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Contributions of C1q, Bacterial Lipopolysaccharide, and Porins during Attachment and Ingestion Phases of Phagocytosis by Murine Macrophages

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In contrast to the S-form of *Salmonella minnesota*, its Re mutant binds to mouse peritoneal macrophages. The binding reaction triggers an oxidative burst, measured by a chemiluminescent reaction. The oxidative burst was abolished in the presence of either purified lipopolysaccharide or porins (outer membrane proteins) extracted from the Re mutant, suggesting that both components are involved in binding of the Re mutant to macrophages. In addition, Fc-recognizing membrane structures on the macrophage surface bind the Re mutant. Preincubation of macrophages with the Re mutant abolishes immunoglobulin G-sensitized erythrocyte-induced chemiluminescence. Macrophages preincubated with immunoglobulin G-sensitized erythrocytes had a low chemiluminescent signal, and after treatment of the cells with the Re mutant, there was an additional, higher signal. Binding of purified C1q to the Re mutant decreased the adherence of the Re mutant to macrophages, resulting in a diminished chemiluminescent signal. Blocking of endogenous macrophage membrane-associated C1q with a monoclonal antibody [F(ab')$_2$ fragment] directed against mouse macrophages (recognizes the A and B chains of C1q) diminished the oxidative burst. Therefore, the endogenous C1q of macrophages also appears to be involved in attachment of the *S. minnesota* Re mutant.

Very little information exists in the literature which describes the interaction of macrophages (Mϕ) with *Salmonella minnesota* in a serum- and antibody-free system. Therefore, we investigated the adhesion, phagocytosis, and induction of the oxidative burst of Mϕ by the wild type and the Re mutant of *S. minnesota*.

Previously, we reported that antibody-independent killing of the serum-sensitive R mutants of *S. minnesota* is initiated through direct binding of C1, the first component of complement (2). This action is due to a high affinity of the Re mutants for binding C1q. In addition, Mϕ are capable of synthesizing C1q, which is then exposed in the membrane of these cells (9). To continue these studies, we wanted to determine if Mϕ with membrane-associated C1q are able to differentiate between the serum-resistant S-form, which does not bind C1q under physiological conditions, and its serum-sensitive Re mutant, which has a strong C1q binding capacity.

(The experiments reported herein represent parts of the Ph.D. dissertation of B.E., Institute of Medical Microbiology, Mainz, Federal Republic of Germany [F.R.G.].)

MATERIALS AND METHODS

Bacterial strains. *S. minnesota* wild type (S-form, SF1111) and the Re mutant (R-595, SF1167) were kindly provided by G. Schmidt, Forschungsinstitut Borstel.

Cultivation. Bacteria were initially subcultured overnight on sheep blood agar plates. For liquid cultures they were inoculated with a sterile loop into 100 ml of nutrient broth (Oxoid, Ltd., Wesel, F.R.G.) and incubated with vigorous shaking at 37°C for 4 to 5 h until exponential growth was reached. The bacteria were pelleted by centrifugation (15 min; 3,500 × *g*), washed twice with 0.01 M phosphate-buffered saline (PBS; pH 7.3), suspended, and adjusted to the desired cell number.

Mϕ. Ten-week-old female mice (strain NMRI; Ivanovas, Kisslegg, F.R.G.) were injected intraperitoneally with 1 ml of thioglycolate broth (Oxoid, Ltd.). Four days later, exudative cells were harvested by washing the peritoneal cavity of each mouse with 5 ml of cooled PBS (4°C), collected by centrifugation (15 min; 250 × *g*), and suspended in culture medium to the desired cell concentration.

Phagocytosis experiments. Assays for phagocytosis were done in Leighton tissue culture tubes. A total of 10^6 Mϕ per ml were suspended in M-199 with Earle salts (M-199; Flow Laboratories, Ltd., Bonn, F.R.G.) with no penicillin, streptomycin, or serum. One milliliter of each cell suspension was added to Leighton tubes and incubated for 60 min at 37°C in the presence of 5% CO_2 to allow the Mϕ to settle. Nonadhering cells were eliminated by washing twice with PBS. The Mϕ were then cultured for 24 h in M-199 and washed twice with PBS prior to phagocytosis experiments. As detected by trypan blue exclusion, more than 95% of the cells were typical, well-spread, viable Mϕ. A 1-ml portion of 3×10^8 bacteria (Re mutant or wild type) was added to the monolayers, and the monolayers were washed at intervals varying between 0 and 90 min. The Mϕ were rinsed six times with PBS to remove extracellular nonadherent bacteria (11). The Mϕ were lysed under hypotonic conditions by adding 1 ml of ice-cold sterile water for 30 min and mixing vigorously to suspend the ingested or cell-associated microorganisms. Lysis of Mϕ was observed directly by phase-contrast microscopy. The viability of bacteria was not affected by these procedures. The number of surviving bacteria was measured by colony counting from appropriate dilutions of the cell lysate after growth overnight on blood agar plates.

Adherence of *S. minnesota* to Mϕ. To determine the optimal number of bacteria for binding to Mϕ, 10^6 Mϕ per ml were suspended in M-199 and added in 300-μl samples to

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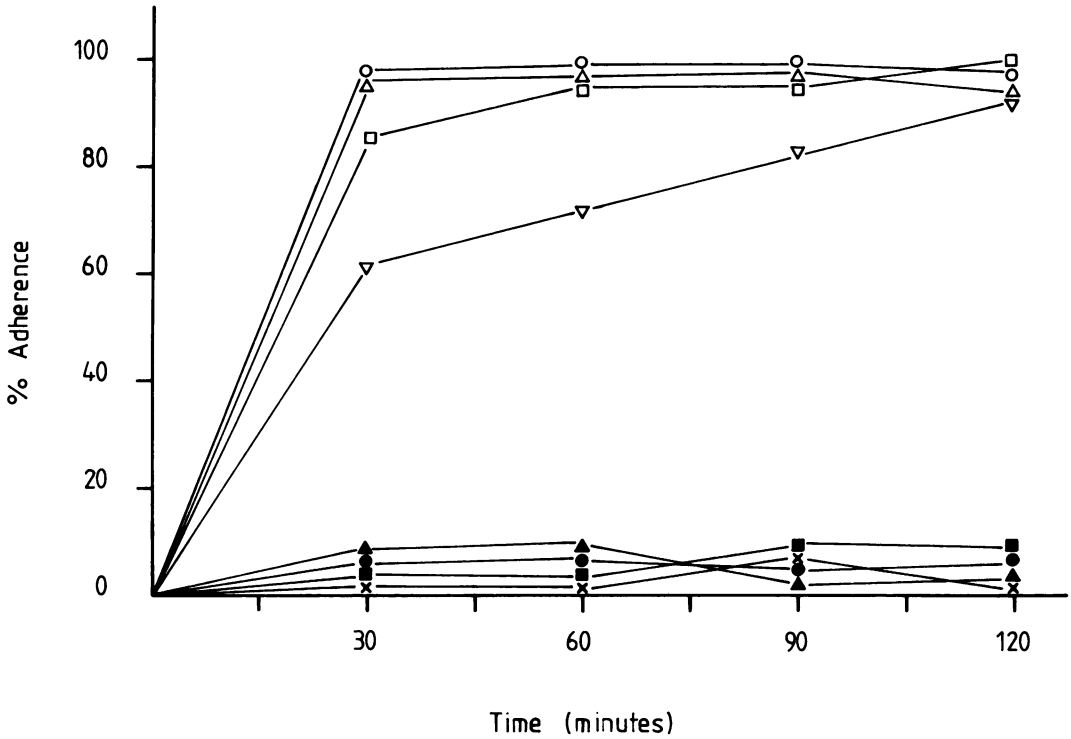

