INFECTION AND IMMUNITY, May 1989, p. 1356–1362
0019-9567/89/051356-07$02.00/0

Vol. 57, No. 5

Role of C1q in Phagocytosis of *Salmonella minnesota* by Pulmonary Endothelial Cells

UNA S. RYAN,* DUANE R. SCHULTZ, JEFFREY D. GOODWIN, JAMES M. VANN, M. P. SELVARAJ, AND M. A. HART

Department of Medicine, University of Miami School of Medicine, P.O. Box 016960, Miami, Florida 33101

Received 12 September 1988/Accepted 28 January 1989

The Re mutant of *Salmonella minnesota* adheres in much greater numbers than the wild type to endothelial cells derived from the bovine pulmonary artery. Since the Re mutant is distinguished from wild-type *S. minnesota* by its ability to bind C1q and since endothelial cells possess receptors for C1q, we examined the role of C1q in the phagocytosis of the *S. minnesota* Re mutant. First, preincubating endothelial cells with C1q-enriched medium resulted in increased adherence of the Re mutant (17.9×10^4 versus 6.6×10^4). Second, preincubating the Re mutant with C1q-enriched medium resulted in increased numbers of adherent bacteria (62.1×10^4 versus 6.6×10^4). Preincubation of both endothelial cells and bacteria with C1q-enriched medium resulted in increased adherence above control levels but less adherence than when either cells or bacteria were preincubated separately in C1q-enriched medium. If serum depleted of C1q was used for preincubation of endothelial cells or bacteria, adherence was reduced below control levels. Thus, C1q plays an important role in the initial steps (recognition, binding, and ingestion) of phagocytosis. Next, the role of C1q was investigated in the respiratory burst response. Levels of superoxide anion released from endothelial cells 15 min after phagocytosis of the Re mutant (100 bacteria per endothelial cell) were assayed by measurement of the superoxide dismutase-inhibitable reduction of ferricytochrome *c*. Superoxide anion release was increased during phagocytosis of the Re mutant (35 nmol of O_2^- per 3×10^6 endothelial cells) and was also elevated above control values by incubation with soluble C1q (10 nmol of O_2^- per 3×10^6 endothelial cells). These results indicate a role for C1q in both the ingestion and the response of endothelial cells to the *S. minnesota* Re mutant.

We have shown previously that endothelial cells (ECs) are capable of phagocytosis in vivo and in vitro (19, 23). Some of the endothelial responses to ingestion of particulates or bacteria are similar to those of professional phagocytes, such as macrophages; these responses include unmasking of an Fc receptor (19), increases in the rates of migration, division, and further phagocytosis (21, 23), and generation of a respiratory burst (23, 30). In this study, we have examined further the adherence and phagocytosis of *Salmonella minnesota* by pulmonary ECs. ECs ingest the Re (rough, core-defective) mutant more readily than the wild type (S or smooth form) does, as do macrophages (8). Since we have shown previously that ECs possess receptors for C1q (37) and since the Re mutant of *S. minnesota* is distinguished from the S type by its ability to bind C1q (immunoglobulin-binding portion of the C1 macromolecular complex) (5, 6), we have focused on the role of C1q both in binding bacteria to the endothelial surface and in triggering the phagocytic responses.

MATERIALS AND METHODS

Preparation of C1q-enriched medium. During these studies, purified human C1q was obtained from Calbiochem (La Jolla, Calif.) and Sigma Chemical Co. (St. Louis, Mo.). Although each preparation at 5 mg/ml contained no immunoglobulin G (IgG) by Ouchterlony analysis with goat anti-human IgG (produced in our laboratories), C1q was applied to a column (1 by 6.5 cm) containing protein A-Sepharose (Pharmacia LKB, Piscataway, N.J.) to ensure the absence of IgG. Each preparation was also tested by Ouchterlony analysis with antisera to normal human serum and to human IgM and C1q. No IgM was detected, and the anti-normal human serum formed a single band which formed a line of identity with the band formed with anti-C1q. In addition, C1q with a high degree of purity was applied to a column (2.5 by 46 cm) containing Sephacryl S-300 Superfine (Pharmacia, Uppsala, Sweden) equilibrated with 0.05 M Tris buffer and 0.3 M NaCl (pH 8.6). Blue dextran 2000 was used to determine the void volume of the column. The C1q eluted as a sharp peak in which molecules with a molecular weight of 400,000 would be expected to elute, indicating that the protein was not aggregated. These preparations of C1q were subsequently used in the experiments described below. C1q was added to medium 199, without serum, at a concentration of 0.38 mg/ml to constitute C1q-enriched medium.

Preparation of C1q-deficient serum. C1q was removed from human serum (35). Whole blood was drawn from a normal individual and allowed to clot for 1 h at 25°C. The clots were then centrifuged at $800 \times g$ for 10 min at 4°C, and the serum was transferred to a centrifuge tube. Four milliliters of 0.1 M EDTA was added to 12 ml of serum, the pH was adjusted to 7.5 with 0.1 N HCl, and the mixture was incubated at 37°C for 10 min. The mixture was then chilled to 4°C, diluted with ice-cold distilled H_2O containing 0.005 M EDTA (pH 7.5) until the relative salt concentration was 0.02 M, and stored overnight at 4°C. The serum was centrifuged at $16,300 \times g$ for 30 min, and the supernatant was removed, leaving most of the C1q in the pellet. The supernatant was reconcentrated to its original volume with an Amicon apparatus (Danvers, Mass.) (membrane size, PM 10; 10,000 molecular weight cutoff). The serum was next incubated with constant stirring overnight at 4°C with 3 g of glutaraldehyde-insolubilized (1) polyclonal goat anti-human C1q serum (Calbiochem). The insolubilized antiserum was removed by centrifugation at $3,000 \times g$ for 10 min at 4°C.

