A Murine Model of Chronic Mucosal Colonization by *Pseudomonas aeruginosa*

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Chronic mucosal colonization by *Pseudomonas aeruginosa* is an integral part of the pathologic process associated with disease due to infection with this organism. We have adapted the streptomycin-treated murine model of chronic mucosal colonization by enteric pathogens to study colonization by *P. aeruginosa*. Mice first received 1 mg of streptomycin per ml of drinking water for 2 to 5 days and then ingested $10^7$ CFU of *P. aeruginosa* per ml of drinking water for a minimum of 5 days. The result of this regimen was chronic mucosal colonization with *P. aeruginosa* for up to 10 weeks, which was determined by fecal cultures and confirmed by culture of the intestines after killing of the experimental animals. Bacterial counts were highest in the cecum and colon, with some evidence for extraintestinal bacterial translocation as well. Use of *P. aeruginosa* mutants deficient in the production of colonization factors such as pili and those dependent on the rpoN gene product resulted in a lower level of chronic colonization. Immune responses to type-specific lipopolysaccharide, pili, and flagellar antigens were measured, and increases in both serum and intestinal antibodies were usually elicited when a strain elaborated a given antigen. This model represents an easy method of routinely achieving chronic mucosal colonization by *P. aeruginosa* and should prove useful for the study of both bacterial virulence factors and host responses associated with this infectious process.

The chronic colonization of the respiratory mucosal surface of cystic fibrosis patients by *Pseudomonas aeruginosa* is well appreciated as an important part of the pathologic process resulting in disease due to infection with this organism (13, 17, 23, 30, 45). Chronic colonization and infection of the ocular mucosa (35, 50, 63) and chronic colonization of the gastrointestinal (GI) mucosa (1, 7, 10, 43) are also acknowledged as important components of *P. aeruginosa* disease, leading to keratitis and bacteremia, respectively. Many investigators have attempted to define the bacterial and host factors involved in mucosal colonization (2, 19, 36-38, 48, 57, 59, 60), but most studies have been confined to measurements of adherence of bacteria in vitro to a variety of targets, including epithelial cells (5, 11, 12, 25, 36, 59), animal organ cultures (37), mucosal proteins (19), and mucins (54, 56, 60, 61). In vitro adherence assays likely measure only one component of colonization, and the actual contribution of adherence to colonization in vivo can only be approximated by these measurements of in vitro adherence.

A reliable animal model wherein chronic colonization of a mucosal surface by *P. aeruginosa* can be established would be useful for quantifying the contribution of various bacterial factors to the colonization process. In addition, an in vivo model would allow assessment of the contribution of host factors, notably immune effectors, in modulating colonization. In the one available model of chronic colonization by *P. aeruginosa*, the bacteria are encased in agar beads and delivered transtracheally to the lungs of rats (6), mice (65), cats (66), or guinea pigs (46). This process bypasses the initial colonization of the mucosal surface and directly establishes a lung infection. Moreover, the contribution of the agar beads to the chronic infectious state is unknown. Other recently developed models have established that colonization of the GI tract of antibiotic-treated rats by *P. aeruginosa* can lead to bacteremia when the animals are made neutropenic (7, 43). No information about the level or duration of GI colonization has been given. Hengtes et al. (20, 21) and Que et al. (51) showed that treatment of mice with streptomycin sulfate eliminated the indigenous GI facultative flora; they also noted changes in the anaerobic flora, and this resulted in colonization for 48 h by *P. aeruginosa* after a single oral dose, but the bacteria failed to multiply. Koch et al. (32) have described a rabbit model of keratitis, but the chronicity of the infection was not determined. Thus, no model that measures chronic colonization of a mucosal surface by *P. aeruginosa* has yet been established.