FIG. 1. Time-dependent adherence of the Re mutant and the S-form of *S. minnesota*. Macrophages were fixed, Gram stained, and then examined by phase-contrast microscopy. Mϕ with asociated bacteria were scored as adherent. Concentrations of bacteria (cells per milliliter) were as follows: Re mutant—(○) 12×10^5; (△) 6×10^5; (□) 3×10^5; (▽) 10^5; wild type—(▲) 12×10^7; (●) 6×10^7; (■) 3×10^7; (×) 10^7.

Lab-Tek tissue culture chamber/slides (Miles Laboratories, Napperville, Ill.). After incubation and washing steps as described in the phagocytosis experiments, Mϕ were incubated with various concentrations of bacteria (Re mutants: 1×10^5 to 12×10^5 cells per ml; wild type: 1×10^7 to 12×10^7 cells per ml) for various periods of time (0 to 120 min). After washing six times with PBS, 20-min fixation with absolute ethanol, and Gram staining, adherent bacteria were examined by phase-contrast microscopy. One hundred Mϕ were counted per slide, and those Mϕ with three or more bound bacteria were scored as positive.

Electron microscopy. Mϕ cultivated for 24 h were infected with either *S. minnesota* Re mutant or the wild type in a ratio of 1:100. After an incubation period of 45 min and washing with PBS, Mϕ were fixed for ultrathin-section preparations with 2.5% glutaraldehyde in PBS for 20 min. The cultures were postfixed with 1% OsO_4 in PBS for 30 min. Samples were dehydrated in a graded ethanol series with uranyl acetate solution and lead citrate. Cells were examined in a Philips electron microscope, model 301. For scanning electron microscopy, cultures were fixed with 2.5% glutaraldehyde, postfixed in 2% OsO_4, dehydrated, critical-point dried, and covered with gold. Samples were viewed in a Philips scanning electron microscope, model 500.

CL. The chemiluminescence (CL) response (electron emission) was assayed in a Bioluminat apparatus (Dr. Berthold Co., Wildbad, F.R.G.) at 37°C. For this assay 3×10^6 to 6×10^6 Mϕ per ml were cultivated on Petriperm culture dishes (Heraeus, Hanau, F.R.G.) in Dulbecco CL-minimum essential medium (Boehringer, Mannheim, F.R.G.) enriched with L-glutamine (584 mg/liter). After 1 h of incubation to allow for adherence, Mϕ were washed twice with PBS (25°C) and removed from the dishes by gently rinsing the plates with cooled PBS (4°C) 10 times with a 5-ml syringe equipped with a 16-gauge needle. The nonadhering cells were collected by centrifugation (15 min; $250 \times g$) and suspended in CL-minimum essential medium to the desired concentration of 2×10^6 cells per ml. The final incubation mixture for the CL assay contained 0.5 ml of Mϕ (2×10^6/ml), 0.1 ml of PBS (or for different assays, the amount of lipopolysaccharide [LPS], porins, and antibody reconstituted to 0.1 ml with PBS), and 0.1 ml of a 0.1% Luminol solution (5-amino-2,3-dihydro-1,4-phtalazinedione; Boehringer). The reaction was initiated at time zero by injection of 0.1 ml of bacteria (6×10^9 cells per ml), 0.1 ml of optimally immunoglobulin G-sensitized sheep erythrocytes (EIgG) (0.625 cells per ml), 0.1 ml of latex, PBS, latex-LPS, LPS bound to erythrocytes (E-LPS), and the Re mutant with bound C1q (Re-C1q). As controls, E, latex, PBS, and phorbol myristate acetate (Sigma Chemie, Munich, F.R.G.) were used. The CL intensity from each sample was measured as counts per minute for a 30-min period. It was confirmed that the viability of Mϕ was not affected by the treatment described above. All CL experiments were repeated four times to confirm the observed changes in CL signals.

Fc rosette formation. Fc rosette activity was tested by rosette formation with EIgG (10^8 ml) after incubation with Mϕ (10^6 ml) for 10 min at 37°C. Mϕ that bound three or more EIgG were counted as positive. A total of 200 Mϕ was counted per incubation mixture (13).

Tests for viability of Mϕ. To determine the extent to which the integrity of the Mϕ was influenced by incubation with the Re mutant, the S-form, or LPS, we looked for dye exclusion

