Lipopolysaccharide-Coated Erythrocytes Activate Human Neutrophils Via CD14 While Subsequent Binding Is Through CD11b/CD18

Annet Troelstra, Lia A. M. de Graaf-Miltenburg, Toon van Bommel, Jan Verhoef, Kok P. M. Van Kessel and Jos A. G. Van Strijp

*J Immunol* 1999; 162:4220-4225; http://www.jimmunol.org/content/162/7/4220

References

This article cites 34 articles, 21 of which you can access for free at: http://www.jimmunol.org/content/162/7/4220.full#ref-list-1

Subscription

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

Permissions

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts

Print ISSN: 0022-1767 Online ISSN: 1550-6606. Copyright © 1999 by The American Association of Immunologists All rights reserved.
Lipoplysaccharide-Coated Erythrocytes Activate Human Neutrophils Via CD14 While Subsequent Binding Is Through CD11b/CD18

Annet Troelstra,1 Lia A. M. de Graaf-Miltenburg, Toon van Bommel, Jan Verhoef, Kok P. M. Van Kessel, and J. a. G. Van Strijp

Interaction of LPS with monocytes and neutrophils is known to occur via CD14 and is strongly enhanced by LPS-binding protein (LBP). Integrins as well as CD14 play a role in the interaction of erythrocytes (E) coated with LPS or whole Gram-negative bacteria with phagocytes. We reasoned that the density of LPS on a particle is an important determinant in these interactions. Therefore, E were coated with different concentrations of LPS (E_{LPS}). The binding of these E_{LPS} to neutrophils was evaluated by flow cytometry. Simultaneously, we measured fMLP receptor expression to evaluate neutrophil activation. E_{LPS} only bound to neutrophils in the presence of LBP. Blocking CD14 inhibited both activation and binding, whereas blocking complement (C) receptor 3 (CR3) inhibited binding but not activation. TNF activation restored E_{LPS} binding in CD14-blocked cells but not in cells in which CR3 was blocked. Salmonella minnesota did bind to neutrophils independent of CR3 or CD14. The addition of LBP enhanced binding twofold, and this surplus was dependent upon CD14 but not on CR3. We conclude that E_{LPS} interact with neutrophils via CD14, initially giving rise to cell activation; subsequently, binding is solely mediated by activated CR3. The Journal of Immunology, 1999, 162: 4220–4225.

Lipopolysaccharide or endotoxin is a major component of the Gram-negative cell wall and is one of the most important mediators of the clinical syndrome of sepsis. Sepsis is a severe situation in which the presence of Gram-negative bacteria in the bloodstream leads to the liberation of LPS from the cell wall. LPS gives rise to cell activation and to the production of TNF and cytokines, finally leading to multiple organ failure and death in 30–50% of cases (1). Until now, several classes of molecules on leukocytes have been recognized as receptors for LPS (2–5).

CD14, a phosphatidyliminostiol-anchored molecule found on monocytes, macrophages, and neutrophils, has been identified as a receptor that interacts with LPS. The interaction between LPS and phagocytes via CD14 is strongly enhanced by LPS-binding protein (LBP) (6).

Complement (C) receptor 3 (CR3) (CD11b/CD18) is a member of the leukocyte integrin family of heterodimeric adhesion molecules, consisting of a common β-subunit and a unique α-subunit. (7). In 1986, a role for integrins in LPS-interaction was found by Wright (8). Each of the three members of the integrin family (CR3, LFA-1, and p150-95) were shown to mediate not only LPS interaction but the binding of Escherichia coli to macrophages as well (9). CR3 is up-regulated and the adhesive capacity of leukocytes is increased after the interaction of CD14 with LPS and LBP (10). Monocytes from CD18-deficient patients or monocytes on which all three members of the β-integrin family were down-modulated were unable to bind E. coli or E coated with LPS (E_{LPS}) (8, 11, 12). From the CD18 receptor family, the binding specificity of CR3 is the most well characterized. Two distinct binding sites on CR3 have been described: 1) a protein-binding-site that binds C3bi, fibrinogen, and Leishmanin glycoprotein 63, and 2) a lipid-binding-site involved in the binding of LPS, lipid A, and lipid IVA (13–15). Experiments with monocytes from CD18-deficient patients suggest no role for CD18 in secretory functions, because these cells were able to respond to LPS with the production of TNF-α and IL-1β; in addition, neutrophils from these patients could be primed by LPS for an enhanced respiratory burst (12).