To address the need for an in vivo model with which to study the propensity of *P. aeruginosa* to colonize mucosal surfaces, we built upon the initial observations of Hengtes and coworkers (21, 51) and tested whether chronic GI colonization by *P. aeruginosa* could be established by varying the parameters of their murine model. We found that a key factor was exposure of mice to *P. aeruginosa* via the drinking water for a minimum of 5 days, after which chronic colonization of the GI tract could be maintained for up to 10 weeks despite an absence of *P. aeruginosa* from the drinking water. We ascertained whether this model could detect differences in the elaboration of bacterial virulence factors by challenging the mice with *P. aeruginosa* strains that were deficient in either pili or other colonization factors, including pili and flagella whose expression is dependent on the alternate sigma factor of RNA polymerase encoded by the *rpoN* gene (26, 67). In addition, we measured local and systemic immune responses to *P. aeruginosa* antigens and confirmed the presence of bacteria in the intestine by culturing organs after killing the experimental animals. In this report, we identify the parameters to obtain chronic GI mucosal colonization by *P. aeruginosa*, and we report the results of our experiments with the different *P. aeruginosa* mutants.

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MATERIALS AND METHODS

Bacterial strains. The clinical isolates of P. aeruginosa used in these studies were stains 15921 and mucoid 2192, obtained from the blood of a bacteremic patient, and the sputum of a cystic fibrosis patient, respectively, and the spontaneous nonmucoid revertant of strain 2192. Strain PAO1 was from our culture collection. It has a banding pattern in pulsed-field gel electrophoresis identical to that reported by other investigators (18, 64) after digestion of chromosomal DNA with restriction endonucleases DpnI and SpeI. Strains PAK and 1244 as well as their respective isogenic mutants deficient in elaboration of the rpoN gene product (26, 56, 67) or pilin (56, 59) were kindly provided by Stephen Lory, University of Washington, Seattle. The mutants were made by insertion of a tetracycline resistance cartridge into either the pilin structural gene or the rpoN gene. All strains were made resistant to streptomycin by growth in increasing concentrations of this antibiotic up to a final concentration of 1 mg/ml. The strains all had comparable growth rates in vitro.

Animal model. Outbred female Swiss-Webster mice 6 to 8 weeks old were housed singly in cages. They were given water containing 1 mg of streptomycin sulfate per ml for a minimum of 48 h; most animals received this regimen for 5 days. The fecal samples used were always less than 4 h old; they were obtained prior to administration of streptomycin and after 48 h to confirm the depletion of the resident gram-negative facultative flora. Samples were collected, weighed, homogenized to 0.1 g/ml in sterile saline, and diluted and plated for bacterial enumeration onto MacConkey agar. Plates were incubated under aerobic conditions at 37°C for 24 to 48 h, and lactose-fermenting and -nonfermenting colonies were counted. Results were calculated as CFU per gram of feces. Mice had an initial count of facultative gram-negative bacteria of 10³ to 10⁵ CFU/g of feces, and this count usually plummeted to <10 CFU/g of feces (lower level of detection) after 48 h of streptomycin treatment. Depending on the batch of mice, we found that up to 10% of animals harbored about 10⁵ CFU of lactose-nonfermenting, streptomycin-resistant strain, which after 48 h of streptomycin treatment. When this organism was encountered, it was eliminated from the GI tract by treatment for 24 to 48 h with trimethoprim-sulfamethoxazole (TMP-SMZ; 2 mg of TMP and 20 mg of SMZ per ml of drinking water); problems occasionally arose when the drugs settled out in the water, and some mice appeared rather sickly after 24 h. Reduction of the antibiotic dose by one-half at this time resulted in resolution of this sickly appearance. After treatment with TMP-SMZ for 48 h, the feces were recultured, and the mice were treated for an additional 24 h with 1 mg of streptomycin sulfate per ml of drinking water. Only healthy-appearing mice showing clearance of the lactose-nonfermenting E. coli were studied further.

