Pseudomonas aeruginosa induces changes in fluid transport across airway surface epithelia

D. J. EVANS,1 P. S. MATSUMOTO,2 J. H. WIDDICOMBE,2,3 C. LI-YUN,1 A. A. MAMINISHKIS,1 AND S. S. MILLER1,4

1School of Optometry and 4Department of Molecular and Cell Biology, University of California, Berkeley 94720; 2Children's Hospital Oakland Research Institute, Oakland 94609; and 3Department of Physiology and Cardiovascular Research Institute, University of California, San Francisco, California 94143

Evans, D. J., P. S. Matsumoto, J. H. Widdicombe, C. Li-Yun, A. A. Maminishkis, and S. S. Miller. Pseudomonas aeruginosa induces changes in fluid transport across airway surface epithelia. Am. J. Physiol. 275 (Cell Physiol. 44): C1284–C1290, 1998.—Fluid transport across cultures of bovine tracheal epithelium was measured with a capacitance probe technique. Baseline fluid absorption (Jv) across bovine cells of 3.2 µl·cm⁻²·h⁻¹ was inhibited by ~78% after 1 h of exposure to suspensions of Pseudomonas aeruginosa, with a concomitant decrease in transepithelial potential (TEP) and increase in transepithelial resistance (Rt). Effects of P. aeruginosa were blocked by amiloride, which decreased Jv by 112% from baseline of 2.35 ± 1.25 µl·cm⁻²·h⁻¹, increased Rt by 101% from baseline of 610 ± 257 Ω·cm², and decreased TEP by 91% from baseline of −55 ± 18.5 mV. Microelectrode studies suggested that effects of P. aeruginosa on amiloride-sensitive Na absorption were due in part to a block of basolateral membrane K channels. In the presence of Cl transport inhibitors [5-nitro-2-(3-phenylpropylamino)-benzoic acid, H₂-DIDS, and bumetanide], chloride secretion across gland acini (14). An important mechanism for fluid removal is amiloride-sensitive Na absorption across airway surface epithelia and, thereby, alter mucociliary clearance and airway colonization by P. aeruginosa and other bacterial pathogens (10).

Mucociliary clearance, an important airway defense against infection, is the mechanism by which bacteria are ensnared in mucus and cleared from the lungs. This process requires ciliary beating, secretion of respiratory mucus by submucosal glands, and regulation of water content of the airway surface liquid (22, 29). The importance of transepithelial fluid movements driven by active ion transport is revealed by the genetic disease cystic fibrosis, in which changes in chloride secretion (30) and active sodium absorption (2) by airway epithelium are associated with inhibition of mucociliary clearance and airway colonization by P. aeruginosa (13, 18). The purpose of this study was to determine whether P. aeruginosa could affect fluid transport across airway epithelia and, thereby, alter mucociliary clearance.

MATERIALS AND METHODS

Bacteria. Strains of P. aeruginosa were stored frozen (−70°C) in trypticase soy broth containing 10% (vol/vol) glycerol. They were grown on a trypticase soy agar surface at 37°C overnight, then resuspended into prewarmed (37°C) sterile normal Ringer solution to a concentration of ~4 × 10⁷ colony-forming units (CFUs)/ml. Filtrates of bacterial cell suspensions were prepared by passage through a 0.2-µm membrane filter (Millipore). Unless otherwise stated, experiments were performed with P. aeruginosa strain PA01.