In an earlier report, we showed that LPS binding to monocytes is completely dependent upon CD14 at concentrations of ≤100 ng LPS/ml (16). With higher concentrations, other interactions, which are LBP-independent, take over. This finding is in accordance with similar studies of cell activation by us and others, for which the same LPS concentration-dependent characteristics were found (17, 18).

To study the role of the surface properties of LPS, we constructed E coated with known amounts of LPS and compared the mechanism of neutrophil binding to free LPS and whole bacteria.

**Materials and Methods**

**Materials**

ReLPS from Salmonella minnesota strain Re595 and TNF were purchased from Sigma (St. Louis, MO). Highly fluorescent rLPS was prepared as described previously (16). BSA was obtained from Organon Technika (Turnhout, Belgium). Recombinant human LBP was a generous gift of Dr. H. Lichenstein (Amgen, Thousand Oaks, CA). The orange fluorescent membrane probe PKH-26 was purchased from Sigma. BODIPY-fMLP (green) and LDS-751 (red) were purchased from Molecular Probes (Eugene, OR).

Copyright © 1999 by The American Association of Immunologists
Table I. Comparison of LPS density on LPS-load erythrocytes and S. minnesota**

<table>
<thead>
<tr>
<th>Method</th>
<th>LPS Density on E&lt;sub&gt;LPS&lt;/sub&gt;&lt;sup&gt;270&lt;/sup&gt;</th>
<th>LPS Density on Salmonella</th>
<th>Ratio Salmonella/E&lt;sub&gt;LPS&lt;/sub&gt;&lt;sup&gt;270&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-LPS in FACS</td>
<td>26,000</td>
<td>16.2</td>
<td>(2.3)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>FITC beads in FACS</td>
<td>38,000</td>
<td>97,000</td>
<td>2.6</td>
</tr>
</tbody>
</table>

**LPS density = number of LPS molecules per surface (μm<sup>2</sup>). The m.w. of LPS = 3,200 (188,000 molecules/fentogram).

Surface of rabbit E (estimated radius of 2.5 μm) = 39.3 μm<sup>2</sup>.

Surface of Salmonella (estimated length of 2 μm, radius of 0.5 μm) = 9.4 μm<sup>2</sup>.

Ratio calculated with LPS density on Gram-negative bacteria as described in literature.

Quantification of bacteria

S. minnesota strain Re595 was grown for 16 h in Mueller-Hinton broth (Oxoid, Hampshire, U.K.). The concentration of bacteria was determined by two completely independent methods. First, a suspension of bacteria was counted microscopically. Second, FITC-labeled standard beads of known concentration (Flow Cytometry Standards, San Juan, NC) were mixed with bacteria and measured in a FACSkan (Becton Dickinson, Mountain View, CA). Beads and bacteria were separately gated on the basis of their forward and side scatter properties and fluorescence. As 10,000 events passed through the count-gate (selecting only beads), the number of bacteria was measured in the same time window. Thereafter, the exact number of bacteria in the starting suspension could be calculated.

Monoclonal Abs

Hybridoma cells secreting the murine mAbs 60bca (IgG1, directed against CD14) and 44a (IgG1, directed against CD11b) were obtained from the American Type Culture Collection (Manassas, VA). Culture supernatants were collected, and mAbs were purified over a protein G column (Pharmacia, Uppsala, Sweden). The production and characterization of mAb 8-2-c1, an IgM anti-LPS mAb, has been described previously (19). Goat anti-mouse FITC was obtained from Dako (Glostrup, Denmark).

