Pseudomonas aeruginosa las and *rhl* quorum-sensing systems are important for infection and inflammation in a rat prostatitis model

Lisa K. Nelson, Genevieve H. D'Amours, Kimberley M. Sproule-Willoughby, Douglas W. Morck and Howard Ceri

Correspondence
Howard Ceri
ceri@ucalgary.ca

Biofilm Research Group, Department of Biological Sciences, University of Calgary, 2500 University Drive NW, Calgary, AB T2N 1N4, Canada

Pseudomonas aeruginosa frequently acts as an opportunistic pathogen of mucosal surfaces; yet, despite causing aggressive prostatitis in some men, its role as a pathogen in the prostate has not been investigated. Consequently, we developed a *Ps. aeruginosa* infection model in the rat prostate by instilling wild-type (WT) *Ps. aeruginosa* strain PAO1 into the rat prostate. It was found that *Ps. aeruginosa* produced acute and chronic infections in this mucosal tissue as determined by bacterial colonization, gross morphology, tissue damage and inflammatory markers. WT strain PAO1 and its isogenic mutant PAO-JP2, in which both the *lasI* and *rhlI* quorum-sensing signal systems have been silenced, were compared during both acute and chronic prostate infections. In acute infections, bacterial numbers and inflammatory markers were comparable between WT PAO1 and PAO-JP2; however, considerably less tissue damage occurred in infections with PAO-JP2. Chronic infections with PAO-JP2 resulted in reduced bacterial colonization, tissue damage and inflammation as compared to WT PAO1 infections. Therefore, the quorum-sensing *lasI* and *rhlI* genes in *Ps. aeruginosa* affect acute prostate infections, but play a considerably more important role in maintaining chronic infections. We have thus developed a highly reproducible model for the study of *Ps. aeruginosa* virulence in the prostate.

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INTRODUCTION

Pseudomonas aeruginosa is a frequent opportunistic pathogen of mucosal surfaces, causing infection and inflammation in the urogenital, gastrointestinal and respiratory tracts. The role of *Ps. aeruginosa* in respiratory and gastrointestinal infections has received considerable attention. However, the contribution of this bacterium to urogenital infections is a topic that is often scarce in the literature. For instance, *Ps. aeruginosa* has been isolated as an aetiological cause of prostatitis in humans, but experimental data concerning *Ps. aeruginosa*-induced prostatitis has yet to be reported (Nickel & Costerton, 1993; Skerk *et al.*, 2004).

Prostatitis is the most common urological complaint in men younger than 50 and, by some estimates, 50 % of men will experience symptoms of prostatitis in their lifetime. The aetiology of prostatitis remains confusing. While 5–10 % of prostatitis cases are believed to have a bacterial origin, some accumulating evidence suggests that this may represent a gross underestimation (Nickel & Moon, 2005; Nickel, 2006). To better understand the initiating factors of

prostatitis, our lab has employed models of bacterial and non-bacterial prostatitis in rats (Ceri *et al.*, 1999b; Lang *et al.*, 2000; Nickel *et al.*, 1990). Using the bacterial prostatitis model, we determined that prostate disease is affected by the virulence factors of *Escherichia coli* and *Proteus mirabilis*, which are the major bacterial contributors to prostatitis (Phan *et al.*, 2008; Rippere-Lampe *et al.*, 2001). *Ps. aeruginosa* is an infrequent cause of bacterial prostatitis (ranges from 0.3 to 3 % of cases) (Skerk *et al.*, 2004; Weidner *et al.*, 1991). Nevertheless, the virulence of *Ps. aeruginosa* in the prostate deserves attention due to the fact that clinical reports indicate that *Ps. aeruginosa* prostatitis is difficult to treat and exceptionally damaging to prostate tissue (Nickel & Costerton, 1993; Wagenlehner & Naber, 2006).

