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Functional Effects of LasR/RhlR on *Pseudomonas aeruginosa* Biofilm Development and Lung Infections in Mice*

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Abstract New strategies are needed for prevention of *Pseudomonas aeruginosa* (*P. aeruginosa*) infections, a widespread disease caused by *P. aeruginosa* with strong drug resistance. The immunoprotective capacity of the receptor of autoinducers protein LasR/RhlR was examined in the BALB/c mice. At first, specialized expression plasmids were developed to facilitate expression of LasR/RhlR proteins in *Escherichia coli* (*E.coli*). Then, biofilms were grown from clinical isolated *P. aeruginosa* PA0305 to investigate the relative contributions of cell signaling for biofilm formation. Morphological characters of biofilm were quantified using Image-Pro Plus software. Fluorescence analysis demonstrated that cell signal molecule LasR/RhlR significantly ($P < 0.05$) influenced development of *P. aeruginosa* biofilm. Active immunization of mice with LasR/RhlR was found to provide significant protection against homologous challenge with *P. aeruginosa* in mice lungs. In 10 days after lungs inoculation, the bacterial clearance rate of the immunized mice was clearly higher than that of non-immunized groups on the basis of microbiological and histological assays. The protective effects of immunization with LasR and RhlR together were the same as the result of LasR or RhlR immunized mice alone. These data indicate that the manner of LasR, RhlR or both is an important determinant of immunoprotection in mice lungs infection.

Key words *Pseudomonas aeruginosa*, quorum sensing system, biofilms, immunization

Although *Pseudomonas aeruginosa* (*P. aeruginosa*) is an opportunistic pathogen, it has always leaded to severe lung degeneration in individuals with cystic fibrosis (CF) [1]. Most of these diseases couldn't be completely cured because *P.aeruginosa* could modify their surrounding environment to construct bacterial biofilms and communicate with each other in the structured macroscopic groups [2]. How *P.aeruginosa* communicate with each other is the focus of many researchers. Quorum sensing (QS) is a signaling process used by *P.aeruginosa* to response to the change of cell density. Recently, genes not only involved in virulence, such as *lasB* and *lecA*, but also coding for secondary metabolits, in pyocyanin and cyanide, have been showed to be controlled by quorum sensing [3,4]. QS system has been well studied and there are at least two complete systems in *P.aeruginosa*. The *las* and *rhl* systems seem to represent a global gene regulation system in *P.aeruginosa*. The *lasR*/*rhlR* genes of *las*/*rhl* systems are activated by *Pseudomonas* autoinducer 1 (PAI-1) and *Pseudomonas* autoinducer 2 (PAI-2), respectively. The LasR and RhlR proteins can bind to these autoinducers and these complexes work as extracellular signals involved in cell-to-cell communication [5]. Although QS has primarily been examined in bacterial communication, a growing number of reports indicated that the *las*/*rhl* are required for maturation of *P.aeruginosa* biofilms [6～8]. However, the current view of quorum sensing circuits in *P. aeruginosa* derives from molecular function of *las*/*rhl* systems. With few exceptions, the vivo characters of autoinducers are well studied[9]. To begin a *vitro* investigation of LasR and RhlR function, the immunoprotection of these proteins were confirmed by the bacterial clearence rate of the immunizated mice

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against experimental pneumonia in mice, for which biofilm-associated pulmonary lesions was developed.

1 Materials and methods

1.1 Materials

PAO1 was a prototypic wild-type strain of *P. aeruginosa* and grown in Luria-Bertani medium, LB medium (containing 1.0% of tryptone, 0.5% of yeast extract, 0.5% of NaCl and adjusted to pH 7.2). Strains PA0315 and PA0205 were obtained from Laboratory Medicine, West China Hospital, Sichuan University, Chengdu, China. Strain PA0305 was a derivative strain of PA0315, contained pEGFP plasmid [10]. Strain PA0205 can develop a mucoid clone on LB agar and is used as an infection strain to challenge mice. Expression vector was constructed by conventional molecular biology techniques[11] and PCR method using pGEX4T-1 (Amersham Pharmacia Biotech). *E. coli* strains DH5α [supE44ΔlacU169 (φ80lacZΔM15) hsdR17recA1 endA1 gyrA96 thi-relA1] and *E. coli* BL21 (DE3)plysS [F- ompT hsdSB (rB-mB-)gal dcm (DE3)pLysS(CamR)] were used as cloning hosts.

