Analysis of Acquisition of *Pseudomonas aeruginosa*
Gastrointestinal Mucosal Colonization and Horizontal Transmission in a Murine Model

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**Background.** Laboratory systems to study bacterial transmission and mucosal colonization leading to infection have not been utilized.

**Methods.** We determined whether transmission of various strains of *Pseudomonas aeruginosa* among individual mice could occur and investigated the properties of such strains in establishing gastrointestinal (GI) mucosal colonization as well as in disseminating systemically after induction of neutropenia.

**Results.** *P. aeruginosa* isolates associated with epidemic spread among patients with cystic fibrosis (CF) readily established GI colonization at higher levels than did strains associated with acute infections in patients without CF, and they outcompeted these strains. Colonization was associated with resistance to bile salts. However, epidemic CF isolates did not disseminate after induction of neutropenia and did not induce as much mucosal pathology as did strains that were capable of disseminating.

**Conclusion.** Murine models can be used to study *P. aeruginosa* transmission and early colonization, and the properties of these strains associated with their known clinical behaviors are mimicked in this setting.

*Pseudomonas aeruginosa* is a major cause of nosocomial infections in patients in the intensive care unit [1, 2] and in patients who have cancer or have undergone bone marrow transplantation [3]. Acute infections, such as ventilator-associated pneumonia and bacteremia caused by *P. aeruginosa*, in these populations are associated with higher mortality than are those infections caused by other bacterial etiologies [4–6]. *P. aeruginosa* also causes chronic lung infections in patients with bronchiectasis or cystic fibrosis (CF) and is associated with poorer prognosis [7].

Because *P. aeruginosa* is, at best, a transient inhabitant of the normal human microbiome, acquisition and mucosal colonization is an initial and crucial step in pathogenesis. In the intensive care unit, acquisition of exogenous *P. aeruginosa* via cross-transmission accounts for the majority of cases of colonization or infection by this organism [8, 9]. In terms of patients with CF, they often acquire *P. aeruginosa* strains from diverse environmental sources outside the hospital [10]. However, well-documented outbreaks involving highly transmissible “epidemic” strains of *P. aeruginosa* have occurred in a number of CF clinics [11–14].

Little is known about these early steps of acquisition, mucosal colonization, and transmission of *P. aeruginosa*, specifically with respect to the epidemic strains. In a murine model of oropharyngeal colonization, chronic *P. aeruginosa* infection has been achieved in transgenic CF mice; however, the colonization levels are too low for quantitative analysis [15, 16]. In the present study, we used a murine model [17] to study gastrointestinal (GI) colonization by *P. aeruginosa*, competitive cocolonization...
between different strains, and horizontal transmission in the setting of antibiotic-induced depletion of the indigenous GI flora. We also evaluated bacterial dissemination after induction of neutropenia. These findings identified and validated a suitable animal model for studying acquisition of *P. aeruginosa* that can be used to define determinants of transmission and mucosal colonization relevant to person-to-person transmission.

### MATERIALS AND METHODS

**Bacterial strains.** The strains used in the study are presented in Table 1. *P. aeruginosa* epidemic strains LES (ie, Liverpool epidemic strain) and C3719 are lipopolysaccharide (LPS) rough, nonmucoid CF respiratory isolates with genomes that have recently been sequenced [22, 23]. Strain PA2192nm, which also has a recently sequenced genome [22], is a nonmucoid variant of mucoid strain PA2192 obtained from a patient with CF who had chronic infection for 8 years. Strains PAO1 (wound isolate) and PA14 (isolated from a burn patient) are well-studied sequenced strains [24, 25], and along with strain PA2192nm, they are referred to as “nonepidemic” strains. All the strains had virtually identical in vitro growth rates, with the exception that the epidemic strains took a slightly longer time to reach log-phase growth (data not shown). Strains were tested for swimming [26] and twitching motility [27] as well as for in vitro cytotoxicity on Caco-2 cells (CytoTox 96; Promega). All of the strains had intact genes for *hslV* (PA0503), *hslU* (PA0504), *hslO* (PA1468610), *pqsA* (PA0996), *pqsB* (PA0997), *pqsC* (PA0998), *pqsD* (PA0999), and *pqsE* (PA1000), as determined by a Basic Local Alignment Search Tool (BLAST) search (data not shown). The presence of *exoS* (PA3841) or *exoU* (PA14 51530) genes was also determined by a BLAST search. LPS glycoforms were analyzed using sodium dodecyl sulfate–polyacrylamide gel electrophoresis [28].

