Bordetella pertussis Lipopolysaccharide Resists the Bactericidal Effects of Pulmonary Surfactant Protein A

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**Bordetella pertussis** Lipopolysaccharide Resists the Bactericidal Effects of Pulmonary Surfactant Protein A

Lyndsay M. Schaeffer,* Francis X. McCormack,† Huixing Wu,† and Alison A. Weiss‡

Surfactant protein A (SP-A) plays an important role in the innate immune defense of the respiratory tract. SP-A binds to lipid A of bacterial LPS, induces aggregation, destabilizes bacterial membranes, and promotes phagocytosis by neutrophils and macrophages. In this study, SP-A interaction with wild-type and mutant LPS of *Bordetella pertussis*, the causative agent of whooping cough, was examined. *B. pertussis* LPS has a branched core structure with a nonrepeating trisaccharide, rather than a long-chain repeating O-Ag. SP-A did not bind, aggregate, nor permeabilize wild-type *B. pertussis*. LPS mutants lacking even one of the sugars in the terminal trisaccharide were bound and aggregated by SP-A. SP-A enhanced phagocytosis by human monocytes of LPS-mutant strains, but only in the absence of functional adenylate cyclase toxin, a *B. pertussis* toxin that has been shown to depress neutrophil activity. We conclude that the LPS of wild-type *B. pertussis* shields the bacteria from SP-A-mediated clearance, possibly by sterically limiting access to the lipid A region. *The Journal of Immunology*, 2004, 173: 1959–1965.

Surfactant protein A (SP-A) is one of four known protein components of the pulmonary surfactant. Previous studies have demonstrated that SP-A<sup>−/−</sup> (knockout) mice have normal baseline pulmonary function (1, 2); however, when challenged with a variety of viruses, bacteria, and fungi, these animals proved to be more susceptible to respiratory infections (3, 4). SP-A is a member of the collectin (collagen-like lectin) family of proteins. Like other collectins, including mannose-binding protein and surfactant protein D, SP-A participates in recognition and clearance of microorganisms (for reviews, see Refs. 5 and 6). The shared structural features of collectins include a globular carbohydrate recognition domain (CRD) at the C terminus, an amphipathic helical neck region, an extended collagen-like domain, and a disulfide bonded N-terminal region. SP-A monomers fold as a triple helix through their collagen-like domains to form trimers, and six trimers assemble into a bouquet of tulips structure, which resembles C1q, the first component of complement (7). The crystal structure of the C-terminal domains of SP-A has been reported recently (8).

SP-A can bind to a variety of microorganisms, including *Escherichia coli* and *Pseudomonas aeruginosa* (9–11), induce aggregation (9, 10, 12), and promote phagocytosis (9, 11, 13). The structural basis of these functions is not completely understood, but evidence that SP-A binds via its CRD region to the lipid A component of LPS suggests a mechanism for interaction with bacterial membranes (10).

The LPS of organisms such as *E. coli* consists of a lipid A domain, a relatively conserved core oligosaccharide, and an O-side chain of repeating sugars. *Bordetella pertussis*, the causative agent of human whooping cough, and several other Gram-negative respiratory pathogens, such as *Moraxella catarrhalis*, *Neisseria meningitides*, and *Haemophilus influenzae*, have an abbreviated LPS structure (14). *B. pertussis* lacks O-side chain (Fig. 1), and in its place has a nonrepeating trisaccharide consisting of α-N-acetylglucosamine, β-2-acetamido-3-acetamido-2,3-dideoxy-mannuronic acid, and β-1-2-acetamido-4-methylamino-fucose (15, 16). Electrophoresis of *B. pertussis* LPS reveals two distinct bands, referred to as bands A and B (16). The slower migrating band A is composed of full-length LPS containing lipid A, core oligosaccharide, and the terminal trisaccharide. Band B migrates faster and is composed of lipid A and core oligosaccharide, but lacks the trisaccharide (16). The simple structure of the *B. pertussis* LPS and the availability of LPS-mutant forms of *B. pertussis* provide a unique opportunity to study the molecular basis of the interaction between LPS and SP-A.

The sugars of the terminal trisaccharide of *B. pertussis* are synthesized and attached to the core by the enzymes encoded in the 12-gene *wlb* operon (17, 18). A number of mutations have been introduced into this operon, leading to the generation of mutant strains lacking one, two, or all three of the terminal sugars. WlbH lacks the terminal sugar (17, 18), MLT7 appears to lack the last two sugars (19), and WlbG and WlbL lack all three sugars (17, 18). Homology studies have suggested that WlbL fails to produce the first sugar in the trisaccharide, while WlbG retains the ability to produce this sugar, but fails to transfer it to the surface of the bacterial cell (18).