1.2 Methods

1.2.1 Purification of LasR/RhlR proteins. To facilitate purification of LasR/RhlR, the *lasR*/*rhlR* genes were first cloned into the GST-Tag vector pGEX4T-1 respectively. pGEX4T-1 contained a GST-coding sequence at the 3′ end of it's multiple cloning site (MCS), thereby generating an LasR/RhlR proteins with GST residues at its C terminus. The *lasR*/*rhlR* genes were amplified from the PAO1 general DNA by using PCR and the following primers lasR-P1: 5′ ATAGAATTCATGGCCTTGGTTGACGGTTT 3′; lasR-P2: 5′ ATACTCGAGTCAGAGAGTAATAA-GACCCA 3′; rhlR-P1: 5′ ATAGAATTCATGAGG-AATGACGGAGGCTT 3′; rhlR-P2: 5′ ATACTCG-AGTTTCAGATGAGACCCAGCGC 3′.The *lasR*/*rhlR* genes were amplified with Pfu DNA polymerase (TaKaRa). Reaction mixtures (50 μl) contained 20 ng of PAO1 general DNA, 2 μmol/L (each) primer, 100 μmol/L (each) deoxynucleoside triphosphate, 4 mmol/L $MgSO_4$, and 1 U of Pfu DNA polymerase in 1× reaction buffer. Mixtures were heated at 94℃ for 3 min before being subjected to 30 cycles of 94℃ for 30 s, 54℃ for 30 s, and 72℃ for 1 min, at last, reaction was finished with 10 min at 72℃. The PCR conditions of *rhlR* were the same as *lasR*. When reactions were finished, PCR products (5 μl) were subsequently analyzed using agarose gel (1.0%) electrophoresis and were purified by using the Omega PCR Purification Kit (TaKaRa). Following digestion with *Eco*R Ⅰ and *Xho* Ⅰ, the PCR products of lasR and rhlR were cloned into *Eco*R Ⅰ-*Xho* Ⅰ-restricted pGEX4T-1 to yield pGLP03 for lasR-pGEX4T-1 and pGRP02 for rhlR-pGEX4T-1. The recombinant plasmids were then transformed into *E. coli* BL21 (DE3) respectively. Overnight cultures of pGLP03 and pGRP02 in LB medium containing appropriate antibiotics (100 mg/L Amp) were diluted 1∶60 into the same medium and incubated for 2 h, at which time isopropyl-β-D-thiogalactopyranoside (IPTG) was added in appropriate final concentration. Four hours later, cells were harvested by centrifugation (8 000 *g* for 10 min), washed twice with 100 ml of phosphate-buffered saline (PBS, pH 7.4), and resuspended in 50 ml of ultrasonic buffer solution (50 mmol/L Tris-HCl, pH 8.0; 2 mmol/L EDTA; 0.1% TritonX-100; and 0.1 mmol/L PMSF). Following ultrasonic treatment (750 W for 4 min), the sonicate solution was centrifuged at 4℃ (10 000 × *g* for 10 min). The pellet (containing bodies) was washed twice with cold PBS (4℃) containing 1% TritonX-100 followed by centrifugation at 10 000 × *g* for 10 min at 4℃. The pellet (inclusion bodies) was solubilized in 2 ml 8 mol/L Urea pH 10.7, 25 mmol/L DTT, 0.1 mmol/L PMSF and renatured for 2 h at 4℃. Then the solution was centrifugated at 10 000 *g* for 10 min and the solubilized proteins were dialysis into cold (4℃) folding buffer for 24 h. The folding buffer was containing 50 mmol/L Tris-HCl (pH 8.0) and 3 mmol/L NaN_3. After the first dialysis, the solubilized proteins were dialyzed into cold (4℃) PBS for 12 h again. The final protein supernatant was appropriately diluted with PBS and applied to a glutathione Sepharose 4B column. After the GST-fused LasR/RhlR protein (GST-LasR; GST-RhlR) bound column was gentle agitated at room temperature, the column was washed with the PBS for three times. The eluate was collected when the column was added glutathione elution buffer and incubated at room temperature for 10 min. Pool all the eluates of different times together as purification products of LasR/RhlR recombination proteins. The purified LasR/RhlR were then used to raise antibodies in BALB/c mice (Labratory Animal Center, Sichuan University, Chengdu, China).

