Pseudomonas aeruginosa induces changes in fluid transport across airway surface epithelia


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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·cm⁻², 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], transport inhibitors [5-nitro-2-(3-phenylpropylamino)-benzoic acid, H₂-DIDS, and bumetanide], P. aeruginosa induced a fluid secretion of −2.5 ± 0.4 µl·cm⁻²·h⁻¹ and decreased Rt without changing TEP. However, these changes were abolished when the transport inhibitors were used in a medium in which Cl was replaced by an impermeant organic anion. Filtrates of P. aeruginosa suspensions had no effect on Jv, TEP, or Rt. Mutants lacking exotoxin A or rhamnolipids or with defective lipopolysaccharide still inhibited fluid absorption and altered biochemical properties. By contrast, mutations in the rpoN gene encoding a σ factor of RNA polymerase abolished actions of P. aeruginosa. In vivo, changes in transepithelial salt and water transport induced by P. aeruginosa may alter viscosity and ionic composition of airway secretions so as to foster further bacterial colonization.

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 (cfu)/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 PAO1.

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

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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-ended 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 menisces 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 epithelium (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 (Vap and Vbl, where TEP = Vap - Vbl). 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 (Rap/Rbl) 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, Vap, and Vbl (Rap/Rbl = Vap/Vbl).

Solutions. Bovine Ringer solution (BRS) contained (in mM) 120 NaCl, 23 NaHCO3, 10 glucose, 5 KCl, 1.8 CaCl2, and 1 MgCl2 (osmolality = 290 ± 5 mosmol). In chloride-free BRS, NaCl was replaced by CH3SO3Na; all other chloride was replaced by gluconate. Osmolality was adjusted to that of 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 × 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 H2DiDS from Molecular Probes (Eugene, OR). Drugs were dissolved directly into Ringer solutions ~30 min before use, except NPPB, which was dissolved in DMSO before it was diluted 1:100 into BRS.

Data analysis. Values are means ± SD unless otherwise stated. Statistical comparisons of differences between means were performed with Student’s t-test, with P < 0.05 considered significant.

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 significant.

Fig. 1. Effects of P. aeruginosa on fluid transport (Jv), transepithelial potential (TEP), and transepithelial resistance (Rt) across bovine airway surface tracheal epithelia (BTE). A: Jv. Positive values indicate fluid absorption. B: TEP (●) and Rt (○). P. aeruginosa [~4 × 10⁷ colony-forming units (cfu)/ml] reduced fluid absorption by ~3 µl·cm⁻²·h⁻¹ (60% inhibition) over 1 h. This change was accompanied by a 240 Ω·cm² (61%) increase in Rt, and a 19 mV (38%) decrease in TEP from baseline values (see Table 1).
2.000 Ω·cm², and decreased TEP from -77 to -7 mV. Subsequent addition of P. aeruginosa caused no further change in steady-state Jv, Rb, 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 -4.8 ± 1.7 mV, increased Rb from 610 ± 105 to 1,226 ± 248 Ω·cm², and decreased Jv from 2.35 ± 0.5 to -0.28 ± 0.6 µl·cm⁻²·h⁻¹ (means ± SE). Changes in Jv, Rb, 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 Vb by -15 mV and Va by -5 mV; TEP decreased correspondingly. Rv/Rb fell from -4 to -2.4. Despite the large change in Rv/Rb, changes in Rb were small (see DISCUSSION). The same tissue was then treated with amiloride (Fig. 3B), which caused large and rapid hyperpolarizations of Va and Vb, a reduction in TEP, and increases in Rv/Rb and Rb. In the continued presence of amiloride, P. aeruginosa (~1 × 10⁸ cfu/ml) did not induce further changes in TEP, Vb, or Vb. Nor was Rv/Rb changed. However, Rb 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.
Fig. 4. Effects of *P. aeruginosa* on $J_v$, TEP, and $R_t$ in presence of chloride (anion) transport inhibitors. A: $J_v$; B: TEP ($\bullet$) and $R_t$ ($\triangle$). Before addition of *P. aeruginosa*, BTE was treated with a cocktail of chloride transport inhibitors. Subsequent addition of bacteria ($\times 4 \times 10^7$ cfu/ml) caused induction of an electrically neutral secretion of chloride and fluid ($-2.8 \mu l \cdot cm^{-2} \cdot h^{-1}$). In 3 experiments, *P. aeruginosa* induced a mean fluid secretion of $-2.5 \pm 0.4 \mu l \cdot cm^{-2} \cdot h^{-1}$ in presence of chloride inhibitors. ba, Basolateral.