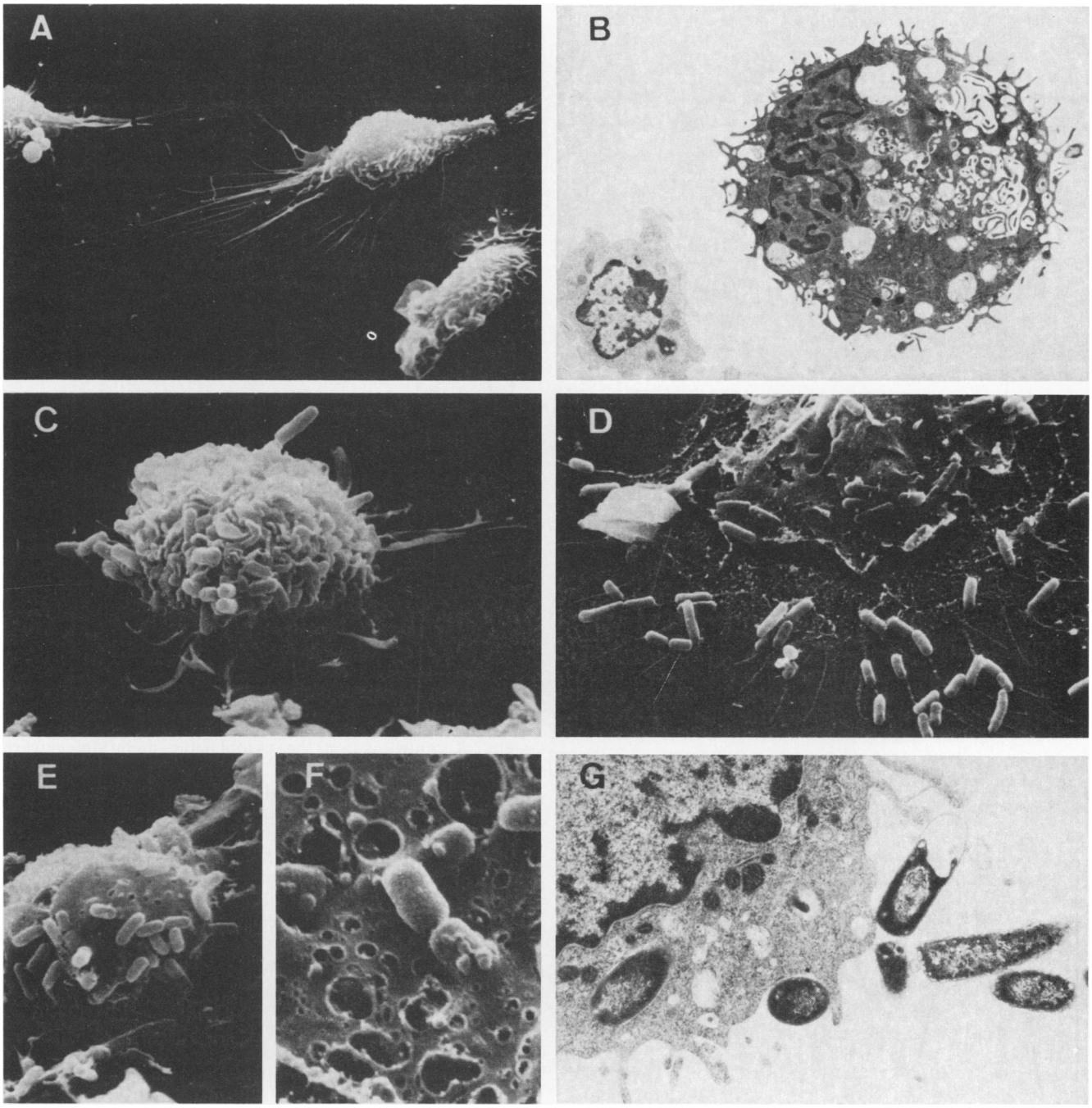

FIG. 2. Scanning electron micrographs and ultrathin sections of Mϕ during different stages of phagocytosis by *S. minnesota*. (A) Scanning electron micrograph of macrophages after incubation with the S-form. No bacteria were attached (×9,240). (B) Ultrathin section of macrophages after incubation with the S-form. Detection of many microvilli of the macrophages (×5,720). Scanning electron micrographs of macrophages during phagocytosis of the Re mutant: (C) attached bacteria on cell surface (×22,994.4); (D) bacteria directed along microvilli (×62,040); (E) adherence of *Salmonella* spp. on cell surface (×21,120); (F) ingestion of bacteria (×73,920). (G) Ultrathin section of Mϕ with the attached and ingested Re mutant (×15,400).

by the cells, as determined with trypan blue solution (0.18% final concentration). Viability was further assessed by measurement of lactate dehydrogenase activity in supernatants by following the rate of oxidation of NADH photometrically at 340 nm according to Wroblewski and LaDue (17).

LPS. Purified LPS from *S. minnesota* Re (List Biologicals Inc., Campbell, Calif.) was suspended in PBS (5 mg/ml) prior to use.

Porins. Porins were purified by a method described by Stemmer and Loos, (submitted for publication). In brief, late-exponential-phase bacteria from a 2-liter culture were suspended in 50 mM HEPES buffer (*N*-2-hydroxyethyl-

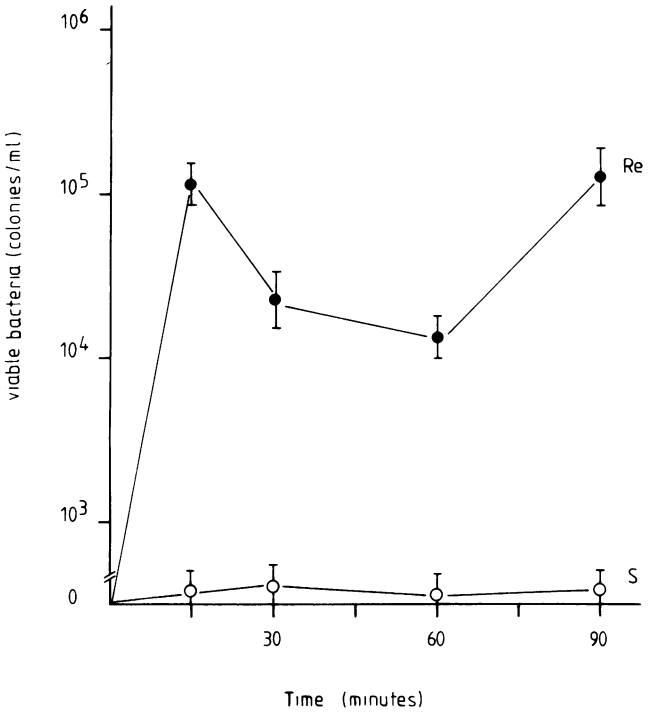

FIG. 3. Time-dependent uptake of *S. minnesota*. A total of 10^6 Mϕ were incubated with 3×10^8 bacteria for various times. After washing six times with PBS, Mϕ were lysed with ice-cold water and viable intracellular bacteria were determined by the amount of cell-forming units. Only the Re mutant was bound. This experiment was repeated three times. The standard deviation for each incubation time is shown.

