, in this study, we sought to determine the effect of IC14, a newly developed recombinant chimeric mAb directed against CD14, on a variety of inflammatory responses in healthy humans injected with a single dose of LPS.
min during the first 2 h after LPS challenge and hourly thereafter. Body temperature was measured orally. Symptoms were registered throughout the study period using a symptom checklist and were scored by incidence and severity (0 = absent, 1 = weak, 2 = moderate, and 3 = severe).

**Assays**

Blood was obtained before the infusion of IC14 or placebo (t = -2 h), at the end of the infusion of IC14 or placebo (t = -1 h), immediately before LPS injection (t = 0 h), and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, and 21 h thereafter. All blood samples (except samples for flow cytometry) were centrifuged at 2000 × g for 20 min at 4°C, and plasma was stored at −20°C until assays were performed.

IC14 and soluble CD14 (sCD14) concentrations were measured in serum, and all other assays were conducted in EDTA-anticoagulated plasma. IC14 levels were measured by an enzyme immunoassay. This assay uses recombinant monomeric sCD14 immobilized on polystyrene plates to capture IC14 from serum. The captured IC14 is detected using a murine anti-human IgG4 Ab conjugated with HRP. IC14 is used as standard. The limit of detection of this assay is 6 ng/ml. sCD14 was measured by enzyme immunoassay using a murine anti-CD14 mAb (2.3 G4C) as capturing and HRP-conjugated human IgG4 Ab as detection reagent. IC14 levels were calculated from a calibration curve, which was prepared using purified recombinant CD14. The assay does not discriminate between free sCD14 and sCD14 complexed with IC14.

Cytokines were determined by specific ELISAs according to the instructions of the manufacturers (with detection limit): TNF-α (TNF; 1.4 pg/ml), IL-6 (0.6 pg/ml), IL-10 (1.2 pg/ml) (all obtained from the Central Laboratory of The Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands), sTNF receptor type 1 (70 pg/ml), and IL-1 receptor antagonist (IL-1RA, 204 pg/ml) (both obtained from R&D Systems, Abingdon, U.K.). Elastase-α-, α1-antitrypsin and lactoferrin levels were measured by specific RIA as described before (12). Ristocetin-induced platelet aggregation of von Willebrand factor was measured as described before (13).

Soluble E-selectin and LPS binding protein concentrations were measured by ELISA exactly as described before (detection limits 57 and 50 pg/ml, respectively) (14, 15). The reagents for this ELISA were provided by W. Buurman (University of Maastricht, Maastricht, The Netherlands). Leukocyte counts and differentials were assessed by a STKR Coulter counter (Coulter, Bedfordshire, U.K.).

Levels of the acute phase proteins albumin, α1-antitrypsin and acid glycoprotein, C-reactive protein, and serum amyloid A were all measured with nephelometric methods on the Behring nephelometer II (Dade Behring, Leusden, The Netherlands). Reagents, standards, and controls were also supplied by Dade Behring. Levels of α1-antichymotrypsin were also determined using the Behring nephelometer II, according to the protocol of ITK Diagnostics (Uithoorn, The Netherlands) using DukO reagents and standard and controls from ITK Diagnostics.

**Flow cytometric analysis**

Saturation of CD14 receptors on circulating monocytes and granulocytes was quantitated in acid-citrate-dextrose-anticoagulated blood at −2, 0, 1, 2, 4, 6, 10, and 21 h relative to LPS injection. All blood samples were immediately placed on ice, and an equal amount of 1% paraformaldehyde solution was added. After lysis of the erythrocytes with isotonic NH4 Cl solution (155 mmol/L NH4Cl, 10 mmol/L KHCO3, 0.1 mmol/L EDTA, pH 7.4), samples were centrifuged at 400 g for 5 min. The remaining cells were washed twice in PBS with 2% w/v normal calf serum and subsequently fixed in PBS, containing 1% w/v BSA, 0.3 mmol/L EDTA, 0.01% w/v sodium azide, and 0.1% paraformaldehyde (final concentration 5 × 10−6 cells/ml). All subsequent procedures were performed at 4°C.