Rabbit E<sub>LPS</sub>

Rabbit E were stained with PKH-26 as described previously (20). In short, rabbit E were washed three times with PBS; next, 5 × 10<sup>6</sup> E were suspended in 0.5 ml of PKH-26 diluent. Subsequently, 0.4 μl of PKH-26 was added to 0.5 ml of diluent, added to the E, and incubated for 20 min at room temperature. The reaction was stopped in HBSS containing 10% BSA for 5 min, and E were washed three times with HBSS/0.1% BSA. Subsequently, E were incubated with increasing concentrations of LPS (1–270 μg/ml) for 60 min at 37°C while being gently shaken and were washed three times with HBSS/0.1% BSA. In parallel, E were incubated with FITC-LPS in equal concentrations and washed; next, the FITC-LPS load was evaluated by flow cytometry. A linear relationship with a correlation coefficient of 0.97 was found between the amount of FITC-LPS added and the observed amount of fluorescence that associates to the E.

Quantitation of LPS on E<sub>LPS</sub> and S. minnesota

As summarized in Table I, we evaluated the number of LPS molecules incorporated into the E membrane by three principally different methods. In experiments concerning the interaction of E<sub>LPS</sub> with neutrophils, the LPS-coated surface of the E will be in contact with the neutrophil. Therefore, the density of LPS molecules per surface is the most relevant parameter in our study. We calculated the surface of a rabbit E to be 9.4 μm<sup>2</sup> (estimated length of 2 μm, estimated radius of 0.5 μm) (22). The LPS density of a Gram-negative bacterium has been the subject of research for several groups and is supposed to be 3 mg of LPS per 10<sup>10</sup> CFU (23–25). The estimated molecular mass of LPS is 3200 g/mol (26, 27); from this, we calculated that ~60,000 molecules of LPS are present per μm<sup>2</sup> on S. minnesota.

Limulus amebocyte lysate (LAL) assay

The LAL assay is a well-accepted method to liberate LPS from membranes or vesicles for LPS quantitation in heated samples (28). The assay was performed according to the manufacturer’s instructions (Chromogenics, Mölndal, Sweden). A standard curve was generated using known concentrations of rLPS. Test samples containing either E<sub>LPS</sub> coated with different amounts of LPS or S. minnesota were analyzed. A density of 97,000 LPS molecules per μm<sup>2</sup> was found for S. minnesota, which is somewhat higher than the density of 60,000 LPS molecules per μm<sup>2</sup> that has been described in other literature (23–25). We found a 2.6 times higher LPS density on S. minnesota than on E<sub>LPS</sub> coated with 270 μg/ml.

Flow cytometric analysis of E<sub>LPS</sub> or bacteria using an anti-LPS mAb

A series of beads coated with different numbers of FITC molecules (7,000–1,800,000) were used as standards. The mean fluorescence of the FITC beads was measured by flow cytometry; a standard curve was obtained. E were coated with 1–30 μg/ml FITC-LPS as described previously, and mean LPS density was determined. Using the standard curve, the amount of FITC molecules per E could be calculated. As our FITC-LPS was labeled with a labeling efficiency of one FITC-molecule per LPS molecule (16), the amount of LPS per E was obtained. LPS load on S. minnesota cannot be obtained using this method. If our calculations use 3 mg LPS/10<sup>11</sup> CFU, as found in other literature, an LPS density ratio of 2.3 times more LPS per surface on S. minnesota compared with E<sub>LPS</sub><sup>270</sup> was found.

Cells

Neutrophils were isolated from peripheral blood obtained from normal human volunteers after informed consent. Blood was collected into heparin tubes (Greiner, Frickenhausen, Germany), diluted 1/1 with PBS (pH 7.4), loaded on a Ficoll (Pharmacia) and Histopaque (density 1.19, Sigma) gradient. After centrifugation for 20 min at 250 × g at room temperature, the neutrophil interface was collected and washed with RPMI 1640 (Life Technologies, Gaithersburg, MD) (pH 7.4) and 0.05% human serum albumin (CLB, Amsterdam, the Netherlands). The remaining E were lysed for 45 s with distilled water, after which concentrated PBS was added to re-establish the isotonic condition.

