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Rapid and Sensitive Method for Evaluating *Pseudomonas aeruginosa* Virulence Factors during Corneal Infections in Mice

MICHAEL J. PRESTON,[1]* SUZANNE M. J. FLEISZIG,[1]† TANWEER S. ZAIDI,[1] JOANNA B. GOLDBERG,[1] VIRGINIA D. SHORTRIDGE,[2] MICHAEL L. VASIL,[2] AND GERALD B. PIER[1]

Channing Laboratory, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts 02115,[1] and Department of Microbiology and Immunology, University of Colorado Health Sciences Center, Denver, Colorado 80262[2]

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A murine corneal scratch model has been used extensively to study various aspects of the pathogenesis of *Pseudomonas aeruginosa*, a common etiologic agent of corneal infections. This model uses mild inhalation anesthetics which keep the animals immobile for a relatively short time and promote the interaction between the infecting organisms and the corneal wound. Under these circumstances, only a small number of *P. aeruginosa* isolates delivered at inocula of $>10^7$ CFU are infectious. We determined that this model is useful for studying other *P. aeruginosa* strains given at lower doses if injectable anesthetics are administered prior to infection to keep the animals immobile for 15 to 30 min. Under these conditions, eight clinical isolates of *P. aeruginosa* tested at doses of 10^8 CFU per eye induced corneal perforation and/or phthisis in C3H/HeN mice. The 50% infective doses of several strains were between 3×10^2 and 1×10^5 CFU per mouse eye. When this modified anesthetic procedure was used to evaluate the roles of different *P. aeruginosa* virulence factors in eye infections, pathology was not observed when eyes were inoculated with 10^8 CFU of strains deficient in production of a complete lipopolysaccharide or the RpoN sigma factor. A strain with a point mutation in the *fur* gene, involved in production of iron-regulated factors, showed decreased virulence, while a mutant deficient in both hemolytic and nonhemolytic phospholipase C was fully virulent. By modifying the anesthesia procedure, the corneal scratch model allows rapid evaluations of the roles of *P. aeruginosa* virulence factors in corneal infections.

Infections of the corneal component of the eye remain a major health problem that can often lead to permanent vision loss. Among bacterial pathogens, *Pseudomonas aeruginosa* is the most frequently isolated organism in clinical cases of ulcerative keratitis associated with contact lens wear (18, 25) as well as from corneal wounds, especially in warmer geographical locations (3, 14, 30). Most studies of the pathogenesis of *P. aeruginosa* corneal infections have used the model initially described by Gerke and Magliocco (7). In this model, scratches are made on the corneas of mice anesthetized with inhalants such as ether, and the challenge strain of *P. aeruginosa* is applied in a small volume (5 μl). However, only a few strains of *P. aeruginosa* have been reported to be pathogenic under these conditions, and inocula of 10^7 to 10^8 CFU are needed to initiate infection (1, 2, 11, 15, 20, 22, 23). The limited number of virulent strains and the need for high-challenge inocula has restricted the use of this model in the evaluation of microbial and host factors involved in the pathogenesis of *P. aeruginosa* corneal infections. The model would be more useful if it allowed comparisons of the virulence of various clinical and laboratory isolates of *P. aeruginosa* and evaluations of changes in virulence for defined genetic mutations introduced into *P. aeruginosa* strains. Moreover, an animal model of infection initiated with small inocula would more closely mimic the situation thought to exist in human infections.

* Corresponding author. Mailing address: Channing Laboratory, 180 Longwood Ave., Boston, MA 02115. Phone: (617) 432-0152. Fax: (617) 731-1541. Electronic mail address (Internet): preston@warren.med.harvard.edu.

† Present address: School of Optometry, University of California, Berkeley, CA 94720.

By increasing the duration of anesthesia with the use of injectable anesthetics, the invasion of *P. aeruginosa* 6294 into corneal epithelial cells 8 to 24 h after in vivo infection has previously been demonstrated by us (6). However, these studies never evaluated the disease process beyond these time points, nor did they evaluate the virulence of a variety of clinical isolates and strains with mutations that could impact upon disease. In this report, we demonstrate the utility of this modified method of anesthesia for evaluating *P. aeruginosa* isolates for virulence in the corneal scratch model and show that a low inoculum of *P. aeruginosa* can initiate disease.