Resident IC14 was detected with FITC-labeled mouse anti-human IgG4 Ab (HP6023; Southern Biotechnology Associates, Birmingham, AL). Total CD14 was determined by adding IC14 in vitro and detecting with HP6023. All analyses were also conducted with the appropriate control Ab (murine FITC-labeled IgG3; Southern Biotechnology Associates). For each test at least 105 cells were analyzed using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Granulocytes and monocytes were identified by forward and side-angle scatter gating. Percentage of saturation was calculated by dividing mean cell fluorescence × 570 nm (MCF) of resident IC14 by the MCF of total CD14, after subtracting the MCF of the IgG3 control from each value.

Expression of CD11b and CD66 on circulating granulocytes was determined. Blood obtained at −2, 0, 2, 4, 6, and 21 h. All blood samples were immediately placed on ice and processed for FACS analysis as described above. All procedures were performed at 4°C. The following Abs were used: FITC-labeled mouse anti-human CD11b and FITC-labeled mouse anti-human CD66 (both obtained from CLB, Amsterdam, The Netherlands). All FACS reagents were used in concentrations as recommended by the manufacturer, and all analyses were conducted with the appropriate control Ab (murine FITC-labeled IgG1; Becton Dickinson, Rutherford, NJ). For each test at least 105 cells were counted. MCF of granulocytes was determined as described above. Data are presented as the difference (linear units) between MCF intensities of specifically and non-specifically stained cells.

**Phagocytosis assay**

Heparinized blood was drawn immediately before and 2 h after the start of the IC14 or placebo infusion (i.e., immediately before LPS injection). Blood was kept at room temperature until use. Phagocytosis by blood monocytes and granulocytes was determined with a commercially available assay according to the instructions of the manufacturer (Phagotest; Orpegen Pharma, Heidelberg, Germany). Briefly, 100 μl of blood was added to 20 μl of FITC-labeled opsonized E. coli and incubated at 37°C for exactly 10 min. Control samples were kept on ice. Then, to suppress fluorescence by membrane-bound bacteria, 100 μl of ice-cold quenching solution (Phagotest) was added, and to stop opsonization, the cells were washed three times with ice-cold wash buffer. Thereafter, lysis buffer was added to the RBC for 20 min. Cells were washed, and DNA staining solution (propidium iodide) was added. Cells were analyzed within 1 h on a FACSscan flow cytometer. Bacteria were excluded by DNA content, stained with propidium iodide, and DNA staining solution. Results are expressed as the percentage of gated cells positive or as MCF.

**Calculations and statistical analysis**

Values are given as mean ± SEM. Pharmacokinetic analyses were performed using standard analysis of area under the serum concentration vs time curve and area under the first moment curve. The terminal elimination half-life was calculated as ln2/kel, where kel is the rate constant for the terminal decline in serum IC14 concentration estimated by log-linear regression. Area under the serum concentration vs time curve and area under the first moment curve were used to estimate clearance, serum mean residence time, and terminal elimination phase volume of distribution. Differences between IC14 and placebo treatments were tested by ANOVA for repeated measures using SPSS for Windows (SPSS, Chicago, IL). Changes of parameters in time were tested using one-way ANOVA. A two-sided p value <0.05 was considered significant. Two sample comparisons were performed using the Student t test. A p value <0.05 was considered significant.

**Results**

**Pharmacokinetics of IC14**

Intravenous infusion of IC14 over 1 h, starting 2 h before LPS administration, resulted in peak IC14 serum concentrations 1 h after the completion of the infusion, i.e., at the time of LPS injection (Fig. 1, upper panel). Table I shows a summary of pharmacokinetic parameters for IC14 in serum.