Cells were washed and resuspended in RPMI 1640 at 1 × 10<sup>7</sup> cells/ml and tested for viability and purity. Viability was always >95% using either trypan blue or propidium iodide exclusion. Purity was checked by microscopy of cytospin slides and was always >98%.

Flow cytometric assay for FITC-LPS binding

In a total volume of 50 μl, 5 × 10<sup>5</sup> neutrophils were incubated with increasing concentrations (1–125 ng/ml) of FITC-LPS in the presence of LPB (100 ng/ml) or HBSS containing 0.1% BSA. For blocking experiments, cells were first incubated for 15 min on ice with 20 μg/ml of the mAbs 60bca (anti-CD14) or 44a (anti-CR3). The mean fluorescence of neutrophils was analyzed by flow cytometry.

Flow cytometric assay for E<sub>LPS</sub> binding and activation

In a total volume of 60 μl, 2 × 10<sup>5</sup> PHK-26-E<sub>LPS</sub> in the presence of 300 ng/ml LPB or HBSS/0.1% BSA, gently spun down for 5 min at 400 rpm, and incubated at 37°C for 30 min. For blocking or activation experiments, cells were preincubated for 15 min at 37°C with 20 μg/ml of mAb or 0.57 nM of TNF-α.

After the reaction was terminated, cells were put on ice and subsequently incubated with 10<sup>-7</sup> M BODIPY-fMLP to determine the level of fMLP receptor expression. As activated neutrophils express more fMLP receptors, we used this as a marker for cell activation. Thereafter, samples were stained with LDS-751 according to the manufacturer’s instructions (Molecular Probes) in a final concentration of 0.05 μg/ml per sample. LDS-751 is a selective red nuclear stain, and was used to distinguish neutrophils from unbound E<sub>LPS</sub>.

Flow cytometric assay for binding of S. minnesota to neutrophils

S. minnesota Re595 was grown overnight at 37°C in Mueller-Hinton broth, harvested, washed in PBS, resuspended in 0.1 M sodium carbonate buffer
at pH 9.6 containing 47 μg/ml FITC (Isomer I; Sigma), incubated for 1 h at 37°C, and washed twice in HBSS. S. minnesota-FITC was incubated in a bacterium to neutrophil ratio of 10:1 or 30:1, and binding to neutrophils was analyzed by flow cytometry.

**Results**

**Binding of FITC-LPS to neutrophils depends upon LBP and CD14**

Histograms of a representative experiment of neutrophils incubated with 42 ng/ml FITC-LPS are shown in Fig. 1A. Only in the presence of LBP (100 ng/ml) was FITC-LPS able to bind to neutrophils. Preincubating the neutrophils with anti-CD14 mAb resulted in a complete inhibition of binding (Fig. 1B). Neither preincubation with anti-CR3 (Fig. 1C) nor preincubation with anti-HLA (data not shown) had any effect on binding, confirming that the binding of FITC-LPS to neutrophils in the presence of LBP is completely CD14-dependent. These results are in complete accordance with literature describing LPS binding to monocytes and macrophages as dependent upon CD14 and LBP (29, 30).

**E LPS binding and activation of neutrophils**

Low binding to isolated neutrophils was observed for E LPS in the absence of LBP and was dependent upon LPS density. A significant enhancement of this binding was seen in the presence of LBP and was dependent upon the LPS load on the E (Fig. 2A).

In addition, with regard to the activation of neutrophils as measured by BODIPY-fMLP binding, we observed an LPS density-dependent activation of neutrophils that was enhanced by the addition of LBP (Fig. 2D).

After preincubation of the neutrophils with either anti-CD14 mAb (Fig. 2B) or anti-CR3 mAb (Fig. 2C), the binding of E LPS was completely blocked. The finding that anti-CD14 inhibited LBP-dependent binding is in accordance with earlier results. The

---

**FIGURE 1.** CD14-dependent binding of FITC-LPS to isolated neutrophils. Fluorescence histograms of a representative flow cytometric experiment of FITC-LPS binding to neutrophils are shown. Blank histograms represent neutrophils without FITC-LPS. A, Neutrophils were incubated with 42 ng/ml FITC-LPS in the presence of 100 ng/ml LBP and allowed to bind for 30 min at 37°C while being gently shaken. B and C, Neutrophils were preincubated with 10 μg/ml anti-CD14 mAb and anti-CR3 mAb at 4°C for 15 min, respectively.

