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

Yoshifumi Imamura,† Katsunori Yanagihara,† Kazunori Tomono, Hideaki Ohno, Yasuhiro Higashiyama, 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...
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 lasI and/or rhlI 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.

ACKNOWLEDGEMENTS

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.

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 × 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>
</table>

*P < 0.05 compared with wild-type.
†P < 0.0001 compared with wild-type.

QS hierarchy, the activity of the rhl system in PAO-JP1 (ΔlasI) is reduced but the activity of the las system in PDO100 (ΔrhlI) is not reduced. We found no difference in viable cell numbers between the three mutant strains, which may suggest that the rhl system is essential for the establishment of chronic respiratory infection.

Chronic inflammation is related to chronic respiratory infection as it causes serious damage to the lung tissue. Histopathological examination showed that the inflammatory cells were more readily recruited to the lungs of mice infected with the wild-type group than to the lungs infected with mutants (Fig. 1). BALF analysis confirmed that the proportions of neutrophils in the mutant-infected groups were lower than for the wild-type-infected group (Table 1). These results support the conclusion that, as for acute infections, in the absence of QS genes P. aeruginosa is attenuated in its ability to cause a severe inflammatory reaction in chronic respiratory infection.

P. aeruginosa elastase, which is under QS control (Van Delden & Iglewski, 1998), is known to affect inflammation in a murine model of chronic P. aeruginosa respiratory infection (Yanagihara et al., 2003). Because the QS-mutant strains produce less elastase than the wild-type, the inflammatory changes in the lungs of those infected with QS-mutant strains may be milder than for the wild-type-infected group. The Pseudomonas autoinducer has been reported to be a potent stimulator of several cytokines in vivo (Smith et al., 2002). Some of these cytokines may attract inflammatory cells to the infection site.

In conclusion, we confirmed the importance of the QS system in chronic P. aeruginosa respiratory infection by using a murine intubation model. By making use of this system, P. aeruginosa gains an advantage in the establishment of chronic respiratory infection, and thus, interference with this system may be a useful target for therapy.

In a model of acute pulmonary infection.

The authors thank Professor B. H. Iglewski (University of Rochester School of Medicine and Dentistry, Rochester, NY, USA) for his assistance with editing the manuscript.