MATERIALS AND METHODS

Bacterial strains and preparation of inocula. *P. aeruginosa* strains (listed in Table 1) were kept frozen at −85°C as individual aliquots in Trypticase soy broth with 15% glycerol. Bacteria from these frozen stocks were inoculated onto either Trypticase soy agar or L agar plates (containing antibiotics for selection when appropriate) overlaid with a 12,000-molecular-weight cutoff dialysis membrane (5). After overnight growth, bacteria were harvested from membranes with a sterile cotton swab and suspended in sterile 1% Proteose Peptone to an optical density at 650 nm of 1.75 to 2.0, depending on the strain used, to achieve a bacterial concentration of approximately 2×10^{10} CFU/ml. Bacteria were serially diluted in 1% Proteose Peptone (saline and buffers lacking protein promote rapid adherence of *P. aeruginosa* organisms to the walls of plastic and glass tubes or each other, resulting in inaccurate determinations of the CFU/ml [19]) to achieve the desired final concentration. The actual bacterial concentration was confirmed by plating appropriate dilutions, in duplicate, on MacConkey agar.

Infection of mice. C3H/HeN and C57BL/6 female mice (5 to 6 weeks old) were obtained from Charles River Breeding Laboratories, Wilmington, Mass. Groups of five mice were given intraperitoneal injections of 0.2 ml of a cocktail containing 6.7 mg of ketamine hydrochloride (Parke-Davis, Morris Plains, N.J.) and 1.3 mg of xylazine (Haver, Shawnee, Kans.) per ml. After the animals were anesthetized, they were placed under a stereoscopic microscope, and three 1-mm-long scratches were made in the corneal epithelium and superficial stroma of each mouse with a 27-gauge needle. Care was taken not to penetrate the stroma to the anterior eye chamber. Bacteria were immediately inoculated onto the

TABLE 1. Bacterial strains used in this study of *P. aeruginosa* virulence factors for corneal infections

Strain	Description	Reference or source
PAO1-V	Wild-type strain, LPS smooth, serogroup O5	M. Vasil
PAO1-I	Wild-type strain, LPS smooth, serogroup O5	B. Iglewski
PAO1-K	Wild-type strain, LPS smooth, serogroup O5	12
AK1012	LPS rough derivative of PAO1-K	12
PAO1 *algC*::tet	*algC* mutant of PAO1-K, LPS rough	4
PAO1 Δ*plcHR* Δ*plcS*	Hemolytic and nonhemolytic phospholipase C deletion mutant	17
PAO1 Δ*phoB*	Deletion mutant in phosphate regulation gene	26
PAO1 *fur*-C6 (A-10-G)	Point mutation in *fur* gene	8
PAO1-NC	*rpoN* insertion mutant	27
6206	Corneal isolate, LPS serogroup O11	Bascom-Palmer Eye Institute (Miami, Fla.)
6073	Corneal isolate, LPS serogroup O11	Bascom-Palmer Eye Institute
6077	Corneal isolate, LPS serogroup O11	Bascom-Palmer Eye Institute
6389	Corneal isolate, LPS serogroup O11	Bascom-Palmer Eye Institute
6382	Corneal isolate, LPS serogroup O11	Bascom-Palmer Eye Institute
6294	Corneal isolate, LPS serogroup O6	Bascom-Palmer Eye Institute
9882-80	Isolate from bacteremic patient, LPS serogroup O11	Brigham and Women's Hospital (Boston, Mass.)
9376-80	Isolate from bacteremic patient, LPS serogroup O11	Brigham and Women's Hospital
Rhodes	Isolate from bacteremic patient, LPS serogroup O1	Brigham and Women's Hospital

abraded cornea in a 5-μl volume dispensed from a micropipette. Mice usually regained consciousness 15 to 25 min after inoculation. In several initial experiments, the contralateral corneas were scratched and 5 μl of sterile 1% Proteose Peptone was dropped onto the abraded corneas, which served as uninfected controls. These control eyes never developed macroscopically visible signs of infection or inflammation. The use of this method of initiating infection for evaluating bacterial invasion of corneal epithelial cells during infection has previously been published (6). All procedures were carried out in accordance with the Association for Research in Vision and Ophthalmology resolution on the use of animals in research and were approved by the Harvard Medical Area Standing Committee on Animals.