The observation that *Ps. aeruginosa* is damaging to prostate tissue may be due to its sizeable genome and large repertoire of virulence factors. Epidemiological evidence has shown that bacteria require a large collection of virulence factors to establish prostate infections as compared to other urological infections such as cystitis and pyelonephritis (Ruiz *et al.*, 2002). Furthermore, prostatitis isolates tend to be better biofilm formers than either cystitis or pyelonephritis isolates (Kanamaru *et al.*, 2006). As such, quorum sensing, which regulates biofilm

Abbreviations: AHL, *N*-acylhomoserine lactone; H&E, haematoxylin and eosin; MN, mononuclear; MPO, myeloperoxidase; p.i., post-infection; PMN, polymorphonuclear; WT, wild-type.

formation and the expression of numerous *Ps. aeruginosa* virulence genes (Juhas *et al.*, 2005), may play an important role in the ability to cause damage to prostate tissue. In support of this idea, it has previously been shown that *Ps. aeruginosa* virulence factors regulated by quorum sensing correlate with disease severity in lung tissue (Jaffar-Bandjee *et al.*, 1995).

There are two quorum-sensing systems in *Ps. aeruginosa* – *las* and *rhl* – which utilize *N*-acylhomoserine lactones (AHLs) as signalling molecules. AHLs, synthesized by bacterial cells, diffuse in and out of cells, binding a transcriptional regulator once a threshold concentration of bacteria is reached. In the *las* system, LasI synthesizes the AHL signal, *N*-3-oxo-dodecanoylhomoserine lactone, and LasR acts as the transcriptional regulator (Gambello & Iglewski, 1991; Passador *et al.*, 1993; Pearson *et al.*, 1994). For the *rhl* system, RhII and RhIR are the synthase and transcriptional regulator, respectively, for the AHL signal, *N*-butyrylhomoserine lactone (Ochsner *et al.*, 1994; Ochsner & Reiser, 1995; Pearson *et al.*, 1995). Binding of the transcriptional regulators in both the *las* and *rhl* quorum-sensing systems induces or represses the expression of many genes, including virulence factors.

The importance of quorum sensing to *Ps. aeruginosa* virulence has been shown in numerous model systems. These include the *Caenorhabditis elegans* model (Tan *et al.*, 1999), the acute pneumonia model (Pearson *et al.*, 2000), and the chronic pneumonia agar bead model (Wu *et al.*, 2001). Since the absence of quorum sensing reduced virulence in all models, including the lung model, we believed that quorum sensing may likewise affect the severity of prostate disease. Consequently, we hypothesized that quorum sensing contributes to inflammation and prostate tissue damage in *Ps. aeruginosa*-induced prostatitis. In a test of this hypothesis, we used our previously established bacterial prostatitis model (Ceri *et al.*, 1999b; Nickel *et al.*, 1990) to induce both acute and chronic infections in the rat prostate with wild-type (WT) *Ps. aeruginosa* strains PAO1 and PA14. In addition, to assess the role of quorum sensing, we used the *Ps. aeruginosa* PAO1 *lasI rhlI* double mutant PAO-JP2 (Pearson *et al.*, 1997), utilized in previous studies (Wu *et al.*, 2001), to infect the rat prostate. The resulting inflammatory response and tissue damage were compared between PAO-JP2 and its isogenic WT PAO1 parent at 2, 8 and 12 days post-infection (p.i.) to represent both acute and chronic infections.

METHODS

Bacteria, media and culture conditions. In this study two well-defined WT laboratory strains of *Ps. aeruginosa*, PA14 and PAO1, as well as the PAO1 isogenic *lasI rhlI* double mutant PAO-JP2 (Pearson *et al.*, 1997), were utilized. All strains were stored at -70°C in Microbank vials (Pro-Lab Diagnostics) according to the manufacturer's instructions. Miller Luria–Bertani agar or Pseudomonas isolation agar (LB and PIA, respectively; Difco) were used to culture

the *Ps. aeruginosa* strains. Second subcultures grown on LB agar were used to establish a culture of 1×10^6 cells ml^{-1} in saline, as determined by McFarland standards, to use as an inoculum for the animal model. Bacterial numbers were later reconfirmed by plate counts on both LB and PIA agar.