**Saturation of CD14 on monocytes and granulocytes**

Infusion of IC14 was associated with a prolonged saturation of both monocyte and granulocyte CD14 (Fig. 1, lower panels). On average, no differences were found between the peak saturation levels of either cell type. Monocyte CD14 saturation peaked 1 h after the completion of IC14 infusion (94.9 ± 1.4%), remaining virtually unchanged for the subsequent 6 h. At 24 h after LPS injection, monocyte CD14 saturation still was 75.1 ± 5.0%. Hence, administration of IC14 was associated with a strong and sustained saturation of CD14 on circulating monocytes and granulocytes.

**Clinical symptoms and vital signs**

LPS administration elicited flu-like symptoms such as fever, chills, headache, nausea, vomiting, myalgia, and backache; all volunteers were symptom-free within 24 h. Infusion of IC14 was not associated with significant adverse effects. Administration of IC14 modestly inhibited the febrile response to LPS. Peak temperatures were
registered 4–5 h after LPS injection (placebo: 38.1 ± 0.19°C; IC14: 37.5 ± 0.15°C; p < 0.05). In addition, IC14 treatment attenuated all LPS-induced clinical symptoms and delayed the time to onset of maximal severity (Table II).

**Cytokines**

LPS administration resulted in transient increases in the plasma levels of TNF (peak at 1.5 h: 1512 ± 463 pg/ml), IL-6 (3 h: 4583 ± 815 pg/ml), IL-10 (3 h: 288 ± 82 pg/ml), sTNF receptor type I (2 h: 3337 ± 135 pg/ml), and IL-1RA (4 h: 33,391 ± 4,929 pg/ml) (all p < 0.05 vs time). IC14 treatment strongly reduced the LPS-induced release of TNF (peak: 43 ± 10 pg/ml, p < 0.001), IL-6 (560 ± 137 pg/ml, p < 0.001), and IL-10 (59 ± 6 pg/ml, p < 0.001) (Fig. 2, upper panels). IC14 only delayed LPS-induced release of the antagonistic members of the cytokine network, sTNF receptor type I secretion, and IL-1RA release (p < 0.05 for both) (Fig. 2, lower panels).

**Granulocyte responses**

Intravenous LPS caused an initial decrease in neutrophil counts (from 2.9 ± 0.2 to 1.4 ± 0.3 × 10⁹/L at 1 h) followed by a neutrophilia (8 h: 17.4 ± 1.2 × 10⁹/L, p < 0.001 vs time). These changes were accompanied by neutrophil activation as reflected by increases in the plasma concentrations of the neutrophil degranulation products elastase-α₁-antitrypsin complexes (from 47.4 ± 1.7 to 209.1 ± 18.9 ng/ml at 3 h, p < 0.001 vs time) and lactoferrin (from 105.1 ± 28.2 to 487.9 ± 85.1 ng/ml at 4 h, p < 0.001 vs time). In addition, LPS induced an up-regulation of the activation markers CD11b and CD66 on granulocytes, peaking at 6 h (MCF: CD11b from 1112 ± 147 to 4210 ± 497, p < 0.05 vs time; CD66 from 120 ± 17 to 364 ± 57, p < 0.05 vs time). IC14 inhibited all granulocyte responses measured. IC14 blunted and delayed the early neutropenia and reduced the subsequent neutrophilia (Fig. 3, upper panel; p < 0.001). The nadir in neutrophil counts occurred only after 2 h (2.7 ± 0.4 × 10⁹/L); maximal neutrophilia was found after 6 h (9.8 ± 0.7 × 10⁹/L). Moreover, IC14 attenuated neutrophil activation, as indicated by abrogated increases in the plasma levels of elastase-α₁-antitrypsin complexes (peak 133.1 ± 15.1 ng/ml, p < 0.001) and lactoferrin (peak 150.8 ± 30.3 ng/ml, p < 0.001) (Fig. 3, upper panels). Furthermore, IC14 reduced the up-regulation of granulocyte CD11b (peak 2714 ± 340, p < 0.001) and CD66 (peak 227 ± 37, p < 0.001) (Fig. 3, lower panels).