1.2.2 SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting. LasR/RhlR were

prepared as described above. LasR/RhlR proteins were electrophoresed on sodium dodecyl sulfate-polyacrylamide gels (with 10% acrylamide in the running gel) as described in reference [11]. Electrophoretically separated proteins were blotted onto a Nylon Transfer membrane (Millipore) at 100 mA constant ampere for 2 h at 4℃. Membranes were processed with skim milk powder (Difco) as blocking step. A mouse anti- *P. aeruginosa* (Chengdu Institute of Biological Products Ministry of Health, Chengdu, China) (diluted 1/200) and a peroxidase-labeled goat anti-mouse immunoglobulin G (Kirkegaard and Perry Laboratories) (diluted 1/1 000) were employed as the primary and secondary antibodies, respectively.

1.2.3 Triparental matings. Introduction of plasmids pGFPuv (Clontech) into *P. aeruginosa* required a triparental mating with employing the helper vector pRK2013 as described previously[10]. Briefly, overnight cultures (100 μl each) of plasmid-containing *E. coli* JM109, pRK2013-containing *E. coli* MM294, and *P. aeruginosa* PA0315 was pelleted together in a microcentrifuge tube, resuspended in 25 μl of LB broth, and spotted onto the center of an LB agar plate. Following incubation overnight at 37℃, bacterial growth was resuspended in 1 ml of LB broth and appropriate dilutions were plated on LB agar containing 50 mg/L kanamycin and 100 mg/L Ampicillin. Plasmid DNA was prepared from *P. aeruginosa* recipients by using the miniprep procedure to confirm successful plasmid transfer.

1.2.4 Development of biofilms in flowing culture. Previous experiments indicated that the development of *P. aeruginosa* biofilm was induced by nitrogen limited medium [12]. So, culture vessels containing flowing medium (1/50 LB) were used for flowthrough biofilm studies. 50 μg/L of kanamycin was added for plasmid maintenance. 6 pieces of silica gel sheets for medical use (offered by Chengdu Chengguang Chemical Co., Chengdu, China) having a surface area of 6×0.36 cm^2 were employed for biofilm cultivation and microscopic analyses. The flow velocity (v) was maintained a rate of 0.14 ml/min, yielding a residence time within the flowcells of 8.5 min. Silica gel sheets were examined by the Olympus BX51 fluorescence microscopy on days 1, 3 and 6.

1.2.5 The action of LasR/RhlR in development of biofilms. To determine whether LasR, RhlR, or both are required for the normal development of the *P. aeruginosa* biofilm, we added one or the other of these proteins into culture medium. After cultured for 1 to 8 days, the fluorescence microscopy was used to observe biofilms clones. Images were collected at 485 nm to record bacteria emitting green fluorescent. Biofilms levels were calculated by value of gray degree in dividing pixel locations in the GFP (green) signal. The images were first converted to binary data and analysis was done using DP Controller 1.65 and Image-Pro Plus. The value of gray degree was showed the level of biofilms on silica gel sheets. Each sheet was detected at different scope in three times.

1.2.6 Inoculating mice with *P. aeruginosa*. Mucoid *P. aeruginosa* clinical strain PA0205 was harvested at late log phase from a shaking incubator at 37℃. The PA0205 cells were washed by PBS 3 times and adjusted to 10^{10} CFU/ml. Mice were anesthetized with propofol injection (0.02 ml/g of body wt intraperitoneally) and a 1-cm skin incision was made just ventral cervical region. A 5-gauge 2 cm blunt needle was used to cannulate the trachea. The *P. aeruginosa* suspending cells were injected 0.1 ml for mice and an aliquot of PBS was used as a control. Following inoculation, the incision was surgically sutured and mice were allowed to recover from the anesthesia.