**FIGURE 2.** Binding of E LPS to neutrophils depends upon both CD14 and CR3. E LPS (coated with different concentrations of LPS, resulting in an LPS density as indicated on the x-axis) were added to neutrophils in the presence of buffer ( ), anti-CD14 mAb ( and ), or anti-CR3 mAb ( and ) and could be identified on the basis of their red fluorescence (LDS-751, nuclear stain); E were labeled orange (PKH-26, membrane stain). The mean fluorescence (FL2, orange) of the neutrophil fraction is shown, representing the amount of E LPS bound to the neutrophils (A, B, and C). Measurement of BODIPY-labeled fMLP binding (FL1) on the same sample was used to determine neutrophil activation (D, E, and F). Data represent the mean of at least five independent experiments ± SEM.
The fact that the LBP-independent part of the binding could be inhibited by anti-CD14 as well is in agreement with our previous finding that for LPS concentrations from 100 to 300-1000 ng/ml, binding is no longer LBP-dependent but is still partially mediated by CD14 (16).

The activation of neutrophils was inhibited by the preincubation of neutrophils with anti-CD14 (Fig. 2E), whereas preincubation with anti-CR3 had no effect on cell activation by E\textsubscript{LPS} (Fig. 2F). Preincubation with anti-HLA, as a control, did not affect binding or activation (data not shown). From these results, we speculate that E\textsubscript{LPS} interact with neutrophils via CD14, leading to the binding of E\textsubscript{LPS} to the activated CR3.

To evaluate whether neutrophil activation could indeed result in E\textsubscript{LPS} binding, we activated the neutrophils with a different agonist, TNF-\alpha. Activation was confirmed by enhanced BODIPY-fMLP binding (Fig. 3D). TNF activation of neutrophils resulted in E\textsubscript{LPS} binding that was independent of LBP (Fig. 3A); however, binding was still dependent upon LPS density. The down-modulated E\textsubscript{LPS} binding to neutrophils after preincubation with anti-CD14 could indeed be restored by TNF-\alpha activation of the neutrophils (Fig. 3B). After preincubation with anti-CR3, the binding of E\textsubscript{LPS} to TNF-\alpha-activated neutrophils did not occur (Fig. 3C). From this observation, we conclude that E\textsubscript{LPS} interacts with the neutrophil via CD14, giving rise to activation (confirmed by enhanced fMLP receptor expression), and that the actual binding is mediated by activated CR3.

S. minnesota-FITC binding to neutrophils

Because it is a particle that is naturally coated with LPS, S. minnesota was used to investigate the receptors involved in association with neutrophils. For E with the highest LPS load, E\textsubscript{LPS}\textsuperscript{270}, we had already observed some LBP-independent binding to neutrophils. This trend was shown to be followed by a significant association of S. minnesota-FITC to neutrophils in the absence of LBP (Fig. 4A). For a bacteria to neutrophil ratio of 10:1 or 30:1, additional LBP enhanced binding by two- to threefold. In this case, microscopic evaluation of our samples revealed that this binding correlates with an association of \(\sim 1-2\) bacteria per cell. Preincubating neutrophils with anti-CD14 mAb inhibited the LBP-dependent part of the binding, whereas anti-CR3 had no effect. These data suggest a role for CD14 in the activation of another receptor that is involved in the actual binding of particles with very high LPS load, such as S. minnesota. Significant LBP-independent binding was observed that was mediated by structures other than CD14 or CR3, as preincubation with mAbs directed at these receptors as well as control Abs (anti-HLA) had no effect on binding.

TNF activation of neutrophils resulted in an association of S. minnesota that was independent of LBP, with mean fluorescence values that were comparable with the values seen for nonactivated cells in the presence of LBP; additional LBP gave no enhancement of binding. This finding indicated that cell activation caused an association of S. minnesota that was completely independent of LBP. Simultaneous TNF activation and blocking of CD14 or CR3 receptors on neutrophils had no effect on the binding of S. minnesota (Fig. 4B). From these results, we conclude that for activated neutrophils, the binding of a particle with a very high LPS density (i.e., S. minnesota) is independent of LBP, CD14, and CR3.