Grading of corneal infection. Infected eyes were graded every 24 to 48 h after inoculation by an investigator who was unaware of the challenge strain or amount of bacteria given. The following grading scheme, which has been previously described (1), was used: grade 0, eye macroscopically identical to the uninfected contralateral control eye; grade 1, faint opacity partially covering the pupil; grade 2, dense opacity covering the pupil; grade 3, dense opacity covering the entire anterior segment; grade 4, perforation of the cornea and/or phthisis bulbi (shrinkage of the eyeball following inflammatory disease). For purposes of calculating 50% infective doses (ID_{50}s), a grade of ≥2 was used to signify infection.

Viable counts of bacteria remaining in eyes after inoculation. To determine the numbers of bacteria remaining in eyes within the first hour after inoculation, *P. aeruginosa* strain 6206, a clinical isolate from an infected human cornea, was inoculated as described above onto scratched mouse eyes at a dose of either 2×10^3 or 1.7×10^6 CFU per eye. At 15, 30, or 60 min after inoculation, five mice that had received each challenge dose were killed by an overdose of carbon dioxide; corneas were dissected from eyes by using a sterile scalpel blade to make a coronal incision at the limbus that extended to approximately the middle of the eye. Corneas were then removed by finishing the cut to the opposite limbus with sterile microdissecting scissors. Corneas were homogenized in 1 ml of sterile 1% Proteose Peptone and serially diluted, and 0.1-ml aliquots were plated on different Trypticase soy agar plates for bacterial enumeration by colony counts. For corneas that had received the low inoculum, three 0.3-ml amounts of homogenized corneas were plated directly on the agar plates.

Immune response to infection. To measure the antibody response to *P. aeruginosa* lipopolysaccharide (LPS), serum samples were collected from animals with macroscopic evidence of infection by nicking their tail veins with a sterile scalpel blade. Serum samples from mice which had received the same challenge inoculum were pooled. Enzyme-linked immunosorbent assays (ELISA) were performed with Immulon II 96-well plates coated with purified LPS (10 μg/ml in 0.04 M phosphate buffer, pH 7.0) and blocked with 5% skim milk. Serum samples were diluted 1:100 in phosphate-buffered saline containing 0.5% Tween 20 and 5% skim milk and directly added in triplicate to sensitized plates. After 2 h at 37°C, wells were washed three times, alkaline phosphatase-conjugated anti-mouse immunoglobulin G (IgG) (γ chain-specific) antibodies were added, plates were incubated at 37°C and then washed again, and the *O-para*-nitrophenol phosphate substrate was added. Color development was measured at 405 nm with an ELISA reader after 1 h of incubation at 37°C. LPSs from *P. aeruginosa* 9882-80 (serogroup O11) and Rhodes (serogroup O1) were purified as previously described (9).

Statistical analysis. The ID_{50} levels and 95% confidence intervals (CI) were calculated by Probit analysis with the Probit 3.SAS program (SAS Proprietary Software Release 6.09; SAS Institute, Cary, N.C.) on a Sun workstation or by Logit analysis with the Systat statistical program (release 5.2.1; Systat, Inc., Evanston, Ill.). For some experiments, the ID_{50} but not the 95% CI could be calculated from the available data by these methods. When a single ID_{50} result is reported for animals observed over the course of 2 to 3 weeks, the ID_{50} was calculated from the data on the day when the maximal pathologic scores were obtained (which yields the lowest or minimal ID_{50} value).