1.2.7 Passive immunization and challenge experiments.

An initial series of challenge studies was performed on non-immunized male BALB/c mice to standardize the animal model. The control group was challenged with *P. aeruginosa* suspending cells containing 10^{10} CFU/ml.

Male BALB/c mice were immunized on day 0, 7 and 14 with 100 μl of PBS containing 10 μg of recombinant LasR, 10 μg RhlR, 10 μg LasR + 10 μg RhlR, or with PBS alone. Antibody title of mice was detected by agar-gel immunodiffusion tests and ELISAs. In brief, 96-well ELISA plates were coated overnight at 4℃ with 50 ng of LasR or RhlR dissolved in 100 μl PBS. After washing, 1∶256 dilution of tested serum samples were added and the plates were incubated at 37℃ for 2 h. Following subsequent 4 times washing, a peroxidase-labeled goat anti-mouse IgG (Kirkegaard and Perry laboratories) were added and incubated at 37℃ for 1 h. The plates were washed again and incubated with 100 μl tetramethylbenzidine substrate. After stopping the reaction with 0.5 mol/L H_2SO_4 the absorbance was measured at 490 nm. A

positive ELISA reading was considered to be 2 standard deviation (SD) above the mean from normal mice sera tested as described above.

On immunized day 28 th, *P. aeruginosa* suspending cells were inoculated mice. After carefully raised, viable bacterial numbers in the lungs were determined. The lungs of 3 mice from each challenge group were removed under sterile conditions. Organs were collected on blood agar. After incubation for 24 h, colonies were counted and the numbers of cfu per gram were determined.

1.2.8 Histologic evaluation. Lungs were perfused and fixed in periodate-lysine-paraformaldehyde for 2 h at 4℃ and embedded in paraffin for frozen sectioning. The tissue was cut into sections 3 μm thick, stained with hematoxylin and eosin (H&E), and evaluated light microscopically. A histologic score for each lung was determined according to the following criteria: 0 = no lung abnormality; 1 = presence of inflammation and granulocytosis involving < 10% of the lung parenchyma; 2 = lesions involving 10% to 50% of the lung; and 4 = lesions involving > 50% of the lung.

1.2.9 Statistical analysis. The viable bacteria were expressed as the mean CFU of independent observations ± the standard deviation. The output of data was analyzed by SPSS for windows 10.0. The statistical significance of the data was determined by *t* test; $P < 0.05$ were considered significant.

2 Results

2.1 Expression of LasR/RhlR in *E. coli*

Sequence analysis of the 720 bp *lasR* and 726 bp *rhlR* fragment in clone pGEX4T-1 displayed the entire *lasR*/*rhlR* gene. The predicted amino acid sequence of the *lasR*/*rhlR* gene gave a 240/242 amino acid protein with a molecular mass about 26 ku. The 720/726 bp fragments are deposited in the EMBL GenBank under accession No. M59425 for *lasR* and AE004768 for *rhlR*. After IPTG induction of *E. coli* BL21 (DE3) cells transformed with the *lasR*/*rhlR* genes respectively, the C-terminally GST-tagged LasR/RhlR protein appeared to be expressed efficiently (Figure 1). The purification of LasR can reach 89.6%, while the RhlR with a yield of 90.0%. The biological activity of LasR/RhlR was confirmed by the immunoblotting method using mouse polyvalent antiserum against *P. aeruginosa* (Figure 1).