**FIGURE 3.** E\textsubscript{LPS} bind to CR3 neutrophils after activation. E\textsubscript{LPS} (loaded with different concentrations of LPS, resulting in a certain LPS density as indicated on the x-axis) were added to neutrophils in the presence of buffer (□) or 300 ng/ml LBP (■), gently spun down for 5 min, and incubated for 30 min at 37°C. Neutrophils were activated with 0.57 nM TNF for 15 min at 37°C and preincubated with buffer (A and D), anti-CD14 mAb (B and E), or anti-CR3 mAb (C and F) where indicated. The mean fluorescence (FL2) of the LDS-751 (FL3)-positive fraction is shown, representing the amount of E\textsubscript{LPS} bound to the neutrophils. Measurement of BODIPY-labeled fMLP binding (FL1) on the same sample was used to determine neutrophil activation (D, E, and F). Neutrophil activation was measured simultaneously from the association of BODIPY-labeled fMLP (FL-1). The same CR3 dependency was found after TNF activation of neutrophils for at least five different donors. As the absolute fluorescence data varied for different donors, one representative experiment of at least five independent experiments is shown for each graph.
FIGURE 4. A, S. minnesota bind to neutrophils in a CD14-dependent fashion. FITC-labeled S. minnesota were incubated with isolated neutrophils in a ratio of 10:1 for 30 min at 37°C in HBSS with (hatched bars) or without (open bars) 300 ng/ml LBP. S. minnesota-FITC association with neutrophils was measured by flow cytometry and presented as mean fluorescence (F1-1). Microscopic evaluation revealed an association of 1–2 bacteria per cell in the samples with LBP. In parallel, neutrophils were preincubated with 10 μg/ml anti-CD14 mAb or anti-CR3 mAb to investigate the role of these receptors. Bars represent the mean (± SEM) of three independent experiments using neutrophils from different donors. B, Binding of S. minnesota to activated neutrophils is independent of LBP, CD14, and CR3. Isolated neutrophils were preincubated for 15 min at 37°C with 0.57 nM TNF-α to allow activation. Subsequently, these neutrophils were incubated with FITC-labeled S. minnesota in a ratio of 10:1 for 30 min at 37°C in HBSS with (hatched bars) or without (open bars) 300 ng/ml LBP. S. minnesota-FITC association with neutrophils was measured by flow cytometry and presented as mean fluorescence (F1-1). In parallel, after incubation with TNF-α, neutrophils were preincubated with 10 μg/ml anti-CD14 mAb or anti-CR3 mAb to investigate the role of these receptors. Bars represent the mean (± SEM) of three independent experiments using neutrophils from different donors.

Discussion

In this paper, we describe experiments investigating the relative role of CD14 and CR3 in the interaction of phagocytes with LPS. E coated with LPS in different densities, and Gram-negative bacteria.

Fluorescein-labeled LPS has been used to study interactions with monocytes, and these interactions were shown to be dependent upon LBP or serum and CD14 (31). The same conditions, as shown in this paper, determine the binding of FITC-LPS to neutrophils. We have shown previously that binding to monocytes was enhanced by LBP or serum for LPS concentrations of ≤100 ng/ml and was completely CD14-dependent and saturable (16). In chemiluminescence experiments with isolated neutrophils, the production of oxygen radicals after priming with 1 ng/ml LPS was also shown to be completely dependent upon LBP and CD14 (16). Consequently, for low LPS in low concentrations, as can be found in the clinical condition of Gram-negative sepsis (32), only CD14 was found to play a role.