**Murine model of GI tract colonization by *P. aeruginosa.*** As described elsewhere [17], C3H/HeN mice (6- to 8-week-old females) were housed in groups of 4 in sterilized cages with sterile filter hoods and were maintained under specific pathogen–free conditions, in compliance with the guidelines of the institutional animal care and use committee of the Harvard Medical Area. Mice were fed sterile water with 2 mg/mL streptomycin and 1500 U/mL penicillin G for 4 days, to deplete indigenous GI flora (as confirmed by bacterial stool cultures [17]). Stable GI colonization by *P. aeruginosa* is not achieved in the presence of indigenous bowel flora [29]. Next, mice were fed sterile water with 1500 U/mL penicillin G and *P. aeruginosa* strains (∼10⁷ cfu/mL for 5 days). Water containing *P. aeruginosa* was changed after 2–3 days to maintain bacterial levels. Stool samples were collected from individual mice on a daily basis, starting 24 h after initiation of *P. aeruginosa* water; weighed; homogenized in 1 mL of 1% proteasepeptone; and serially diluted and plated on cetrimide agar to quantify bacterial levels. The presence of green oxidase-positive colonies was used to confirm *P. aeruginosa* GI colonization.

**Murine model of *P. aeruginosa* cocolonization.** To evaluate *P. aeruginosa* strain competition during murine GI colonization, one epidemic strain and one nonepidemic strain were mixed together in sterile water with 1500 U/mL penicillin G at ∼10⁷ cfu/mL for each strain and were fed to 8 mice treated with antibiotics. Levels of GI colonization were measured daily for 5 days. Cetrimide agar with or without 0.060 mg/mL gentamicin was used to grow colonies of the epidemic strains at 37°C for 48 h. We could not find antibiotics to select for growth of the nonepidemic strains and against the epidemic strains. To quantify the nonepidemic strains, we used a significant colony growth differential on cetrimide agar between these and the epidemic strains. All colonies of the nonepidemic strains were macroscopically visible by 24 h of incubation, whereas colonies of the epidemic strains were not visualized until 48 h after incubation. Therefore, we counted the colonies that were macroscopically visible on cetrimide agar at 24 h as those of the nonepidemic strains, and we further confirmed that the colonies that emerged between 24 and 48 h of incubation were

### Table 1. Bacterial Strains Used

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype and phenotype</th>
<th>LPS structure</th>
<th>Motility</th>
<th>exoS or exoU</th>
<th>Origin and/or reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAO1</td>
<td>Wild-type wound isolate, Cm susceptible</td>
<td>LPS smooth, complete core</td>
<td>Yes</td>
<td>Yes</td>
<td>No a</td>
</tr>
<tr>
<td>PAO1 <strong>Δ<em>galU</em></strong></td>
<td><strong>Δ<em>galU</em> mutant</strong></td>
<td>LPS rough, incomplete outer core</td>
<td>NA</td>
<td>NA</td>
<td>No b</td>
</tr>
<tr>
<td>PA14</td>
<td>Wild-type isolate from a burn patient</td>
<td>LPS smooth, complete core</td>
<td>Yes</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>PA2192nm</td>
<td>Nm variant of mucoid CF isolate PA2192</td>
<td>LPS rough, complete core</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>LES</td>
<td>Epidemic CF isolate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>LPS rough, complete core</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>C3719</td>
<td>Epidemic CF isolate&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>LPS rough, complete core</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
</tbody>
</table>

**NOTE.** CF, cystic fibrosis; Cm, chloramphenicol; LPS, lipopolysaccharide; NA, not available; nm, nonmucoid.

<sup>a</sup> [18].