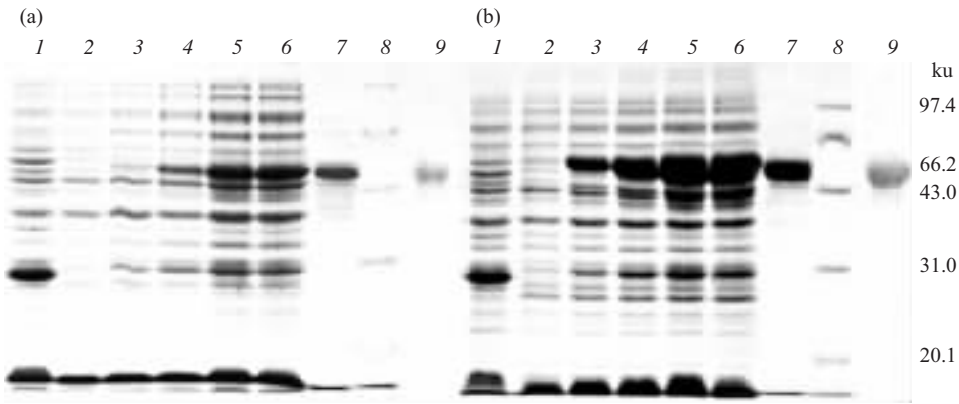

Fig. 1 Whole-cell protein profiles (SDS-PAGE) of *E. coli* BL21 (DE3) with pGLP03 (a) or pGRP02 (b)
(a) *1*: *E. coli* BL21 (DE3) with pGEX4T-1; *2*: *E. coli* BL21 (DE3) with pGLP03 before IPTG induction; *3*, *4*, *5* and *6*: 1, 2, 4 and 6 h after induction respectively; *7*: The purified LasR; *8*: Protein molecular low mass marker; *9*: Western immunoblot of LasR. (b) *1*: *E.coli* BL21 (DE3) with pGEX4T-1; *2*: *E. coli* BL21 (DE3) with pGRP02 before IPTG induction; *3*, *4*, *5* and *6*: 1, 2, 4 and 6 h after induction respectively; *7*: The purified RhlR; *8*: Protein molecular low mass marker; *9*: Western immunoblot of RhlR.

2.2 Development and morphology of biofilms

After 24 h of the inoculation period, the PA0305 biofilms were consisted of a sparse layer of cells, but no obvious structure of biofilms was occurred yet. (Figure 2a). By day 3, there was a monolayer of cells between the colonies (Figure 2b). When the strain was cultured with silica gel sheets in medium for 6 days, PA0305 had developed a very thick biofilms coating bacteria itself (Figure 2c).

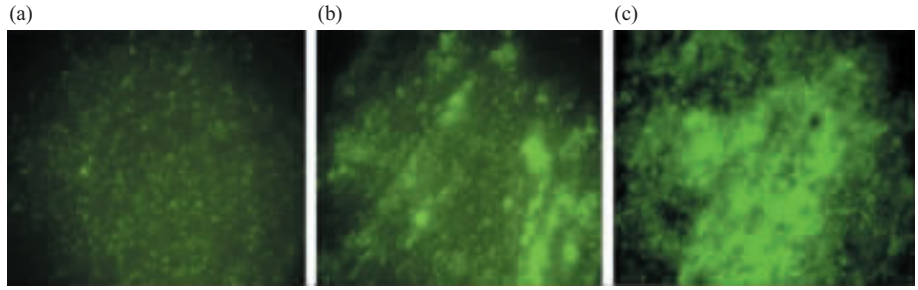

Fig. 2　The structure of PA0305 biofilms formed on the surface of silica gel sheets

(a), (b) and (c): PA0305 biofilms formed for 1 day, 3 days and 6 days. Magnification, ×400.

2.3　The action of LasR/RhlR in development of biofilms

The activities of the bactericidal biofilm with GST (10 mg/L) as control and with LasR (10 mg/L) or RhlR (10 mg/L) or both of them (5 mg/L each) are illustrated in Figure 3. Exposure to LasR/RhlR resulted in a > 40% developing rate in the PA0305 when compared with control (P <0.05) at the end of 3 days. No matter LasR or RhlR effected alone or together, the result was the same (P > 0.05). In fluorescence intensity, there was no significant difference between the biofilm with or without LasR/RhlR on any of the 6 days (P > 0.05).