RESULTS AND DISCUSSION

Evaluation of clinical isolates. We first evaluated the abilities of six *P. aeruginosa* strains isolated from corneal infections and two strains isolated from bacteremic patients to cause disease after a dose of 10^8 CFU per eye had been administered. Seven of the eight strains tested were from serogroup O11; this serogroup antigen is expressed by about 50% of all clinical isolates of *P. aeruginosa* from corneal infections (16). Strains 6206, 6294 (serogroup O6), 6073, 6077, 6389, 6382, 9882-80, and 9376-80 initiated infections leading to infectious grades of 3 or higher by 48 h in all challenged mice. These results established that various corneal and noncorneal isolates of *P. aeruginosa* were pathogenic in this eye infection model when the modification of longer duration of anesthesia was employed. In addition, when large bacterial inocula were used in this model, strains causing significant disease (scores of >3 in all mice) were identified within 2 days.

Quantitative measurement of infectious dose. We examined the course of corneal infection over 19 days for one corneal isolate, *P. aeruginosa* 6206, by inoculating mice with doses ranging from 1×10^1 to 6.5×10^5 CFU per eye (Table 2). This strain initiated infections yielding grades of ≥2 by 5 days postchallenge in four of five mice inoculated with 7.9×10^2 CFU and in two of five mice inoculated with 9.3×10^1 CFU per eye (ID_{50} on day 5, 2.7×10^2 CFU; 95% CI, 5.5×10^1 to 1.3×10^3 CFU). In addition, the ID_{50}s calculated on day 2 and day 19 were the same (1.0×10^3 CFU per eye) and within the 95% CI of the minimal ID_{50}, which indicated that the infection was sufficiently established by 48 h to make this early time point useful for quantitative evaluations of the virulence of *P. aeruginosa* strains. A second corneal isolate, strain 6389, had a minimal ID_{50} on day 5 of 2.5×10^4 CFU per eye (95% CI, 2.5×10^3 to 1.6×10^5 CFU) in this model.

To determine whether these results were unique to C3H/HeN mice, we determined the ID_{50}s for two *P. aeruginosa* strains, 6206 and 6294, in C57BL/6 mice. The minimal ID_{50}s

TABLE 2. Course of infection in the eyes of mice challenged with *P. aeruginosa* 6206 in the modified corneal scratch model

Infecting dose (CFU)	Ocular scores on day[a]:				
	1	2	5	11	19
6.5×10^5	3, 3, 3, 3, 2	3, 3, 3, 3, 3	3, 3, 3, 3, 3	3, 3, 3, 3, 3	4, 4, 4, 4, 4
9.7×10^4	3, 3, 3, 2, 2	3, 3, 3, 3, 3	3, 3, 3, 3, 3	3, 3, 3, 2, 2	4, 4, 4, 4, 4
8.8×10^3	2, 1, 1, 1, 1	3, 3, 1, 1, 1	3, 3, 3, 2, 0	3, 2, 2, 2, 0	4, 4, 1, 1, 0
7.9×10^2	2, 2, 1, 1, 1	3, 3, 2, 2, 1	3, 3, 3, 2, 0	3, 3, 2, 2, 0	4, 4, 4, 1, 0
9.3×10^1	1, 1, 0, 0, 0	2, 1, 1, 0, 0	3, 3, 0, 0, 0	3, 2, 0, 0, 0	4, 4, 0, 0, 0
1.0×10^1	1, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0	0, 0, 0, 0, 0

[a] Ocular grading scores are defined in Materials and Methods.

were 1.1×10^4 CFU per eye for strain 6206 and 5.9×10^4 CFU per eye for strain 6294 (no 95% CI for either strain). Thus, the point estimates for the ID_{50}s for *P. aeruginosa* 6206 and 6294 were also low in C57BL/6 mice. Both C3H/HeN and C57BL/6 mice are naturally susceptible to *P. aeruginosa* corneal infection according to the genetic studies of Hazlett et al. (10).

