Role of *Pseudomonas aeruginosa* quorum-sensing systems in a mouse model of chronic respiratory infection

Yoshifumi Imamura,† Katsunori Yanagihara,† Kazunori Tomono, Hideaki Ohno, Yasuhiro Hagishiyama, Yoshitsugu Miyazaki, Yoichi Hirakata, Yohei Mizuta, Jun-ichi Kadota, Kazuhiro Tsukamoto and Shigeru Kohno

Second Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8501, Japan

The role of quorum-sensing systems in a mouse model of chronic *Pseudomonas aeruginosa* infection was studied. A chronic *P. aeruginosa* respiratory infection model was established by placement of a tube pre-coated with strain PAO1 (wild-type) or a quorum-sensing mutant, namely PAO-JP1 (ΔlasI), PDO100 (ΔrhlI) or PAO-JP2 (ΔlasI/ΔrhlI), in the bronchus. At day 14 after infection, the numbers of viable bacteria in the quorum-sensing-mutant groups were lower than in the wild-type group. Histopathological examination showed milder inflammatory changes in the lungs infected with the mutant groups compared with the wild-type group. In the bronchoalveolar lavage fluid from the quorum-sensing-system-mutant groups the proportion of neutrophils was lower than in wild-type group. These findings indicate that the quorum-sensing system plays an important role in chronic *P. aeruginosa* respiratory infection.

**INTRODUCTION**

*Pseudomonas aeruginosa* is a major pathogen known to cause chronic respiratory infection in patients with morbidities such as cystic fibrosis, bronchiectasis and diffuse panbronchiolitis. *P. aeruginosa* controls its gene expression in response to cell density by a quorum-sensing (QS) system (de Kievit & Iglewski, 2000) and through this produces many extracellular virulence factors to promote survival in various environments such as the human respiratory tract.

*P. aeruginosa* has two major QS systems, *las* (Gambello et al., 1993) and *rhl* (Ochsner & Reiser, 1995). The *las* system is controlled by two regulatory proteins, LasI and LasR. LasI is the autoinducer synthase, which is responsible for the synthesis of N–(3-oxododecanoyl) homoserine lactone (3O-C12-HSL), while LasR is a transcriptional activator protein (Gambello & Iglewski, 1991; Pearson et al., 1994). In a similar fashion, the *rhl* QS system is controlled by the regulatory proteins RhlI and RhlR; RhlI is the autoinducer synthase, responsible for the synthesis of N-butyryl-homoserine lactone (C4-HSL), and RhlR is the transcriptional activator protein (Pearson et al., 1995). The *las* and *rhl* systems are linked to each other, with the *las* system dominant over the *rhl* system in the QS hierarchy (Latifi et al., 1996; Pesci et al., 1997).

The role of the QS system *in vivo* has been studied in several animal models. *P. aeruginosa* strains lacking QS genes have a reduced ability to cause acute pneumonia, bacteraemia and mortality in neonatal mouse models of acute pulmonary infection (Pearson et al., 2000; Tang et al., 1996). The QS system also operates in a chronic lung infection model, as a lasI/rhlI double mutant was unable to establish chronic infection in a rat model (Wu et al., 2001). However, whether this attenuation occurs in other animal models or with other QS-mutant strains remains unknown. In the present study, we investigated the role of the QS system in a murine model of chronic *P. aeruginosa* respiratory infection by using single *lasI* and *rhlI* mutants and a *lasI/rhlI* double mutant.

**METHODS**

**Laboratory animals.** Eight-week-old, male, C57BL/6, specific pathogen-free mice were purchased from Charles River (Yokohama, Japan). All animals were housed in a pathogen-free environment and received sterile food and water in the Laboratory Animal Center for Biomedical Science at Nagasaki University. The experimental protocol was approved by the Ethics Review Committee for Animal Experimentation at our institution.

**Bacterial strains and culture conditions.** Four *P. aeruginosa* strains, PAO1 (wild-type), PAO-JP1 (ΔlasI), PAO-JP2 (ΔlasI/ΔrhlI) and

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†These authors contributed equally to this work.

Abbreviations: BALF, bronchoalveolar lavage fluid; QS, quorum sensing.
PDO100 (ΔrhlI), were kindly provided by Professor Iglewski (University of Rochester School of Medicine and Dentistry, Rochester, NY). For each experiment, strains were plated from 10% skimmed milk (Yukijirushi) stocks maintained at −70 °C. All cultures were grown at 37 °C, and liquid cultures were shaken at 250 r.p.m.

**Murine model of chronic P. aeruginosa respiratory infection.** We used a modified mouse model of chronic respiratory P. aeruginosa infection (Yanagihara et al., 1997). Disposable sterile plastic cut-down intravenous catheters (3 Fr, 1.0 mm diameter; Atom Co.) were used for tracheal intubation. The tubes were 3-0 mm long, with slits made at the proximal end to prevent blockage by oral secretions. Bacteria were grown from frozen stocks overnight on trypticase soy agar and a swab of bacteria was resuspended in trypticase soy broth to an optical density of 0·1 at 660 nm and grown at 37 °C with shaking to an optical density of 0·5 at 660 nm. Bacteria were pelleted and resuspended in PBS, and dilutions were adjusted to 2×10⁷ c.f.u. ml⁻¹.