Animal model. The rat model of prostatitis described previously (Ceri *et al.*, 1999a, b; Lang *et al.*, 2000; Nickel *et al.*, 1990; Phan *et al.*, 2008) was employed in this study. In brief, male Sprague–Dawley rats of approximately 300 g were obtained from the Life and Environmental Sciences Animal Resource Centre at the University of Calgary. The rats were maintained in polycarbonate box cages on aspen chip bedding and housed at $20 \pm 2^{\circ}\text{C}$, $40 \pm 10\%$ relative humidity with 12 h of daily illumination. Animals were provided rat chow and water *ad libitum*. Rats were anaesthetized with 4% halothane prior to transurethral catheterization with a lubricated sterile PE10 polyethylene feeding tube. A volume of 0.2 ml of bacterial suspension, prepared as above, was injected at the base of the prostate. *Ps. aeruginosa* strain PAO1 or PAO-JP2 was instilled into eight rats per experimental time point (2, 8 and 12 days; $n=48$). *Ps. aeruginosa* strain PA14 was also instilled into six rats per experimental time point (2, 8 and 12 days; $n=18$) (data not shown). Control rats were catheterized in the same manner, but injected with 0.2 ml sterile saline. For the acute infection model, animals were asphyxiated with CO_2 on day 2 after infection; animals with chronic infections were sacrificed by the same method at day 8 or 12 p.i. Approval for this study was granted by The Life and Environmental Sciences Animal Care Committee in accordance with guidelines of the Canadian Council of Animal Care.

Tissue preparation. The ventral prostate was aseptically removed from the animal, photographed and weighed. The tissue was divided into four pieces: one was homogenized in sterile saline to determine bacterial counts per mg tissue, one fixed in 10% neutral-buffered formalin for histological processing, one stored on ice for immediate myeloperoxidase (MPO) activity determinations, and one immediately frozen and stored at -70°C for later cytokine analysis.

Histological sections. The prostate tissue was fixed as above and embedded in paraffin. Sections of 5 μm thickness were stained with standard haematoxylin and eosin (H&E) protocols. Sections were scored blindly according to the criteria established by a veterinary anatomical pathologist. Acute sections (2 days) were scored as follows: grade 0, a normal appearance; grade 1, oedema and infiltrates of polymorphonuclear (PMN) leukocytes in the prostate stroma; grade 2, PMN leukocytes in the prostate acini; grade 3, loss of epithelial and/or basement membrane architecture in the prostate acini; grade 4, haemorrhage; and grade 5, tissue necrosis. Chronic sections (8 and 12 days) were graded as microscopically showing: grade 0, a normal appearance; grade 1, oedema and PMN or mononuclear (MN) leukocytes in prostate stroma; grade 2, PMN or MN leukocytes in the prostate acini; grade 3, loss of epithelial and/or basement membrane architecture in the prostate acini; grade 4, necrosis accompanied by PMN and MN leukocytes and/or fibrosis; grade 5, necrosis with no evidence of tissue repair and absence of PMN and MN leukocytes.

MPO assay. Prostate tissue was homogenized in hexadecyltrimethyl ammonium bromide (HTAB) buffer. The homogenates were then centrifuged at 13 000 g for 2.5 min and the supernatants mixed with *o*-dianisidine in phosphate buffer and assayed at 450 nm. The activity was recorded per mg protein (Mullane *et al.*, 1985).

Cytokine (IL-1 β and Gro/CINC-1) assays. Tissue to be screened for cytokine levels was homogenized in PBS containing 2 μl protease inhibitor cocktail (Sigma) per ml of homogenate. The homogenates were centrifuged at 1000 g for 10 min and supernatants used for

assays. Assays were performed using commercial ELISA kits for rat IL-1 β (eBiosciences) and rat Gro/CINC-1 (R&D Systems) according to the manufacturers' instructions.