To establish chronic GI colonization after antibiotic treatment, groups of five mice housed individually were given 10⁷ CFU of streptomycin-resistant P. aeruginosa per ml of drinking water that also contained 1 mg of streptomycin per ml for a minimum of 5 days. Preliminary studies indicated that <5 days of treatment did not routinely result in chronic colonization, and no benefit was noted from exposure of the mice to the bacteria for a longer period. Determination of the bacterial density in the water indicated that all of the strains increased in cell density, some by as much as 10-fold, during the 5-day period. However, this did not appear to affect the actual numbers of organisms delivered to the GI tract inasmuch as there were comparable levels of P. aeruginosa in the GI tract during the initial 5-day period of feeding of this organism, as determined by quantitative fecal cultures. After 5 days, the water containing bacteria was replaced with water containing 0.1 mg of streptomycin per ml.

Measurement of bacterial colonization. Quantitative levels of bacterial colonization were estimated by fecal culture as described above. Homogenates were plated onto MacConkey agar for detection of the reacquisition of lactose-fermenting E. coli and onto cetrimide agar with 0.1 to 1 mg of streptomycin per ml for quantifying the level of P. aeruginosa colonization. No evidence for reacquisition of any strain of E. coli was obtained, including reacquisition of the lactose-nonfermenting, streptomycin-resistant strain, which could be differentiated from P. aeruginosa by morphology on MacConkey agar. On occasion, the presence of Proteus mirabilis was noted on the cetrimide plates, and in these cases the values for that individual mouse were not used in our calculations. The mean and standard error of quantitative culture results for individual mice given identical treatments were calculated and recorded.

Chronic GI colonization was confirmed at the end of the experimental period. Mice were killed, and bacterial levels were measured by quantitative cultures of homogenates of the duodenum, ileum, cecum, and colon after removal of as much of the intraluminal contents as feasible by gentle scraping and washing. Translocation of bacteria from the GI tract was assessed by culture of the mesenteric lymph nodes, spleen, and liver. Organs were removed, weighed, homogenized in tryptic soy broth, diluted, and plated for bacterial enumeration. Results were expressed as mean CFU per gram of tissue.

Immunologic determinations. The serum and mucosal immune responses to P. aeruginosa antigens at the end of the period of chronic colonization were assessed by enzyme-linked immunosorbent assay as described elsewhere (4, 47). Antigens used included type-specific lipopolysaccharides (LPS) prepared in our laboratory by the phenol-water extraction procedure (69), flagella (type a, provided by S. Lory), and pilin from strains PAK and PAO1 (provided by William Paranchych, Edmonton, Alberta, Canada). Serum samples were obtained at the end of the experiment by nicking the tail with a razor. Samples from individual mice treated identically were pooled for serologic analyses. Mucosal antibodies to LPS were measured in the remainder of the fecal homogenates used for bacterial enumeration after centrifugation and sterilization via filtration. Isotypes were determined with heavy-chain-specific secondary antibody conjugates to murine immunoglobulin M (IgM), IgG, and IgA. Titers were determined as the highest post-colonization dilution of serum or fecal homogenate reacting threefold more strongly than a comparable dilution of pre-colonization serum or fecal homogenate.

Immunohistologic detection of P. aeruginosa. Samples of intestinal tissues were obtained after killing of mice chronically colonized with P. aeruginosa for 21 days. The tissue samples were snap-frozen in liquid nitrogen, sectioned on a cryostat, fixed for 5 s in acetone at room temperature, and rinsed in phosphate-buffered saline (PBS). Endogenous peroxidase was inactivated by incubation for 20 min at room temperature in 1 part hydrogen peroxide and 3 parts methanol. After being washed in PBS, samples were incubated in 5% normal goat serum for 20 min at room temperature, washed in PBS, and allowed to react with 1:20 dilutions of either normal rabbit serum or rabbit serum specific for the LPS of the colonizing strain. This preparation was left
washed and stored at room temperature. For long-term colonization, whether by the pilin-deficient or the rpoN-deficient strain, each point indicates the mean number of CFU per gram of feces from four or five mice; error bars represent standard errors.