**sCD14 levels**

LPS infusion resulted in an increase of sCD14 concentrations (from 1.42 ± 0.12 to 1.92 ± 0.36 μg/ml at 24 h, p < 0.001 vs time). IC14 administration was associated with an earlier and more pronounced rise in IC14 levels (peak 3.46 ± 0.81 mg/L at 1 h) relative to LPS administration (from 2.9 to 1 h relative to LPS administration). Data are derived from eight subjects.

**Endothelial cell activation**

LPS administration resulted in endothelial cell activation as evidenced by increases in the plasma concentrations of von Willebrand factor (from 87.5 ± 15.8% to 360.1 ± 39.7% at 6 h, p < 0.001 vs time) and soluble E-selectin (from 77.5 ± 54.1 to 357.9 ± 68.8 ng/ml at 6 h) (p < 0.001 vs time). Treatment with IC14 reduced the increases in both markers of endothelial cell activation. Peak von Willebrand levels in subjects treated with IC14 were 240.0 ± 30.2% (p < 0.05) (Fig. 5, middle panel), peak soluble E-selectin concentrations were 224.8 ± 51.1 ng/ml (non-significant) (Fig. 5, lower panel).

**Acute phase proteins**

LPS elicited an acute phase response as reflected by increases in the plasma concentrations of C-reactive protein, serum amyloid A, α₁-antitrypsin, α₁-antichymotrypsin, α₁-acid glycoprotein, and LPS binding protein (Table III), all p < 0.05. The release of albumin was reduced over time (p < 0.05). IC14 attenuated the increase in serum amyloid A and C-reactive protein levels (p < 0.05) but only moderately influenced the change in other acute phase proteins (non-significant, Table III).

### Table I. Pharmacokinetic parameters of IC14 in serum after single i.v. administration followed by single dose i.v. administration of endotoxin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SEM</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax (mg/L)</td>
<td>10.81 ± 0.45</td>
<td>9.42–12.64</td>
</tr>
<tr>
<td>Area under the serum concentration vs time curve (mg*h/ml)²</td>
<td>95.46 ± 7.95</td>
<td>69.41–139.14</td>
</tr>
<tr>
<td>t₁/₂ (h)</td>
<td>6.06 ± 0.26</td>
<td>5.3–7.5</td>
</tr>
<tr>
<td>Mean residence time total (h)</td>
<td>8.94 ± 0.40</td>
<td>7.8–11.27</td>
</tr>
<tr>
<td>Clearance (L/h/kg)</td>
<td>0.011 ± 0.001</td>
<td>0.007–0.014</td>
</tr>
<tr>
<td>Terminal elimination phase volume of distribution (L/kg)</td>
<td>0.095 ± 0.006</td>
<td>0.078–0.124</td>
</tr>
</tbody>
</table>

*IC14 (1 mg/kg) was infused over 1 h, from −2 to −1 h relative to LPS administration. Data are derived from eight subjects.

² From 0 to infinity.
Phagocytosis

CD14 is involved in the phagocytosis of bacteria by various cell types (16, 17). Hence, blocking CD14 may have an undesired inhibitory effect on the capacity of immunocompetent cells to phagocytose bacteria. Therefore, we were interested to determine the effect of IC14 infusion on the phagocytosis of FITC-labeled \textit{E. coli} by circulating monocytes and granulocytes. Infusion of placebo did not influence phagocytosis (Table IV). Infusion of IC14 was associated with a modest reduction in the capacity of monocytes and granulocytes to phagocytose \textit{E. coli}, when phagocytosis was expressed as MCF (reflecting the relative number of bacteria phagocytosed per cell; Table IV). IC14 did not change the number of monocytes or granulocytes that phagocytosed \textit{E. coli} (Table IV).