Statistical analysis. Group data are expressed as means $\pm$ SEM. Figures and statistical analyses were compiled using GraphPad Prism v4.02 software (GraphPad Software). Bacterial counts and MPO readings were log₁₀-transformed. Following this, bacterial counts, MPO assays and cytokine ELISAs were analysed by one-way ANOVA and Tukey's multiple comparison test. *P*-values <0.05 were considered statistically significant.

RESULTS

Bacterial numbers

In order to establish the rat prostate as a model for *Ps. aeruginosa* infections and to examine how quorum sensing affects *Ps. aeruginosa*'s ability to infect the prostate, *Ps. aeruginosa* strains WT PA14 (data not shown), WT PAO1 (PAO1) and an isogenic quorum-sensing *lasI rhII* double mutant (PAO-JP2) were used to establish acute and chronic infections in the rat prostate. Infections were defined as acute at 2 days and chronic after 8 days infection based on morphological paradigms of acute and chronic prostatitis in man (Ceri *et al.*, 1999a), which are recapitulated in the rat model of prostatitis at these time points (Ceri *et al.*, 1999b; Lang *et al.*, 2000; Nickel *et al.*, 1990; Phan *et al.*, 2008; Rippere-Lampe *et al.*, 2001). In accordance with a previous study (Phan *et al.*, 2008), a 12 day chronic time point was added to look for the possibility of healing in cases where the loss of virulence factors resulted in a reduced potential to move on to a chronic infection.

Ps. aeruginosa PAO1 was able to initiate an infection in the rat ventral prostate and to sustain consistent bacterial numbers over 12 days infection (Fig. 1a). Bacteria were confined to the prostate and no spread of *Ps. aeruginosa* to the spleen, kidneys, liver or bladder was seen at day 2 or day 8 p.i. (data not shown). Comparing the infectious ability of WT *Ps. aeruginosa* to its isogenic quorum-sensing-deficient strain, bacterial counts in prostate tissue revealed that PAO1 and PAO-JP2 differed only at the chronic stage of infection. No statistical differences between PAO1 and PAO-JP2 were detected at 2 days p.i. (Fig. 1a). However, at both 8 and 12 days p.i., significantly fewer bacteria were recovered from the prostate in the PAO-JP2 group than the PAO1 group ($P<0.05$ and $P<0.01$, respectively). In addition, while no statistical differences were found between the number of bacteria recovered from the prostate 2, 8, or 12 days p.i. in the PAO1 group, bacterial numbers at 12 days p.i. were significantly lower than bacterial numbers recovered at 2 days p.i. in the PAO-JP2 group ($P<0.05$). This indicates that the quorum-sensing-deficient PAO-JP2 bacteria, but not the PAO1 WT bacteria, were cleared from the prostate by the host immune system.

Fig. 1. Severity of prostate infections in *Ps. aeruginosa*-infected rats. Prostates were infected with sterile saline (●), PAO1 (■), or PAO-JP2 (▲) for 2, 8 or 12 days as described in Methods. (a) Bacterial numbers in prostate tissue. (b) Weight of prostates. ** $P<0.01$, *** $P<0.001$ for PAO1 or PAO-JP2 vs control; # $P<0.05$, ## $P<0.01$, ### $P<0.001$ for PAO1 vs PAO-JP2.

Gross morphology

Prostate weight was determined as a quantifiable measure of oedema. At all time points, PAO1- and PAO-JP2-infected prostates weighed more than controls, but the difference in weight was only statistically significant for chronic PAO1 infections (Fig. 1b). This is because prostate weight in PAO1 infections increased with time; prostate weight at 8 and 12 days p.i. was statistically higher than 2 days p.i. ($P<0.01$ and $P<0.001$ respectively, Fig. 1b). Conversely, PAO-JP2 never reached significant differences in prostate weight from control values and at day 12 p.i. prostate weight in the PAO-JP2 group was lower than the PAO1 group by approximately threefold ($P<0.001$, Fig. 1b).