Cell culture. Primary cell cultures of bovine airway surface tracheal epithelia (BTE) were obtained as described previously (32). Briefly, strips of tracheal epithelium were pulled away from underlying tissues, and epithelial cells were isolated by overnight digestion (4°C) with 0.05% (wt/vol) protease (type XIV, Sigma Chemical) in PBS. The next day, epithelial cells were freed from the tissue strips by vigorous shaking (30 s), and the action of protease was terminated by addition of FCS. Cells were pelleted by centrifugation (200 g, 10 min) and resuspended in the appropriate cell culture medium (32). Cells were plated at a density of 10⁶ cells/cm²

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
onto 12-mm-diameter, 0.4 µm-pore-size Transwell filters (Costar, Cambridge, MA) coated with a thin film of human placental collagen (20 µg/ml). Cells were grown at an air-liquid interface (no medium added to the mucosal surface) and fed every alternate day. Confluent sheets of airway surface epithelium developed after ~7 days of culture at 37°C in an atmosphere of 5% CO2-95% air. For most experiments, epithelial sheets were 8–17 days old. For BTE, transepithelial resistance (Rt) was 490 ± 250 Ω·cm² and transepithelial potential (TEP) was −66 ± 18 mV (means ± SD, n = 8 tracheae).

Fluid transport. Fluid transport (Jv), TEP, and Rt were measured with a double-sided capacitance probe technique as previously described (13). Briefly, a sheet of epithelium was mounted between two Lucite half-chambers. The chambers were perfused for 4 min with control Ringer solution and then sealed. Capacitance probes were placed over the fluid menisci in the reservoirs on either side of the epithelium to detect changes in fluid height caused by transepithelial fluid transport. TEP was recorded continuously, and Rt was determined at 1-min intervals from the voltage deflections caused by transepithelial current pulses of known magnitude.

After Jv stabilized, the solution in the chambers was completely exchanged with fresh Ringer solution. Provided this control solution change did not appreciably affect Jv, Rt, or TEP, P. aeruginosa cell suspensions were then added to both sides of the epithelia (bilateral addition avoided artifacts associated with changes in surface tension). Tissues were exposed to bacteria for ~1 h. In some experiments, airway epithelia were pretreated with ion transport inhibitors and/or substituted media before the addition of bacteria. Control experiments ensured that the inhibitors and solutions did not affect bacterial or tissue viability. In other control experiments (not shown), Jv, Rt, and TEP of the BTE remained stable over ~6 h, in which multiple control solution changes were performed.

Microelectrodes. As described previously (16), calomel electrodes in series with Ringer-agar bridges were used to measure TEP, and the signals from intracellular microelectrodes were referenced to the apical or basal bath to measure the apical and basolateral membrane potentials (Vb and Va, where Vb = −Ve − Vm). Microelectrodes were made from fiber-filled borosilicate glass tubing of 0.5 mm ID and 1 mm OD (Sutter Instrument, Novato, CA). They were backfilled with 150 mM KCl and had resistances of 100–170 MΩ.

Silver-silver chloride wire was used to make the electrical connection between the microelectrode and the electrode amplifier (Axoprobe 1A, Axon Instruments). The Rt and the apparent ratio of the apical to basolateral membrane resistance (Rab/Rab) were obtained by passing bipolar 4-µA current pulses across the tissue for 3 s every 30 s and measuring the resulting changes in TEP, Vb, and Vb (Rab/Rab = Vb/Vb). Solutions. Bovine Ringer solution (BRS) contained (in mM) 120 NaCl, 23 NaHCO3, 10 glucose, 5 KCl, 1.8 CaCl2, and 1 MgCl2 (osmolarity = 290 ± 5 mosmol). In chloride-free BRS, NaCl was replaced by CH3SO3Na; all other chloride was replaced by gluconate. Osmolarity was adjusted to that of chloride-free BRS using mannitol. Bicarbonate- and chloride-free BRS was the same as chloride-free BRS, except NaHCO3 was replaced by sodium HEPES. Solutions were equilibrated with 5% CO2-10% O2-95% N2 to pH 7.4, except for bicarbonate- and chloride-free BRS, which was equilibrated with 10% O2-90% N2 (pH 7.4). Addition of P. aeruginosa to Ringer solutions had no detectable effect on osmolarity.