<sup>b</sup> Liverpool clone.

<sup>c</sup> Manchester clone.
equivalent in number to those found on cetrimide with gentamicin used to select for the epidemic strains.

**Murine model of horizontal transmission of P. aeruginosa.** Horizontal transmission among mice within a cage was analyzed by placing one mouse that was previously colonized with *P. aeruginosa* into a cage with 3 other antibiotic-treated mice that were not given *P. aeruginosa*. To prevent transmission of *P. aeruginosa* via drinking water, acidified water (0.2 mol/L sodium acetate [pH 4.0]) was used and monitored daily by culture on cetrimide agar. Cages and bedding were changed daily. Stool samples were collected from each mouse daily for 5 consecutive days and were processed to quantify colonization levels after introduction of the colonized mouse.

**Murine model of *P. aeruginosa* GI translocation.** After *P. aeruginosa* colonization was established, one 200-µg dose of monoclonal antibody RB6-8C5 specific for the Ly6 antigen on polymorphonuclear leukocytes was administered intraperitoneally [17]. The drinking water was replaced with sterile water containing 1500 U/mL penicillin G. For 7 days, mice were monitored twice daily for morbidity and mortality. Previous experience has documented that no deaths occur after 7 days in this experimental setting. Moribund mice were euthanized, and their spleens, along with those of mice found dead between observation periods, were resected, homogenized in 1 mL of 1% proteose peptone, serially diluted, and plated on tryptic soy agar (TSA) (1.5% agar in tryptic soy broth), MacConkey agar, and cetrimide agar. The presence of green oxidase–positive colonies on cetrimide agar was used as confirmation of dissemination of *P. aeruginosa*. In a report presented elsewhere, Koh et al [17] showed that when *P. aeruginosa* translocates from the GI tract into the systemic circulation, the organism can be recovered not only from the blood and liver but also from the spleen.

**Bile salt susceptibility assay.** *P. aeruginosa* was grown on TSA at 37°C overnight. Colonies were suspended in phosphate-buffered saline (PBS) to a concentration of ∼10^6 cfu/mL. One hundred microliters of bacterial suspension were placed, in triplicate, in a sterile 96-well microtiter plate. Then, 100 µL of 1% sodium deoxycholate (Sigma) in PBS was added to individual wells. Next, 100 µL of 2% bovine serum albumin (BSA) in PBS was added to the other wells with 100 µL of the bacterial suspension to serve as negative controls. The plate was incubated at 37°C for 4 h with gentle shaking, and samples were serially diluted, plated on TSA, and incubated at 37°C for bacterial enumeration.

**Serum susceptibility assay.** One hundred–microliter aliquots of tryptic soy broth–grown *P. aeruginosa* that were diluted in PBS to contain ∼10^6 cfu were placed, in triplicate, in individual wells of sterile 96-well plates. Serum from a healthy human was diluted in PBS to 10%. Heat-inactivated (at 56°C for 30 min) serum and PBS without serum served as controls. Equal volumes (100 µL) of serum and bacterial suspensions were mixed and incubated at 37°C for 1 h with gentle shaking. Samples were serially diluted, plated on TSA, and incubated overnight at 37°C for enumeration.

**Histologic analysis of the murine GI tract.** Neutropenic C3H/HeN mice colonized with *P. aeruginosa* strains PA14 or LES were euthanized at 0, 24, or 42 h after neutropenia was induced, whereas mice colonized with strain LES were also euthanized at 72 or 96 h. After sacrifice, ceca were resected and fixed in Bouin’s solution, and sections were stained with hematoxylin–eosin and then reviewed by a veterinary pathologist. Gross histopathology of the GI tract was also recorded.

