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Septicemia-Inducing *Escherichia coli* O115:K"V165"F165$_1$ Resists Killing by Porcine Polymorphonuclear Leukocytes In Vitro: Role of F165$_1$ Fimbriae and K"V165" O-Antigen Capsule

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***Escherichia coli* O115:K"V165":F165$_1$ wild-type strain 5131 survives in the bloodstream of experimentally inoculated gnotobiotic pigs and induces septicemia, whereas its afimbriate (F165$_1$-negative) Tn*phoA* mutant M48 and its acapsular (K"V165"-negative) spontaneous mutant 5131a are both nonpathogenic. We evaluated the role of the mannose-resistant F165$_1$ fimbrial system and of the O-antigen K"V165" capsule in resistance to phagocytosis by porcine polymorphonuclear leucocytes (PMNLs) in vitro. F165$_1$-positive strains (5131 and 5131a) attached to and were ingested by PMNLs at a significantly higher level than afimbrial mutant M48 ($P < 0.001$) after 1 h of incubation. During incubation of these strains with PMNLs for up to 6 h, parental strain 5131 resisted killing whereas afimbriate mutant M48 and acapsular mutant 5131a were gradually killed and were found at significantly lower numbers than the parental strain 5131 at 2 ($P < 0.05$) and 6 ($P < 0.001$) h. When bacteria were opsonized with normal pig serum, the afimbriate and acapsular mutants survived less well than when the bacteria were nonopsonized. Upon examination by electron microscopy of PMNLs after 2 h of incubation with bacteria, structurally normal bacteria were observed more often within phagosomes of PMNLs incubated with the parental strain than within phagosomes of PMNLs incubated with the afimbriate or the acapsular mutant. The extracellular oxidative response (as tested by release of hydrogen peroxide) of PMNLs stimulated by phorbol myristate acetate was completely inhibited by F165$_1$-positive strains but only partially inhibited by the afimbriate mutant. These results suggest that the F165$_1$ fimbrial system may mediate adherence of *E. coli* O115 to PMNLs. Survival of the parental strain in the presence of PMNLs, which may be intracellular, is at least partially due to the presence of the F165$_1$ fimbrial system and of the O-antigen capsule K"V165". Furthermore, the presence of the F165$_1$ fimbrial system may contribute to the bacterial inhibition of the oxidative response of porcine PMNLs.**

Extraintestinal infections due to *Escherichia coli* include septicemia and polyserositis, newborn meningitis, and urinary tract infections in humans and other animals (8, 34, 39). Virulence-associated factors of *E. coli* causing septicemia include mannose-resistant fimbriae, cytotoxins, aerobactin and ColV plasmids, outer membrane proteins, lipopolysaccharide (LPS), and capsule and O-antigen capsule (8, 33, 34). These virulence-associated factors allow the bacteria to invade the host and escape its defense mechanisms by inducing their resistance to the bactericidal effect of complement, phagocytic killing, and growth in body fluids with a low concentration of free iron (3, 39). Furthermore, in most virulent gram-negative bacteria, the negative charge and hydrophobicity associated with fimbrial antigens, capsular antigens (K antigen, slime, and other polysaccharides such as O-antigen capsule), and smooth LPS increase the capacity of the bacteria to avoid phagocytic recognition and intracellular killing (30, 33, 35).

Mannose-specific type 1 fimbriae and other adhesins have been associated with increased adherence of bacteria to a number of different eukaryotic cells by means of specific receptors present on these cell surfaces (1, 22, 29, 40). Thus, phagocytes can recognize specific structures expressed on bacterial surfaces; this nonopsonic recognition termed lectinophagocytosis allows the phagocytes to bind and, subsequently, ingest the bacteria by lectin-sugar interactions (35). However, specific ligand-receptor interactions mediated by type 1 fimbriae do not always ultimately lead to phagocytic killing of bacteria (29). In fact, there is no consensus of opinion on the role of type 1 fimbriae, either in resistance to phagocytosis or in pathogenicity. These fimbriae may be disadvantageous for the bacteria in the presence of phagocytes, but other reports indicate that they could also be advantageous for bacteria which are entering the circulatory system or located within the phagocytes (1, 21, 29).

Phagocytes kill invading microorganisms by oxygen-dependent or oxygen-independent mechanisms. The former, also referred to as the respiratory burst, is characterized by an increased oxygen uptake, production of the superoxide anion (O_2^-) and hydrogen peroxide, and formation of hydroxide radicals. Hydrogen peroxide, myeloperoxidase, and a halide form a powerful antimicrobial system (7, 20). Furthermore, a fraction of the O_2^--forming NADPH oxidase is localized in the plasma membrane of phagocytes. Activation of this oxidase can result in the extracellular release of oxygen metabolites, which could play a role in the killing of nonphagocytized bacteria (2).