Bacterial adherence. As described previously (7), tissue culture medium was removed, and confluent cell sheets were washed once with 500 µl of sterile BRS. P. aeruginosa suspension (200 µl containing ~4 x 10⁷ bacteria) was added to the apical surface and incubated for 1 h at 37°C. Bacteria were removed, and the epithelial surface was washed gently three times with BRS (500 µl). The tissue was excised from the Transwell filter, washed once more, then homogenized for 15 min at room temperature in 1 ml of 0.25% Triton X-100 in trypticase soy broth. This lysed the epithelial cells and released bacteria into the medium without affecting bacterial viability. Counts of the viable bacteria in the lysate were performed. In each experiment, three tissue sheets were used to assess the binding for each bacterial strain. Comparisons between strains were made on the same day to control for variability in binding that occurs on cells of different age and from different sources. Representative experiments are presented in RESULTS. Experiments were repeated up to three times with similar results.

Ion transport inhibitors. Amiloride and bumetanide were obtained from Sigma Chemical, 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) from Research Biochemicals International (Natick, MA), and H2-DIDS from Molecular Probes (Eugene, OR). Drugs were dissolved directly into Ringer solutions using mannitol. Bicarbonate- and chloride-free BRS was replaced by gluconate. Osmolarity was adjusted to that of BRS using mannitol. Bicarbonate- and chloride-free BRS was equilibrated with 5% CO2-10% O2-90% N2 (pH 7.4). In chloride-free BRS, NaHCO3 was replaced by CH3SO3Na; all other chloride was replaced by gluconate. Osmolarity was adjusted to that of chloride-free BRS using mannitol. Bicarbonate- and chloride-free BRS was the same as chloride-free BRS, except NaHCO3 was replaced by sodium HEPES. Solutions were equilibrated with 5% CO2-10% O2-85% N2 to pH 7.4, except for bicarbonate- and chloride-free BRS, which was equilibrated with 10% O2-90% N2 (pH 7.4). Addition of P. aeruginosa to Ringer solutions had no detectable effect on osmolarity.

Bacterial adherence. As described previously (7), tissue culture medium was removed, and confluent cell sheets were washed once with 500 µl of sterile BRS. P. aeruginosa suspension (200 µl containing ~4 x 10⁷ bacteria) was added to the apical surface and incubated for 1 h at 37°C. Bacteria were removed, and the epithelial surface was washed gently three times with BRS (500 µl). The tissue was excised from the Transwell filter, washed once more, then homogenized for 15 min at room temperature in 1 ml of 0.25% Triton X-100 in trypticase soy broth. This lysed the epithelial cells and released bacteria into the medium without affecting bacterial viability. Counts of the viable bacteria in the lysate were performed. In each experiment, three tissue sheets were used to assess the binding for each bacterial strain. Comparisons between strains were made on the same day to control for variability in binding that occurs on cells of different age and from different sources. Representative experiments are presented in RESULTS. Experiments were repeated up to three times with similar results.

Ion transport inhibitors. Amiloride and bumetanide were obtained from Sigma Chemical, 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB) from Research Biochemicals International (Natick, MA), and H2-DIDS from Molecular Probes (Eugene, OR). Drugs were dissolved directly into Ringer solutions using mannitol. Bicarbonate- and chloride-free BRS was replaced by gluconate. Osmolarity was adjusted to that of BRS using mannitol. Bicarbonate- and chloride-free BRS was equilibrated with 5% CO2-10% O2-90% N2 (pH 7.4). In chloride-free BRS, NaHCO3 was replaced by CH3SO3Na; all other chloride was replaced by gluconate. Osmolarity was adjusted to that of chloride-free BRS using mannitol. Bicarbonate- and chloride-free BRS was the same as chloride-free BRS, except NaHCO3 was replaced by sodium HEPES. Solutions were equilibrated with 5% CO2-10% O2-85% N2 to pH 7.4, except for bicarbonate- and chloride-free BRS, which was equilibrated with 10% O2-90% N2 (pH 7.4). Addition of P. aeruginosa to Ringer solutions had no detectable effect on osmolarity.