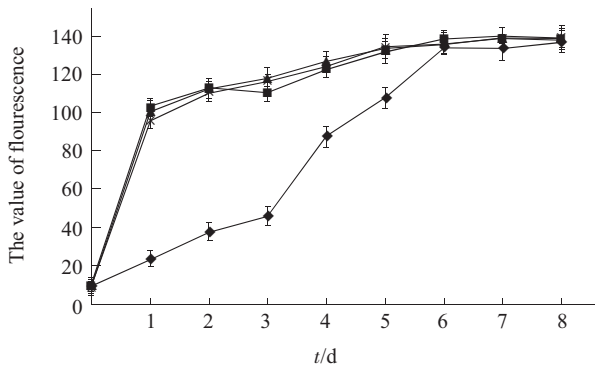

Fig. 3　The influence of LasR/RhlR on development of a mature biofilms in PA0305

The value of fluorescence showed the process of biofilm formation. There was no difference between LasR, RhlR and LasR+RhlR groups in fluorescent value, but all groups were clearly different from control group before 3 days.■—■: LasR(10 mg/L); ▲—▲: RhlR(10 mg/L); ×—×: LasR+RhlR(5 mg/L each); ◆—◆: Control (GST 10 mg/L).

2.4　Antibody response of mice to immunization with recombinant LasR/RhlR proteins

Mice were immunized three times at one-week intervals with recombinant LasR/RhlR proteins, or a combination of both proteins. A humoral immune response to these proteins was documented by double immunodiffusion measurement of antibodies in blood samples that obtained from representative animals 6 days after each immunization. Antibody levels after the third immunizations were the same in animals immunized with a single protein and in those immunized with both LasR and RhlR.

2.5　Immunoprotection of mice with LasR/RhlR

In ELISA tests, both recombinant proteins reacted strongly with the immunized serum. In total, 92 mice serum scored positive in the ELISA based on native control. However, mice immunized subcutaneously with recombinant LasR/RhlR plus incomplete Freund's adjuvant had no better survival rate than control. At 10 days after *P. aeruginosa* inoculation, survival in animals vaccinated with one or both proteins was 97.5% (93.7% to 100%), compared with 96.8% (90% to 100%) in the control group. This difference was no statistically significant in the experiment (P > 0.05).

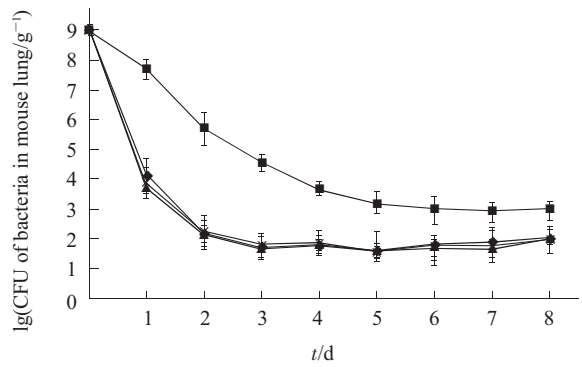

Fig. 4　Protection provided by recombinant LasR and RhlR in *P.aeruginosa*

Mice were immunized subcutaneously with LasR, RhlR, LasR+RhlR, or PBS (control) and challenged with *P. aeruginosa* on day 28. The numbers of CFU in the lungs of immunized and control mice on day 38 are shown. Data represent the means of three experiments and 3 mice per group in each experiment. ◆—◆: LasR immunization; ▲—▲: RhlR immunization; ×—×: LasR+RhlR immunization; ■—■: Control.

However, when mice were challenged with 1.0 × 10^9 CFU/mL *P. aeruginosa*, the bacterial number was recovered on every 24 h in the lungs. The mice immunized with both LasR/RhlR showed significantly fewer organisms in the lungs when compared with non-immunized controls, like mice immunized with LasR or RhlR alone. Under these experimental conditions, the lungs were protected against the challenge dose (Figure 4).

2.6 Histopathologic changes in experimental *P. aeruginosa* infection and immunized mice

Consistent with the bacterial clearance rate in mice lung, interstitial inflammation and fibrosis were observed in BALB/c mice 10 days after an injection of *P. aerugionosa*. The histopathologic examination showed a strong inflammatory response. Histologic abnormalities of the lung included increased interstitial wall thickness, interstitial mononuclear cell infiltrates, number of fibroblasts, and interstitial collagen deposition with distortion of normal lung architecture (Figure 5c). These histologic changes, however, were markedly decreased in extent and severity when evaluated in immunized mice. In the immunized mice, which were given LasR/RhlR, lymphocyte infiltration into the lung parenchyma and granuloma formation were less marked than in control group (Figure 5b). Although the histologic scores were higher than those of the normal group (Figure 5a), they were also significantly lower than those of control group (Table 1). While there was no difference in lung structure between normal group and immunized mice without challenge.