Discussion

Sepsis is associated with excessive activation of a number of host mediator systems, including the cytokine network, leukocytes, and the vascular endothelium, each of which can contribute to the development of tissue injury (18, 19). Binding to CD14 is considered to be the common pathway to induction of the innate immune response to a variety of microbial pathogens. Studies have shown that absence or blocking of CD14 protected mice, rabbits, and monkeys from toxicity associated with LPS administration (9, 11, 20), whereas mice with overexpression of CD14 had an increased susceptibility to LPS-induced shock (21). This study is the first to describe the effect of an anti-CD14 mAb in humans. Treatment with IC14 reduced clinical signs and symptoms induced by LPS such as fever, headache, and vomiting. In this respect, it should be noted that administration of E5531, a lipid A analog functioning as an LPS antagonist, also led to a decrease in clinical symptoms (22) but that in previous studies neither complete neutralization of endogenous TNF by infusion of a recombinant TNF receptor fusion protein (23, 24) nor treatment with the anti-inflammatory cytokine IL-10 (25) or reconstituted high density lipoprotein (26) influenced LPS-induced clinical symptoms. Moreover, inflammatory responses such as cytokine release and granulocyte activation were strongly inhibited by IC14.

In accordance with in vitro and animal data, IC14 inhibited the proinflammatory cytokine release induced by i.v. LPS nearly completely (4, 7, 11). However, the release of the anti-inflammatory cytokine type I-sTNF receptor and IL-1RA was only delayed.

<table>
<thead>
<tr>
<th>TABLE II. Effect of IC14 on incidence, mean severity, and time of peaking of endotoxin-induced clinical symptoms (^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptoms</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Fever</td>
</tr>
<tr>
<td>Headache</td>
</tr>
<tr>
<td>Chills</td>
</tr>
<tr>
<td>Myalgia</td>
</tr>
<tr>
<td>Backache</td>
</tr>
<tr>
<td>Nausea</td>
</tr>
<tr>
<td>Vomiting</td>
</tr>
</tbody>
</table>

\(^a\) The total number of volunteers suffering from a specific symptom, mean maximum severity (0 = absent, 1 = mild, 2 = moderate, 3 = severe) and time of maximum severity (hours, relative to LPS injection) are summarized.

\(^b\) Mean severity is temperature in °C.

FIGURE 2. Mean (±SE) circulating concentrations of TNF, IL-6, and IL-10 (upper panels) and sTNFR1 and IL-1RA (lower panels). IC14 (1 mg/kg; ◦) or placebo (●) was given i.v. from -2 to -1 h relative to endotoxin injection (4 ng/kg; t = 0 h). The \(p\) value indicates difference between IC14 and placebo.
study did not address the mechanism(s) underlying these differential effects of IC14 on agonistic and antagonistic cytokine response. We previously found that reconstituted high density lipoprotein, which, like IC14, interferes with the bioavailability of LPS, also had a more profound inhibitory effect on TNF release than on release of sTNF receptors and IL-1RA during human endotoxemia (26). These data suggest that either CD14-independent pathways are involved in sTNF receptor and IL-1RA release elicited by i.v. LPS and/or that the threshold dose at which LPS triggers these anti-inflammatory mediators is lower than the threshold LPS dose that induces TNF release. Similarly, LPS-induced clinical symptoms, which were not completely prevented by IC14, may use CD14-independent mechanisms and/or require lower LPS concentrations. In support of the former possibility are earlier findings of CD14-independent pathways for LPS and lipoarabinomannan-induced activation of monocytes in vitro (27, 28) and for LPS in mice in vivo (20).