RESULTS

Effects of P. aeruginosa on Jv. The data shown in Fig. 1 illustrate the effects of P. aeruginosa on Jv, Rt, and TEP across a sheet of BTE. Jv decreased from −5 to ~2 µl·cm⁻²·h⁻¹, Rt increased from 390 to 630 Ω·cm², and TEP decreased from −50 to −31 mV. In six similar experiments the bacteria-induced decrease in Jv was...
bath before addition of bacteria. Amiloride alone decreased J_v, effects. Amiloride blocked these bacteria-induced effects. Amiloride blocked these bacteria-induced effects.

**Fig. 2.** Effect of amiloride on P. aeruginosa-induced changes in J_v, R_t, and TEP across BTE. A: J_v. Positive values indicate fluid absorption. B: TEP (•) and R_t (△). Amiloride (20 µM) was added to apical (ap) bath before addition of bacteria. Amiloride alone decreased J_v by 3.6 µl·cm⁻²·h⁻¹ (82% inhibition), increased R_t by 1,480 Ω·cm² (3-fold), and decreased TEP by 70 mV (91%). In absence of amiloride, P. aeruginosa (~4 × 10⁷ cfu/ml) caused little or no further changes in J_v, R_t, or TEP.

2.5 ± 1.4 µl·cm⁻²·h⁻¹ from a baseline absorption of 3.2 ± 1.3 µl·cm⁻²·h⁻¹, the increase in R_t was 302 ± 164 Ω·cm² from a baseline of 628 ± 215 Ω·cm², and the decrease in TEP was 19 ± 9 mV from a baseline of −45 ± 19 mV.

Blockade by amiloride. Figure 2 shows an experiment in which apical amiloride (20 µM) decreased J_v from 4.4 to 0.8 µl·cm⁻²·h⁻¹, increased R_t from 520 to 2,000 Ω·cm², and decreased TEP from −77 to −7 mV. Subsequent addition of P. aeruginosa caused no further change in steady-state J_v, R_t, or TEP. Two other experiments produced practically the same result. In other experiments (not shown), removal of amiloride resulted in recovery of tissue responses to P. aeruginosa similar to those shown in Fig. 1. With 50 µM amiloride (n = 3) the results were indistinguishable from those with 20 µM. For all six experiments, amiloride reduced TEP from 55 ± 7.6 to −8.4 ± 1.7 mV, increased R_t from 610 ± 105 to 1,226 ± 248 Ω·cm², and decreased J_v from 2.35 ± 0.5 to −0.28 ± 0.6 µl·cm⁻²·h⁻¹ (means ± SE). Changes in J_v, R_t, and TEP induced in BTE by P. aeruginosa in the presence of amiloride, 0.1 ± 0.3 µl·cm⁻²·h⁻¹, −44 ± 38 Ω·cm², and −2 ± 1.5 mV (means ± SE), respectively, were not significantly different from zero and were significantly less than the values obtained in the absence of amiloride (see above).

Figure 3 shows continuous intracellular microelectrode records obtained while P. aeruginosa was added to the tissue and removed, amiloride was applied, and P. aeruginosa was readded. As shown in Fig. 3A, P. aeruginosa depolarized V_B by ~15 mV and V_A by ~5 mV; TEP decreased correspondingly. R_A/R_B fell from ~4 to ~2.4. Despite the large change in R_A/R_B, changes in R_t were small (see Discussion). The same tissue was then treated with amiloride (Fig. 3B), which caused large and rapid hyperpolarizations of V_A and V_B, a reduction in TEP, and increases in R_A/R_B and R_t. In the continued presence of amiloride, P. aeruginosa (~1 × 10⁷ cfu/ml) did not induce further changes in TEP, V_A, or V_B. Nor was R_A/R_B changed. However, R_t declined, suggesting that P. aeruginosa may have decreased the shunt resistance. Similar effects of P. aeruginosa were seen in other experiments in which we were not able to obtain a continuous record but had to make separate impalements before and after addition of P. aeruginosa.
secretion was accompanied by a 27% decrease in $R_t$ from a baseline of 327 ± 101 Ω·cm². Chloride channel blockers in combination with replacement of chloride by methylsulfate ($n = 3$) or replacement of chloride and bicarbonate ($n = 3$) completely blocked the induction of secretion and resistance changes induced by P. aeruginosa (data not shown).