RESULTS

Chronic colonization by clinical isolates of P. aeruginosa. We examined the ability of P. aeruginosa 2192 (mucoid and nonmucoid), PAO1, and 15921 to establish chronic colonization in the GI tract of streptomycin-treated mice for 20 to 25 days (Fig. 1). During the initial 5-day period, when drinking water contained P. aeruginosa, counts were high (10^5 to 10^6 CFU/g of feces; not shown), presumably as a result of passage of live organisms through the GI tract. After the switch to drinking water without P. aeruginosa, the counts fell to 10^2.5 to 10^4.5 CFU/g of feces and generally remained at this level for the remainder of the observation period.

Colonization by mutant strains. Strains PAK and 1244 and their pilin-deficient and rpoN-deficient mutants were next examined for the effect on chronic colonization of the loss of pili and rpoN-dependent colonization factors. In addition, mice colonized with the PAK series of strains were examined two or three times a week for 10 weeks to determine whether long-term colonization of the GI tract could be established by P. aeruginosa. As shown in Fig. 2 and 3, both the pilin-deficient and the rpoN-deficient strains colonized the animals at a lower level than did the parental strains. The CFU per gram of feces achieved by the mutant strains after 5 days of feeding of bacteria was not significantly different (P > 0.05, Mann-Whitney U test) from the value for the parental strains, indicating a comparable degree of inoculation of the GI tract by these different strains. After removal of bacteria from drinking water, the wild-type strains again achieved chronic colonization levels of 10^5.5 to 10^6 CFU/g of feces; these levels persisted for 10 weeks in animals colonized by the PAK series of strains (Fig. 3). The pilin and rpoN mutant strains colonized at only 1 to 10% of these levels; some mice (about 40%) actually cleared these organisms, while others maintained a low level of chronic colonization.

Repeat experiments with the wild-type PAK and 1244 strains confirmed that long-term colonization (6 to 10 weeks) could routinely be established by P. aeruginosa in the GI tract of streptomycin-treated mice (results not shown).

Presence of bacteria in tissues. Counts of P. aeruginosa measured in the various segments of the intestine and in extraintestinal tissues after killing of the experimental animals are shown in Fig. 4 for animals whose fecal counts are shown in Fig. 1 and 2 (except those infected with nonmucoid strain 2192). Laboratory error (improperly prepared agar...
plated) prevented accurate determination of levels of colonization by the strain PAK series after 10 weeks. For most strains, counts were the highest in the cecum and colon, as has been observed by other investigators during short-term GI colonization by *P. aeruginosa* (21, 51). The levels in these tissues (10^2 to 10^4 CFU/g of tissue) were close to those found in feces (10^2 to 10^4 CFU/g of feces). Thus, fecal measurements were likely an accurate approximation of the colonization level in the tissues.

The results of cultures to detect *P. aeruginosa* in the spleen and/or mesenteric lymph nodes of animals after killing are shown in Fig. 4. Liver homogenates were uniformly sterile when plated onto tryptic soy agar (results not shown). This finding indicates that *P. aeruginosa* probably can breach the intestinal barrier and translocate to extraintestinal tissues in this model. The mice showed no obvious signs of systemic infection; the level of infection of extraintestinal tissues was low, and such infection was not found at all in most of the mice examined (one mouse each had *P. aeruginosa* 15921 or mucoid 2192 in the spleen; two mice had positive mesenteric lymph node cultures for *P. aeruginosa* PAO1 and 1244; three mice had mesenteric lymph nodes positive for the *P. aeruginosa* mucoid 2192).