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E. coli isolates of serogroup O115:K"V165" have been associated with septicemia and polyserositis in naturally infected calves and pigs and in experimentally inoculated pigs (8, 33, 34). These isolates express various virulence-associated factors, which include the fimbrial antigens $F165_1$ and $F165_2$ (18), the K"V165" O-antigen capsule, the smooth LPS characteristic of the O115 serogroup, the aerobactin system, and the ColV plasmid (8, 33, 34). The $F165_1$ fimbriae belong to the P fimbria class, being most similar to F11, but possess a Prs-like adhesin (group III G adhesin) similar to that found on human uropathogenic *E. coli*. This adhesin agglutinates sheep, human A_1P_1, and pig erythrocytes and recognizes the globopentasyceramide containing the GalNAc-α-(1-3)-β-GalNAc moiety of the Forssman antigen (9, 18, 27, 28). By using Tn*phoA* and spontaneous mutants, we have demonstrated that both the K"V165" O-antigen capsule and the $F165_1$ fimbrial system are required not for initial intestinal colonization but for systemic survival of bacteria in experimentally inoculated pigs (33, 34). Thus, the purpose of this study was to evaluate the role of these determinants in the resistance of *E. coli* O115:K"V165" to phagocytosis in vitro.

(These results were presented at the 92nd General Meeting of the American Society for Microbiology, New Orleans, La., 26 to 30 May 1992 [32].)

MATERIALS AND METHODS

Bacterial strains and growth conditions. Strains 5131 and 862B are pathogenic and nonpathogenic *E. coli* of serogroups O115:K"V165":$F165_1$ and O115:K^-:F^-, respectively (34). They were isolated at the Faculté de Médecine Vétérinaire, Université de Montréal, Saint-Hyacinthe, Québec, Canada, from piglets with septicemia and diarrhea. Nonpathogenic strain 862B was used as a negative control in our phagocytic killing assay and electron microscope analysis described below. Strain M48 is a Tn*phoA* mutant obtained from the parental strain 5131 and is nonpathogenic for experimentally inoculated pigs (17, 34). The Tn*phoA* insertion in this mutant has been located in the *A* gene of the *f165*$_1$ operon. Thus, this mutant no longer produces the $F165_1$ fimbrial system; it is negative for mannose-resistant hemagglutination of sheep erythrocytes and for fimbrial antigen $F165_1$ as assessed by slide hemagglutination, immunoelectron microscopy, Western blot (immunoblot), and immunodot with anti-$F165_1$ serum. In addition, this mutant is less hydrophobic than its parental strain 5131 but identical to this parental strain in all other respects, including in vitro growth rate (17, 34). Strain 5131a is a spontaneous O-agglutinable mutant from the parental strain 5131 (8) and is also nonpathogenic for experimentally inoculated pigs (33). It produces a markedly reduced amount of O-antigen capsule K"V165".

Strains were grown on minimal Davis liquid medium (Difco Laboratories, Detroit, Mich.) plus Casamino Acids (MD-1 medium) for 18 h at 37°C to facilitate the expression of $F165_1$ fimbriae (10).

Opsonization of bacteria. Bacteria were incubated for 15 min at 37°C in 50% fresh serum obtained from clinically healthy pigs. The opsonized bacteria were washed in phosphate-buffered saline (PBS; 0.1 M, pH 7.4).

Preparation of porcine PMNLs. Blood from three clinically healthy pigs was collected from the jugular vein by using Vacutainer blood collection tubes (Becton Dickinson, Rutherford, N.J.), containing sodium heparin, and pooled. Polymorphonuclear leukocytes (PMNLs) were extracted by dextran sedimentation and hypotonic lysis as described previously (25). They were allowed to adhere to plastic tissue culture plates in 5% CO_2 for 2 h at 37°C or maintained in suspension for the different assays. The viability of infected or noninfected PMNLs determined by trypan blue exclusion was approximately 98% after 2 h of incubation and approximately 95% after an additional 6 h incubation.

Phagocyte-bacterium interaction. The attachment to and ingestion of bacteria by PMNLs were determined by the fluorescence quenching technique described by Hed (19). Briefly, 2×10^5 PMNLs in Dulbecco's modified Eagle's medium (GIBCO Canada Inc., Burlington, Ontario) supplemented with 20 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid; D-MEM-H medium; GIBCO) and 10% heat-inactivated fetal calf serum (GIBCO) were incubated in each of the eight wells of Lab-Tek chamber slides (Nunc, Inc., Naperville, Ill.) in 5% CO_2 for 2 h at 37°C. The chambers were washed with balanced salt solution (Pharmacia LKB Biotechnology, Inc., Piscataway, N.J.) to eliminate nonadherent cells. A volume of 270 μl of fresh D-MEM-H medium and 30 μl of a suspension containing 10^8 nonopsonized bacteria ml^{-1} labelled with fluorescein isothiocyanate (Sigma Chemical Co., St. Louis, Mo.) was added to each chamber, and the slides were incubated as described above for 30 min or 1 or 2 h. After washing the slides with balanced salt solution, adhered and ingested bacteria were examined with a Leitz diaplan microscope (Leitz, Wetzlar, Germany). After the addition of crystal violet, the attached but not the ingested bacteria lost their fluorescence. The results are expressed as the mean of associated or ingested bacteria per PMNL for 100 PMNLs examined in each of three experiments.