Allen et al. (20) have identified and mutated the *waaF* gene of *B. pertussis*. This gene is responsible for the addition of a heptose molecule at an early step in the synthesis of the core oligosaccharide (20). Mutation of this gene results in a truncated LPS molecule with only lipid A, a single ketodeoxyoctulosonic acid (Kdo) residue, and the first heptose residue of the core oligosaccharide (20). The LPS from *WaaF* mutants is smaller than band B LPS and fails to react with mAbs against band B.

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**Abbreviations used in this paper:** SP-A, surfactant protein A; BG, Bordet-Gengou; CRD, carbohydrate recognition domain; FHA, filamentous hemagglutinin; H-H, HBSS buffered with 10 mM HEPES; Kdo, ketodeoxyoctulosonic; MOI, multiplicity of infection; SS, Stainer-Scholte; SS-BG, SS broth overlaid on BG agar.
The PCR product was cloned into the TOPO 2.1 TA cloning vector (Invitrogen). We have examined the LPS structural elements required for SP-A binding, aggregate, opsonize, and kill B. pertussis.

Materials and Methods

Bacterial strains and growth conditions

Plasmids and bacterial strains used in this study are described in Table I. B. pertussis strains containing a plasmid encoding GFP introduced by electroporation (22) were used, except where indicated. B. pertussis strains were grown on Bordet Gengou (BG) agar (BD Biosciences, Sparks, MD) or in Stainer-Scholte (SS) broth overlaid on BG agar (SS-BG), as described previously (30). All other strains were grown on Luria-Bertani agar plates. Antibiotics were used at the following concentrations: gentamicin sulfate, 50 μg/ml; nalidixic acid, 30 μg/ml; kanamycin, 50 μg/ml; chloramphenicol, 15 μg/ml; and ampicillin, 100 μg/ml.

SDS-PAGE and immunoblotting

SDS-PAGE was performed by the method of Laemmli (31) with the modifications of Peppler (32). Proteins were transferred to Immobilon-P polyvinylidene difluoride membranes (Millipore, Bedford, MA) and probed with the primary Ab at 1/1000. mAbs were: band A LPS, G10F8C3 (33), and band B LPS, BL-8 (35); adenylate cyclase toxin, 9D14 (36).

Generation of a waaF mutant strain

The WaaF strain was generated in a BP338 background using a similar strategy to that used previously (20). Primers 5'-CACCGGACCCGGGAGAT-3' and 5'-GCTTCGCCGCACCTTCCGCATAGG-3' were used to generate an 859-bp internal fragment of the waaF gene by PCR. The PCR product was cloned into the TOPO 2.1 TA cloning vector (Invitrogen, Carlsbad, CA). This plasmid was digested with HindIII and a 3-kb HindIII fragment from pUW2138, encoding gentamicin resistance, and the OriT region (to allow for mobilization from E. coli into B. pertussis), or gent/OrfT, was inserted to generate plasmid pLMS023 (Fig. 2). LMS023 was mobilized into B. pertussis by triparental mating, as previously described (37). A kanamycin- and gentamicin-resistant colony was selected and screened for loss of band A LPS by Western blot using a mAb to band A and B LPS (19).

Mutation of the adenylate cyclase toxin gene

Adenylate cyclase toxin mutants were generated, as previously described, using suicide plasmid pKC109 (26). Loss of adenylate cyclase toxin expression was confirmed by Western blotting using mAb 9D14 (36).

Isolation and characterization of LPS

Mutant and wild-type LPS was isolated by incubating boiled bacteria in PBS with 0.1 mg/ml proteinase K (Sigma-Aldrich, St. Louis, MO) for 1.5 h at 50°C, followed by acetone precipitation. The LPS was separated on 16% SDS-PAGE, followed by silver staining using the Bio-Rad silver stain kit (Bio-Rad, Hercules, CA), according to the manufacturer’s instructions. Purified LPS for ELISA was prepared using a modification of the Tri-Reagent (Molecular Research Center, Cincinnati, OH) method previously reported by Yi and Hackett (38). Following Tri-Reagent extraction, samples were treated with proteinase K, as above, and then re-extracted with Tri-Reagent to ensure removal of all bacterial protein.