**Antibody responses to colonization.** Serum titers of antibody to LPS and pili of *P. aeruginosa* are shown in Table 1 for animals colonized by strain PAO1 and by the PAK and 1244 series of strains. Pili from strain PAK were used with samples from animals colonized by strain 1244, since preliminary tests indicated that this pilus preparation, and not that of strain PAO1, reacted well with sera from these animals. Levels of serum IgG to type-specific LPS were high; serum IgM responses were more modest but increased among most groups of animals. Serum IgA titers were also increased, but there was some variance among the different groups of mice in terms of their IgA response to colonization. There were no differences between the antibody response to LPS of mice colonized by the pilin- and rpoN-deficient PAK strains and that of mice colonized by the parental isolate despite the lower levels of colonization by the former strains. The mutant 1244 strains did provoke a somewhat lower serum antibody response than did the parental strain. As expected, serum immune responses to pili were essentially absent in animals colonized by pilin- and rpoN-deficient strains but were strong among animals colonized with the parental strains.

The reactivities of pre- and postcolonization sera at dilutions of 1:10 and 1:100 against type a flagella are shown in Fig. 5. Animals colonized by the rpoN-deficient strains exhibited no immune response to flagella, whereas animals colonized by the pilin-deficient strains had a modest but detectable response. Strong serum immune responses to flagella were noted among animals colonized by the PAK and 1244 parent strains, while animals colonized by strain PAO1 showed a modest response to this flagellar preparation.

Mucosal levels of IgG and IgA antibody to LPS, as measured in the fecal homogenates, were modestly increased at the end of the colonization period in all animals (titers of 10 to 40 from a 0.1-g/ml homogenate; results not shown). Responses to pili and flagella were not determined for fecal homogenates.

**Immunohistologic appearance of *P. aeruginosa* in colonized tissues.** The location of the colonizing *P. aeruginosa* cells...
was visualized in sections of the cecum of animals chronically colonized for 3 weeks (Fig. 6) after reaction with LPS-specific rabbit antibody. Clumps of bacteria were trapped within the mucus layer, while few cells were associated with the underlying epithelial cells. Examination of the entire luminal portion of five separately stained sections revealed no more than 10 to 20 rod-shaped, stained cells associated with epithelial cells. Most sections included clumps of darkly stained, rod-shaped cells embedded in a lightly staining mucus layer. Sections that were allowed to react with normal rabbit serum showed no obvious darkly stained, rod-shaped organisms in the mucus layer (Fig. 6).

DISCUSSION

In this report, we demonstrate the feasibility of chronically colonizing the murine GI tract with a variety of strains of *P. aeruginosa*. Levels of colonization over 3 to 10 weeks were comparable among wild-type mucoid and nonmucoid strains. Interruption of the pilin gene or the rpoN gene reduced the level of colonization, and 40% of mice colonized by these mutant strains eliminated the organisms. Serum immune responses to LPS, pil, and flagella were noted in mice colonized with strains that elaborated these antigens, whereas strains that did not produce pil and/or flagella did not provoke host immune responses to these antigens. Histologic analysis showed collections of rod-shaped cells reacting with specific antibody to LPS of *P. aeruginosa* within the mucus overlying the epithelial cells of the GI tract; thus, the organisms were likely growing as microcolonies within the mucus layer.