**Measurement of cytokine responses in murine GI tissue.** Mice colonized with either strain PA14 or LES, as well as noncolonized control mice, were made neutropenic and then euthanized 42 h later. After the mice were euthanized, ceca were resected and their contents gently flushed out, and cecal tissue was washed in modified Eagle medium (Gibco) and then homogenized in 1 mL of ice-cold PBS with protease inhibitors (complete Mini; Roche Diagnostics) and 0.5% Triton-X100 (Amresco). The sample was centrifuged (at 5000 g at 4°C for 5 min), tumor necrosis factor (TNF)–α and interleukin (IL)–1β levels in supernatant were measured by enzyme-linked immunosorbent assays (eBioscience), and protein concentrations were measured using the BCA Protein Assay Kit (Pierce).

**Statistical analyses.** Pairwise comparisons between strains were analyzed by Mann-Whitney U tests, with the exception that competitive cocolonization data were analyzed with Wilcoxon signed rank test. Survival data were analyzed by the log-rank test. Bonferroni’s correction was used when multiple comparisons were performed. One-way analysis of variance (ANOVA) with Dunnett’s multiple comparison was used for analysis of results from the bile salt susceptibility and serum susceptibility assays. As appropriate, nonnormally distributed data were log transformed. Kruskal-Wallis tests with Dunn’s multiple comparison were used to analyze proinflammatory cytokine levels. Correlations between TNF-α and IL-1β in each mouse were analyzed using Spearman’s correlation test.

**RESULTS**

**Comparative GI colonization levels of different strains.** *P. aeruginosa* strains PAO1, PA14, LES, C3719, and PA2192nm all colonized the murine GI tract. One day after the start of oral feeding of bacteria, the number of colony-forming units of the epidemic strains LES and C3719 per gram of stool were higher than those of the other strains (Figure 1A) (P < .01, by Mann-Whitney U test with Bonferroni’s correction). By day 5, strains PAO1, PA14 and PA2192nm reached colonization levels comparable to those of strain C3719, but all still colonized at a lower level than did strain LES (Figure 1B) (P < .01).
Association of resistance to bile salts with GI colonizing capacity. *P. aeruginosa* strain PAO1ΔgalU, which lacks the outer core oligosaccharide of LPS [20], is unable to colonize the murine GI tract [17]. Because strains C3719 and PA2192nm have a full LPS outer core but lack LPS O-side chains [22, 30], as does strain LES (S. Lory, unpublished data), we hypothesized that their LPS structure conferred resistance to bile salts and, potentially, the ability to colonize the GI tract. We found that strain PAO1ΔgalU was significantly more susceptible to bile salts, compared with all the other strains tested (Figure 1C) (*P*. < .01, by ANOVA with Dunnett’s multiple comparison), indicating that possession of an intact LPS outer core, even in the absence of LPS O-side chains, conferred sufficient resistance to bile salts to allow for GI colonization by *P. aeruginosa*.

Serum susceptibility assay. LPS smooth strains PAO1 and PA14 were more serum resistant than the LPS rough strain PA2192nm, whereas epidemic strains LES and C3719 and strain PAO1ΔgalU, which are also LPS rough strains, were significantly more serum-susceptible than strain PA2192nm (Figure 1D) (*P*. < .01, by one-way ANOVA with Dunnett’s multiple comparison).

Competitive colonization between the epidemic and non-epidemic strains. When the epidemic strains LES or C3719 were administered concurrently with the nonepidemic strains PA14 or PA2192nm, the epidemic strains colonized 38 of 40 mice at higher levels than did the nonepidemic strains throughout the 5-day observation period (Figure 2A–E, for day 5) (data not shown for days 1–4) (*P*. < .05, by Wilcoxon signed rank test). Cocolonization by strains C3719 and PAO1 on day 5 showed that only 4 of 8 mice had higher levels of the epidemic strain, and the difference was not significant (Figure 2F). Competitive indices between epidemic and nonepidemic strains are also shown (Figure 2G). When comparing the GI colonization levels in mice monocolonized by the nonepidemic strains PAO1,
PA14, and PA2192nm (Figure 1B) with the levels of these strains in mice cocolonized along with the epidemic strains LES and C3719 (Figure 2A–F), the levels of the nonepidemic strains were significantly lower in the cocolonized mice than in the monoclonized mice (P < .01, Mann-Whitney U test), with the single exception of strain PAO1 in mice cocolonized with epidemic strain C3719. Notably, the 2 epidemic strains achieved comparable levels of GI colonization, regardless of whether the animals were monoclonized with these 2 strains (Figure 1) or cocolonized along with nonepidemic strains (Figure 2).