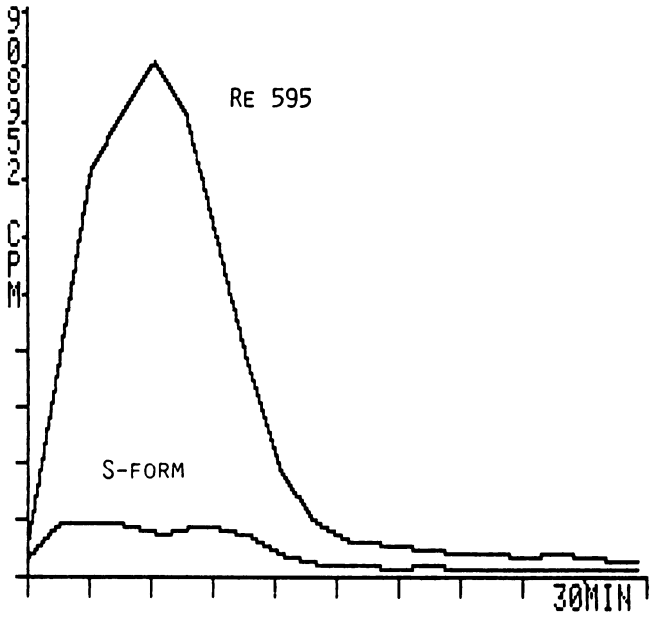

FIG. 4. CL of peritoneal Mϕ during phagocytosis of *S. minnesota* (S = wild-type; Re = core-defective mutant). A total of 10^6 Mϕ were stimulated with 6×10^8 bacteria.

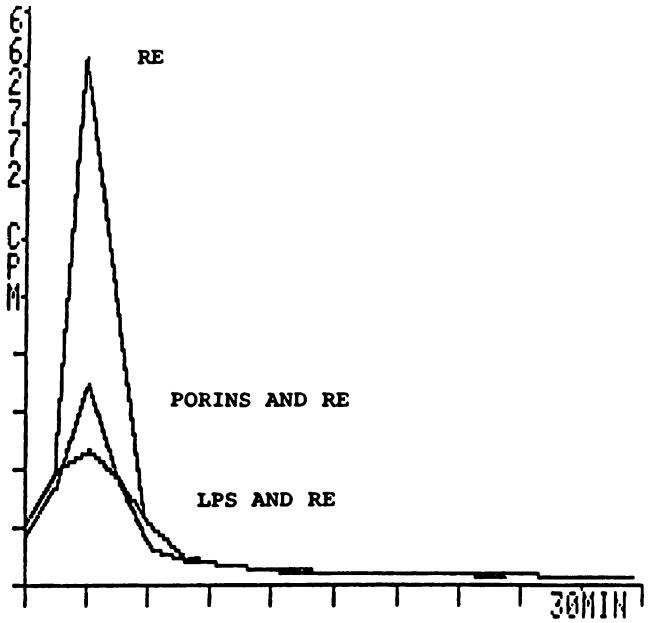

FIG. 5. CL of peritoneal Mϕ (10^6) preincubated with either porins (final concentration, 4 μg/ml) or Re LPS (final concentration, 300 μg/ml) for 30 min at 37°C; then the macrophages were incubated with 6×10^8 cells of the Re mutant.

piperazine-*N'*-2-ethanesulfonic acid), pH 7.4, with 1 mg each of DNase and RNase (Sigma). The cells then were disrupted by French pressure cell treatment at 0°C, and after different steps of centrifugation the cytoplasmic membranes were extracted with 2% Triton X-100. After a subsequent lysozyme and papain treatment, the material was adjusted to 20 mM HEPES, pH 8.2, and mixed with solid *n*-octyl glucoside and urea. From this solution the porins were further purified by the Fast Protein Liquid Chromatography System on a column containing Mono Q, a strong anion exchanger (Pharmacia, Freiburg, F.R.G.). The purified porins had two major proteins with apparent molecular weights of 36,000 and 39,000 after analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions.

C1q. Human C1q was purified by the method of Stemmer and Loos (15), using the Fast Protein Liquid Chromatography System FPLC. Protein concentration was determined by the method of Lowry et al. (10), using bovine serum albumin as the standard.

Anti-C1q F(ab')$_2$. The monoclonal rat antibody to mouse Mϕ (no. 146), which reacts with human C1q and is directed against the globular, Fc-recognizing portions of the molecule (A and B chains), was used (5).

RESULTS

Binding and phagocytosis of bacteria by Mϕ. After Mϕ were incubated with either the Re mutant or the S-form, the attachment of the mutant was much greater, even when 10 times higher concentrations of the S-form were used (Fig. 1). The uptake of the mutant in different stages was shown by electron microscopy and scanning electron microscopy (Fig. 2). Figure 3 shows that the ingestion of bacteria was observed only for the Re mutant. During the first 15 min of incubation of Mϕ with 3×10^8 cells of the Re mutant, $1.4 \times$