Phagocyte killing assay. The number of live bacteria (in CFU per milliliter), after incubation of PMNLs with nonopsonized or opsonized bacteria, was determined as described previously (21). Briefly, a volume of 1 ml of a suspension of 2×10^6 PMNLs ml^{-1} in D-MEM-H medium supplemented with 10% heat-inactivated fetal calf serum was distributed in each well of Nunclon 24-well tissue culture plates (GIBCO) and incubated as described above. Nonadherent cells were removed by washing the wells with balanced salt solution, and 0.9 ml of fresh D-MEM-H medium was added to adherent cells. A volume of 0.1 ml of a suspension containing 10^8 CFU of bacteria ml^{-1} grown in MD-1 medium for 18 h at 37°C was added to PMNL cultures to give a bacterium-to-phagocyte ratio of approximately 10:1. To avoid interference due to extracellular proliferation of the microorganisms, spectinomycin (Sigma; final concentration, 3 μg ml^{-1}) was added to the wells to limit bacterial growth (21, 25). In parallel, a volume of 1 ml of a suspension containing 10^7 CFU of bacteria ml^{-1} grown as described above and used as a control was incubated in D-MEM-H medium containing the same concentration of spectinomycin but in the absence of PMNLs. At the beginning of the experiment (time zero), a volume of 0.1 ml was immediately removed from each well, serially diluted, and plated onto brain heart infusion agar (Difco) to obtain the preincubation colony count. The tissue culture and control plates were incubated in 5% CO_2 for 1, 2, or 6 h at 37°C. To release intracellular bacteria after incubation, 0.1% Triton X-100 (Fisher Scientific, Fair Lawn, N.J.) was added to each well, and bacterial counts were performed as they were at time zero. All data are the means of three or four independent experiments done in triplicate.

Electron microscope analysis. (i) PMNL monolayer. A volume of 1 ml of a suspension of 2×10^6 PMNLs ml^{-1} extracted as described above were allowed to adhere to 13-mm round, plastic Thermanox discs (Nunc) in wells of 24-well Nunclon tissue culture plates (GIBCO). Two hours after incubation as described above, nonadherent cells were re-

moved by washing, and 0.9 ml of fresh medium was added to the wells. A volume of 0.1 ml of a suspension containing 10^9 CFU of opsonized bacteria ml^{-1} grown overnight in MD-1 liquid medium was added to each well. Two hours after incubation in 5% CO_2 at 37°C, the wells were washed twice in PBS (pH 7.2) at 4°C, and the adherent PMNLs were fixed for 2 h at 4°C in 2.5% glutaraldehyde diluted in PBS. After subsequent washing and postfixation with 1% osmium tetroxide for 1 h, the PMNLs on the plastic disc were dehydrated in acetone and embedded in Epon (JEMBED 812 embedding kit; J. B. EM Services Inc., Montréal, Quebec, Canada). Thin sections were stained with uranyl acetate and lead citrate and examined with a transmission electron microscope (Philips 201) at an accelerating voltage of 60 kV.

(ii) PMNL suspension. A volume of 0.5 ml of a suspension containing 10^9 CFU of opsonized bacteria ml^{-1} grown as described above was added to 4.5 ml of a suspension of 10^6 PMNLs ml^{-1}. Two hours after incubation in 5% CO_2 at 37°C, the mixture was centrifuged at 3,200 × *g* for 4 min and the pellet was washed twice in PBS (pH 7.2) at 4°C, fixed in 2.5% glutaraldehyde diluted in PBS for 10 min at 4°C, and then fixed further at room temperature. After being washed, the phagocytes were processed for electron microscopy analysis as described above.