This C1q-deficient serum was tested in an Ouchterlony

* Corresponding author.

assay against rabbit anti-human C1q serum (center well), as were the normal serum, diluted supernatant, concentrated supernatant, and C1q precipitate, diluted 1:3 (outer wells). Immunoprecipitation was seen only between antibody and complete normal serum and between antibody and pelleted antigen.

Spectrophotometric readings (optical density at 280 nm) were taken of the normal and C1q-deficient sera to adjust for equal protein concentrations.

EC culture. ECs were obtained from the bovine main pulmonary artery by methods that avoid exposure to proteolytic enzymes both during isolation and throughout subculture, as described previously (26, 28). The ECs were grown in Ryan red medium (26) without antibiotics and were used in passages 12 through 18. The cells maintained the cobblestone monolayer morphology throughout and were characterized every five passages by electron microscopy, angiotensin-converting enzyme activity, factor VIII antigen reactivity as detailed before (20, 24, 27, 29), and by uptake of fluorescent acetylated low-density lipoprotein measured at passage 3 (36).

Culture and labeling of *S. minnesota*. *S. minnesota* strains (S form and Re mutant) were a gift from M. Loos, Johannes Gutenberg University, Mainz, Federal Republic of Germany. The Re mutant cultured on plates of tryptic soy broth with 5% sheep blood was used to inoculate 100 ml of nutrient broth. The broth was shaken overnight at 37°C and centrifuged at 800 × *g* for 15 min, the supernatant was removed, and the bacteria were suspended in phosphate-buffered saline (PBS) (1.5 mM KH_2PO_4, 8.05 mM Na_2HPO_4, 136.8 mM NaCl [pH 7.3]); this process was repeated two times. A hemacytometer count was made on this suspension.

Tritiated thymidine (100 μl) was added to 30 ml of nutrient broth. The bacteria (10^8 Re mutants) were added to the broth, and the suspension was placed in a 37°C bath and shaken vigorously for 5 h. The bacteria were then centrifuged at 800 × *g* for 15 min and suspended in PBS. The process of centrifugation and suspension were repeated two times. Hemacytometer and scintillation counts were made on the suspension, and the specific activity was calculated. The Re mutant organisms were kept at 4°C until used to prevent further proliferation.

Phagocytosis experiments. ECs were seeded on cover slips placed in 24-well Falcon plates (Becton Dickinson Labware, Oxnard, Calif.) and cultured for 3 days at 37°C in Ryan red medium. At 24 h before the experiment, the medium was removed and replaced with medium with no antibiotics.

The assay was performed in two 24-well tissue culture plates containing 15-mm-diameter circular glass cover slips on which ECs had been grown to confluence (3×10^5/cover slip). Just before the experiment, the ECs were washed three times at 37°C with PBS.

One control and five experimental conditions were used for this experiment as follows: (i) ECs plus Re mutant plus medium with 10% normal human serum; (ii) ECs pretreated for 1 h with C1q-enriched medium plus Re mutant; (iii) ECs plus Re mutant pretreated for 1 h with C1q-enriched medium; (iv) ECs pretreated for 1 h with C1q-enriched medium plus Re mutant pretreated for 1 h with C1q-enriched medium; (v) ECs pretreated for 1 h with C1q-deficient medium plus Re mutant; and (vi) ECs plus Re mutant pretreated for 1 h with C1q-deficient medium. The inoculum was 3×10^7 Re mutant organisms or approximately 100 Re mutant organisms per EC. A preincubation period of 1 h was used to treat the ECs and the Re to the excess or the absence of C1q. Preincubation of the Re mutant was carried out at 4°C; this temperature prevents further growth but does not affect the binding capacity of C1q (6). After incubation at 37°C, the cover slips were removed and placed into scintillation vials. NaOH (1 N, 0.3 ml) was added to solubilize the ECs and to release [^{3}H]thymidine from the bacteria. HCl (0.3 ml, 1 N) was added 10 min later to neutralize the NaOH. Four milliliters of liquid scintillation cocktail was administered to each vial, and all vials were counted in a scintillation counter. The numbers of cell-associated Re mutant organisms were calculated by using the specific activity of the [^{3}H]thymidine-labeled *S. minnesota* preparations.