Human SP-A

Human SP-A was purified from lung washings of patients with the lung disease alveolar proteinosis, as previously described (29). SP-A preparations contained 140–190 pg of LPS/μg of SP-A, as determined by the Limulus Amebocyte Lysate (BioWhittaker, Rockland, MD).

Table I. Bacterial plasmids and strains used in this study

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<td>Kpn</td>
<td>Klebsiella pneumoniae, human fecal isolate, susceptible to SP-A-mediated killing</td>
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</table>

* Gent', gentamicin resistant; OrfT, origin of transfer P conjugation; Cm', chloramphenicol resistant; Kan', kanamycin resistant; Na'f', nalidixic acid resistant; Strep', streptomycin resistant; amp', ampicillin resistant; cyc, adenylate cyclase toxin operon.
Aggregation by SP-A

Bacteria were grown overnight in SS-BG and suspended to an OD₆₀₀ of 0.6 in SS salts. SS contains sufficient calcium (0.135 mM) to promote calcium-dependent SP-A activity. SS salts are SS broth lacking l-cysteine, FeSO₄, ascorbic acid, nicotinic acid, and glutathione; this medium will not support replication of the bacteria. Purified human SP-A was then added to a final concentration of 5 μg/ml. Samples were incubated at 37°C, shaking at 150 rpm, and the OD₆₀₀ was determined at the indicated times.

Binding of SP-A to LPS

The ability of SP-A to bind directly to LPS was examined by ELISA. Briefly, Pro-bind 96-well plates (BD Biosciences) were coated with 1 μg of purified LPS in coating buffer (1.59 g of Na₂CO₃, 2.93 NaHCO₃/L in H₂O, pH 9.6) for 2 h at 37°C. Between all steps, wells were washed with PBS with 0.5% Tween. Wells were blocked with PBS with 1% BSA for 30 min at 37°C, incubated with 50 μg/ml SP-A in wash buffer for 1 h at 37°C, and probed with a 1/1000 dilution of a polyclonal rabbit anti-human SP-A Ab (39) for 1 h at room temperature, followed by alkaline phosphatase-conjugated goat anti-rabbit Ab (Valent Pharmaceuticals, Costa Mesa, CA). The reaction was developed using the Sigma Fast p-nitrophenyl phosphatase substrate (Sigma-Aldrich). Absorbance was read at 405 nm. Wells were repeated in triplicate, and three independent experiments were performed.

Microscopic examination of SP-A killing

Bacterial membrane integrity was assessed by staining with the membrane-impermeant fluorescent probe, propidium iodide (Molecular Probes, Eugene, OR). Bacteria (not containing plasmid-encoded GFP) were harvested from 16-h BG or Luria-Bertani agar plates and suspended in SS salts at an OD₆₀₀ of 0.07. Samples were incubated with unlabeled SP-A at 100 μg/ml for 1 h at 37°C with shaking in a 5-ml polystyrene tube. An equivalent volume of propidium iodide diluted in SS salts was added to a final concentration of 30 μM, and samples were stained for 15 min in the dark. Bacteria were pelleted, suspended in SS salts to one-tenth the original volume, and 7 μl was mounted on a glass slide. Samples were observed by phase-contrast microscopy to identify bacterial cells and under fluorescent microscopy using the Texas Red filter (excitation 560 nm, emission 630 nm) to assess viability.

Bacterial cultures for phagocytosis studies

Growth conditions can influence virulence factor expression. To address this issue, phagocytosis studies were performed using bacteria harvested from both broth and agar cultures. For liquid cultures, bacteria expressing GFP were grown overnight in SS-BG, pelleted, and suspended in fresh SS broth, and the OD₆₀₀ was determined. Bacteria were washed three times in HBSS (BioWhittaker) buffered with 10 mM HEPES (Sigma-Aldrich) (H-H) and suspended to an OD₆₀₀ of 1.0 in H-H. For agar-grown cultures, bacteria were grown for 24 h on BG agar plates and harvested in SS broth using a Dacron fiber-tipped swab. The OD₆₀₀ was determined, and the bacteria were pelleted and suspended to an OD₆₀₀ of 1.0 in H-H.

For SP-A opsonization, SP-A was added to bacteria at 30 μg/ml and incubated for 15 min at 37°C, while control bacteria were incubated without SP-A. The samples were diluted to 400 μl and added to wells containing adherent phagocytes.