Although monocytes are considered the main producers of cytokines during endotoxemia, granulocytes have been implicated in the pathogenesis of tissue injury during overwhelming sepsis by virtue of their capacity to release potent proteinases and oxygen radicals (29). In vitro, blocking CD14 inhibits granulocyte responses induced by LPS (30). In rabbits, anti-CD14 treatment attenuated both the initial leukocytopenia and the subsequent leukocytosis after i.v. administration of LPS (9). In accordance, IC14 strongly reduced leukocyte responses elicited by i.v. LPS in healthy humans. IC14 not only inhibited the LPS-induced early

![Graphs showing neutrophil counts and degranulation markers](image1)

![Graph showing circulating concentrations of sCD14](image2)

![Graph showing endothelial activation](image3)
IC14 INHIBITS LPS-MEDIATED INFLAMMATORY RESPONSES IN HUMANS

Table III. Effect of IC14 on endotoxin-induced acute phase proteins

<table>
<thead>
<tr>
<th>Protein</th>
<th>Placebo and Endotoxin (n = 8)</th>
<th>IC14 and Endotoxin (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Time&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>−2 h</td>
<td>21 h&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>C-reactive protein (mg/L)</td>
<td>0.6 ± 0.2</td>
<td>56.2 ± 7.2</td>
</tr>
<tr>
<td>Serum amyloid A (mg/L)</td>
<td>2.9 ± 0.2</td>
<td>194.7 ± 16.5</td>
</tr>
<tr>
<td>Albumin (g/L)</td>
<td>44 ± 1</td>
<td>39.8 ± 1.5</td>
</tr>
<tr>
<td>α&lt;sub&gt;1&lt;/sub&gt;-antitrypsin (g/L)</td>
<td>1.16 ± 0.03</td>
<td>1.3 ± 0.04</td>
</tr>
<tr>
<td>α&lt;sub&gt;1&lt;/sub&gt;-antichymotrypsin (g/L)</td>
<td>0.32 ± 0.01</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>α&lt;sub&gt;1&lt;/sub&gt;-acid glycoprotein (g/L)</td>
<td>0.64 ± 0.04</td>
<td>0.79 ± 0.04</td>
</tr>
<tr>
<td>LPS binding protein (ng/ml)</td>
<td>3.3 ± 0.9</td>
<td>17.6 ± 5.1</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean values ± SE of acute phase proteins. IC14 or placebo was infused from −2 to −1 h relative to endotoxin injection (1 mg/kg). ANOVA was performed using results from time points −2, 4, 6, 8, 10, and 21 h; only results at time points −2 and 21 h are shown.

<sup>b</sup> Time in hours relative to LPS administration.

<sup>c</sup> p < 0.05 for 21 h vs −2 h for all proteins.

<sup>d</sup> p < 0.05 for IC14 vs placebo at 21 h.

neutropenia and later neutrophilia, but also the activation of neutrophils, as monitored by the plasma concentrations of elastase-α<sub>1</sub>-antitrypsin complexes and lactoferrin, indicative of neutrophil degranulation (12, 31), and the expression of CD11b and CD66 on the surface of circulating granulocytes. The vascular endothelium plays a pivotal role in the inflammatory reaction to infection (18, 19). Endothelial cells lack CD14 on their surface. Instead, endothelial cells may be stimulated via LPS-sCD14 complexes or by LPS indirectly, via products of myeloid cells, i.e., cytokines (2, 10, 32). Increased levels of sCD14 have been found in sepsis patients and were associated with mortality (33, 34). In addition, sCD14 levels were increased at the site of inflammation in meningitis (35) and lung (36). In accordance with these results we found that endotoxin induced release of sCD14. However, sCD14 levels were twice as high after IC14 treatment. Because the capture ELISA for sCD14 does not discriminate between sCD14-IC14 complexes and monomeric sCD14, we consider it highly likely that the elevated sCD14 levels in IC14-treated subjects reflect sCD14-IC14 complexes. The fact that IC14 treatment was associated with an inhibition of LPS-induced endothelial cell activation as reflected by attenuated increase in the plasma levels of von Willebrand factor suggests that the sCD14-IC14 complexes were not biologically active. Furthermore, the fact that IC14 inhibits the interaction between cell surface CD14 and LPS would also imply that this Ab inhibits the interactions between sCD14 and LPS. It is difficult to directly investigate the biological activity of sCD14-IC14 complexes in blood samples obtained from our volunteers because free sCD14 can either inhibit or enhance LPS responses, depending on the cell type studied (34, 37–39), and because serum obtained from normal subjects after LPS injection by itself inhibits cytokine production (40).