Oxidative burst assay. The phagocytic activity of PMNLs was assessed by measurement of hydrogen peroxide (H_2O_2) release determined by the extinction of scopoletin (Sigma) fluorescence as described by Root et al. (38). Briefly, a volume of 4.5 ml of a suspension containing 10^6 PMNLs ml^{-1}, 4 μM scopoletin, and 22 nM horseradish peroxidase (Sigma) in PBS (pH 7.4) plus 0.1% (wt/vol) gelatin (Fisher) was incubated in 5% CO_2 for 5 min at 37°C before the addition of 0.5 ml of a suspension of 10^9 CFU of nonopsonized or opsonized bacteria ml^{-1} or 1 μg of phorbol myristate acetate (PMA; Sigma) ml^{-1}, the latter being used as a positive control. The addition of PMA to the suspension of resting PMNLs mimics the process of phagocytosis, resulting in an increased oxidative burst (5). In some experiments, PMA was added either at the same time as the bacteria, or 20 min before the bacteria. A volume of 0.5 ml of the mixture was removed at different times and centrifuged at 6,000 × *g* for 4 min at room temperature. The supernatant was monitored immediately for fluorescence in a TKO 100 Fluorometer (Hoefer Scientific Instruments, San Francisco, Calif.). The results are the mean of three to five independent experiments and are expressed as nanomoles of H_2O_2 per 10^6 PMNLs, determined from a standard curve with H_2O_2 reagent (Fisher).

Statistics. Student's *t* test for paired samples was used for statistical analysis (42). Differences were considered to be significant when *P* values of <0.05 were obtained.

RESULTS

Interaction between porcine PMNLs and *E. coli* O115 strains. Optimum adherence and ingestion of bacteria by PMNLs was observed from 1 to 2 h after incubation in 5% CO_2 at 37°C (data not shown). We chose 1 h as a convenient incubation period when PMNL-associated and -ingested bacteria could be counted easily. Both the parental strain 5131 and the acapsular mutant 5131a adhered to and were ingested by PMNLs at a significantly higher level than the afimbrial mutant M48 ($P < 0.001$). These results suggest that PMNLs may express receptors for the $F165_1$ adhesin which would lead to lectinophagocytosis of bacteria producing these fimbriae. Of the $F165_1$-positive strains, the number of associated or ingested bacteria of acapsular mutant 5131a was significantly

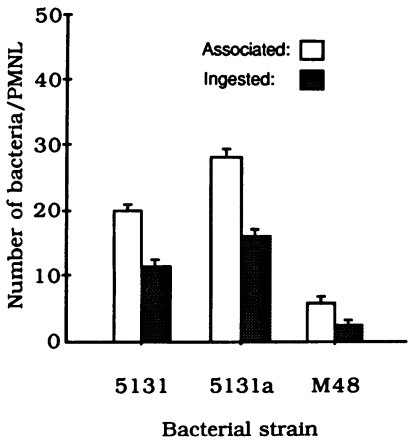

FIG. 1. Adherence and ingestion of *E. coli* O115 strains incubated with porcine PMNLs. Fluorescein isothiocyanate-labelled bacteria (10^7 CFU) were incubated for 1 h with approximately 10^5 PMNLs. The number of associated bacteria on 100 phagocytes was counted, and after the addition of crystal violet, the number of ingested bacteria was determined. The results are expressed as means of associated or ingested bacteria per PMNL ± standard deviation of three independent experiments.

greater than that of parental strain 5131 ($P < 0.001$; Fig. 1). The absence of the K"V165" O-antigen capsule in mutant 5131a could increase exposure of $F165_1$ fimbriae or other determinants which promote association of bacteria with and ingestion by PMNLs.

Bactericidal effect of PMNLs on *E. coli* O115 strains. The bactericidal assay was performed at a bacterium-to-phagocyte ratio of approximately 10:1 in the presence of spectinomycin, the latter being used to limit bacterial growth. Preliminary experiments had shown that, at a spectinomycin concentration of 3 μg ml^{-1}, PMNL viability was not affected and bacterial numbers remained constant for each of the tested strains in the absence of PMNLs (negative control) at 1, 2, and 6 h after incubation in 5% CO_2 at 37°C (data not shown).

After incubation of bacteria with PMNLs, the total numbers of viable bacteria of parental strain 5131 remained constant for 6 h, even though bacteria of this strain adhered to and were ingested by PMNLs (Fig. 2A). The numbers of viable bacteria of nonpathogenic strain 862B rapidly decreased over the same time. The numbers of viable bacteria of the acapsular mutant 5131a, which more readily adhered to and was ingested by PMNLs than the parent strain, decreased after incubation with the PMNLs and were significantly different from those of the parental strain 5131 at 2 ($P < 0.05$) and 6 ($P < 0.001$) h after incubation with PMNLs (Fig. 2A). Surprisingly, the numbers of viable bacteria of the afimbrial mutant M48, which adhered to and was ingested by PMNLs significantly less well than the parental strain 5131, also decreased to a greater extent than they did for the acapsular mutant, during the incubation with PMNLs, and were significantly different from those of the parental strain at 2 ($P < 0.05$) and 6 ($P < 0.001$) h. These results suggest that the presence of both the K"V165" O-antigen capsule and the $F165_1$ fimbrial system are important in the resistance of parental strain 5131 to killing by PMNLs, probably by different mechanisms.