Determination of the extent of phagocytosis of the Re mutant was achieved with antibiotics to eliminate extracellular adherent Re mutant organisms. ECs grown to confluence on circular cover slips in 24-well plates were incubated at 37°C and 5% CO_2 for 1 h with 10 or 100 Re mutant organisms per EC in Ryan red medium. Cultures were then washed three times with Hanks balanced salt solution and assayed for CFU of Re mutant organisms or cultures were incubated 1 h further in Ryan red medium containing 87 μg of ampicillin per ml and 50 μg of gentamicin per ml. Antibiotic-treated cultures were washed three times in Hanks balanced salt solution and then harvested to assay CFU. CFU were assayed by placing the cover slip containing the monolayer in 1.0 ml of sterile distilled water to lyse the ECs. The lysate was then diluted appropriately and applied to agar spread plates which were incubated overnight at 37°C before the colonies were counted. To determine the effectiveness of the antibiotic treatment, endothelial monolayers were fixed with 0.5% paraformaldehyde in PBS for 30 min to render the cells incapable of phagocytosis. The fixed cultures were inoculated with the Re mutant and processed as described above. The antibiotic treatment resulted in elimination of 99.99% of CFU. In lieu of the data (11, 33) showing that the antibiotics used do not enter cells, we can conclude that the viability of *S. minnesota* Re internalized by the ECs was not affected by the antibiotics. Additional confirmation that bacteria were indeed intracellular, rather than associated with the cell surface, was achieved by scanning electron microscopy to visualize intracellular bacteria (see below).

Scanning electron microscopy. Cells to be prepared for scanning microscopy were grown on segments of glass cover slips (≈10 by 10 mm). The osmium-thiocarbohydrazide impregnation method was used (14, 25). Primary fixation in 2.5% glutaraldehyde in 0.05 M sodium cacodylate with 6% sucrose (pH 7.4) was followed by two cycles of fixation in osmium tetroxide (1%) in distilled water and thiocarbohydrazide (1% in distilled water) with thorough washing with distilled water between each step. The preparations were dehydrated through an ethanol series and subsequently critical point dried with Freon 113-CO_2. The critical-point-dried glass pieces were glued, cell surface up, onto aluminum stubs with silver paint. The cells were examined, without further coating with metal, in an ISI-DS-130 scanning electron microscope at 20 or 30 kV.

Assay for superoxide anion release. Superoxide anion (O_2^-) was assayed by measurement of the superoxide dismutase (SOD)-inhibitable reduction of ferricytochrome *c* (15). Readings were taken at 550 nm on a double-beam spectrophotometer (LAMBDA 3B; The Perkin-Elmer Corp., Norwalk, Conn.). The total volume of the reaction mixture was 8 ml. The final concentrations used were 40 μg of SOD per ml, 70 μM ferricytochrome *c*, and 68 pM C1q solution. The following samples were assayed: ECs plus reaction buffer plus ferricytochrome *c*; ECs plus reaction buffer plus ferricy-