Using C3H/HeN mice, we quantified the virulence of two clones of *P. aeruginosa* PAO1 (serogroup O5), which is commonly used in pathogenesis studies (Table 3). Strain PAO1-V, from our laboratory, had a minimal ID_{50} of 2.5×10^3 CFU per eye (95% CI, 0 to 2.5×10^4 CFU) in one experiment and in a subsequent determination a minimal ID_{50} of 5×10^2 CFU per eye, which was not significantly different from the first determination (the point estimate for the second determination was within the 95% CI of the first determination). It is interesting that another variant of strain PAO1, supplied by B. Iglewski, Rochester, N.Y. (strain PAO1-I), had a minimal ID_{50} of 2.5×10^6 CFU per eye (95% CI, 2.5×10^5 to 1.6×10^7 CFU) in C3H/HeN mice and in a repeat determination a minimal ID_{50} of 1.0×10^5 CFU per eye (no confidence intervals). Both of these values are significantly different ($P < 0.05$) from the ID_{50} of strain PAO1-V. Thus, the modified model was useful for revealing differences in the virulence of clonally related strains. A third variant of strain PAO1, strain PAO1-K, had an ID_{50} in C57BL/6 mice of 1.3×10^5 CFU per eye (95% CI, 3.7×10^4 to 4.5×10^5 CFU), a value comparable to that of strain PAO1-I. These results demonstrate again the well-established principle that phenotypic changes can occur in strains maintained and passaged in different laboratories.

Viable counts of bacteria surviving in eyes during the first hour of infection. Although the results discussed above showed that a relatively low ID_{50} level was achieved in the modified corneal scratch model, we sought to determine why even lower ID_{50} levels could not be achieved. We speculated that this effect was due to the rapid clearance of most bacteria from the corneal surface shortly after their application, which leaves only a small residual inoculum, the "effective inoculum," to infect the corneal surface. Bacterial survival on and in the cornea during the immediate postchallenge period was thus quantified after a low-level (2×10^3 CFU per eye) or high-level (1.7×10^6 CFU per eye) dose of *P. aeruginosa* 6206. Following challenge at either dose, less than 20% of bacteria could be recovered 15 min after inoculation (Fig. 1), a period when the animals remained anesthetized and thus neither moved nor blinked, which would have facilitated corneal clearance of bacteria. Sixty minutes after infection, a time during which the animals were awake and blinking for approximately 30 to 45 min, the CFU of *P. aeruginosa* remaining were further reduced to 9% of the initial inoculum in each case. The fact that similar proportions of bacteria were cleared over the first hour following both low- and high-challenge inoculation indicated that comparable volumes of liquid containing different concentrations of bacteria were rapidly cleared from corneas, with over 80% of the clearance occurring during anesthesia. Thus, an inoculum of 10 to 20% of the total bacterial count applied to each eye appears to be the minimal dose or effective inoculum of bacteria that can cause infection in this model. The fact that some mice became infected after challenge with 10^2 CFU of *P. aeruginosa*, an effective inoculum of only 10 to 20 CFU, indicates very high levels of pathogenic potential for some *P. aeruginosa* strains in this model.

Immune response to infection. Previous works have established the pattern of immune responses to *P. aeruginosa* antigens during corneal infection with strain 19660 (20, 22, 23). In this study, we sought to determine whether *P. aeruginosa*-specific serum IgG could be elicited after infection with low-level challenge doses. Previous studies demonstrated that intraperitoneal injections of 10^3 or fewer live or heat-killed *P. aeruginosa* organisms do not evoke IgG responses in mice (13, 21). We measured the levels of IgG to serogroup O11 LPS in sera from infected mice following challenge with strain 6206. We used serogroup O1 LPS as the control antigen. The results (data not shown) indicated that vigorous serum IgG antibody responses to serogroup O11 LPS were detectable in those mice which showed macroscopic signs of infection following challenge with as few as 10^2 CFU of *P. aeruginosa*, thereby supporting the contention that bacteria are capable of sufficient multiplication in eyes to evoke significant *P. aeruginosa*-specific