To mimic the phagocytosis of septicemia-inducing *E. coli* O115 strains in vivo, bacteria were opsonized with normal pig serum for 15 min and the experiment was repeated. Preliminary experiments had shown that opsonization of parental

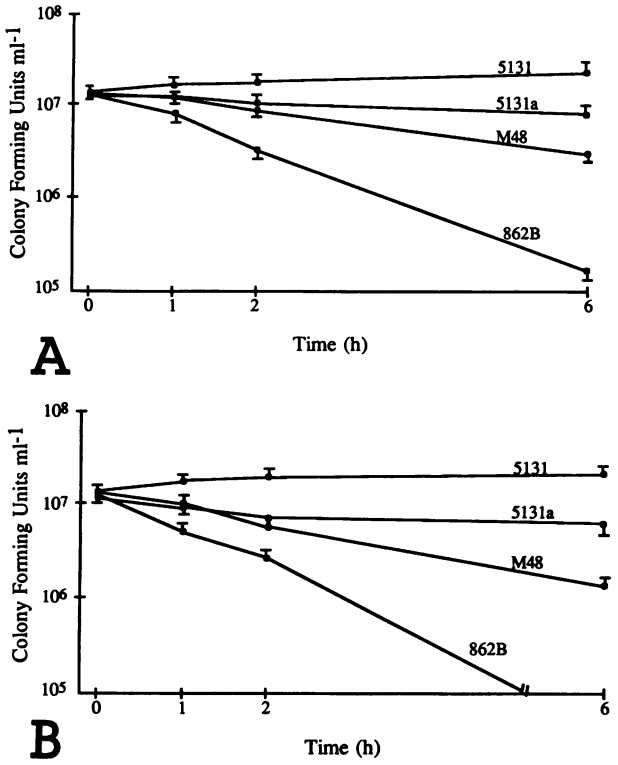

FIG. 2. Viability of *E. coli* O115 strains incubated with porcine PMNLs. Nonopsonized (A) or opsonized (B) bacteria (10^7 CFU) were incubated with approximately 10^6 porcine PMNLs or without these phagocytes (not shown) as described in Materials and Methods. At time zero, a sample was immediately removed, diluted, and plated on brain heart infusion agar to obtain the initial CFU per milliliter. The results are expressed as means of three or four independent experiments (each done in triplicate) ± standard deviation.

strain 5131 and its mutants increased the number of PMNL-associated and -ingested bacteria in vitro (data not shown).

Nevertheless, the numbers of the parent strain 5131 remained relatively constant during 6 h of incubation in the presence of PMNLs (Fig. 2B). The numbers of viable bacteria of the afimbrial and acapsular mutants decreased during the 6 h of incubation with PMNLs in a manner similar to that of the nonopsonized bacteria, although to a greater extent, and were significantly different from the numbers of the parental strain at 2 h of incubation ($P < 0.05$) and 6 h after incubation ($P < 0.001$; Fig. 2B). These results demonstrate that opsonization of bacteria does not affect the resistance of parental strain 5131 to killing by PMNLs but slightly accentuates the ability of the phagocytes to kill this strain when it lacks the K"V165" O-antigen capsule or the F165$_1$ fimbrial system.

Electron microscopic analysis of bacterial phagocytosis. To confirm the resistance of parental strain 5131 to phagocytosis and the destruction of the afimbrial and acapsular mutants by PMNLs, phagocytes used as a monolayer or in suspension were examined by electron microscopy 2 h after incubation with bacteria. At this time, morphologically intact bacteria of strain 5131 had been taken up and were accumulating within the phagosomes of PMNLs either as a monolayer or in suspension (Fig. 3A). In contrast, only dispersed bacteria with some cellular debris were observed within phagocytes incubated with the afimbrial mutant M48 (Fig. 3B) or the acapsular mutant 5131a (data not shown). Few bacteria were observed within phagocytes incubated with nonpathogenic strain 862B, although some material resembling bacterial remnants could be observed within numerous PMNL phagosomes (not shown).