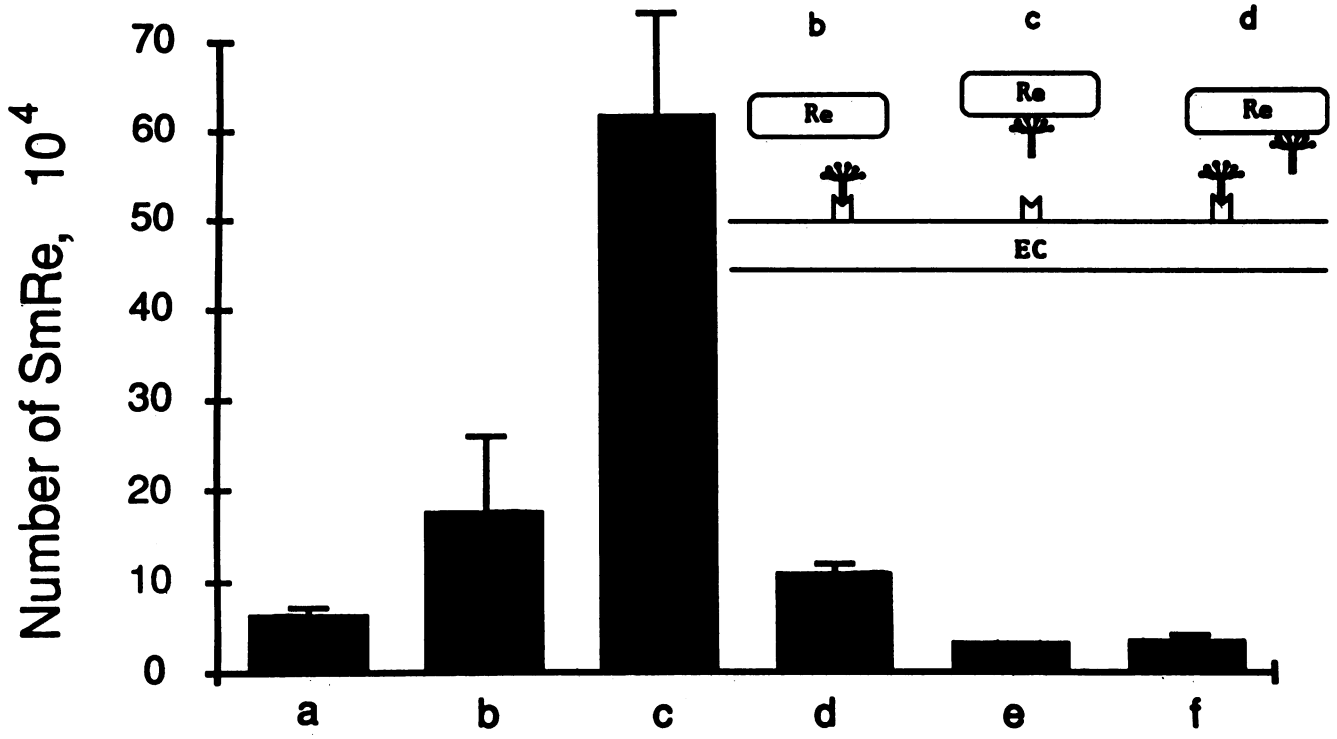

FIG. 1. Effect of preincubation with C1q-enriched or C1q-deficient medium on adherence of *S. minnesota* by ECs. Bars: a, ECs plus the Re mutant plus medium with 10% normal human serum; b, ECs pretreated for 1 h with C1q-enriched medium plus the Re mutant; c, ECs plus the Re mutant pretreated for 1 h with C1q-enriched medium; d, ECs pretreated for 1 h with C1q-enriched medium plus the Re mutant pretreated for 1 h with C1q-enriched medium; e, ECs pretreated for 1 h with C1q-deficient medium plus the Re mutant; f, ECs plus the Re mutant pretreated for 1 h with C1q-deficient medium. To test whether the mean Re adherence of the control (bar a) was significantly different from those of bars b, c, d, e, and f, a one-way ANOVA was applied. It was found that $P < 0.001$ when bars b and d were tested against the control, and $P < 0.005$ when bars c, e, and f were tested. The inset diagram depicts binding of C1q via collagenous tail to the endothelial surface and via the globular heads to the bacterial surface. SmRe, *S. minnesota* Re.

tochrome *c* plus SOD; ECs plus reaction buffer plus ferricytochrome *c* plus C1q solution; ECs plus reaction buffer plus ferricytochrome *c* plus C1q solution plus SOD.

RESULTS

Bar a (Fig. 1) shows that 6.6×10^4 Re mutant organisms adhered to ECs incubated in medium containing 10% normal human serum in 1 h. If the ECs were preincubated for 1 h with C1q-enriched medium (0.38 mg/ml, no serum) and then exposed to the bacteria for 1 h (Fig. 1, bar b), adherence was increased (17.9×10^4 Re mutant organisms). Preincubation of the Re mutant for 1 h with C1q-enriched medium (bar C) produced a dramatic increase in adherence (62.1×10^4). When both ECs and the Re mutant were pretreated with medium enriched with C1q, binding was enhanced above control levels (11.1×10^4) but was lower than the rates obtained when either bacteria (bar b) or ECs (bar c) were exposed to C1q before coincubation (bar d). However, when either the ECs (bar e) or the bacteria (bar f) were incubated in C1q-deficient medium (M199 with 10% C1q-depleted normal human serum), adherence was reduced (3.5×10^4 and 3.7×10^4, respectively) when compared with controls. The S form (wild type) of *S. minnesota* does not bind to ECs (21).

Since bactericidal activity of ECs is associated with the release of reactive oxygen metabolites (30), the effect of C1q on the respiratory burst response of ECs to phagocytosis of the Re mutant was examined. The release of superoxide anion from ECs was markedly increased as a result of phagocytosis of the Re mutant (Fig. 2). C1q in solution was capable of increasing the release of O_2^- approximately 10-fold above that of unstimulated cells (Fig. 2).

We used two approaches to determine whether the bacteria were fully internalized or simply adherent to ECs. Firstly, CFU of intracellular *S. minnesota* Re were determined by elimination of extracellular adherent *S. minnesota* Re with antibiotics (Table 1). With an inoculum of 100 *S. minnesota* Re per EC, 3.41×10^3 *S. minnesota* Re were internalized, compared with 2.12×10^5 total cell-associated *S. minnesota* Re (Table 1). Fixed EC cultures inoculated with 100 *S. minnesota* Re per EC and not treated with antibiotics had 9.43×10^4 CFU. This value was reduced to 7.8 CFU with antibiotic treatment, indicating the antibiotics were greater than 99.99% effective in 1 h. Secondly, EC monolayers after incubation with Re for 1 h were fixed and examined by scanning electron microscopy (Fig. 3). Bacteria were clearly seen both during ingestion and after intracellular sequestration. The steps in internalization of bacteria seem to involve binding (Fig. 3a), ruffling of the EC membrane (Fig. 3b), penetration of the bacteria end on (Fig. 3c), followed by complete resealing of the cell membrane. Intracellular bacteria close to the surface could still be resolved (Fig. 3d). These steps resemble the internalization of *Staphylococcus aureus* by ECs (21).

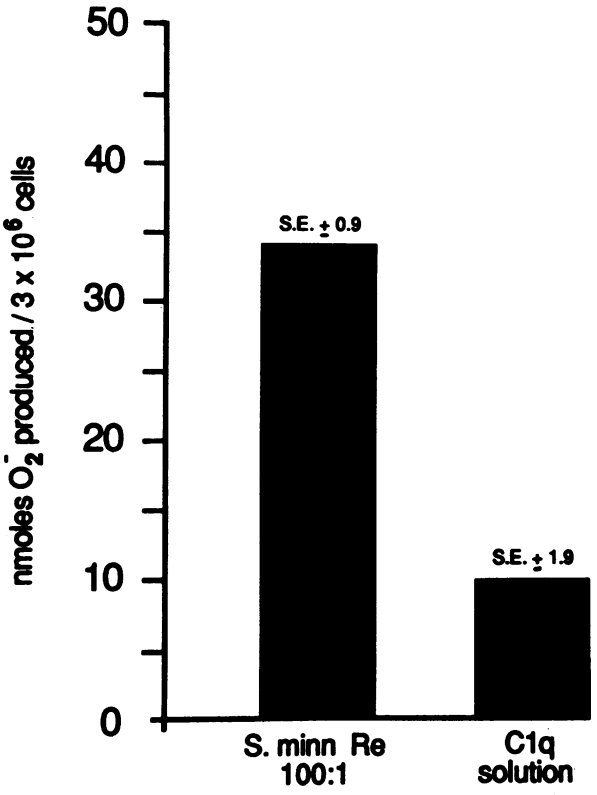

FIG. 2. Superoxide anion production by ECs exposed to the Re mutant of *S. minnesota* (S. minn Re) 100 bacteria per EC) and in response to C1q in solution (0.09 mg/ml); both incubations were for 20 min. S. E., Standard error.