TABLE 3. ID_{50}s of selected wild-type and mutant strains of *P. aeruginosa*

Strain	ID_{50}(s)[a]	95% CI[b]	Day(s) of minimal ID_{50} determination
PAO1-V	2.5×10^3, 5×10^2	0–2.5×10^4	3, 9
PAO1-I	2.5×10^6, 1.0×10^5	2.5×10^5–1.6×10^7	3, 5
PAO1-K	1.3×10^5	3.7×10^4–4.5×10^5	2
PAO1 *algC*::tet	$>10^8$		ND[c]
AK1012	$>10^8$		ND[c]
PAO1-NC	$>10^8$		ND[c]
PAO1 Δ*plcHR* Δ*plcS*	1.5×10^2		7
PAO1 Δ*phoB*	6.3×10^3		5
PAO1 *fur*-C6 (A-10-G)	1.3×10^5	5.2×10^4–2.1×10^6	5

[a] Determined in C3H/HeN mice, except for those determined in C57BL/6 mice (PAO1-K, PAO1 *algC*::tet, and AK1012).

[b] 95% CIs could not be calculated for all strains with the data available.

[c] ND, not determined.

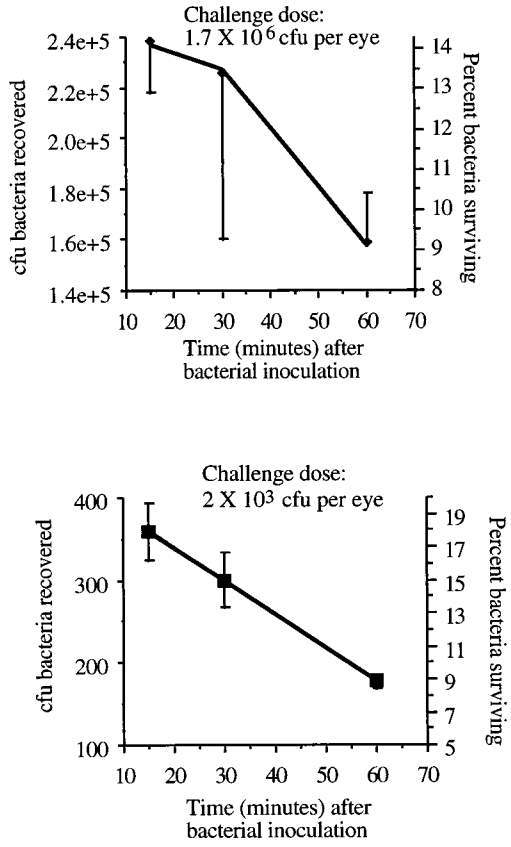

FIG. 1. Clearance of *P. aeruginosa* 6206 from the eyes of mice infected for the indicated times by using injectable anesthetics in the corneal scratch model. Each point represents the mean CFU of *P. aeruginosa* recovered from the infected eyes of five mice; error bars represent the standard errors of the means. In the upper panel, error bars are shown in only one direction for clarity.

antibody responses. Little or no IgG in sera from infected mice was reactive with serogroup O1 LPS. The specificity of antibody responses for serogroup O11 LPS indicated that the disease observed in mice infected with low-level doses of *P. aeruginosa* was indeed due to *P. aeruginosa* challenge with strain 6206.

Evaluation of virulence of selected mutant strains of *P. aeruginosa* in the modified corneal scratch model. To evaluate the utility of this model in determining changes in pathogenicity as a result of the introduction of genetic mutations in *P. aeruginosa*, we measured the virulence of various mutant derivatives of strain PAO1 (Table 3). One type of mutant had mutations in the *algC* gene involved in synthesis of a complete LPS structure. We tested two derivatives of strain PAO1-K with mutations in the *algC* gene (4). Strain PAO1-NC had a mutation in the *rpoN* gene encoding the alternative σ^{54} component of RNA polymerase (28). Mutations in either *rpoN* or *algC* were expected to severely compromise infectivity. Additional mutants of strain PAO1-V carrying mutations with less predictable effects on pathogenesis were also evaluated (Table 3). One mutant was missing the structural genes for both the hemolytic and nonhemolytic phospholipases (17, 29), a second mutant was missing the *phoB* gene involved in regulation of phosphate-responsive genes (26), and a third mutant had a point mutation in the *fur* gene that resulted in a change from the alanine at position 10 to a glycine (A-10-G) (8). The *fur* gene encodes a repressor that negatively regulates the expression of two siderophores (pyochelin and pyoverdin) and exotoxin A (24).