The intubation procedure was performed under pentobarbital anaesthesia. The blunt end of the inner needle of an intravenous catheter (Angiocath, Becton Dickinson) was inserted through the oral cavity with the outer sheath and the attached tube at the tip. The tube was advanced through the vocal cords into the trachea. After the inner needle was removed, 50 μl of P. aeruginosa (1×10⁸ c.f.u.) was inoculated through the outer sheath, followed by a gentle push of the outer sheath to place the tube in the main bronchus. No animals died of subsequent infection and infection was restricted to the lung.

**Bacteriological examination.** The animals were sacrificed by cervical dislocation on day 14 after inoculation. The tube was removed and the lungs were excised under aseptic conditions. The lungs were homogenized in 1·0 ml of PBS and cultured quantitatively by serial dilution on *Pseudomonas* isolation agar (Difco).

**Lung lavage, tissue fixation and histopathological staining of tissue sections and leukocytes.** These procedures were performed as described previously (Kaneko et al., 2003). Briefly, mice were sacrificed on day 14 after inoculation. The chest was opened to expose the lungs and a disposable sterile plastic cut-down intravenous catheter was inserted into the trachea. Bronchoalveolar lavage was performed three times sequentially using 1 ml saline each time and the recovered fluid fractions were pooled for each animal. Leukocytes in the bronchoalveolar lavage fluid (BALF) samples obtained from each mouse were washed and counted with a haemocytometer. For differential cell counts, cells were centrifuged onto a slide in a table-top centrifuge at 1000 g for 1 min and the slides were stained with May-Giemsa stain, and differential cell counts were performed by counting 100 cells.

**Statistical analysis.** All data were expressed as mean ± SEM. Differences between groups were examined for statistical significance using the Student’s t-test. A P value less than 0·05 denoted the presence of a statistically significant difference.

**RESULTS**

**Bacteriological examination**

The numbers (mean ± SEM) of viable bacteria in the lungs of the mice 14 days after inoculation were as follows for the QS mutants: PAO-JP1, 3·03 ± 0·37 log₁₀ c.f.u. lung⁻¹; PAO-JP2, 3·01 ± 0·32 log₁₀ c.f.u. lung⁻¹; PDO100, 3·50 ± 0·27 log₁₀ c.f.u. lung⁻¹. These were significantly lower than the counts for the wild-type PAO1, 4·71 ± 0·30, log₁₀ c.f.u. lung⁻¹.

**Histopathological examination**

The effect of QS mutants on inflammatory changes in the animal model was assessed by microscopic examination. This showed that for the wild-type-infected animals recruitment of inflammatory cells occurred in the peribronchial wall (Fig. 1A). This was markedly decreased in the QS-mutant groups (Fig. 1B–D).

**Cell counts in BALF**

No significant difference was observed in the total cell counts for the BALF specimens (Table 1). However, the proportion of neutrophils was significantly lower in QS-mutant-infected groups than in the wild-type-infected group (Table 1).

**DISCUSSION**

In this study, we compared the pathogenesis of *P. aeruginosa* QS mutants with that of the wild-type strain in a mouse model of chronic respiratory infection. Mutants that lacked the *las*I and/or *rhl*I genes were cleared more efficiently from the lungs of mice compared with the wild-type strain as their viable cell numbers were significantly lower than those of the wild-type. As the *las* system supersedes the *rhl* system in the...
In conclusion, we confirmed the importance of the QS cells to the infection site. This system may be a useful target for therapy.

The authors thank Professor B. H. Iglewski (University of Rochester School of Medicine and Dentistry, Rochester, NY, USA) for providing P. aeruginosa strains, and Dr. F. G. Issa (Word-Medex, Sydney, Australia) for his assistance with editing the manuscript.

**Acknowledgements**

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**References**


**Table 1. BALF total cell number and cell composition**

Results are expressed as mean ± SEM (n = 5).

<table>
<thead>
<tr>
<th>P. aeruginosa strain</th>
<th>10^4 x Total cells (cells ml^-1)</th>
<th>Macrophages (%)</th>
<th>Lymphocytes (%)</th>
<th>Neutrophils (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAO1 (wild-type)</td>
<td>21.7 ± 4.1</td>
<td>45.5 ± 7.8</td>
<td>21.5 ± 0.7</td>
<td>33.0 ± 7.1</td>
</tr>
<tr>
<td>PAO-JP1 (ΔlasI)</td>
<td>22.7 ± 6.9</td>
<td>83 ± 3.0</td>
<td>14.0 ± 4.0</td>
<td>3.3 ± 3.2†</td>
</tr>
<tr>
<td>PAO-JP2 (ΔlasI/ΔrhlI)</td>
<td>17.5 ± 5.7</td>
<td>71.0 ± 27.7</td>
<td>26.0 ± 24.3</td>
<td>3.3 ± 3.2†</td>
</tr>
<tr>
<td>PDO100 (ΔrhlI)</td>
<td>6.5 ± 3.3</td>
<td>85.3 ± 2.3*</td>
<td>14.3 ± 2.1</td>
<td>1.0 ± 0.0†</td>
</tr>
</tbody>
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*P < 0.05 compared with wild-type.
†P < 0.0001 compared with wild-type.