For LPS concentrations of >100 ng/ml, binding to monocytes is still partially CD14-dependent but is not LBP-dependent (16). For an E coated with LPS or a Gram-negative bacterium, one might expect a local LPS concentration or density that is relatively high; therefore, other receptors than CD14 might be involved. We found that S. minnesota has an LPS density that is 2.6 times higher than our E with maximal LPS coat, E_{LPS}^{270}, according to the LAL assay and 2.3 times higher according to the FITC beads method. According to the method using anti-LPS Abs, E_{LPS}^{270} had an LPS density that was 16.2 times higher than S. minnesota. However, we think that the latter method is too dependent upon the form in which the LPS is presented, which may explain the large difference with the other two methods used. The results for S. minnesota (obtained by LAL assay) were not significantly different from the literature. We found a density of 97,000 molecules per μm², whereas a density of 60,000–180,000 is described in the literature (23–25, 33).

The binding of E coated with different amounts of LPS to isolated neutrophils was dependent upon LBP and was prevented by either anti-CD14 or anti-CR3 mAbs. Anti-CD14 preincubation prevented activation by E_{LPS}; preincubation with anti-CR3 did not affect neutrophil activation. This observation led to our hypothesis that E_{LPS} activate neutrophils via CD14 and subsequently bind to the activated CR3. This hypothesis was confirmed by the observation that E_{LPS} bound to neutrophils activated by TNF-α (an LPS-independent activating agent) that were preincubated with anti-CR3. This study did not bind to activated neutrophils preincubated with anti-CR3. Therefore, an activating role is proposed for CD14 LPS in the presence of LBP, although the actual binding of E_{LPS} was mediated by the activated and up-regulated CR3. These findings illustrate a difference in the mechanism of interaction for free LPS and the LPS present on a particle.

Our finding that E_{LPS} binding was mediated by CR3 is in accordance with the fact that monocytes or macrophages isolated from CD18-deficient patients are defective in the binding of E_{LPS} or E. coli (12). LPS stimulation of phagocytes from CD18-deficient patients still results in the production of TNF-α and IL-1β, and the neutrophils of these patients can be primed for an oxidative burst. Furthermore, Zarewych et al. recently showed that, indeed, LPS in the presence of serum or LBP induced a physical association between CD14 and CR3, leading to a CR3-mediated attachment of neutrophils to surfaces followed by a dissociation of CD14-CR3 complexes (34). These investigators suggested that CD14 serves as a scout for CR3, which, in turn, is responsible for transmembrane signaling and attachment of the cell.

We demonstrated an association of S. minnesota with neutrophils in the presence of buffer only. This association could be enhanced twofold by additional LBP.

The LBP-dependent part of the binding was inhibited by preincubating the cells with anti-CD14, indicating CD14-mediated binding. LBP-independent binding was not mediated by CD14. This corresponds with our previous finding that for high LPS concentrations, binding was only partially dependent upon CD14 (16). However, in contrast to E_{LPS}, no reduction of LBP-dependent S. minnesota interaction with neutrophils was seen after preincubation with anti-CR3. LBP-independent binding was also not affected by anti-CR3. This illustrates that most of the LBP-dependent binding of S. minnesota is mediated by CD14. Recently,
Grunwald et al. also showed a CD14-dependent interaction of monocytes with E. coli that was dependent upon serum or LBP that could be inhibited by anti-CD14 mAb, excess soluble sCD14, or excess LPS. A mAb directed at CR3 did not inhibit binding and ingestion. In the hands of these investigators, a significant background binding of E. coli was also seen (35). However, microscopic evaluation of our samples revealed an association of ~1–2 bacteria per cell. As observed after classical opsonization with C and Abs, this association is only marginal compared with the association of 7–8 bacteria per cell (36).

Our data show that structures other than CR3 and CD14 account for the binding of S. minnesota on activated cells, and this binding is independent of LBP. This finding is in contrast to E. coli that bound to CR3 after LBP-dependent neutrophil activation via CD14 or via direct TNF stimulation of the neutrophil.

The results described in this paper suggest that the way in which LPS is presented determines the mechanism of binding to neutrophils. Interaction of free LPS with neutrophils is completely mediated via CD14 and LBP, whereas for whole bacteria, the interaction is mainly independent of LBP and CD14. For E coated with LPS, a two-step mechanism is proposed: neutrophils are activated via CD14 and subsequently binding is mediated by CR3.

References