Phagocytosis by monocytes

Monocytes were isolated from the blood of healthy adult volunteers, as previously described (24), and suspended to 6 × 10⁷/ml in H-H with autologous serum (0.1%). One-milliliter samples were added to sterile 24-well tissue culture dishes containing glass coverslips and incubated for 1 h at 37°C, 5% CO₂, to allow for adherence. Bacteria were added at a multiplicity of infection (MOI) of ~5; the plates were centrifuged for 5 min at 640 × g to facilitate contact between bacteria and adherent monocytes and incubated for 1 h at 37°C with 5% CO₂ to allow phagocytosis to occur.

Phagocytosis by neutrophils

Neutrophils were isolated from the blood of healthy adult volunteers, as previously described (22), and suspended to 2 × 10⁷/ml in HBSS (BioWhittaker) buffered with 10 mM HEPES (Sigma-Aldrich) and BSA (0.25%). One-milliliter samples were plated and incubated to allow for adherence, as described above. Bacteria were added to achieve an MOI of ~5, centrifuged for 5 min at 640 × g to facilitate contact between bacteria and adherent neutrophils, and incubated for 1 h at 37°C with 5% CO₂ to allow phagocytosis to occur.

Staining and preparation of slides

Following phagocytosis, samples were incubated with ethidium bromide, as previously described (22). Slides were observed by fluorescence microscopy under a GFP filter. The numbers of adherent and internalized bacteria were counted for 100 phagocytes per coverslip, performed in triplicate, with at least three independent repetitions.

Statistical analyses

All experiments were performed at least in triplicate. Data were analyzed using Student’s t test.

Results

Generation of a waaF mutation in B. pertussis

Disruption of the B. pertussis waaF gene, responsible for addition of the second heptose in LPS biosynthesis, results in a deep-rough LPS, containing the Kdo residue and a single heptose residue of the core oligosaccharide (20). To generate a waaF mutant in the BP338 background, suicide plasmid pLMS023 containing an 859-bp internal fragment of the waaF gene (Fig. 2A) was introduced into BP338. Colonies were screened for loss of band A LPS expression by Western blot, and LPS isolated from a band A negative strain was analyzed by SDS-PAGE. The mutant expressed a single band of LPS that migrated faster than both band A and B LPS (Fig. 2B), consistent with the truncated LPS of a waaF mutant (20).

FIGURE 2. Characterization of LPS expression. A. Suicide plasmid pLMS023 containing nt 73–932 of waaF was mobilized into wild-type B. pertussis, and colonies were selected for kanamycin and gentamicin resistance, indicative of integration into the B. pertussis chromosome. B. Silver-stained SDS-PAGE of B. pertussis LPS, in which the wild-type strain, BP338, expresses predominantly band A LPS, and BP347, the Bvg mutant, expresses predominantly band B LPS. LPS from the WaaF mutant fails to comigrate with either band A or B. C. Band A and B expression. LPS from whole bacteria was separated on 16% SDS-PAGE gels and transferred to polyvinylidene difluoride membranes. A and B, Immunoblot probed with mAb to band A; band B, immunoblot probed with mAb to band B. WT, wild-type strain BP338; Bvg⁺, strain BP347.
Characterization of LPS expression from wild-type and LPS mutants

Previous studies have shown that although the LPS of wild-type B. pertussis, BP338, consists of almost entirely band A LPS, some strains fail to complete their LPS molecules and express a mixture of band A and B LPS (19). Band A expression was examined by Western blot (Fig. 2C). Only the wild-type strain, BP338, reacted with Ab BL-2, a mAb specific to band A (34). No band A expression was observed for BP347, the Bvg mutant (Fig. 2C, Bvg−). Bvg mutants lack a transcription factor that regulates virulence factor expression (27). BP347 was previously reported to express a mixture of band A and B LPS (19), and the lack of band A expression led us to characterize the LPS expressed by other mutants used in this study.

As expected, none of the LPS mutants produced full-length band A LPS (Fig. 2C). Band B expression was characterized using mAb BL-8 (35) specific for band B LPS. The wild-type strain reacted only weakly with the band B mAb (Fig. 2C, band B), consistent with Fig. 2B, in which this strain produced predominantly full-length LPS. In contrast, the Bvg mutant, which failed to produce band A LPS, showed strong reactivity with the band B mAb. Mutants WlbG and WlbL, which are only capable of producing band B LPS, also displayed strong reactivity with mAb BL-8. Band B was produced by mutants WlbH and ML7T, but to a lesser extent than the WlbG and WlbL mutants, consistent with previous studies suggesting that these mutants produce band B LPS, as well as truncated band A LPS (17–19). The WaaF mutant showed no reactivity with the band B monoclonal, confirming this mutant is unable to produce full-length core oligosaccharide.