The acute phase response is an important pathophysiologic phenomenon that is considered to replace the normal homeostatic mechanism with new set points that presumably contribute to defensive or adaptive capabilities (41). IC14 administration modestly attenuated the LPS-induced changes in serum amyloid A- and C-reactive protein, whereas the levels of the other acute phase proteins were not significantly influenced by IC14. In transgenic CD14-deficient mice, no change was found in the LPS-induced increase in serum amyloid A, LBP, fibrinogen, and ceruloplasmin (42). In C3H/HeJ mice, which are deficient for TLR4, the LPS-induced increase in serum amyloid A was completely abolished (42). Taken together, the induction of acute phase proteins seems to be partly CD14 independent and may be dependent on another TLR4 using signal pathway.

It should be noted that our results do not discriminate between mechanisms involving direct effects of LPS on target cells (via other CD14-independent pathways) or secondary effects of LPS-induced release of mediators occurring via CD14-independent mechanisms. The marked reduction of LPS-induced TNF release by IC14 may have contributed to the inhibition of the activation of other inflammatory pathways considering that neutralization of endogenous TNF activity inhibited these responses in endotoxemic humans (43, 44).

The results presented herein provide the first proof of principle in humans that an anti-CD14 Ab can diminish inflammatory responses induced by i.v. LPS. However, it should be noted that the role of CD14 in bacterial clearance, which, given the nature of our

Table IV. Effect of IC14 on phagocytosis of FITC-labeled E. coli by monocytes and granulocytes

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Placebo and Endotoxin (n = 8)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>IC14 and Endotoxin (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time&lt;sup&gt;b&lt;/sup&gt;</td>
<td>Time&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Monocytes</td>
<td>% phagocytosis</td>
<td>48.4 ± 7.9</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>960 ± 145</td>
</tr>
<tr>
<td>Granulocytes</td>
<td>% phagocytosis</td>
<td>68.8 ± 7.5</td>
</tr>
<tr>
<td></td>
<td>MCF</td>
<td>1373 ± 208</td>
</tr>
</tbody>
</table>

<sup>a</sup> Mean value ± SEM are given.

<sup>b</sup> t = 0 h, before IC14 administration; t = 2 h, 2 h after start of the 1-h infusion of IC14, prior to LPS.

<sup>c</sup> p < 0.05 for t = 2 h vs t = 0 h.
experiments in healthy humans, could not be investigated in this study, is not unequivocal. After infection with *E. coli*, CD14-deficient mice had a greatly reduced bacteremia and bacterial load in lungs compared with wild-type mice (20), whereas after infection with *S. aureus* bacterial load in liver, spleen, and blood were similar (45). Rabbits pretreated with anti-CD14 Abs before pulmonary inoculation with *E. coli* had increased bacterial outgrowth in the lung compared with control animals, but systemic inflammatory responses were prevented (46). Whether blocking CD14 on cells at the site of an infection significantly attenuates the induction of the human innate immune response to invading bacteria, thereby impairing antibacterial host defense, remains to be established. In our study, we were reassured to find that IC14 only modestly influenced phagocytosis of *E. coli* by monocytes and granulocytes, indicating that in vivo CD14 may not be indispensable for an adequate ing of microorganisms by cells that are part of the first line of defense. Our results indicate that blocking CD14 reduces LPS responsiveness in humans in vivo, and suggests that blocking IC14 might be of benefit in patients with evidence of systemic inflammation due to Gram-negative infection.

References