DISCUSSION

ECs have been reported to phagocytize and, in some instances, to kill a variety of bacteria (19, 30). However, the exact mechanisms involved in recognition, binding, uptake, and sequestration of the bacteria are not known nor are the precise signals known which are responsible for triggering bactericidal activities. In this study, we examined the role of C1q in the adherence of *S. minnesota* by pulmonary ECs and in the EC response to phagocytosis. Neither $C1r_2$ nor $C1s_2$ of macromolecular C1 appeared to be involved here, since the inhibitor of activated C1 ($C\bar{1}$) was present in the normal and C1q-depleted sera (data not shown).

ECs can discriminate between the Re and S forms of *S. minnesota*, showing a preferential uptake of Re (30). This provides an interesting parallel with macrophages which also predominantly bind the Re form (8) and raises the question of the mechanisms responsible for preferential binding of the Re form. Most S strains of gram-negative bacteria are resistant to the killing effects of normal serum, whereas the corresponding rough forms are killed by serum (16–18). Clas et al. (6), in $C\bar{1}$ fixation and transfer tests, showed that $C\bar{1}$ bound to various serum-sensitive gram-negative bacteria and could be transferred to EAC4 cells. Although both *S. minnesota* S and Re forms could bind $C\bar{1}$, the Re form bound four times more per bacterium than the S form. The percentages of effective C1q molecules detected on the cells and in the fluid phase of the S and Re forms correlated with the data obtained with macromolecular $C\bar{1}$. By using C1q, rabbit anti-C1q, and ferritin-labeled goat anti-rabbit IgG, it was shown further (5) by electron microscopy that C1q is bound only by the Re form and not by the serum-resistant S form of *S. minnesota*. Therefore, by three different methods, $C\bar{1}$ fixation and transfer assays, immunofluorescence, and electron microscopy, C1q is bound only by the Re form and not by the serum-resistant S form of *S. minnesota*. Similar findings were reported with *Escherichia coli* (3, 4). When a C1q-deficient human serum was used, neither the S nor the Re strain was affected by this treatment. The addition of C1q with a high degree of purity to the deficient serum restored the bactericidal activity for the Re mutant. There was no effect with normal human serum deficient of C1q chromatographically or with patient serum deficient in C1q. In fact, two binding sites appeared to exist on the bacterium for C1. Tenner et al. (34) showed that several *E. coli* strains directly activated purified C1 in the absence of antibody and C1 inhibitor. Both C1q and C1s were bound, but not C1r. The C1s-binding site on *E. coli* J5 was only demonstrated when C1s was offered to *E. coli* J5 together with C1q and calcium. In the above studies, only dimeric C1s bound to the microbes. None of these studies addressed which end of C1q bound to the *E. coli*. A clinical isolate of *E. coli* (12) was able to bind human C1q (as part of macromolecular $C\bar{1}$) in the absence of IgG. The C3 convertase was formed on the surface by sequential interaction with functionally purified human $C\bar{1}$, C4, and C2 with or without pretreatment of the bacterium with purified normal human IgG. Both were phagocytized by neutrophils. Therefore, *E. coli* and human $C\bar{1}$, C4, C2, and C3 in the absence of IgG facilitated opsonization, phagocytosis, and intracellular killing by the peripheral leukocytes.

Clearly what distinguishes Re and S appears to be the ability of the Re form to bind C1q. The experiments (Fig. 1) in which bacteria or ECs were preincubated with C1q-enriched or C1q-deficient serum substantiate the role of C1q in the initial binding and recognition phases of phagocytosis by ECs. Previously, ECs have been shown to possess C1q receptors (37). Thus, ECs exhibit the same preference as macrophages.

Whereas the macrophage produces C1q and this molecule may directly mediate binding and subsequent ingestion of the Re mutant (8), in ECs the mechanism seems to be more complicated. Our data suggest that C1q mediates binding of the Re mutant. Preincubation of ECs with medium enriched with C1q, which we have previously shown to bind to EC

TABLE 1. Internalization of labeled *S. minnesota* by pulmonary ECs

Inoculum (no. of RE/EC)	CFU/well (mean ± SEM) (*n*) after treatment[a]		
	No antibiotics and ECs not fixed	Antibiotics and ECs not fixed	Antibiotics and ECs fixed
10	$(3.73 \pm 0.19) \times 10^4$ (3)	$(1.95 \pm 0.02) \times 10^2$ (2)	0.17 ± 0.17 (2)
100	$(2.12 \pm 0.43) \times 10^5$ (2)	$(3.41 \pm 0.27) \times 10^3$ (4)	7.8 ± 7.1 (2)

[a] Values shown were taken after ECs (3×10^5 per well) were lysed. Antibiotics eliminate extracellular adherent *S. minnesota* Re.