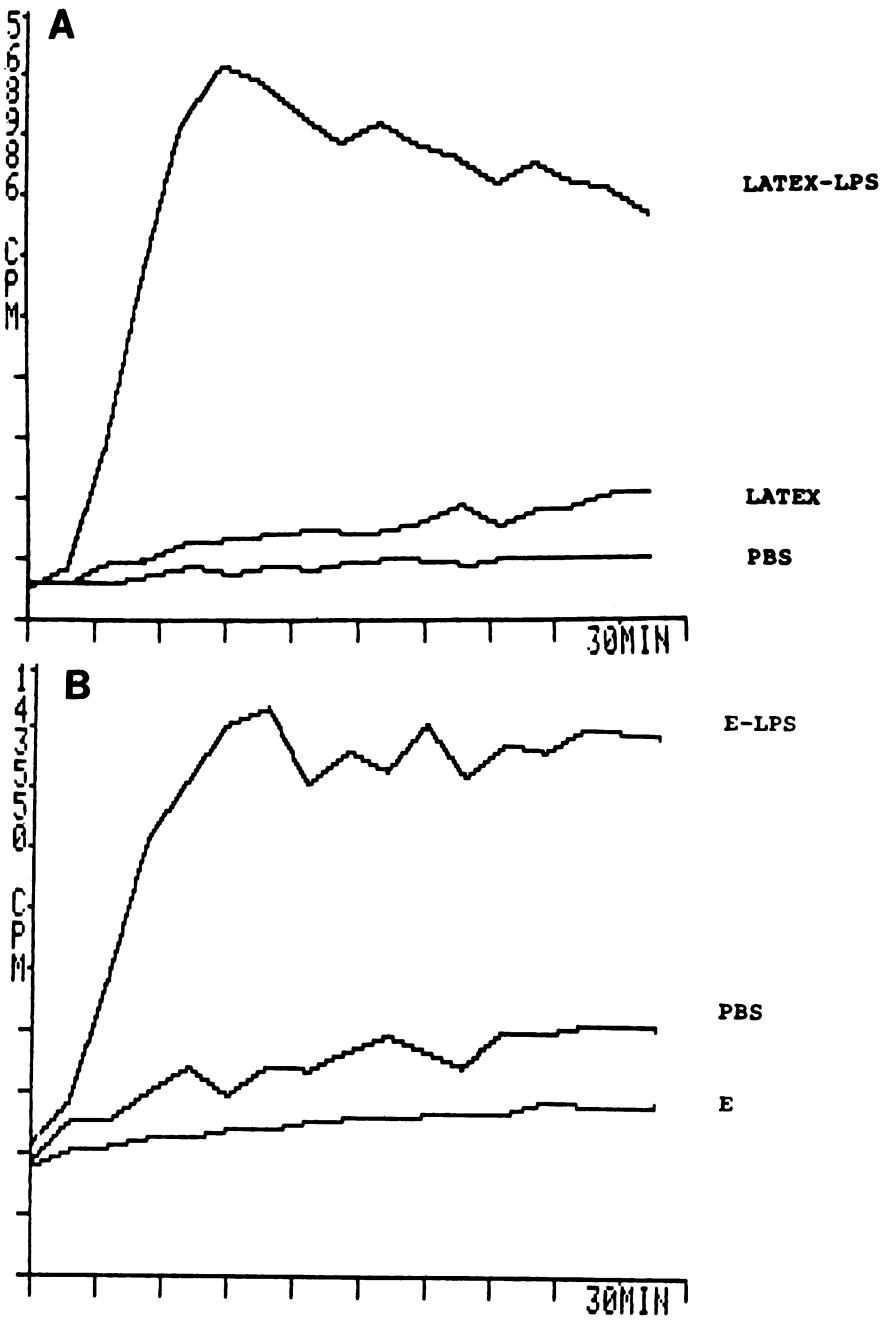

FIG. 6. CL induced through (A) LPS bound to latex, latex, and PBS or (B) erythrocytes (E), PBS, and E-LPS.

10^5 bacteria were taken up. After 60 min, ingested bacteria were reduced to 7.4×10^4 cells. The observed increase in number of colonies after this time probably can be explained by an increase in number of organisms which were not killed. There was no significant binding of the S-form to the Mϕ.

Induction of CL by bacteria and modulation by LPS and porins. Next we determined whether attachment of the bacteria to Mϕ leads to the generation of an oxidative burst, measured as a CL signal. Figure 4 shows that the S-form induced a low CL signal, while the Re mutant induced a high one. This corresponded with the binding affinity of the two forms. Heating of rough mutants of *S. typhimurium* and *S. minnesota* for 45 min at 70°C diminished the CL signal induced by this bacteria in polymorphonuclear leukocytes (PMN) (7). Accordingly, we heated the Re mutant for 45 min at 70°C, and this completely abolished the ability of the Re mutant to induce CL (experiment not shown). This indicates that Mϕ-activating surface structures were removed or impaired by heat.

The oxidative burst measured as counts per minute in the CL assay was inhibited if Mϕ were preincubated (30 min, 37°C) with final concentrations of 4 μg of porin or 300 μg of LPS per ml (Fig. 5). The inhibition was found to be dose

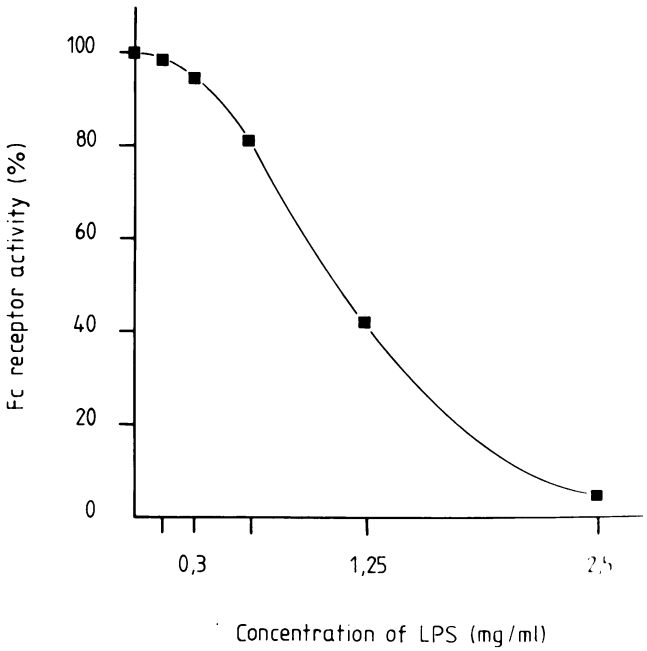

FIG. 7. Decreased Fc receptor activity due to various concentrations of LPS derived from the Re mutant. Mϕ were incubated for 1 h at 37°C with various concentrations of LPS; the cells were then washed, and rosette formation was measured with EIgG.

dependent. Furthermore, simultaneous incubation of Mϕ with a mixture of Re mutant LPS and porins resulted in an increased inhibition compared with Re mutant LPS and porins alone (data not shown). Thus, both components may be involved in the attachment of bacteria to the Mϕ. To confirm whether Re mutant LPS binds to Mϕ and if there is an oxidative burst in Mϕ induced by multimeric Re mutant LPS, Re mutant LPS was bound to latex beads or erythrocytes mimicking Re mutant LPS-coated bacteria. Re mutant LPS bound to a carrier was able to induce a CL signal (Fig. 6A and B). In experiments with S-LPS-coated beads, a markedly reduced CL signal was observed which was comparable to that of the S-form. S-LPS did not prevent Re mutant-induced CL. Therefore, we conclude that LPS binds directly to Mϕ. These experiments show that the induction of the oxidative burst is observed when LPS is presented in a multivalent form such as by LPS-coated latex beads or erythrocytes or by intact bacterial surfaces such as the Re mutants of *S. minnesota*. Similar results were obtained with porins on latex beads; namely, the porins coupled to latex beads induced a CL response. Porins not bound to latex gave no CL response.