Our success at establishing long-term colonization by *P. aeruginosa* in the murine GI tract contrasts with the outcome of efforts by previous workers to establish *P. aeruginosa* GI colonization in streptomycin-treated mice (21, 51). We are fairly certain that the difference is due to the method of exposure of the animals to the bacterial challenge. The previous studies (21, 51) used an intragastric bolus dose of *P. aeruginosa*, and the bacteria were cleared by 48 h. In contrast, we fed mice continuously for 5 days with *P. aeruginosa* in their drinking water. All of the strains studied survived in the water at levels at least as high as the inoculating level, and some even proliferated. In contrast, when *E. coli* is fed to mice in a similar fashion, there is a decrease in numbers of CFU per milliliter in the drinking water over 24 to 48 h (unpublished observation). When we fed mice *P. aeruginosa* for <5 days, the majority of the animals were not colonized, while feeding for longer than 5 days offered no discernible advantage over feeding for 5 days. Also, in some preliminary studies, we noted that chronic colonization did not take place in the presence of the indigenous bowel flora. Itoh et al. (27) observed that gnotobiotic mice could be colonized by *P. aeruginosa* at a level comparable to that achieved in our model. They found that colonization of the gnotobiotic mice with a combination of bacteroides, lactobacilli, and clostridia obtained from conventional mice, but not from limited-flora mice, resulted in clearance of *P. aeruginosa* within 14 days. Also, neither bacteroides and lactobacilli together nor bacteroides and clostridia together interfered with colonization by *P. aeruginosa*. Therefore, it is not clear whether treatment with streptomycin enhances susceptibility to *P. aeruginosa* colonization solely via elimination of the gram-negative facultative flora of the bowel or whether qualitative and/or quantitative changes in the anaerobic flora are also essential to susceptibility to colonization. We did not look for changes in the anaerobic flora; however, Hentges et al. (20) have shown that streptomycin treatment of mice decreases the frequency of isolation of *Corynebacterium*, *Streptococcus*, *Bifidobacterium*, *Clostridium*, and anaerobic *Lactobacillus* species from cecal contents. Streptomycin also causes an increase in water content and pH of cecal contents and a decrease in the concentration of short-chain volatile fatty acids. The effects of these changes on susceptibility of streptomycin-treated mice to *P. aeruginosa* colonization is unknown, but they may be important.

One issue that arises with regard to animal models is their true relatedness to the human disease that they are intended to mimic. As noted above, GI colonization is an important component of *P. aeruginosa* infection of humans, especially in patients with conditions predisposing to immune suppression. Use of our model to understand the bacterial and host factors critical to GI colonization, along with determination of the specificity, isotype, and mechanism of action of immune effectors that could reduce or prevent GI colonization, could lead to new strategies for the prevention of *P. aeruginosa*-related disease emanating from the GI tract.

Whether GI colonization in this model mimics respiratory colonization in cystic fibrosis patients is more speculative. In vitro studies by Ramphal and coworkers (52-56) and Reddy (58) have suggested that a specific interaction of *P. aeruginosa* with tracheobronchial mucins is a key component of bacterial colonization in cystic fibrosis. Sajjan et al. (60) have shown that *P. aeruginosa* binds equally well to human small-intestinal mucin and to respiratory mucin from patients with cystic fibrosis under certain conditions; under other conditions, however, these authors found no specificity for mucins compared with other glycoproteins. The differences between the claims of investigators for a specific interaction of *P. aeruginosa* with mucins and the work of Sajjan et al. (60) can probably be explained by differences in technique. The latter researchers showed that in vitro binding of *P. aeruginosa* PAO1 to human small-intestinal mucin and cystic fibrosis-associated tracheobronchial mucin was comparable at bacterial inputs of between $10^8$ and $6 \times 10^8$ and that the level of this binding was significantly higher than that of binding to bovine serum albumin-coated and uncoated wells. Whether this is a specific lectin-like interaction or is a nonspecific trapping due to hydrophobic forces is not clear. It is likely that colonization of a mucosal surface is via entrapment of *P. aeruginosa* in mucus, and this may be comparable in both our murine model and human disease.

The finding that cystic fibrosis tracheobronchial mucin and human small-intestinal mucin bind or trap *P. aeruginosa* comparably suggests that GI colonization may mimic respiratory colonization to a sufficient degree to make the study of the former relevant to the latter. In addition, the finding that pilin- and rpoN-deficient strains produce a lower level of colonization than do parent strains is consistent with the observation by almost all workers that both pilin and an rpoN-regulated nonpilus adhesin are involved in the in vitro adherence of *P. aeruginosa* to mucins (53, 54, 56, 58, 60). Since rpoN-deficient strains have multiple defects, we cannot attribute their reduced level of colonization solely to the lack of colonization factors. For example, these strains are also glutamine auxotrophs (26, 67), and nutritional deficiencies could contribute to lower colonization levels via lower in vivo growth rates or lower metabolism.

