Med Microbiol Immunol (2009) 198:113–121
DOI 10.1007/s00430-009-0111-z

ORIGINAL INVESTIGATION

Influence of *Pseudomonas aeruginosa* quorum sensing signal molecule *N*-(3-oxododecanoyl) homoserine lactone on mast cells

Hongtao Li · Lili Wang · Lu Ye · Yan Mao · Xuhua Xie · Chao Xia · Jia Chen · Zimin Lu · Jianxin Song

Received: 16 December 2008 / Published online: 1 April 2009
© Springer-Verlag 2009

Abstract Quorum sensing system is a cell-to-cell communication system that plays a pivotal role in virulence expression in bacteria. Recent advances have demonstrated that the *Pseudomonas aeruginosa* quorum sensing molecule, *N*-3-oxododecanoyl homoserine lactone ($3OC_{12}$-HSL), exerts effects on mammalian cells and modulates host immune response. Mast cells (MCs) are strategically located in the tissues that are constantly exposed to external stimulus. Therefore, it is very much possible that $3OC_{12}$-HSL may interact with MCs. Little is known, however, about specific effects of $3OC_{12}$-HSL on MCs. To address this, we investigated the influence of $3OC_{12}$-HSL on cell viability, apoptosis, intracellular calcium and cytokine release in MCs. We found that at high concentrations (100 μM), $3OC_{12}$-HSL inhibited proliferation and induced apoptosis in P815. The $3OC_{12}$-HSL treatment significantly increased intracellular calcium release in both P815 and HMC-1. We also observed that $3OC_{12}$-HSL-induced histamine release and degranulation in HMC-1 cells. Furthermore, $3OC_{12}$-HSL-induced IL-6 production at lower concentrations (6.25–12.5 μM) but steadily reduced IL-6 production at high concentration (50–100 μM). These data demonstrate that *P. aeruginosa* $3OC_{12}$-HSL affects MCs function.

Keywords *Pseudomonas aeruginosa* · Quorum sensing system · Homoserine lactone · Mast cells · Apoptosis · Calcium · Cytokines

H. Li · L. Wang · L. Ye · Y. Mao · X. Xie · C. Xia · J. Chen · J. Song (✉)
Department of Infectious Diseases,
Tongji Hospital, Wuhan, China
e-mail: Songsingsjx@sina.com

H. Li
e-mail: lhtmed@yahoo.com.cn

Z. Lu
Department of Pharmacology, Tongji Medical College,
Huazhong University of Science and Technology,
430030 Wuhan, China

Introduction

Quorum sensing system (QSs) is a bacterial cell-to-cell communication signal system based on small diffusible molecules called autoinducers (AIs) [1, 2]. Through QSs, bacteria can keep track of their cellular density and regulate their behavior as a group according to their population density [1, 2]. In Gram-negative bacteria, the most common type of AIs are acylated homoserine lactones (AHLs) [1, 2]. *P. aeruginosa*, a common gram-negative pathogen, produces two major AHLs: *N*-(3-oxododecanoyl)-L-homoserine lactone ($3OC_{12}$-HSL) and *N*-butanoyl-L-homoserine lactone (C_4-HSL), both AHLs play a central role in orchestrating the expression of virulence genes and participate in development of biofilms [1–5]. Recently, an increasing body of evidence shows that the *P. aeruginosa* QS signal molecule $3OC_{12}$-HSL, but not C_4-HSL, can interact with different eukaryotic cells and modulate immune response, including the regulation of inflammatory cytokines expression and the promotion of apoptosis [6–8]. These findings suggested that $3OC_{12}$-HSL may act as a modulator of the host immune system in the context of *P. aeruginosa* infection.

Mast cells (MCs) are a group of immune cells that have been shown not only to function as key effector cells of allergic inflammation, but also to play a pivotal role in infectious diseases [9–11]. However, only a few of studies

have explored the interaction between *P. aeruginosa* and MCs. Recently, an in vivo study using MC-deficient mice demonstrated that activated MCs are crucial for controlling of *P. aeruginosa* skin infection [12]. Moreover, increased MC numbers are found in *P. aeruginosa*-infected lungs and a relatively higher percentage of activated MCs are observed in cystic fibrosis (CF) airways, strongly suggesting that MCs play a pivotal role in *P. aeruginosa* infection [13, 14]. Most importantly, the MC count is notably lower in infections by QS-deficient *P. aeruginosa* than those of wild type [15], indicating that QS signals may be important in MC involvement with *P. aeruginosa* infections.

Mast cells are derived from hematopoietic stem cells, and abundantly reside and mature in the tissues that are constantly exposed to the external environment such as lung, intestine and skin [10, 11, 16]. Since MCs strategically locate in the places where bacterial infections most likely occur, they are assumed to frequently come into contact with and respond to bacteria or bacteria-related factors.

Although the exact concentrations of QS signals in vivo during *P. aeruginosa* infection are unknown, a handful of studies have demonstrated that biologically active levels of $3OC_{12}$-HSL are detected in sputum from CF patients [17–19]. Owing to sensitivity limitations, however, the measurements used in these studies were considered to underestimate the local AHL concentrations at the site of infection [20]. Moreover, an in vitro study has shown that *P. aeruginosa* biofilms can produce $3OC_{12}$-HSL at a concentration approximately 600 μM, which is 6- to 10-fold higher than that of $3OC_{12}$-HSL used to exhibit immune response to host cells [6, 20]. Therefore, it is reasonable to speculate that MCs in the vicinity of infection region are frequently exposed to QS signals.