**Horizontal transmission of P. aeruginosa strains.** All the P. aeruginosa strains could be transmitted from a single colonized mouse placed into a cage with 3 uncolonized, antibiotic-treated mice (Figure 3). For all strains, GI colonization levels in mice within the same cage established an equilibrium, increasing until day 2 or 3 (with levels ranging from 10^3 to 10^7 cfu/g of stool, depending on the strains) and then decreasing by day 5 (with levels ranging from 10^3 to 10^6 cfu/g of stool). P. aeruginosa was never detected in the acidified drinking water.

**Systemic spread of P. aeruginosa after induction of neutropenia.** Seven of 8 mice monoclonized with strain PAO1 and all 8 mice monoclonized with strain PA14 died or became moribund after neutropenia was induced (Figure 4A). Five of 8 mice monoclonized with strain PA2192nm died, and the overall duration of survival was significantly longer than that of mice monoclonized with strains PAO1 or PA14 (Figure 4A) (P < .05, by log-rank test with Bonferroni’s correction). None of 8 mice monoclonized with epidemic strains LES or C3719 died (Figure 4A). Strain PA2192nm was isolated from the spleen of the dead or moribund mice, albeit at a level significantly lower than that of strains PAO1 or PA14 (Figure 4B) (P < .05, by Mann-Whitney U test with Bonferroni’s correction). GI colonization levels in all the surviving mice were comparable to those achieved before induction of neutropenia (data not shown).

**Cecal histopathologic findings in neutropenic mice.** At 42 h after induction of neutropenia, the GI tracts of mice colonized by strain PA14 were macroscopically edematous and hyperemic (Figure 5A). The GI tracts of mice that had been neutropenic for 42 h and colonized by strain LES (Figure 5B) or of mice colonized with strain PA14 before the induction of neutropenia appeared to be normal and comparable to those of uncolonized...
neutropenic mice (data not shown). Histopathologic analysis of the ceca from neutropenic mice colonized with strain PA14 revealed shortening and flattening of the villi along with marked sloughing of the epithelial cells (Figure 5C and 5E). No abnormalities were observed in mice colonized with strain PA14 just before induction of neutropenia (data not shown), nor were any abnormalities observed in mice colonized with strain LES at 42 h (Figure 5D and 5F) or up to 96 h after induction of neutropenia (data not shown).

**Cecal TNF-α and IL-1β concentrations in neutropenic mice.** Animals colonized with strain PA14 had significantly higher tissue levels of TNF-α and IL-1β than did neutropenic mice colonized with strain LES (Figure 6) (P < .05, by Kruskal-Wallis tests with Dunn’s multiple comparison test). Tissue levels of TNF-α and IL-1β in each neutropenic mouse colonized with strain PA14 were significantly correlated (P < .05, by Spearman correlation test) (data not shown).