Bacterial factors. A mutant strain of P. aeruginosa (PG201 rhlR) that is deficient in the production of rhamnolipid (and elastase) toxins (20) decreased $J_v$ from 8.0 to 3.9 µl·cm⁻²·h⁻¹, increased $R_t$ from 300 to 440 Ω·cm², and decreased TEP from −82 to −59 mV. An exotoxin A mutant (PA103 tox::Ω) decreased $J_v$ from 3.7 to 0.1 µl·cm⁻²·h⁻¹, increased $R_t$ from 780 to 850 Ω·cm², and decreased TEP from −56 to −49 mV. Similar results were obtained in a second experiment with each mutant. Thus rhamnolipids, elastase, and exotoxin A may not be involved in the actions of P. aeruginosa on $J_v$, $R_t$, and TEP. Filtrate of a P. aeruginosa suspension had little or no effect on $J_v$, $R_t$, or TEP, causing a small increase in $J_v$ of 0.7 ± 0.6 µl·cm⁻²·h⁻¹ from a baseline absorption of 3.9 ± 2 µl·cm⁻²·h⁻¹, a decrease in $R_t$ of 10 ± 26 Ω·cm² from a baseline of 440 ± 317 Ω·cm², and a decrease in TEP of 4 ± 3 mV from a baseline of −57 ± 22 mV ($n = 3$).

P. aeruginosa may need to bind to airway surface epithelial cells to alter fluid transport and bioelectrical properties. Accordingly, we tested the effects of mutants lacking various attachment factors. The effects of these mutants on $J_v$, $R_t$, and TEP are summarized in Table 1.

Table 1. Baseline values of $J_v$, $R_t$, and TEP across BTE and changes induced by P. aeruginosa and some of its mutants

<table>
<thead>
<tr>
<th>P. aeruginosa</th>
<th>$J_v$, µl·cm⁻²·h⁻¹</th>
<th>$R_t$, Ω·cm²</th>
<th>TEP, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA01</td>
<td>3.2 ± 0.5</td>
<td>628 ± 88</td>
<td>−45 ± 8</td>
</tr>
<tr>
<td>AK 1012</td>
<td>3.8 ± 0.6</td>
<td>340 ± 25</td>
<td>−68 ± 6</td>
</tr>
<tr>
<td>PAK</td>
<td>2.7 ± 0.8</td>
<td>639 ± 105</td>
<td>−68 ± 6</td>
</tr>
<tr>
<td>PAK-N1</td>
<td>4.9 ± 0.9</td>
<td>323 ± 55</td>
<td>−60 ± 8</td>
</tr>
</tbody>
</table>

Values are means ± SE. $J_v$, fluid absorption; $R_t$, transepithelial resistance; TEP, transepithelial potential; BTE, bovine tracheal epithelia. For $J_v$, a minus sign indicates a decrease in absorption; for TEP, a minus sign indicates a decrease in TEP. *Not significantly different from zero; †significantly different from parent strain.
significantly affect P. aeruginosa binding. The adherence of P. aeruginosa PA01 was 1,175,000 ± 127,000 (SE) cfu/tissue, whereas in the presence of amiloride, PA01 adherence was 1,050,000 ± 162,000 cfu/tissue (n = 3).