Studies of Fc-recognizing and other membrane structures during phagocytosis. Rosette formation with EIgG was used to measure Fc receptor activity of Mϕ. Preincubation of Mϕ (30 min, 37°C) with various concentrations of purified LPS of the Re mutant resulted in a dose-dependent inhibition of EIgG uptake by Mϕ (Fig. 7). The concentrations of LPS used in these experiments did not affect the viability of the Mϕ as tested by trypan blue exclusion and by the release of lactate dehydrogenase (control Mϕ [10^6] without LPS: 2.6 ∓ 0.7 mU of lactate dehydrogenase per ml; Mϕ [10^6] with 250 mg of LPS: 3.3 ∓ 0.7 mU of lactate dehydrogenase per ml). Since uptake of EIgG by Mϕ also triggers the respiratory burst, we tested whether EIgG-induced CL could be prevented by preincubation of the Mϕ with the Re mutant and vice versa. Figure 8A shows that preincubation of Mϕ with the Re mutant abolished the subsequent EIgG CL. However, in Mϕ preincubated with EIgG, an additional but weaker CL signal was observed by subsequent treatment with the Re mutant (Fig. 8B). This indicated that the Re mutant recognizes additional binding sites on the Mϕ surface in addition to those recognized by EIgG. In additional experiments it was shown that, in Re mutant-treated Mϕ, a CL signal was induced by phorbol myristate acetate (data not shown).

Endogenous C1q in attachment and blockade of the oxidative burst induced by the Re mutant. Corresponding to the low or high binding affinity of C1q to the S-form or the Re mutant (2), preincubation of bacteria with human C1q (15 min, 30°C, 10^3 effective molecules per cell) diminished the binding rate of the Re mutant to Mϕ. This was determined by phase-contrast microscopy and the CL assay (Fig. 9A and B). Similiar results were obtained with C1q on EIgG rosette formation. Preincubation of the Mϕ with the $F(ab')_2$ fragment (final concentration, 25 μg/ml) of a monoclonal antibody which was produced against mouse Mϕ and which recognizes the A and B chains of purified C1q led to a blockade of the oxidative burst induced by the Re mutant (Fig. 10). Preincubation with immunoglobulin G (final concentration, 625 μg/ml) or another unrelated antibody did not block this signal. C1q or the $F(ab')_2$ fragment of the monoclonal antibody alone did not induce any CL.

DISCUSSION

The phagocytic process involves, first, contact between microbial agents and the receptors of phagocytic cells and, second, adhesion of the microbe. The results of the adhesion (Fig. 1) and phagocytosis (Fig. 3) experiments, and the electron microscopic determinations (Fig. 2), show the importance of cell wall structure in interaction of *Salmonella* sp. strains with Mϕ: the rough Re mutant of *S. minnesota* lacking sugars in the cell wall LPS adhered and was taken up by mouse Mϕ. Adherence and phagocytosis of the smooth wild type were not observed. In agreement with Friedberg and Shilo (4), who studied the interaction of mouse peritoneal Mϕ and guinea pig PMN with strains of *S. typhimurium*, a complete core appears to be important for resisting ingestion and for intracellular survival. The presence of the O-specific side chains may contribute further resistance.

It is known from studies on PMN that phagocytosis initiates oxidative metabolism, resulting in increased hexose monophosphate shunt activity and generation of activated oxygen products such as singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radicals. The formation of these products is accompanied by emission of energy in the form of light, termed chemiluminescence (CL) (1, 3). Our studies showed that, with thioglycolate-elicited peritoneal Mϕ, the S strain of *S. minnesota* which did not bind directly to the Mϕ did not induce a CL signal. In contrast, the Re mutant rapidly bound to Mϕ induced high peak values of CL (Fig. 1 and 4). Huixiu et al. published similar results investigating PMN and different *Salmonella* sp. strains (7). Preincubation of the Mϕ with isolated porins and purified LPS both from the Re mutant abolished the oxidative burst induced by the Re mutant. Thus, it appear that both LPS and outer membrane components are involved in attachment of Re mutants to the phagocytic cell. These results agree with the studies of Henricks et al. (6) with human PMN and *Escherichia coli*. They showed diminished phagocytic, che-