**DISCUSSION**

We conducted the present study to validate a murine model related to host acquisition and infection by *P. aeruginosa*, using GI colonization and horizontal transmission to investigate these early steps in the infectious process. Mice treated with antibiotics became rapidly colonized in the GI tract by all the *P. aeruginosa* strains tested and could facilely transmit them to their antibiotic-treated, noncolonized cage mates. The epidemic strains achieved higher GI colonization levels than did the non-epidemic strains and outcompeted them in the cocolonization setting. We also used polymorphonuclear leukocyte depletion, a risk factor for systemic nosocomial infections by *P. aeruginosa*, to study the association of colonization and dissemination. We found that the epidemic strains failed to disseminate from the GI tract, which was an interesting corollary to the localized, nondisseminating nature of lung infections that develop in pa-
Figure 4. A, Survival curves of C3H/HeN mice monoclonized with *P. aeruginosa* strain PAO1, PA14, PA2192nm, Liverpool epidemic strain (LES), or C3719, followed by monoclonal antibody RB6-8C5–induced neutropenia (n = 8 per strain). *P* < 0.01 and **P** < 0.05, compared with mice colonized with strain PAO1 or PA14; *P* < 0.05, compared with mice colonized with strain PA2192nm, by log-rank test with Bonferroni’s correction. B, Bacterial levels of *P. aeruginosa* strains isolated from the spleens of moribund or dead mice after gastrointestinal colonization followed by the induction of neutropenia (n = 7, n = 8, and n = 5 for strain PAO1, PA14, and PA2192nm, respectively). *P* < 0.05, by Mann-Whitney U test with Bonferroni’s correction.

Figure 5. Gross anatomy and microscopic histologic findings of the murine (C3H/HeN) gastrointestinal (GI) tract after prior monoclonization with *P. aeruginosa* strain PA14 (A, C, and E) or Liverpool epidemic strain (LES) (B, D, and F) and subsequent monoclonal antibody RB6-8C5–induced neutropenia. Mice were euthanized, and sections were obtained 42 h after induction of neutropenia. A, Diffuse erythema in noncontiguous areas of the GI tract, notably in the small intestine as well as the tip of the cecum. C and E, Shortening and flattening of the villi along with marked sloughing of the epithelial cells are clearly seen. B, D, and F, Normal macro- and microscopic appearances. Magnification: ×100 (C and D) and ×400 (E and F).

tients with CF who are infected with these strains. This murine model of acquisition, colonization, transmission, and dissemination of *P. aeruginosa* can now provide further opportunities to gain insights into why certain *P. aeruginosa* strains may achieve a competitive advantage and/or have a predilection for specific clinical manifestations of infection.

We utilized a GI model of colonization to study *P. aeruginosa* epidemic strains that are responsible for chronic lung infections, because the GI tract is an ideal mucosal surface for serial, quantitative analysis of *P. aeruginosa* colonization. It is also an important source of this organism that can ultimately lead to colonization of the respiratory tract [31]. It is plausible that a fecal-oral transmission route contributes to spreading strains of *P. aeruginosa* among patients with CF (eg, via oropharyngeal or upper GI tract colonization, followed by aspiration to the lungs), because it has been shown that such patients have high fecal burdens of *P. aeruginosa* [32]. In populations without CF, a significant portion of cases of *P. aeruginosa* ventilator-associated pneumonia in the intensive care unit have been shown to be caused by the endogenous strains that originally existed in the GI flora of the patients [33]. On the basis of these observations, *P. aeruginosa* GI colonization appears to be highly relevant to *P. aeruginosa* colonization/infection in the respiratory tract.

Although our animal model is not representative of the hygienic conditions in most hospitals, we do demonstrate that, in a laboratory setting, *P. aeruginosa* can be transmitted from a carrier to an antibiotic-treated, noncolonized host without contaminated water serving as a source—most likely via a fecal-oral route, an important mode of transmission readily achieved even in a modern hygienic environment [34]. In fact, although water is a well-established environmental source of *P. aeruginosa*...
in hospitals [35–38], several recent studies of outbreaks of P. aeruginosa nosocomial infections have reported a lack of a persistent environmental source [11, 39, 40], suggesting some other method of horizontal transmission between infected and noninfected individuals.

It is not clear why the epidemic strains spread so well among patients in CF clinics in England and Wales [39–41]. Using a laminar flow model of survival of bacteria in aerosols, Clifton et al [42] did not find a difference among epidemic and nonepидemic P. aeruginosa isolates, but they did find that mucoid strains of P. aeruginosa had better overall survival in the aerosols, with survival affected by temperature and humidity. Our finding that the epidemic strains colonized the GI mucosal surface better than the nonepidemic strains may partly explain the epidemic capacity of the strains. Moreover, these epidemic strains appear to be genetically different from other strains [18, 43], with strain LES harboring a large number of phage-encoded pathogenicity islands [43].