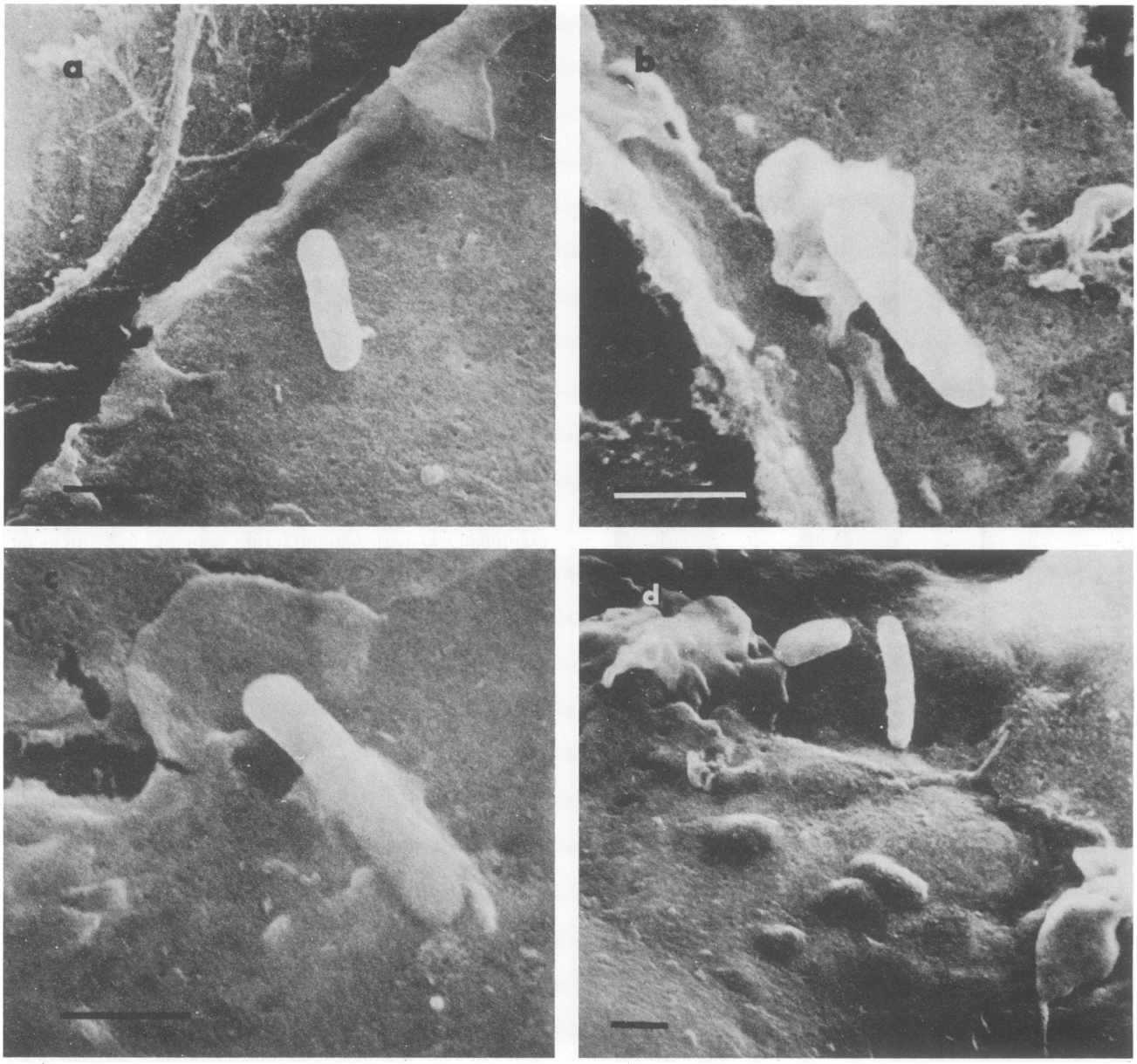

FIG. 3. Scanning electron micrographs showing binding of the Re mutant to the surface of ECs (a), ruffling of the endothelial membrane (b), internalization of bacteria end on (c), and sequestration within the EC (d). Bars, 1 μm.

surface high-affinity binding sites (37), increased adherence, as measured by cellular association of tritiated bacteria (Fig. 1, bar b). Furthermore, preincubation of the Re mutant with C1q-enriched medium also increased adherence (Fig. 1, bar c). We had originally anticipated that preincubation of both bacteria and ECs with C1q might result in blocking of binding sites available for interaction. However, this was not the case, and adherence was in fact enhanced above control values (Fig. 1, bar d). The increased ingestion can be explained in a number of ways. Firstly, the binding of C1q to its receptor on the EC is via the collagenlike portion (37), a mechanism which results in exposure and availability of the IgG-binding heads. On the other hand, binding of C1q to the Re mutant occurs at least in part via the globular heads, leaving part of the collagenlike tail exposed, but binding may also occur via the C1s subunit (7). Thus, there are a number of possibilities for aggregation of C1q on the bacterial surface with concomitant possibilities for multiple interactions with receptors or charged domains on the ECs. It should also be pointed out that while the data presented here indicate the importance of C1q in recognition, binding, and ingestion of the Re mutant of *S. minnesota* by pulmonary ECs, other mechanisms not investigated here might be expected to play a role.