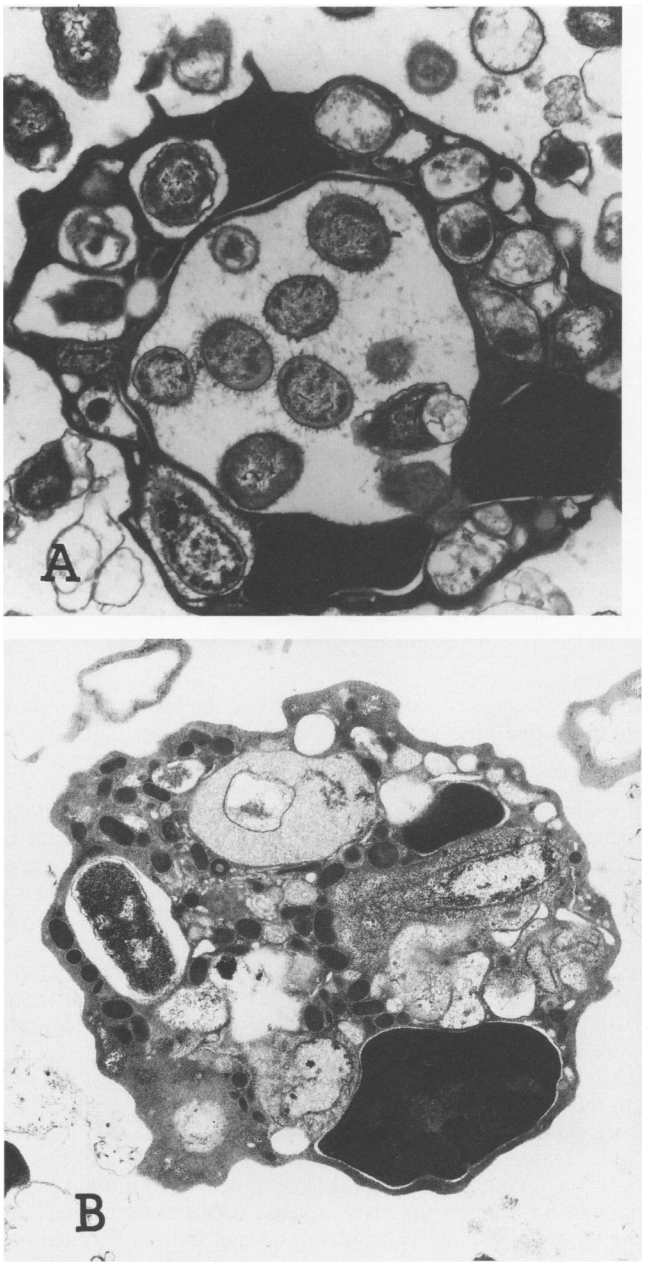

FIG. 3. Electron microscopic analysis of phagocytosis by porcine PMNLs. Approximately 10^6 PMNLs in suspension was incubated for 2 h with 10^8 CFU of the parental strain 5131, the acapsular mutant 5131a, the afimbrial Tn*phoA* mutant M48, or the nonpathogenic wild-type strain 862B as described in Materials and Methods. Transmission electron microscopy was performed by standard techniques. The parental strain 5131 accumulated within phagosomes of PMNLs (A), whereas debris was observed in PMNLs incubated with the afimbrial mutant M48 (B), the acapsular mutant 5131a (not shown), or the nonpathogenic strain 862B (not shown).

Oxidative burst response by PMNLs incubated with *E. coli* O115 strains. Many bacteria have developed different strategies for avoiding the bactericidal effect of phagocytes (5). One

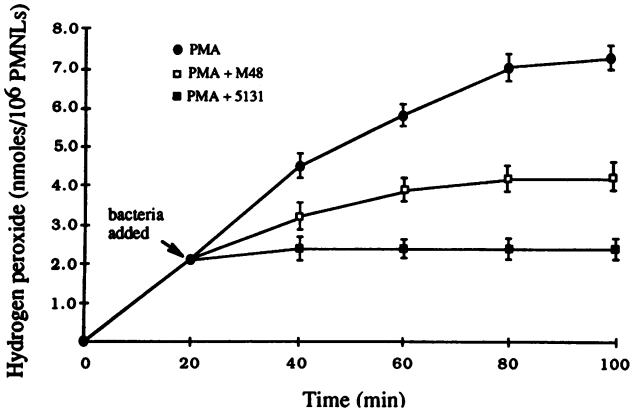

FIG. 4. Hydrogen peroxide release by porcine PMNLs. PMA (1 μg ml^{-1}) was added to PMNLs 20 min prior to the addition of bacteria of the parental strain 5131, its afimbrial mutant M48, or its acapsular mutant 5131a (not shown). The release of H_2O_2, measured as the decrease in relative fluorescence of scopoletin (4 μM) in the presence of horseradish peroxidase (22 nM), was monitored as described in Materials and Methods. The results are expressed as the mean values of four different tests ± standard deviations.

of these strategies involves the ability of the bacteria to reduce the oxidative burst, even after attachment to or ingestion by phagocytic cells. In preliminary experiments, we observed that neither the parental strain 5131, its acapsular mutant 5131a, nor its afimbrial mutant M48 stimulated an extracellular oxidative response in PMNLs, as measured by reduction of scopoletin by released H_2O_2. In contrast, PMNLs incubated in the presence of PMA resulted in a high level of H_2O_2 release. These data suggest that *E. coli* of serogroup O115 may actively inhibit an oxidative response from porcine PMNLs.