Histology

Prostate sections from saline controls and acute and chronic PAO1 infections were examined by H&E staining to assess tissue damage resulting from WT *Ps. aeruginosa* infections. The prostate gland contains numerous acini (sac-like ducts, Fig. 2a) within stromal tissue. Normally, the acini and stroma are clear of leukocytes and there is little stromal tissue between acini, as seen in the saline-

Fig. 2. Histology of prostate infections by *Ps. aeruginosa*. Sections stained as described in Methods showing typical appearance of (a) saline controls, (b) PAO1 (the asterisk indicates area displaying haemorrhage) and (c) PAO-JP2-infected prostate tissue in acute infections. Histology of chronic infections is seen in (d) saline controls, (e) PAO1-infected tissue (arrow indicates sites of necrosis) and (f) PAO-JP2-infected tissue. Magnification $\times 180$.

injected controls (Fig. 2a, d). In tissue sections from both acute and chronic PAO1 infections the acini and the stroma were filled with leukocytes and the stroma was oedematous (Fig. 2b, e). Haemorrhage was also observed in acute PAO1 sections (Fig. 2b, see asterisk). Loss of tissue integrity in the epithelium and basement membrane of the prostate acini was particularly evident in chronic sections, with some areas of the chronic sections displaying clear signs of necrosis (Fig. 2e, arrow). The histopathological damage of all tissue sections was assessed and graded based on the grading scheme described in Methods. This grading scale was different from that used to assess prostate tissue sections from *E. coli* and *Pr. mirabilis* infections in our previous studies (Phan *et al.*, 2008; Rippere-Lampe *et al.*, 2001). The change of scale was a reflection of the fact that prostate disease with *Ps. aeruginosa* follows a markedly more severe course than that of *E. coli* or *Pr. mirabilis*. Consequently, a veterinary pathologist aided in a new scale design and blinded tissue grading for PAO1- and PAO-JP2-infected prostates. Grades were given on a scale of 0–5. A grade of 3 was considered moderate inflammation, whereas a grade below 3 was considered mild inflammation and a grade above 3 was categorized as severe inflammation. None of the control rats scored in the moderate to severe range of inflammation, whereas the percentage of PAO1-infected rats scoring in the moderate to severe range was 83 %, 100 % and 80 %, respectively, for 2, 8 and 12 days p.i. (Fig. 3). Quorum-sensing mutations in *Ps.*

aeruginosa resulted in reduced tissue damage as compared to WT during acute and chronic infections. In acute infections, haemorrhage was present in nearly all PAO1-infected prostates, while virtually absent in PAO-JP2-infected prostates (Fig. 2b, asterisk, and Fig. 2c). Furthermore, fewer infiltrating PMNs and fewer changes to acinar architecture were observed in acute PAO-JP2 sections. During 8 and 12 day chronic infections, necrosis was primarily observed in PAO1-infected prostates (Fig. 2e, see arrow); in the rare case where necrosis was observed in PAO-JP2-infected prostates, it was accompanied by evidence of tissue healing. Some PAO-JP2 sections were characterized by moderate inflammation, but by day 12 many sections showed mild inflammation with only a few PMNs or MNs in the stromal tissue (Fig. 2f). As such, the percentage of PAO1 rats scoring in the moderate to severe range of inflammation was greater than the percentage of PAO-JP2 rats at all time points (Fig. 3).