Binding and aggregation of B. pertussis in suspension by SP-A

To examine the ability of SP-A to promote aggregation, bacterial suspensions were incubated with or without SP-A in SS salts, and the OD was monitored. In the absence of SP-A, none of the strains examined demonstrated a significant drop in OD_{600} (data not shown), indicating that the bacteria remained in suspension throughout the course of the experiment. This same trend was observed for two strains expressing wild-type LPS, BP338 (Fig. 3A) and BP536 (Fig. 3B), in the presence of SP-A. However, a drop in OD_{600} was seen after the addition of SP-A for all of the LPS mutants, indicating that SP-A induced the formation of aggregates of bacteria large enough to settle out of suspension. These results indicate that SP-A is unable to aggregate wild-type B. pertussis; however, strains lacking one or more of the three terminal sugars of the LPS molecule are rapidly aggregated in the presence of SP-A.

To determine whether aggregation was due to a direct interaction between SP-A and the LPS of B. pertussis, we performed an ELISA to measure the amount of SP-A bound by purified LPS. The LPS of wild-type and the WlbG mutant strains of B. pertussis was purified and used to coat wells of a Pro-bind assay plate. The hydrophobic coating ensures that the purified LPS bound to the plates in the correct orientation. Wells were incubated with either SP-A (50 μg/ml) or wash buffer and probed with Ab to SP-A. For LPS from the wild-type strain, the absorbance when SP-A was present was not different from wells incubated with buffer alone (Fig. 3C). In contrast, a significant increase (p < 0.05) in absorbance was observed with LPS from the WlbG strain in the presence of SP-A. These results indicate that SP-A bound to the mutant LPS, but not to the wild-type LPS.

Taken together, these data indicate that the terminal trisaccharide of the B. pertussis LPS molecule blocks the binding of SP-A to wild-type B. pertussis.

Effects of SP-A on attachment and phagocytosis of B. pertussis by human monocytes

Previous studies have demonstrated a role for SP-A in enhancing the uptake of a variety of opsonized particles by human monocytes and macrophages (13, 40, 41). We examined the effects of SP-A opsonization on the adherence and phagocytosis of wild-type and LPS-mutant strains of B. pertussis by human monocytes (Fig. 4).

As seen in previous studies (24), phagocytosis of unopsonized B. pertussis by human monocytes is relatively inefficient, with less than one internalized bacterium per monocyte when experiments are conducted at an MOI of 5 (Fig. 4). In the absence of SP-A, internalization of wild-type and mutant bacteria was similar. Addition of SP-A did not affect the number of wild-type bacteria internalized by human monocytes. In contrast, a 2-fold increase in phagocytosis was observed for the LPS mutants opsonized with SP-A (p < 0.05). No differences were observed in the number of extracellular adherent bacteria (data not shown).

SP-A has been reported to promote phagocytosis by opsonization and by directly stimulating phagocytic cells (42). The finding that the presence or absence of SP-A had no effect on phagocytosis of the wild-type strain indicates that direct stimulation of host cells...
by SP-A is not responsible for the increase in phagocytosis that was observed.

The bacterial adhesin filamentous hemagglutinin (FHA) has been shown to mediate bacterial attachment to human cells (23, 43). FHA exists as a membrane-bound protein, and in addition, soluble FHA is released into the culture supernatant by a proteolytic event mediated by the B. pertussis protease, SphB1 (44). Previous studies (24) examined phagocytosis of broth-grown bacteria, which were centrifuged and washed several times. Washing could influence the ratio of surface-associated FHA to soluble FHA. We found that bacteria harvested from agar displayed increased adhesion to monocytes compared with the broth-grown cells (data not shown). However, phagocytosis of broth-grown cells was similar to that seen in Fig. 4 for agar-grown cells, and while addition of SP-A promoted internalization of LPS mutants, it did not alter internalization of the wild-type strain.