**DISCUSSION**

Airway epithelia exhibit baseline absorption of fluid. The inhibition of absorption by amiloride implicates active sodium transport as a driving force (13, 32). Here, we show that this fluid absorption is inhibited by P. aeruginosa, which also raises R_v and decreases TEP. These effects were achieved by adding bacteria to apical and basolateral cell surfaces. This was done for two reasons: 1) to avoid artifacts in recorded J_v changes and 2) because exposure of basolateral cell surfaces to bacteria may occur frequently in vivo because of inflammation and tissue injury. Recently, it was shown that interaction with basolateral cell surfaces may be significant in the pathogenesis of P. aeruginosa infections (6). Amiloride blocked all the effects of P. aeruginosa without altering bacterial binding, suggesting that this pathogen acts by inhibiting one or more components of the amiloride-sensitive sodium transport pathway. These findings are consistent with previous studies showing that P. aeruginosa decreased short-circuit current and reduced net sodium absorption across short-circuited airway epithelial cells (9, 26). Bacterial cells were required to alter J_v, R_v, or TEP; filtrates of P. aeruginosa suspensions were ineffective. Loss of production of rhamnolipids, LPS O antigen, and exotoxin A did not affect these actions of P. aeruginosa. By contrast, a mutant unable to express adhesins under transcriptional control of the α factor RpoN not only showed markedly reduced binding to the airway epithelial cells but also had no effect on fluid absorption. Thus direct interaction of bacterial cells with the airway epithelium may be needed for P. aeruginosa to inhibit fluid absorption.

Possible mechanisms. Net transepithelial fluid movement across airway surface epithelium reflects the balance of active secretory and absorptive solute transport. Thus inhibition of fluid absorption across airway epithelium by P. aeruginosa could be due to a reduction of active solute absorption and/or an increase in secretion. In airway epithelia, major solute transport processes are amiloride-sensitive active sodium absorption and active secretion of chloride (27), the former inhibited by amiloride and the latter by bumetanide, NPPB, and DIDS. As in our previous studies on human tracheal cells (13), pretreatment of BTE with amiloride brought J_v to approximately zero while increasing R_v and decreasing TEP, consistent with its known inhibitory action on apical membrane sodium channels (1). In Fig. 3B the hyperpolarization of V_A and the large increase in R_A/R_B in the presence of an increase in R_v point to amiloride's predominant action being on apical membrane sodium channels. In the presence of amiloride, P. aeruginosa had no effect on J_v. Taken together these results indicate that this pathogen acts predominantly by inhibiting active sodium absorption rather than by stimulating a secretory process.

Microelectrode studies were used to help determine the site(s) of action of P. aeruginosa on active sodium absorption. In the continuous record of Fig. 3A, R_v did not change significantly. However, we believe that the lower-than-normal R_v and TEP of this tissue were due to edge damage. If this is so, the resulting high shunt conductance would have obscured the effects of changes in R_A and R_B on R_v. Pooled results from all tissues showed that P. aeruginosa increased R_v from ~500 to ~800 Ω cm². This increase in R_v coupled with the 50% decrease in R_A/R_B (Fig. 3A) suggests a decrease in basolateral membrane conductance. In particular, the finding that V_A depolarizes more than V_B implicates closure of potassium channels as the predominant action of P. aeruginosa on R_v and R_A/R_B; potassium channels provide the predominant conductance of the basolateral membrane of airway epithelia (28). However, one tissue showed a P. aeruginosa-induced depolarization of 11 mV in V_B and a hyperpolarization of 6 mV in V_A. This result is best explained by a simultaneous block of apical membrane sodium channels and basolateral potassium channels. In the other two experiments, V_A and V_B depolarized, presumably because a large depolarizing shunt current from the basolateral side obscured any block of apical membrane sodium conductance by P. aeruginosa.

P. aeruginosa may also have effects on ion transport processes other than electrogenic sodium absorption. Specifically, in tissues treated with chloride transport blockers (Fig. 4), P. aeruginosa induced a fluid secretion that was accompanied by a decrease in R_v but no change in TEP, suggesting induction of an electrically neutral secretory process. This fluid secretion and R_v change were not seen when chloride transport blockers were used in combination with removal of chloride or chloride plus bicarbonate, suggesting that chloride was the secreted anion. The identity of the secreted cation was not investigated.