To determine whether this absence of reaction by PMNLs was due to an active inhibition by the bacteria and whether the $F165_1$ fimbrial system and the K"V165" O-antigen capsule were involved in an inhibitory effect, we examined the effect of these strains on the extracellular oxidative response of PMNLs stimulated by PMA. When nonopsonized bacteria and PMA were added to PMNLs simultaneously or when the PMNLs were treated with PMA for 20 min prior to the addition of bacteria, the parental strain 5131 or its acapsular mutant 5131a appeared to inhibit the H_2O_2 release to a greater extent than the afimbrial mutant M48 did, but no significant difference was observed between these strains (results not shown).

Since preliminary experiments showed that bacterial opsonization resulted in greater association with and ingestion by PMNLs of parental strain 5131 and its mutants and less variability in the bacterium-phagocyte interaction of the different strains, we examined the effect of the opsonized strains on the release of H_2O_2 by PMNLs stimulated with PMA. When the bacteria and PMA were added simultaneously to PMNLs, we observed a complete inhibition of H_2O_2 release by PMNLs incubated with the parental strain 5131 or its acapsular mutant 5131a. In contrast, only partial inhibition of H_2O_2 release by PMNLs incubated with the afimbrial mutant M48 was observed (results not shown). Similarly, when PMNLs were treated with PMA for 20 min prior to the addition of bacteria, the addition of the parental strain 5131 or its acapsular mutant 5131a (not shown) resulted in a total inhibition of H_2O_2 release (Fig. 4). In contrast, only partial inhibition of H_2O_2 release, significantly different from that of the parental strain 5131 ($P < 0.05$), was observed for the afimbrial mutant M48. These results suggest that the absence of extracellular oxidative response of PMNLs after the addition of $F165_1$-positive strains, as measured by H_2O_2 release, is partially due to an active inhibition by these bacteria. This inhibition may be partially due to the presence of the $F165_1$ fimbrial system. This may account for the resistance of the parental strain to phagocytic killing.

DISCUSSION

We have demonstrated that septicemic *E. coli* O115:K"V165":$F165_1$ adheres to and is taken up by porcine PMNLs but is not killed in the presence of these phagocytes for at least 6 h. The adherence of $F165_1$-positive strains (parental strain 5131 and acapsular mutant 5131a) to PMNLs to a greater extent than that of the afimbriate mutant M48 in the absence of opsonins suggests the presence of $F165_1$ receptors on porcine PMNLs and mediation of lectinophagocytosis by the $F165_1$ fimbrial system. These results may also reflect changes in the phagocyte-bacterium interaction due to changes in bacterial cell surface properties resulting from the absence of the $F165_1$ fimbrial system. Wild-type parent strain 5131 produces $F165_1$ fimbriae which belong to the P fimbrial class but express an adhesin related to the Prs adhesin (16, 18). Thus, porcine PMNLs may express receptors which could be of great importance in bacterial recognition during phagocytosis of $F165_1$-positive or Prs-positive bacteria.

In general, after attachment of bacteria to phagocytes, usually mediated by antibodies and/or complement directed against the bacterial surface, the bacteria are engulfed into phagosomes (phagocytosis) and killed through oxygen-dependent or oxygen-independent bactericidal activity (7, 23). Thus, the survival of bacteria in the presence of, or after ingestion by, phagocytes is a prerequisite for intracellular growth and could be explained by at least one or more of the four distinct mechanisms which can be induced by an intracellular parasite: (i) bacterial multiplication in phagolysosomes due to resistance to bactericidal activity, (ii) bacterial escape from the phagosomes into the cell cytoplasm, (iii) blockage of phagosome-lysosome fusion, or (iv) inhibition of induction of the oxidative burst (6, 15, 36).

Our results provide evidence that *E. coli* O115:K"V165" parental strain 5131 can be ingested but not killed and probably accumulates in the phagosomes of porcine PMNLs. The presence of both the $F165_1$ fimbrial system and the K"V165" O-antigen capsule is necessary for the complete resistance of the parental strain 5131 to phagocytic killing by PMNLs. The difference between the ability of the opsonized parental strain 5131 and that of its afimbrial mutant M48 to modulate hydrogen peroxide release after the addition of PMA suggests that the presence of the $F165_1$ fimbrial system is at least partly responsible for the decrease in the capacity of porcine PMNLs to undergo respiratory burst activity, thus leading to bacterial resistance to phagocytic killing.