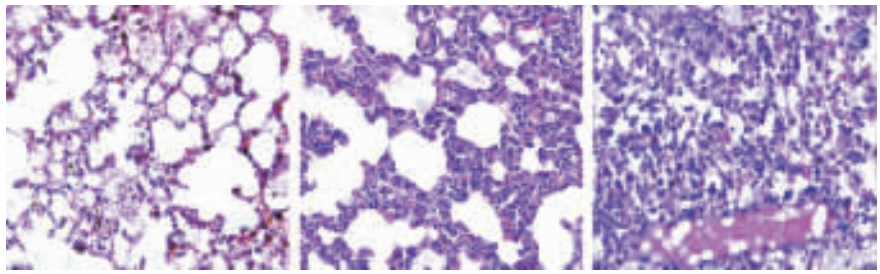

Fig. 5 H&E staining of lungs sections in mice

(a), (b) and (c): The photomicrographs of normal; immunized and challenged lungs in mice. Magnification, ×400.

Table 1 Comparison of pathologic change in mice lungs

Group	*n*	Ⅰ(%)	Ⅱ(%)	Ⅲ(%)	Ⅳ(%)	Value
A	12	11(91.7)	1(8.3)	0(0)	0(0)	1
B	20	18(90.0)$^{\triangle}$	2(10.0)$^{\triangle}$	0(0)$^{\triangle}$	0(0)$^{\triangle}$	2$^{\triangle}$
C	20	1(5.0)**	3(15.0)	9(45.0)	7(35.0)**	49**
D	20	1(5.0)*	13(65.0)*	6(30.0)*	0(0)	25*

A: Normal group; *B*: Immunized LasR/RhlR group; *C*: *P.aeruginosa* challenged group; *D*: Immunized-challenged group. Group *B* compared with normal group (*A*), $^{\triangle}P > 0.05$; Group *D vs* normal group (*A*), $^{*}P < 0.05$; Group C compared with the other groups, $^{**}P < 0.05$.

3 Discussion

The LasR and RhlR proteins belong to the LuxR family of transcription regulators whose members bind to specific DNA sequences, called lux boxes[13]. In the case of *P. aeruginosa*, these specific sequences have been called *las* boxes[14]. Quorum-sensing systems in *P. aerugionosa* including the LasR-LasI system and the RhlR-RhlI system are global regulators of gene expression. The *lasR* and *rhlR* genes encode the *N*-acyl-homoserine lactone-dependent transcriptional regulators LasR and RhlR, respectively. Acyl-HSL signaling is an important virulence factor in *P. aeruginosa*[15]. LasR is a 3OC12-HSL-responsive transcriptional regulator and trig for quorum-activated gene expression[16]. RhlR is a C4-HSL-responsive transcription factor[17]. Both of the acyl-HSLs can diffuse through the cell envelope, so a critical cell population density is required to produce signals at

levels sufficient for quorum-controlled gene regulation[18]. The *rhlR* gene is encoded immediately downstream of the *rhlAB* operon and has been reported to be activated by LasR[19,20]. A *P. aeruginosa lasR* mutant did not express RhlR during grown on Luria-Bertani (LB) medium[21].

Several recent studies have begun to elucidate the molecular mechanisms that effect development of *P.aeruginosa* biofilms [22,23]. By testing the biofilms density, we were able to examine the contributions of LasR/RhlR in the process of biofilms maturation. First, we have observed a number of differences between biofilms grown with or without LasR/RhlR. The differences in initial attachment and growth rate of *P.aeruginosa*, biofilm development and cell density, immunoprotection and bacteria challenge, were detected *in vitro* and *in vivo* tests. Unlike previous results[24], which suggested that, cell signaling is not required for biofilm formation, in our flow system, the addition of LasR/RhlR promoted bacterial biofilms formation and our data suggest that cell signaling plays an important role in the development of the biofilm. Second, the focus of our study is to identify proteins that are relevant to host immunity. The most direct method available for assessing the relevance of a protein is to determine whether immunization with that protein is able to alter the disease in an animal model. LasR/RhlR were expressed as a recombinant protein in *E. coli* and immunogenicity of them were detected in mice. The immunoprotective tests showed LasR/RhlR could increase the bacterial clearance rate in mouse lungs and may be work as immunoprotective antigen to develop the genetic engineering vaccine.