MPO

Levels of MPO (an indicator of host response and inflammation; Mullane *et al.*, 1985) were compared between PAO1 and PAO-JP2 infections. MPO levels were significantly higher at 2, 8 and 12 days p.i. in the PAO1 group compared to the control group (Fig. 4a). This indicated that *Ps. aeruginosa* colonization of the prostate was able to induce host inflammatory responses. The

Fig. 3. Percentage of PAO1-, PAO-JP2- and saline-infected rats that displayed moderate to severe inflammation (grades 3, 4 and 5) at 2, 8 and 12 days. Prostate sections were processed, stained and graded as described in Methods. (a) Acute infections. (b) Chronic infections.

absence of quorum sensing in *Ps. aeruginosa* PAO-JP2 led to decreased inflammation in the host during chronic infections, but not in acute infections (Fig. 4a). In the PAO1 group, MPO levels remained relatively constant across all time points assayed (Fig. 4a). In contrast, during PAO-JP2 infections MPO levels dropped with time, decreasing by nearly 1 log from 2 days to 12 days p.i. Due to this decline, MPO levels differed significantly between PAO-JP2 and PAO1 at 12 days p.i. ($P < 0.05$).

Cytokine assays

While a comprehensive screen of cytokines and chemokines was not undertaken in this study, we chose two well-established markers of inflammation, IL-1 β and Gro/CINC-1 (the rat equivalent of human chemokine IL-8), to gauge inflammation. Both of these inflammatory markers have been shown to be elevated during prostate infections (Phan *et al.*, 2008). Based on these markers, and in agreement with the MPO assay, WT *Ps. aeruginosa* induced inflammation in the prostate. Compared to the control, a significant increase in IL-1 β was observed 2 days p.i. with PAO1 (Fig. 4b). Gro/CINC-1 similarly increased over the control in the PAO1 group at 2 days p.i., but unlike IL-1 β , Gro/CINC-1 was also important early in chronic infections as it was statistically higher than the control at 8 days p.i. (Fig. 4b, c). Comparing the quorum-sensing mutant to WT, levels of IL-1 β did not differ significantly between PAO1 and PAO-JP2 at any time point assessed (Fig. 4b). Gro/CINC-1 levels were not statistically different between PAO1 and PAO-JP2 at 2 and 8 days p.i., but by 12 days p.i., Gro/CINC-1 levels in PAO1-infected rats were significantly higher than in PAO-JP2-infected rats (Fig. 4c). In addition, the decline in Gro/CINC-1 levels was more pronounced in the PAO-JP2 group than the PAO1 group. When comparing Gro/CINC-1 levels at 2, 8 and 12 days p.i., a significant decrease was only observed

between 2 and 12 days p.i. in PAO1-infected rats ($P < 0.05$). In contrast, in PAO-JP2-infected rats, Gro/CINC-1 levels at 8 days had already decreased significantly from levels measured at 2 days ($P < 0.01$); at 12 days, the decrease from 2 days was even more evident ($P < 0.001$) (Fig. 4c).

DISCUSSION

Our results detail the virulence capability of *Ps. aeruginosa* in the prostate; to our knowledge, this is the first report of its kind. In this study, we employed our previously established bacterial prostatitis model (Ceri *et al.*, 1999b; Nickel *et al.*, 1990; Phan *et al.*, 2008; Rippere-Lampe *et al.*, 2001) to study *Ps. aeruginosa* infections. We were able to successfully establish acute and chronic infections in the rat prostate with WT *Ps. aeruginosa*. WT strain PAO1 resulted in significantly greater bacterial colonization, more severe histological changes and significantly greater inflammation than saline-injected controls (Figs 1–4). This would appear to be typical for laboratory strains of *Ps. aeruginosa*, as the WT PA14 strain produced very similar results in this model (data not shown). Importantly, characteristic hallmarks of inflammation in the prostate (Ceri *et al.*, 1999a) were present during infections by both WT *Ps. aeruginosa* strains.