Our findings in the cocolonization experiments also correlated with the clinical observation that strain LES can “superinfect” a CF-affected lung previously colonized by other P. aeruginosa strains and can outcompete the original strains [44]. The inability of the epidemic strains to disseminate, even in the presence of neutropenia, fits with the chronic and localized nature of lung infections by these strains that are observed in patients with CF. These in vivo characteristics are associated with the serum susceptibility of the strains, a well-known property of CF isolates obtained from chronically infected patients [45–47]. Thus, it appears that strains of P. aeruginosa do not need to synthesize O-antigens to initiate mucosal colonization or resist host innate immunity in the GI tract and lung.

Our findings are also somewhat comparable to those obtained with strain LES in a rat model of chronic lung infection [23]. In this setting, strain LES was recovered from cointected mice at lower levels than strain PAO1 but at higher levels than strain PA14 [23]. Also, the distribution of these nonepidemic strains in the lungs was different from that of strain LES. Overall, both models showed that the epidemic isolates can be highly successful in establishing tissue colonization and can outcompete some nonepidemic isolates.

Antibiotic depletion of the indigenous murine GI microbial flora that is needed to establish P. aeruginosa GI colonization is likely analogous to what occurs in hospitalized patients without CF who are exposed to broad-spectrum antibiotics. Whether antibiotic treatment of patients with CF affects acquisition of P. aeruginosa colonization of their lungs is difficult to ascertain because of the highly variable nature of clinical care of these patients early in life. Studies have shown that treating patients with CF by using antibiotics directed against Staphylococcus aureus leads to earlier acquisition of P. aeruginosa [48, 49] and that, although the increasing use of early, intensive, and routine antibiotic treatments in young patients with CF after initial acquisition of P. aeruginosa does lead to temporary eradication, the vast majority of these patients acquire new strains of P. aeruginosa after the conclusion of therapy [50]. Thus, it appears that use of antibiotics to condition a mucosal surface for P. aeruginosa colonization may reflect a common situation encountered in clinical medicine.

In conclusion, we developed and validated a murine model of P. aeruginosa acquisition, using GI colonization and horizontal transmission. Both epidemic and nonepidermic strains were readily acquired and horizontally transmitted to noninfected mice, and epidemic strains showed a superior ability to monoclonize and cocolonize the GI mucosa, compared with noncolonized controls (n = 4). Mice were euthanized 42 h after MAb administration, and ceca were harvested to measure the tissue levels of cytokines. Lines denote median values; boxes, 25th and 75th percentiles; and error bars, 10th and 90th percentiles. * P < .05, by Kruskal-Wallis test with Dunn’s multiple comparison post hoc test.

Figure 6. Levels of tumor necrosis factor (TNF)-α (A), and interleukin (IL)-1β (B) in the ceca of C3H/HeN mice with a gastrointestinal tract monoclonized by either Pseudomonas aeruginosa strain PA14 (p = 8) or P. aeruginosa strain LES (n = 6), followed by monoclonal antibody (MAb) RB6–8C5–induced neutropenia. Mice that were treated with antibiotics but not given any P. aeruginosa were also induced neutropenic to serve as noncolonized controls ( ). Mice were euthanized 42 h after MAb administration, and ceca were harvested to measure the tissue levels of cytokines.
other strains. The epidemic strains failed to disseminate, even in the setting of neutropenia, consistent with the actual clinical properties of these strains. Further use of this model may provide critical insights into these early steps in bacterial pathogenesis and, possibly, help to develop novel means for therapy and infection control.

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References

25. Liberati NT, Urbach JM, Miyata S, et al. An ordered, nonredundant library of Pseudomonas aeruginosa strain PA14 transposon insertion mutants. Proc Natl Acad Sci U S A 2006;103:2883–8.