The goal of this study was to provide evidence to illumine the function of LasR/RhlR in development of *P. aeruginosa* biofilms and immunoprotective capacity of LasR/RhlR. Further studies are needed to determine whether the addition of LasR/RhlR is cross-active with other genes in *P. aeruginosa*. Since less than 100% of the immunized animals were protected, there is a need for studies directed toward defining how the immunity could be improved. In addition, more reliable and efficient expression systems are needed for production of LasR/RhlR. Studies are needed to determine whether LasR/RhlR can be further purified while retaining their immunoprotective properties.

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QS系统中 LasR 和 RhlR 蛋白对铜绿假单胞菌生物被膜形成的影响以及免疫原性研究 *

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摘要 为探讨铜绿假单胞菌 PAO1 中 *lasR* 和 *rhlR* 基因表达产物的分子生物学特性，研究它们对铜绿假单胞菌生物被膜形成的影响以及对小鼠的免疫保护效果，采用聚合酶链式反应 (PCR) 方法扩增铜绿假单胞菌标准株 PAO1 中的 *lasR* 和 *rhlR* 基因，全自动荧光测序仪测序，并用 Blast 方法检测克隆片段. 利用 pGEX4T-1 载体分别构建 *lasR*/*rhlR*-pGEX4T-1 重组质粒，在大肠杆菌 BL21(DE3)中诱导表达，并经过免疫印迹实验验证其生物学活性. 用硅胶膜培养法建立生物被膜模型，诱导转入了 pGFPuv 质粒的铜绿假单胞菌 PAG0305 形成生物被膜，并测定 LasR 蛋白和 RhlR 蛋白对生物被膜形成的影响. 同时用纯化的重组蛋白免疫小鼠，菌落计数法检测免疫组和对照组鼠肺对铜绿假单胞菌的清除率. 以 PAO1 染色体 DNA 为模板的 PCR 结果显示，*lasR* 的全基因序列为 720 bp，*rhlR* 基因序列为 726 bp，经序列分析和同源性比较分别与 GenBank 中 *lasR*/*rhlR* 基因 (登录号：M59425； AE004768) 的同源性为 100%. 大肠杆菌 BL21 (DE3) 分别转化重组质粒 *lasR*/*rhlR*-pGEX4T-1 后，经 IPTG 诱导和 SDS- 聚丙烯酰胺凝胶电泳分析，表达的融合蛋白分子质量均为 54 ku 左右，与预期蛋白质分子质量相同. 荧光显微镜观察和测定结果表明，在硅胶膜上 PAG0305 能够形成典型的发荧光的生物被膜，LasR 或 RhlR 蛋白 (10 mg/L) 存在的情况下，PAG0305 生物被膜的形成速度在前三天比对照组平均提高 40.77%，而且两蛋白单独存在与同时存在时的作用相同. 体内实验中，免疫小鼠肺部对铜绿假单胞菌的清除率显著高于未经免疫的正常组 ($P < 0.05$). 上述结果表明：构建的 *lasR*/*rhlR*-pGEX4T-1 重组质粒能够在大肠杆菌 BL21(DE3)中成功地表达并具有生物学活性. LasR/RhlR 蛋白在体外模型中能够加快铜绿假单胞菌生物被膜的形成速度，是调节铜绿假单胞菌生物被膜形成的重要因素之一. 免疫结果表明，重组蛋白对小鼠表现出一定的保护作用，这为进一步开展疫苗研究奠定了基础.

关键词 铜绿假单胞菌，数量感知系统，生物被膜，免疫原性
学科分类号 Q93，R3

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