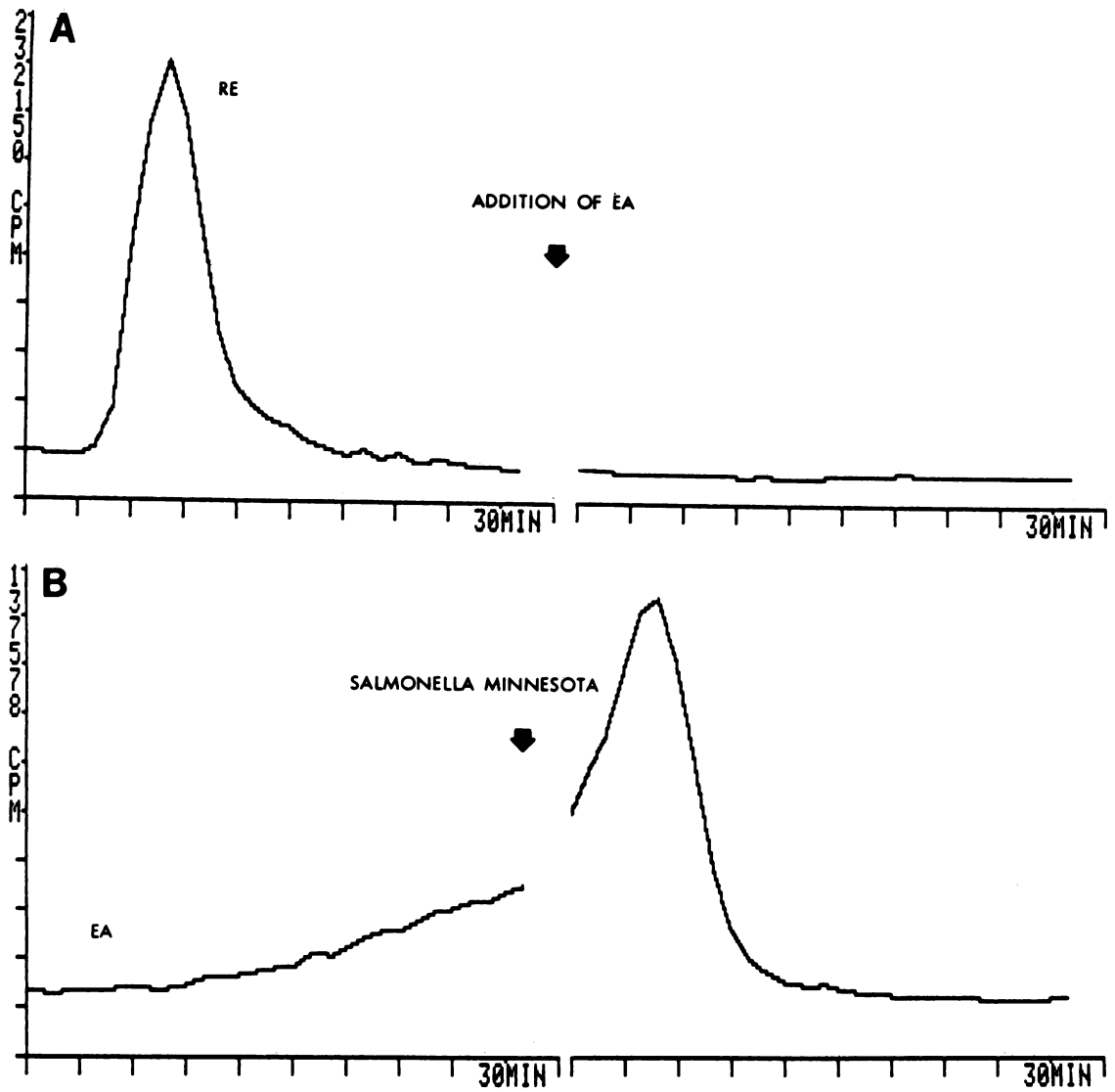

FIG. 8. CL of Mϕ after stimulation with (A) the Re mutant (first stimulus) and EIgG (second stimulus) and (B) EIgG (first stimulus) and the Re mutant (second stimulus).

motactic, and metabolic activities of PMN after incubation with a cell wall preparation of *E. coli* containing mostly lipid A. Proctor (14) also published evidence that PMN did not show CL when challenged with endotoxin or lipid A. In 1985, Lian and Pai (8) showed inhibition of CL in PMN by plasmid-mediated outer membrane proteins of *Yersinia enterocolitica*. The results in Fig. 6A and B show that LPS, bound to either latex or erythrocytes, induced a CL signal. Therefore, we conclude that LPS offered in a polymeric form such as E-LPS or LPS-coated beads or in the form of intact bacteria binds directly to Mϕ and induces the oxidative burst.

According to Vogel et al. (16), LPS induces a profound inhibition of Fc-mediated phagocytosis in LPS-responsive Mϕ of C3H/HeN mice. Furthermore, low concentrations of LPS stimulate phagocytosis in Mϕ of nonresponder C3H/HeJ mice. It was suggested by these authors that the lipid A moiety of the LPS may be responsible for the enhancement or inhibition of Fc-mediated phagocytosis because polymyxin B, which forms stable molecular complexes with the lipid A portion of LPS (12), counteracts these reactions. In our experiments (Fig. 7), LPS derived from the Re mutant of *S. minnesota* caused a dose-dependent inhibition of Fc receptor activity on Mϕ as measured by uptake of EIgG. The induction of the oxidative burst because of EIgG was prevented by preincubating Mϕ with the Re mutant but not with the S-form. These cells, however, were still sensitive to phorbol myristate acetate stimulation. In contrast, Mϕ preincubated with EIgG produced an additional CL signal after a subsequent treatment with the Re mutant. From these observations we conclude that the Re mutant binds to both Fc-recognizing membrane structures and additional membrane constituents. This interpretation is strengthened by our data (Fig. 5) showing that bacterial porins are also involved in attachment of the Re mutant to Mϕ.

Clas and Loos (2) showed a low binding affinity of C1q to the S-form and a strong affinity to the Re mutant. Therefore,

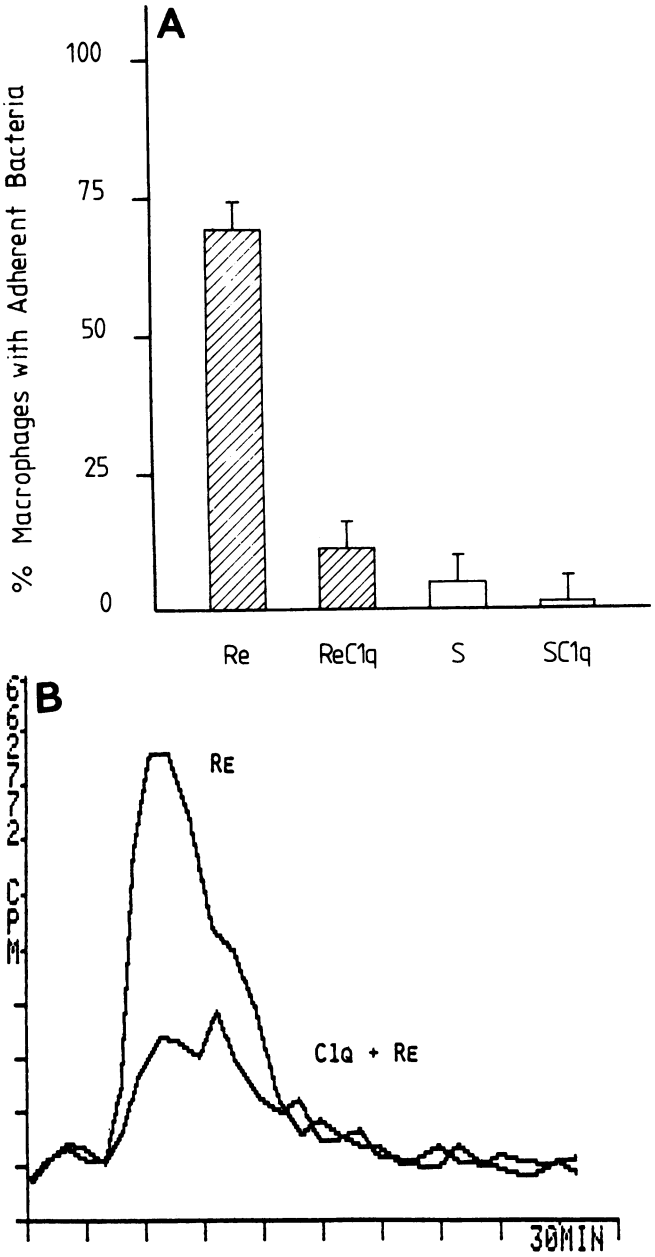

FIG. 9. Decrease in both the binding rate (A) and the CL signal (B) by preincubation of bacteria with purified C1q.