There are other similarities between the murine model of chronic GI colonization and chronic colonization of the mucosal surface by *P. aeruginosa* in cystic fibrosis patients. In both cases, once chronic colonization is established,
FIG. 6. Immunohistochemical staining of a section of the cecum of mice colonized for 21 days by *P. aeruginosa* 1244 (b) or for 70 days by *P. aeruginosa* PAK (c). Sections were reacted with sera specific for the homologous LPS. (a) Section of cecum obtained from a colonized mouse and allowed to react with normal rabbit serum.
organisms are recovered without evidence of clearance despite an intense immune response to bacterial antigens (16, 22, 44, 45). In this study, few *P. aeruginosa* organisms were observed closely associated with epithelial cells; rather, they were seen as microcolonies trapped within the overlying mucus layer. The microcolony mode of growth was described by Lam et al. (33) and was suggested to be part of the bacterium’s ability to resist host defenses, particularly phagocytosis (29, 68). Histochemical staining of specimens obtained at autopsy from cystic fibrosis patients studied by Baltimore et al. (3) as well as scanning and transmission electron microscopy of cystic fibrosis lung tissue obtained at autopsy or after heart-lung transplantation (28) showed that *P. aeruginosa* was mostly associated with the secretions from the surfaces of the airways. Baltimore et al. (3) noted that in airways with diameters of 1 mm or greater (bronchioles and bronchi), where epithelium remained intact, the organisms did not appear to interact with the epithelium. Studies by Nelson et al. (41) showed in vitro adherence of *P. aeruginosa* to mucin monolayers; Plotkowski et al. (48) demonstrated adherence of *P. aeruginosa* to mucus and not to cell surfaces of frog palates and further showed that epithelial respiratory cells from cystic fibrosis patients did not bind *P. aeruginosa* better than did comparable cells from non-cystic fibrosis subjects (49). In this latter study, Plotkowski et al. also showed that primary cultures of nasal polyp cells from cystic fibrosis and non-cystic fibrosis individuals bound *P. aeruginosa* in the extracellular matrix, similar to what we observed in the mouse intestine. Therefore, in the chronically colonized murine GI tract, the presence of bacteria mostly in the secretions in the lumen indicates a close relatedness of this model, in terms of microanatomic site and mechanism, to colonization of the lung of cystic fibrosis patients.

We are unaware of any histologic studies examining *P. aeruginosa* in the GI tract of humans; however, the GI tract can be a portal of entry of *P. aeruginosa* into the mesenteric lymph nodes and systemic circulation in the presence of underlying susceptibility, such as that associated with burn injury and neutropenia (1, 7, 10, 24, 31, 42). In addition, work in our laboratory indicates that *P. aeruginosa* interacts with ocular mucus of both humans and animals in a manner comparable to that seen in vitro with respiratory mucus (14, 15). Finally, the common mucosal immune system (9, 39) and the putative role of local immunity in modulating mucosal colonization (39, 40) could potentially make the murine model of chronic GI colonization suitable for studies of the impact of preexisting immune effectors and immune modulators on colonization by *P. aeruginosa*.

In conclusion, we have developed a murine model of chronic mucosal colonization by *P. aeruginosa* that should be useful for in vivo evaluation of the contribution of bacterial and host factors to colonization. While effective immunotherapeutic agents that control bacteremia are being developed and investigated by a number of researchers (8, 34, 62), few studies have examined the mechanisms involved in preventing the initial bacterial colonization of a mucosal surface. Obviously, one of the most effective strategies for the prevention of infection would be to inhibit initial bacterial mucosal colonization as much as possible. Our model provides an opportunity to examine the manner in which *P. aeruginosa* interacts with a mucosal surface in vivo for long periods and to study interventions with therapeutic promise.

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