The *rpoN* mutant strain, PAO1-NC, and both of the LPS-deficient strains (AK1012 and PAO1 *algC*::tet) caused no response greater than a grade of 1 over 3 weeks in any mouse given a dose as high as 10^8 CFU per eye (Table 3). In contrast, both the phospholipase-deficient strain, PAO1 Δ*plcHR* Δ*plcS*, and the phosphate-regulatory mutant strain, PAO1 Δ*phoB*, were as virulent as the parental PAO1-V strain (minimal ID_{50}s of both were within the 95% CI for the parental strain), which indicates that neither the hemolytic or nonhemolytic phospholipase C nor the *phoB* regulatory gene was required for virulence in the modified corneal scratch model. Strain PAO1 *fur*-C6 (A-10-G) had a significantly increased ID_{50} over that of the parental strain ($P \leq 0.05$), which indicates some compromise in virulence due to this mutation in the *fur* gene. It was of interest that a single point mutation in this one gene in *P. aeruginosa* PAO1-V resulted in a strain whose ID_{50} was comparable to those of the PAO1-I and PAO1-K clonal variants (Table 3). Small genetic differences between otherwise identical strains could be the basis for the differences in virulence measured among parental PAO1 isolates in this model.

We have shown here that modification of the previously described corneal scratch model (1, 2, 7, 10, 11) by the use of injectable anesthetics to provide a longer period of immobility of the experimental animals results in a model that is much more amenable to the study of bacterial pathogenesis. By a different method of anesthesia, every *P. aeruginosa* strain isolated from human infections that was tested caused severe eye damage at an inoculum of 10^8 CFU, and selected strains of *P. aeruginosa* from a variety of sources had ID_{50}s in the range of 3×10^2 to 1×10^5 CFU per eye. This model can be used to quickly screen mutant strains for reductions in virulence simply by challenging animals initially with a high-level dose (10^8 CFU per eye) of bacteria to determine whether virulence has been affected. Strains that were highly compromised in virulence, such as the *rpoN* and LPS mutants tested here, caused no observable corneal damage at a dose of 10^8 CFU per eye. Those strains that were still virulent according to high-dose testing were further tested with a range of doses to determine the ID_{50}. We found that neither the hemolytic nor nonhemolytic phospholipase C played a prominent role in this eye infection model. Nor did it appear that a *P. aeruginosa* gene involved in responding to phosphate levels (*phoB*) made a significant contribution to virulence in this model. The Ala-10→Gly mutation in the PAO1 *fur*-C6 mutant results in derepression of siderophore and exotoxin A production in iron-sufficient environments (8, 24). It might therefore seem counterintuitive that the C6 mutant is less virulent than the parental strain, which expresses exotoxin A only in environments limited in iron. However, the Fur protein has more global effects on gene regulation, and it is clear that *fur* mutants are considerably more susceptible to oxidative stress (e.g., H_2O_2) than are the corresponding wild-type strains (8).

We also found that minimal or near minimal ID_{50} levels were achieved by 2 to 5 days after infection, which makes the model useful for rapid evaluation of pathogenicity. The corneal infection model can also be used to determine the steps in the pathogenesis of eye infections. Indeed, we have already shown by using injectable anesthetics in the corneal scratch model that *P. aeruginosa* invades corneal epithelial cells (6), which suggests a role in pathogenesis for this interaction between bacteria and the host.

The corneal scratch model closely parallels events thought to occur during wound infections of human eyes, from which *P. aeruginosa* is among the most commonly isolated pathogens (3,

14, 30). The scratch model may also be representative of ulcerative keratitis associated with contact lens wear. In both experimental animal infections and human cases, *P. aeruginosa* is able to rapidly establish an infectious process that can lead to loss of vision and/or the entire eye if proper treatment is not instituted (14). The need for effective therapies of human infections to help save visual acuity in infected eyes is pressing, and the modified mouse corneal infection model should be useful for evaluating pharmacologic interventions.

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