we studied the interaction of both strains with Mϕ in the presence of purified C1q. Preincubation of bacteria with highly purified C1q diminished both the binding rate and consequently the CL signal (Fig. 9). Recently, it was reported that peritoneal Mϕ synthesize C1q and that endogenous C1q, the Fc-recognizing subcomponent of C1, is expressed in the membrane of Mϕ (9). Preincubation of Mϕ with the $F(ab')_2$ fragment of a monoclonal antibody which recognizes the A and B chains of purified C1q (5) diminished the Re mutant induction of the oxidative burst. Thus, endogenous C1q, which is expressed in the membrane of Mϕ and functions there as an Fc receptor (9), may be one constituent on Mϕ surfaces involved in the binding of gram-negative bacteria, which have a strong binding capacity for C1q. These experiments contribute to our understanding of how smooth or rough bacteria interact with Mϕ. Currently we are studying the interaction of Mϕ with mutants of *S. typhimurium* which have different deficiencies in their outer membrane components to better understand phagocyte-bacteria relationships.

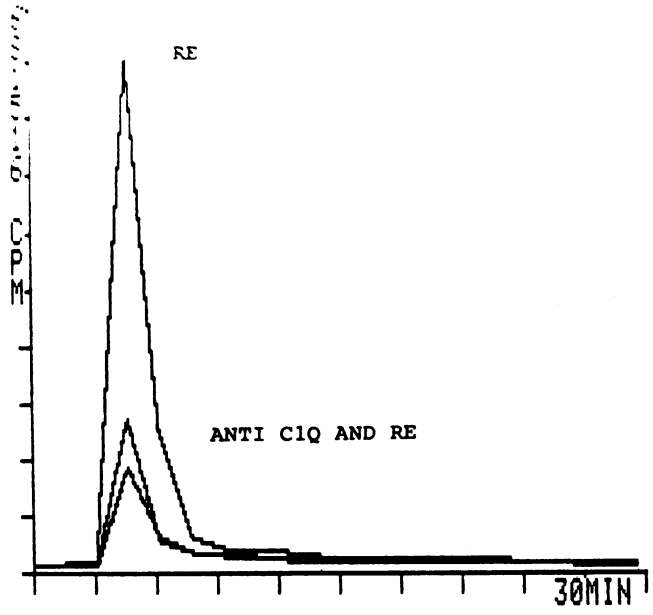

FIG. 10. Mϕ preincubated with various concentrations of the $F(ab')_2$ fragment of anti-C1q (protein concentration, 200 μg/ml) at 50 and 100 μl (30 min, 37°C). The inhibition of CL was dependent on the concentration of $F(ab')_2$.

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LITERATURE CITED

1. **Allen, R. C., R. L. Stjernholm, and R. H. Steele.** 1972. Evidence for the generation of an electronic exitation state(s) in human polymorphonuclear leukocytes and its participation in bactericidal activity. Biochem. Biophys. Res. Commun. **44**:679–684.
2. **Clas, F., and M. Loos.** 1981. Antibody-independent binding of the first component of complement (C1) and its subcomponent C1q to the S- and Re-forms of *Salmonella minnesota*. Infect. Immun. **41**:1138–1144.
3. **DeChatelet, L. R., G. D. Long, P. S. Shirley, D. A. Bass, M. J. Thomas, F. W. Henderson, and M. S. Cohen.** 1982. Mechanism of the luminol dependent chemiluminescence of human neutrophils. J. Immunol. **129**:1589–1593.
4. **Friedberg, D., and M. Shilo.** 1970. Role of cell wall structure of *Salmonella* in the interaction with phagocytes. Infect. Immun. **2**:279–285.
5. **Heinz, H.-P., H. Dlugonska, E. Rüde, and M. Loos.** 1984. Monoclonal anti-mouse macrophage antibodies recognize the globular portions of C1q, a subcomponent of the first component of complement. J. Immunol. **133**:400–404.
6. **Henricks, P. A. J., M. E. van der Tol, R. M. W. M. Thyssen, B. S. van Asbeck, and J. Verhoeff.** 1983. *Escherichia coli* lipopolysaccharides diminish and enhance cell function of human polymorphonuclear leukocytes. Infect. Immun. **41**:294–301.

7. **Huixiu, J., O. Stendahl, and L. Edebo.** 1984. Activation of polymorphonuclear leukocytes in Salmonella. Acta Pathol. Microbiol. Immunol. Scand. Sect. B **92**:107–113.
8. **Lian, C.-J., and C. H. Pai.** 1985. Inhibition of human neutrophil chemiluminescence by plasmid-mediated outer membrane proteins of Yersinia enterocolitca. J. Immunol. **49**:145–151.
9. **Loos, M.** 1983. Biosynthesis of the collagen-like C1q molecule and its receptor functions for Fc and polyanionic molecules on macrophages. Curr. Top. Microbiol. Immunol. **102**:1–56.
10. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
11. **Menzel, J., H. Jungfer, and D. Gemsa.** 1978. Contribution of immunoglobulins M and G, complement, and properdin to the intracellular killing of *Escherichia coli* by polymorphonuclear leukocytes. Infect. Immun. **19**:659–666.
12. **Morrison, D. C., and D. M. Jacobs.** 1976. Binding polymyxin B to the lipid A protein of bacterial lipopolysaccharide. Immunochemistry **133**:813–818.
13. **Müller, W., H. Hanauske-Abel, and M. Loos.** 1978. Biosynthesis of the first component of complement by human and guinea-pig peritoneal macrophages: evidence for an independent production of C1 subunits. J. Immunol. **121**:1578.
14. **Proctor, R. A.** 1979. Endotoxin in vitro interactions with human neutrophils: depression of chemiluminescence, oxygen consumption, superoxide production, and killing. J. Immunol. **25**:912–921.
15. **Stemmer, F., and M. Loos.** 1984. Purification and characterization of human, guinea-pig and mouse C1q by fast protein liquid chromatography (FPLC). J. Immunol. Methods **74**:9–16.
16. **Vogel, S. N., S. T. Marshall, and D. L. Rosenstreich.** 1979. Analysis of the effects of lipopolysaccharide on macrophages: differential phagocytic responses of C3H/HeN and C3H/HeJ macrophages in vitro. Infect. Immun. **25**:328–336.
17. **Wroblewski, F., and J. S. LaDue.** 1955. Lactic dehydrogenase activity in blood. Proc. Soc. Exp. Biol. Med. **90**:210.