Bacterial factors. P. aeruginosa may induce changes in ion-coupled fluid transport through a variety of mechanisms, including toxin secretion or binding to an epithelial cell surface receptor. Our data show that if a toxin is involved, it is not secreted into the surrounding environment before airway cell contact, since the filtrate of a bacterial suspension did not affect ion or fluid transport. This does not rule out toxin involvement; however. Some toxins are only secreted by bacteria on host cell contact (type III secretion), a form of delivery identified in P. aeruginosa and other gram-negative bacteria (31).

A glycolipid hemolysin of P. aeruginosa has been shown to reduce sodium absorption and unidirectional chloride fluxes across human bronchial epithelium (26). Low concentrations of purified P. aeruginosa rhamnolipids decrease amiloride-sensitive short-circuit current across sheep tracheal epithelium (9). Finally, P. aeruginosa exotoxin A has been shown to increase fluid absorption across distal airways in vivo (21). In the present study a rhamnolipid-deficient (and elastase-deficient) mutant of P. aeruginosa and an exotoxin A-deficient mutant inhibited fluid absorption and al-
tered the bioelectrical properties of BTE. Thus, although we cannot preclude a role for these toxins in mediating fluid transport changes in vivo, our data indicate that P. aeruginosa can alter ion-coupled fluid transport in airway cells without these toxins.

Inhibition of fluid absorption by P. aeruginosa requires RpoN, a σ factor of RNA polymerase that controls the transcription of an unknown number of genes (11), one of which may code for a toxin secreted in response to epithelial cell contact. However, in agreement with the results of others (23), the rpoN mutant of P. aeruginosa (PAK-N1) showed markedly lowered adherence (16-fold lower than its parent strain PAK).

Thus adherence would seem necessary to effect changes in Jv and bioelectrical properties, and RpoN is known to control expression of adhesins, such as pili and flagella. The critical level of adherence needed to alter ion-coupled fluid transport may be 5–10% of wild type, since the LPS mutant (AK1012) showed fivefold lower adherence than its parent but had the same effects on ion and fluid transport. An additional possibility is that binding via RpoN-controlled adhesins is required to affect ion and fluid transport.

Thus the P. aeruginosa-induced changes in fluid transport are not mediated by rhamnolipids, elastase, exotoxin A, or LPS O antigen. However, they do require the presence of whole bacterial cells and involve expression of genes controlled by the bacterial factor RpoN.

Clinical implications. The P. aeruginosa-induced alterations in salt and water transport across airway epithelium could affect further bacterial colonization in one of two ways. First, decreased fluid absorption would dilute the mucous secretions, thereby reducing the clearance of mucus and entrapped P. aeruginosa (17). The resulting buildup of mucus, albeit dilute, could also encourage colonization by increasing the number of binding sites for P. aeruginosa (23).

Second, P. aeruginosa could promote further colonization by altering the salt content of airway surface liquid. Joris et al. (15) reported that cystic fibrosis airway surface liquid has higher-than-normal NaCl levels (120 vs. 80 mM). More recently, it was demonstrated that the killing ability of antimicrobials secreted by the airway epithelium was inhibited at higher salt levels (8, 24).

Bacterial inhibition of active sodium absorption could increase bacterial colonization by elevating NaCl concentrations in the airway surface liquid.

We thank Kefu Yu for expert technical assistance and Dr. Suzanne Fleischig for helpful discussions and advice. We also acknowledge and thank Drs. Barbara Iglewski, Stephen Lory, and Urs Ochsner for generating P. aeruginosa mutant strains.

This work was supported by a grant from Cystic Fibrosis Research (Palo Alto, CA), a grant from the American Lung Association of California to D. J. Evans, and National Heart, Lung, and Blood Institute Grant HL-42368 to J. H. Widdicombe.

Address for reprint requests: D. J. Evans, School of Optometry, 387A Minor Hall, University of California, Berkeley, CA 94720-2020.

Received 17 March 1998; accepted in final form 25 J une 1998.

REFERENCES