The role of quorum sensing in *Ps. aeruginosa* during acute and chronic prostatitis was also ascertained in this study. It was determined that at the acute stage of infection, 2 days p.i., bacterial numbers and prostate weight were comparable between PAO1 and PAO-JP2 (Fig. 1). These results suggested that during acute infections, bacterial clearance and prostate oedema did not differ significantly between WT and quorum-sensing-deficient strains of *Ps. aeruginosa*. However, upon examination of tissue histology, it became apparent that PAO1 acute infections took a more severe course than PAO-JP2. While inflammation was

Fig. 4. Host inflammatory response to prostate infections by *Ps. aeruginosa*. Prostates were infected with sterile saline (●), PAO1 (■) or PAO-JP2 (▲) for 2, 8 or 12 days as described in Methods. (a) MPO, (b) IL-1β, and (c) Gro/CINC-1 levels in homogenized prostate tissue. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ for PAO1 or PAO-JP2 vs control; # $P < 0.05$, ## $P < 0.01$ for PAO1 vs PAO-JP2.

evident in tissues from both PAO1- and PAO-JP2-infected rats, tissue damage and haemorrhage was seen almost exclusively in the PAO1 group, resulting in a more severe grade of histopathology (Figs 2a–c, 3). Despite this, the inflammatory response in the host remained comparable between PAO1 and PAO-JP2 (Fig. 4). Therefore, our prostate model determined that quorum sensing in acute *Ps. aeruginosa* infections affects the level of tissue damage, but not the level of bacterial colonization or inflammation.

We have previously shown that acute prostate infections are more drastically affected by virulence factors of *E. coli* and *Pr. mirabilis*. The absence of the ZapA virulence factor in *Pr. mirabilis* resulted in less severe histological changes and significantly reduced bacterial colonization and host

inflammation (Phan *et al.*, 2008). Mutations of cytotoxic necrotizing factor 1 (CNF1) in *E. coli* also reduced histological damages (Rippere-Lampe *et al.*, 2001) and host inflammation, although the reduction in inflammation was not statistically significant (M. Lang, A. D. O'Brien & H. Ceri, unpublished data). Therefore, in comparison to virulence factors in other uropathogens, quorum sensing in *Ps. aeruginosa* does not appear to be as important for establishing acute infections.

Differences in bacterial clearance and inflammation between PAO1 and PAO-JP2 were more pronounced at the chronic stage of infection, i.e. at 8 and 12 days p.i. As compared to its WT counterpart, strain PAO-JP2 was markedly less able to maintain itself within the prostate and produce chronic prostatitis. In chronic infections, bacteria persisted at levels close to the acute disease in prostates infected with PAO1 (Fig. 1a). Conversely, bacteria began to clear in prostates infected with PAO-JP2. With the decline of colonizing bacteria in PAO-JP2, oedema subsided; however, oedema remained elevated in PAO1-infected rats (Fig. 1b). In line with this trend, the severity of tissue damage persisted in PAO1 chronic infections, whereas PAO-JP2-infected tissue showed less damage and signs of healing (Figs 2d–f, 3). Inflammatory markers (MPO, IL-1β and Gro/CINC-1) also remained high in PAO1-infected rats, but not in PAO-JP2-infected rats (Fig. 4). Based on these results, we can conclude that quorum sensing is important for chronic infections; when *Ps. aeruginosa* lacked the *lasI* and *rhlI* quorum-sensing systems, bacteria were cleared, inflammation subsided and the tissue began to heal.