(n = 3). In control experiments (not shown) the repeated addition of *P. aeruginosa* to BTE induced changes in $J_v$, $R_t$, and TEP similar to those induced during the first exposure.

Role of chloride. The experiment shown in Fig. 4 is representative of three similar experiments in which we used NPPB (100 µM, mucosal), H$_2$-DIDS (500 µM, mucosal), and bumetanide (500 µM, basolateral) to inhibit known anion transport pathways (27). In the example shown, these blockers decreased $J_v$ from 4.5 to 0.5 µl cm$^{-2}$ h$^{-1}$, increased $R_t$ from 310 to 419 Ω cm$^2$, and decreased TEP from −70 to −14 mV. Subsequent addition of *P. aeruginosa* to the tissue in the presence of the chloride transport inhibitor cocktail caused little change in TEP but induced a significant fluid secretion of $-2.8 \mu l \cdot cm^{-2} \cdot h^{-1}$ (Fig. 4). In three experiments the mean fluid secretion induced by *P. aeruginosa* was $-2.5 \pm 0.4 \mu l \cdot cm^{-2} \cdot h^{-1}$. This bacteria-induced fluid secretion was accompanied by a 27% decrease in $R_t$ from a baseline of 327 ± 101 Ω cm$^2$. 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 rhIR) 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 cm$^2$, 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 cm$^2$, 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 cm$^2$ from a baseline of 440 ± 317 Ω cm$^2$, 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. Strain AK1012, an LPS mutant of strain PA01 (deficient in expression of LPS O antigen and partially deficient in LPS core) (12), exhibited significantly lower adherence to BTE than strain PA01 (adherence was 23,000 ± 10,000 and 125,000 ± 22,000 cfu/tissue for AK1012 and PA01, respectively (n = 3)). However, it caused changes in $J_v$, $R_t$, and TEP that were not significantly different from the parent strain PA01. The PAK strain (Table 1, row 3) had the same effects on $J_v$, $R_t$, and TEP as PA01. However, its mutant (PAK-N1; Table 1, row 4), which cannot express adhesins under RpoN control (11), showed significantly reduced adherence (2,700 ± 1,300 vs. 43,000 ± 12,000 cfu/tissue for the parent strain (n = 3)) and had no significant effects on $J_v$, $R_t$, and TEP. Because amiloride completely blocked the effects of *P. aeruginosa* on $J_v$, $R_t$, and TEP (Fig. 2), the effects of amiloride (50 µM) on *P. aeruginosa* adherence were also examined. Amiloride did not

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 cm$^2$</th>
<th>TEP, mV</th>
</tr>
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<tr>
<td></td>
<td>Baseline</td>
<td>Δ</td>
<td>Baseline</td>
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<tr>
<td>PA01</td>
<td>6</td>
<td>3.2 ± 0.5</td>
<td>-2.5 ± 0.6</td>
</tr>
<tr>
<td>AK1012</td>
<td>3</td>
<td>3.8 ± 0.6</td>
<td>-3.1 ± 0.2</td>
</tr>
<tr>
<td>PAK</td>
<td>4</td>
<td>2.7 ± 0.8</td>
<td>-1.4 ± 0.3</td>
</tr>
<tr>
<td>PAK-N1</td>
<td>4</td>
<td>4.9 ± 0.9</td>
<td>-0.8 ± 0.3</td>
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</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_i 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_i, 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_i 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_i 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_i did not change significantly. However, we believe that the lower-than-normal R_i 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_i. Pulled results from all tissues showed that P. aeruginosa increased R_i from ~500 to ~800 Ω·cm². This increase in R_i 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_B depolarizes more than V_A implicates closure of potassium channels as the predominant action of P. aeruginosa on R_i 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_i but no change in TEP, suggesting induction of an electrically neutral secretory process. This fluid secretion and R_i 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 that may also have effects on ion transport processes other than electrogenic sodium absorption. Specifically, 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 $J_v$ 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 clearance of secretions 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.

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