The role of C1q in phagocytosis of the Re mutant is not restricted to the initial stages of recognition and binding but also appears to be a key contributory factor in triggering the respiratory burst. It has been shown previously that ECs can release O_2^- and that this can be stimulated by calcium ionophore and phorbol esters which act synergistically (15, 30). The present data show that the release of superoxide anion by ECs is also stimulated both by ingestion of Re and by C1q in solution (Fig. 2). There is a precedent for this in the literature (32). Although production of superoxide or

hydrogen peroxide was not specifically shown, particle-bound C1q has been shown to stimulate oxidative metabolism in human polymorphonuclear leukocytes in the absence of immunoglobulins, as evidenced by measurement of hexose monophosphate shunt activity and the induction of a chemiluminescent response (32). The magnitude of the chemiluminescent response was dependent on the input of particle-bound C1q. C1q also augmented the chemiluminescent response of polymorphonuclear leukocytes to aggregated IgG (32).

Because adherence is a necessary first step in the process of phagocytosis, C1q may act as an opsonin in the phagocytosis of the Re mutant by ECs. As has been pointed out previously (22), ECs and macrophages possess a number of interesting functional parallels, even if the mechanisms whereby the functions are manifested may not be identical.

Thus, ECs have the capability of participating in activities normally attributed to professional phagocytes. ECs are not usually motile cells, although in culture, ingestion of polystyrene spheres will induce increases in migration, division, and phagocytic behavior (30). However, it is likely that if ECs play a role in phagocytic and bactericidal activities in vivo, they are restricted to clearing localized vascular obstructions and infections. There are a number of instances in which such a role could be of importance; bacteria are found within ECs in subacute bacterial endocarditis (13) and in cerebral vascular infections and are commonly associated with the intimal lining of vascular prostheses (2, 10) and bioprosthetic valves (9). Uptake of bacteria by ECs could be of pathophysiologic significance whether or not the bacteria are killed. Uptake without killing would result in protection from professional phagocytes and a safe harbor from antibiotic action (31), tending to prolong infection, provide resistance to antibiotic therapy, and ultimately lead to bacteremia. On the other hand, depending on the species, strain, and size of inoculum, endothelial bactericidal or bacteriostatic actions could provide a localized second line of defense, particularly in blocked or slowflow vessels such as might be found in the septicemia associated with adult respiratory distress syndrome.

ACKNOWLEDGMENTS

It is a pleasure to thank Patricia Arnold for excellent technical assistance.

This work was supported by Public Health Service grants HL 21568 and HL 33064 from the National Institutes of Health and grant CTR 814 from the Council for Tobacco Research.