Notably, our results on chronic WT *Ps. aeruginosa* infections in the rat prostate are supportive of the human experience with this disease. That is, our observations confirm clinical findings that *Ps. aeruginosa* is particularly damaging to prostate tissue (Wagenlehner & Naber, 2006). Indeed, in this study, a new tissue-grading scale was developed to account for the increased damage observed in *Ps. aeruginosa* tissues as compared to the tissue histology observed in our previous work with *E. coli* and *Pr. mirabilis* (Phan *et al.*, 2008; Rippere-Lampe *et al.*, 2001). Prostate necrosis and changes in acini architecture were observed much more frequently in tissue sections from chronic *Ps. aeruginosa* infections than infections with *E. coli* or *Pr. mirabilis*. Also, prostate acini were observed to shrink during *E. coli* and *Pr. mirabilis* infections, but in chronic *Ps. aeruginosa* infections, the acini remained enlarged and filled with PMNs (Fig. 2e). Nevertheless, while the histological presentation of *Ps. aeruginosa* differed from that of *E. coli* between day 2 and day 8 p.i., it is important to note that hallmarks of acute and chronic prostate disease remained (Ceri *et al.*, 1999a). Interestingly, our results revealed additional differences between *Ps. aeruginosa* and other uropathogens during chronic infection, which have not previously been reported in clinical findings. As with typical chronic prostate infections in humans, the weight of the rat prostate decreased during chronic *E. coli* and *Pr.*

mirabilis infections (Ceri *et al.*, 1999a; Phan *et al.*, 2008; Rippere-Lampe *et al.*, 2001). In this study, prostate weight continually increased during chronic *Ps. aeruginosa* infections (Fig. 1b), indicating sustained oedema. Together, these observations imply that, compared to the more common *E. coli* and *Pr. mirabilis* prostate infections, chronic infections with *Ps. aeruginosa* follow a different trajectory, one which perhaps involves a more persistent and rigorous inflammatory response.

The persistent inflammatory response and damage to prostate tissue occurring in *Ps. aeruginosa* prostate infections is likely attributable to the virulence factors regulated by quorum sensing. Evidence of *in vivo* secretion of three virulence factors regulated by quorum sensing – exotoxin A, elastase and alkaline protease – has previously been shown (Jaffar-Bandjee *et al.*, 1995). Moreover, three separate studies have shown that the level of these virulence factors correlates with disease severity in mucosal tissues (Grimwood *et al.*, 1993; Jaffar-Bandjee *et al.*, 1995; Storey *et al.*, 1991). These quorum-sensing-controlled enzymes, therefore, likely contribute to the pathogenesis of *Ps. aeruginosa*. Supporting this, it has been shown that chronic infections in the lungs of rats with *Ps. aeruginosa* mutants defective for either exotoxin A or elastase result in reduced epithelial damage to the lungs (Woods *et al.*, 1982). Thus, taken together, our data support the importance of quorum sensing in the regulation of virulence *in vivo* and further demonstrate the robustness of our model for the study of prostatitis and infection and inflammation at mucosal surfaces in general.

In addition to detailing *Ps. aeruginosa*-induced prostatitis, our study is distinct because chronic *Ps. aeruginosa* infections are rarely studied in mucosal models without having to resort to foreign bodies. Previous reports on the role of quorum sensing in *Ps. aeruginosa* infections have been generated in lung models where *Ps. aeruginosa* is implanted in agar beads in order to establish chronic infections (Wu *et al.*, 2001). Foreign-body models in the lung have produced reproducible pathology-mimicking mucosal disease (Cash *et al.*, 1979; Gallant *et al.*, 2000; Lindsey *et al.*, 2008; Pedersen *et al.*, 1990); however, there have been a number of reports identifying problems with foreign-body models, including bypassing initial *Ps. aeruginosa* colonization and tissue injury (Pedersen *et al.*, 1990; Starke *et al.*, 1987), technical difficulties (Starke *et al.*, 1987) and foreign-body inflammation (Thomassen *et al.*, 1984). Therefore, our study comments on quorum sensing from the unique perspective of a mucosal model system that does not require a foreign body. Under these conditions, we determined that quorum sensing affects the severity of *Ps. aeruginosa* prostate infections, particularly in the chronic phase. Thus, in all, we have developed a highly reproducible model system of chronic *Ps. aeruginosa* infections that can be used to elucidate the roles of *Ps. aeruginosa* virulence factors at mucosal surfaces.

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