Adenylate cyclase toxin antagonizes SP-A-mediated phagocytosis of B. pertussis by human neutrophils

Opsonization with SP-A did not increase phagocytosis of wild-type B. pertussis or the LPS mutants by neutrophils (Fig. 5). However, the adenylate cyclase toxin of B. pertussis elevates cAMP levels in eukaryotic cells and has been shown to cripple the phagocytic abilities of human neutrophils (23, 45). Phagocytosis is increased several-fold for strains that fail to express the toxin or in the presence of neutralizing Abs to adenylate cyclase toxin (23, 45). Because the presence of functional adenylate cyclase toxin could impair SP-A-mediated opsonization, we generated LPS/ad-

![Image 85x95 to 261x206](image1)

![Image 102x643 to 245x742](image2)

![Image 318x145 to 544x319](image3)

![Image 1963](image4)

![Image 1964](image5)

![Image 533](image6)
Influence of SP-A on bacterial viability

Previous work has shown that both SP-A and surfactant protein D are able to directly inhibit the growth of some Gram-negative bacteria via a mechanism that is distinct from their macrophage-dependent antimicrobial functions (29). Growth inhibition appears to be due to permeabilization of the bacterial membrane by a CRD-mediated mechanism. To determine whether SP-A could kill B. pertussis, bacterial viability was assessed using the membrane-impermeant DNA-specific dye, propidium iodide. B. pertussis appeared as small coccobacilli, smaller than Klebsiella pneumoniae when viewed by phase microscopy, and most of the bacteria were viable following growth under control conditions (Fig. 7, Bacteria alone). Following incubation with SP-A, the SP-A-susceptible K. pneumoniae strain and the WlbG mutant formed aggregates, and many of the bacteria stained with propidium iodide, consistent with cell death (Fig. 7, Bacteria + SP-A). In contrast, the wild-type B. pertussis did not aggregate in the presence of SP-A and did not stain extensively with propidium iodide. These results suggest that B. pertussis mutants that bind SP-A are susceptible to SP-A-mediated killing.

Discussion

In this study, the ability of SP-A to mediate the clearance of B. pertussis was examined. SP-A was unable to bind to or aggregate B. pertussis strains expressing wild-type LPS. However, binding and SP-A-mediated aggregation were observed for five independent mutants in the same biochemical pathway (LPS synthesis) from two independent parental lines. Furthermore, ELISA studies with purified LPS suggest that SP-A binds directly to mutant, but not wild-type LPS. SP-A has been reported to bind to the lipid A portion of LPS (10), and it is possible that one or more of the three terminal sugars may sterically hinder access of SP-A to lipid A; however, these sugars may block binding to other structures as well. Mutant WlbH, lacking only the terminal sugar, was aggregated by SP-A. However, whether the terminal sugar is sufficient for preventing SP-A binding is unclear, because the WlbH mutant is inefficient at adding the terminal sugars to the core and produces a considerable amount of band B LPS (Fig. 2C).

The failure of wild-type B. pertussis LPS to be recognized by SP-A not only provides the organism from the agglutinating functions of SP-A, but also serves to protect the bacteria from SP-A-mediated phagocytosis. SP-A has been reported to have direct stimulatory effects on phagocytic cells (42); however, SP-A only promoted phagocytosis of mutants capable of binding SP-A. These data suggest that enhanced phagocytosis of the LPS mutants was due to the opsonic effects of SP-A, and not a direct stimulatory effect of SP-A on the monocytes. Wild-type LPS also provided bacteria from SP-A-mediated phagocytosis by neutrophils, but only in the absence of the B. pertussis adenylate cyclase toxin. Adenylate cyclase toxin has been shown to inhibit the phagocytic capability of neutrophils (23, 45), and it was necessary to inactivate the adenylate cyclase toxin of LPS mutants before any effect of SP-A could be observed.

SP-A has been shown to kill bacteria by increasing membrane permeability (29). A mutant B. pertussis strain that was aggregated in the presence of SP-A was also susceptible to SP-A-mediated permeability, as demonstrated by propidium iodide staining; however, the wild-type strain was not affected by SP-A. We conclude that the antimicrobial activity of SP-A is ineffective against B. pertussis strains expressing wild-type LPS. We speculate that SP-A resistance contributes to the ability of B. pertussis to evade the immune defenses of the respiratory mucosa. It is of interest that the LPS of a number of other Gram-negative respiratory pathogens, including M. catarrhalis, N. meningitides, and H. influenzae (14), have short or absent O-chains and may, therefore, have susceptibilities to SP-A that differ from those observed for E. coli and P. aeruginosa. Studies of SP-A-resistant and susceptible bacteria may yield insights into the molecular basis of SP-A/LPS interactions.

References