Among other mannose-resistant fimbrial antigens, the nonfimbrial adhesin 1 (NFA-1) of urinary isolates also mediates bacterial attachment to human PMNLs but, in contrast to $F165_1$ fimbriae, triggers the oxidative burst in these phagocytes, which results in ingestion and killing of the microorganisms (13). Strains from human urinary tract infection, expressing globotetrasylceramide-sensitive adhesins specific for globoseries glycolipid receptors (P fimbriae), poorly bind to and activate human PMNLs. However, coating of PMNLs with globoseries glycolipids results in improved binding of such strains and also in increased oxidative activity (40). Similarly, genetically cloned *E. coli* strains expressing either S adhesin or

P fimbriae together with its adhesin are more readily phagocytosed by, and induce a higher oxidative response in, human PMNLs than the nonadherent strain (41). Bacteria expressing these fimbriae are associated with newborn meningitis and urinary tract infection, respectively, in humans. However, a genetically cloned strain expressing the S adhesin together with S fimbriae adheres to PMNLs but is less phagocytosed than the clone expressing S adhesin but not S fimbriae and does not induce an oxidative response (41). Thus, it appears that the presence of certain fimbriae such as S, irrespective of the presence of the fimbrial adhesin, may be involved in uptake and subsequent oxidative response of PMNLs. In contrast, we demonstrate that the presence of the $F165_1$ fimbrial system is responsible for a decreased oxidative response, even though bacteria have been taken up by porcine PMNLs. Similarly, certain outer membrane proteins may be responsible for a diminished oxidative response in human PMNLs which have ingested *Yersinia enterocolitica* (26).

The presence of K"V165" O-antigen capsule also seems to be involved in the resistance of *E. coli* O115 to phagocytosis. This O-antigen capsule was previously shown to increase the resistance of bacteria to the bactericidal effect of serum in vitro and to enhance pathogenicity in vivo (33). Our present results show that the nonopsonized acapsular mutant adhered to and was ingested by porcine PMNLs in higher numbers and killed to a greater extent from 2 to 6 h after incubation with the phagocytes than its nonopsonized parental strain 5131. On the other hand, the acapsular mutant demonstrated a greater amount of exposed fimbrial material than its parent strain on immunofluorescence microscopy with anti-$F165_1$ serum (personal observation). It is possible that the loss of the K"V165" O-antigen capsule increased exposure of $F165_1$ fimbrial epitopes and consequently resulted in greater attachment to and ingestion by PMNLs, with subsequent killing.

Surface polysaccharides termed K antigens (capsule and microcapsule), such as K1 capsule of isolates from newborn meningitis, and O-antigen capsule may influence the resistance of bacteria to complement or to phagocytic killing (14, 21, 33, 37). Resistance to phagocytosis may be induced by the hydrophilicity and negative charge of polysaccharide capsule, characteristics which reduce the surface tension at the interface between the phagocytic cells and the bacteria (31). The negative charge on these cells would result in mutual repulsion, reducing the contact between them. Furthermore, certain polysaccharide capsules may inhibit induction of cytokine mediators which enhance bacterial killing in the environment of the phagocytes (4).

Opsonization of bacteria with normal porcine serum does not seem to enhance phagocytic killing of the parental strain 5131. In contrast, the opsonized afimbrial and acapsular mutants were killed to a significantly greater extent 6 h after incubation with PMNLs than the parent strain 5131 ($P < 0.001$). The killing of opsonized mutants incubated with PMNLs appeared to be greater than that of the same mutants when nonopsonized. Complement opsonization of the afimbrial or the acapsulate mutants could have sensitized these mutants to the bactericidal effect of PMNLs in a manner which would have enhanced their phagocytic killing. It is possible that changes in the outer membrane surface structures of these mutants facilitate complement attachment to their extracellular exposed structures and, consequently, increase phagocyte-bacterium interaction which results in phagocytic killing. In addition, some reports indicated that opsonization of bacteria such as *Staphylococcus aureus* and *E. coli* by immunoglobulins and complement components is required for optimal phagocytic bactericidal activity (11, 24) and may contribute to an increased oxidative response from human PMNLs (12).

Thus, killing of the afimbrial mutant by porcine PMNLs could involve an oxygen-dependent mechanism, whereas killing of the acapsular mutant could involve an oxygen-independent mechanism.

In conclusion, this study provides evidence that septicemia-inducing *E. coli* of serotype O115:K"V165":H51:$F165_1$ resists phagocytosis by porcine PMNLs in vitro. We have demonstrated that although the $F165_1$ fimbrial system of *E. coli* O115:K"V165" appears to mediate bacterial adherence to phagocytes, this fimbrial system participates in the resistance to phagocytic killing of bacteria incubated with porcine PMNLs, possibly in part because of the inhibition of hydrogen peroxide release by these phagocytes. The K"V165" O-antigen capsule could provide an additional mechanism of bacterial resistance to phagocytic killing. Thus, we have elucidated in part a mechanism for the role of the $F165_1$ fimbrial system in the resistance of *E. coli* of serogroup O115 to phagocytic killing by porcine PMNLs. However, further studies to define the observed inhibitory activity of the $F165_1$ fimbrial system on the oxidative burst of these PMNLs are needed.

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