LITERATURE CITED

1. **Avrameas, S., and T. Ternynck.** 1969. The cross-linking of proteins with glutaraldehyde and its use for the preparation of immunoadsorbents. Immunochemistry **6**:53–66.
2. **Bergamini, T. M., D. F. Bandyk, D. Govostis, H. W. Kaebnick, and J. B. Towne.** 1988. Infection of vascular prostheses caused by bacterial biofilms. J. Vasc. Surg. **7**:21–30.
3. **Betz, S. J., and H. Isliker.** 1981. Antibody-independent interactions between *E. coli* J5 and human complement components. J. Immunol. **127**:1748–1754.
4. **Betz, S. J., N. Page, C. Estrade, and H. Isliker.** 1982. The effect of specific antibody on antibody-independent interactions between *E. coli* J5 and human complement. J. Immunol. **128**: 707–711.
5. **Clas, F., J. R. Golecki, and M. Loos.** 1984. Electron microscopic study showing the antibody independent binding of C1q, a subcomponent of the first component of complement, to serum-sensitive salmonellae. Infect. Immun. **45**:795–797.
6. **Clas, F., and M. Loos.** 1981. Antibody-independent binding of the first component of complement (C1) and its subcomponent C1q to the S and R forms of *Salmonella minnesota*. Infect. Immun. **31**:1138–1144.
7. **Clas, F., G. Schmidt, and M. Loos.** 1985. The role of the classical pathway for the bactericidal effect of normal sera against gram-negative bacteria. Curr. Top. Microbiol. Immunol. **121**:19–72.
8. **Euteneuer, B., S. Störkel, and M. Loos.** 1985. Differences in attachment and phagocytosis of *Salmonella minnesota* strains (S form, Re mutant) by mouse peritoneal macrophages: participation of endogenous C1q and bacterial surface components (LPS, porins). Curr. Top. Microbiol. Immunol. **121**:85–97.
9. **Fernández-Guerrero, M. L., J. M. Muelas, J. M. Aguado, G. Renedo, J. Fraile, F. Soriano, and E. de Villalobos.** 1988. Q fever endocarditis on porcine bioprosthetic valves. Ann. Intern. Med. **108**:209–213.
10. **Fry, W. J.** 1972. Vascular prosthesis infections. Surg. Clin. N. Am. **52**:1419–1424.
11. **Johnson, J. D., W. L. Hand, J. B. Francis, N. K. Thompson, and R. W. Corwin.** 1980. Antibiotic uptake by alveolar macrophages. J. Lab. Clin. Med. **95**:429–439.
12. **Leist-Welsch, P., and A. B. Bjornson.** 1982. Immunoglobulin-independent utilization of the classical complement pathway in opsonophagocytosis of *Escherichia coli* by human peripheral leukocytes. J. Immunol. **128**:2643–2651.
13. **MacNeal, W. J., M. J. Spence, and A. E. Slavkin.** 1943. Early lesions of experimental endocarditis lenta. Am. J. Pathol. **19**: 735–749.
14. **Malick, L. E., and R. B. Wilson.** 1975. Modified thiocarbohydrazide procedure for scanning electron microscopy: routine use for normal, pathological, or experimental tissues. Stain Technol. **50**:265–269.
15. **Matsubara, T., and M. Ziff.** 1986. Superoxide anion release by human endothelial cells: synergism between a phorbol ester and a calcium ionophore. J. Cell. Physiol. **127**:207–210.
16. **Muschel, L. H., and J. S. C. Fong.** 1977. Serum bactericidal activity and complement, p. 137–158. *In* R. A. Good and S. B. Day (ed.), Biological amplification systems in immunology. Plenum Publishing Corp., New York.
17. **Muschel, L. H., and L. L. Larsen.** 1970. The sensitivity of smooth and rough gram-negative bacteria to the immune bactericidal reaction. Proc. Soc. Exp. Biol. Med. **133**:345–348.
18. **Rowley, D.** 1968. Sensitivity of rough gram-negative bacteria to the bactericidal action of serum. J. Bacteriol. **95**:1647–1650.
19. **Ryan, U. S.** 1986. The endothelial surface and responses to injury. Fed. Proc. **45**:101–108.
20. **Ryan, U. S.** 1986. Immunofluorescence and immunocytochemistry of endothelial surface antigens. J. Tissue Cult. Methods **10**:27–30.
21. **Ryan, U. S.** 1987. Endothelial cell activation responses, p. 3–33. *In* U. S. Ryan (ed.), Pulmonary endothelium in health and disease. Marcel Dekker, Inc., New York.
22. **Ryan, U. S.** 1988. The macrophagelike properties of endothelial cells. News Physiol. Sci. **3**:93–96.
23. **Ryan, U. S.** 1988. Phagocytic properties of endothelial cells, p. 33–49. *In* U. S. Ryan (ed.), Endothelial cells, vol. III. CRC Press, Boca Raton, Fla.
24. **Ryan, U. S., E. Clements, D. Habliston, and J. W Ryan.** 1978. Isolation and culture of pulmonary artery endothelial cells. Tissue Cell **10**:535–554.
25. **Ryan, U. S., and M. A. Hart.** 1986. Electron microscopy of endothelial cells in culture. II. Scanning electron microscopy and OTOTO impregnation method. J. Tissue Cult. Methods **10**:35–36.
26. **Ryan, U. S., and G. Maxwell.** 1986. Isolation, culture and subculture of bovine pulmonary artery endothelial cells: mechanical methods. J. Tissue Cult. Methods **10**:3–5.
27. **Ryan, U. S., and L. J. Mayfield.** 1986. Assay and computation of angiotensin converting enzyme activity of endothelial cells. J. Tissue Cult. Methods **10**:15–25.
28. **Ryan, U. S., M. Mortara, and C. Whitaker.** 1980. Methods for microcarrier culture of bovine pulmonary artery endothelial cells avoiding the use of enzymes. Tissue Cell **12**:619–635.
29. **Ryan, U. S., J. W. Ryan, C. Whitaker, and A. Chiu.** 1976.

Localization of angiotensin converting enzyme (kininase II). II. Immunocytochemistry and immunofluorescence. Tissue Cell **8**:125–146.

30. **Ryan, U. S., and J. M. Vann.** 1987. Endothelial cells: a source and target of oxidant damage, p. 963–968. *In* M. G. Simic, K. A. Taylor, and A. F. Ward (ed.), Oxygen radicals in biology and medicine. Plenum Publishing Corp., New York.
31. **Ryan, U. S., and J. M. Vann.** 1988. Cultured endothelial cells as probes for in vivo biology, p. 49–59. *In* A. M. Goldberg (ed.), Alternative methods in toxicology, vol. 6. Mary Ann Liebert Inc., New York.
32. **Schrieber, R. D.** 1984. The chemistry and biology of complement receptors, p. 115–143. *In* H. J. Müller-Eberhard and P. A. Meischer (ed.), Complement. Springer-Verlag, Berlin.
33. **Sutter, E.** 1952. Multiplication of tubercle bacilli within phagocytes cultivated in vitro, and effect of streptomycin and isonicotinic acid hydrazide. Am. Rev. Tuberc. **65**:775–776.
34. **Tenner, A. J., R. J. Ziccardi, and N. R. Cooper.** 1984. Antibody-independent C1 activation by E. coli. J. Immunol. **133**:886–891.
35. **Volanakis, J. E., and R. M. Stroud.** 1972. Rabbit C1q: purification, functional and structural studies. J. Immunol. Methods **2**:25–34.
36. **Voyta, J. C., D. P. Via, C. E. Butterfield, and B. R. Zetter.** 1984. Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein. J. Cell Biol. **99**:2034–2040.
37. **Zhang, S. C., D. R. Schultz, and U. S. Ryan.** 1986. Receptor-mediated binding of C1q on pulmonary endothelial cells. Tissue Cell **18**:13–18.