Given MCs function as sentinel cells in the locations that are frequently exposed to external stimulus and that relatively higher level of AHLs are excepted to be found in infected areas, we wondered whether $3OC_{12}$-HSL directly influences MCs. In the present study, we investigated the possible effects of $3OC_{12}$-HSL on MC P815 and HMC-1. Our results suggest that *P. aeruginosa* quorum sensing signal $3OC_{12}$-HSL induces apoptosis and intracellular calcium release in P815. $3OC_{12}$-HSL also induced degranulation and histamine secretion in HMC-1. These data demonstrate that *P. aeruginosa* $3OC_{12}$-HSL influences MCs function.

Materials and methods

AHL synthesis

Pseudomonas aeruginosa $3OC_{12}$-HSL was synthesized in the Department of Pharmacology, Tongji Medical College, according to the method described by Chhabra [21]. The synthesized compound was purified by liquid chromatography to homogeneity as determined by high-pressure liquid chromatography. Molecular structure was confirmed by proton nuclear magnetic resonance (NMR) spectroscopy, and its biological activity was checked according to the bioassay method as described in [22]. The dried AHLs were stored at −20 °C and was dissolved in Me_2SO at 200× the desired concentration before further experimentation.

Cell culture

The murine mast cell line P815 (ATCC TIB-64) and human mast cell line HMC-1 were cultured with RPMI 1640 and IMDM, respectively, both mediums were supplemented with 10% FCS (Hyclone), 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin. Cells were maintained in 75-cm^2 tissue culture flasks at 37 °C in a 5% (v/v) CO_2, water-saturated atmosphere. Cultured cells at a density of 5×10^5 cells/ml were incubated with the serum-free basal medium for 12 h and washed twice before challenge. For challenge experiments, cells were exposed to various concentrations of $3OC_{12}$-HSL (6.25–100 μM). At indicated times following incubation, the cultures were centrifuged at 450 g for 10 min at 4 °C. Culture supernatants were collected and stored at −80 °C for further experiments.

Cell viability assay

Viability of cells treated with $3OC_{12}$-HSL was measured using the WST-1 Cell proliferation and cytotoxicity assay kit (Beyotime, Haimen, China) in accordance with the manufacturer's instructions. At the indicated time points, 10 μl of this reagent was added to each well containing 100 μl of cell suspension with different concentrations of $3OC_{12}$-HSL and incubated for an additional 1 h. The absorbance at 450 nm was monitored and the reference wavelength was set at 630 nm. The percent viability of cells was calculated by comparison to that of untreated control cells.

Apoptosis assay

Cell apoptosis was evaluated by flow cytometry utilizing Annexin V-FTIC apoptosis Kit (Keygen Biotech, Nanjing, China). Briefly, P815 cells (5×10^5 cells/ml) were incubated with various concentrations of $3OC_{12}$-HSL for 4 h. After the challenge, cells were collected and then washed twice with phosphate buffered saline (PBS, 137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4). Collected cells were suspended and labeled with annexin V-FTIC and propidium iodide (PI) for 15 min at 25 °C in the dark. Cells were then analyzed by flow cytometry (FACSAria, BD Bioscience, USA) with Diva Cytometer

Software (BD Bioscience, USA). Cells that stained positive for annexin V were counted as apoptotic.

Calcium analysis

To monitor the effect of $3OC_{12}$-HSL on calcium release, P815 cells were loaded with 5 μM of the membrane-permeable calcium indicator fluo-3-acetoxymethyl (AM) ester (Beyotime, Haimen, China) for 40 min at 37 °C in Hank's buffer. After loading with the fluo-3 dye, cells were washed with Hank's solution and suspended in D-Hank's that lacked calcium and contained 5 mM EGTA. The cell suspensions were transferred into 35 mm glass-bottom dishes for analysis. Fluorescence measurements were performed using an Olympus Fluoview-500 confocal system equipped with a 40× objective. Fluo-3 was excited by argon laser light at 488 nm and fluorescence was measured at wavelengths of 515 nm. At 20 s after fluorescence recording started, different treatments were given and fluorescence was recorded for 200 s. In this study, 512 by 512 pixels *X-Y* images were acquired at a rate of 1 s per line. Image processing and analysis were performed by using Olympus Fluoview software. Intracellular calcium change ($F/F0$) were expressed as average fluorescence (F) divided by background fluorescence ($F0$) [23].

Transmission electron microscopy (TEM)

About 2×10^6 cells of each specimen were collected and prepared for TEM analysis. Briefly, cells were centrifuged and washed twice. After 2 h of fixation in 2.5% glutaraldehyde, cells were washed twice with 0.1 M PBS and then fixed in 1% osmic acid for 30 min. Following gradient dehydration, samples were infiltrated in acetone/resin (1/1 v/v) and resin both for 2 h. After embedding and polymerization at 80 °C for 10 h, samples were cut into ultrathin sections. Sections were then stained by uranyl acetate and lead citrate for imaging with transmission electron microscope (FEI Tecnai G^2 12).

ELISA assay

IL-4, IL-6 (R&D systems, USA) and histamine (Uscnlife, USA) released from P815 or HMC-1 cells in response to $3OC_{12}$-HSL were measured by using ELISA kits, according to the manufacturer's instruction.

Statistical methods

Data are expressed as mean ± SD representing three times of independent experiments performed in duplicate. Statistical significance between means was analyzed by one-way analysis of variance or the Student's *t* test. A P value of less than 0.05 was taken as statistically significant.

Results

Purity and biological activity of $3OC_{12}$-HSL

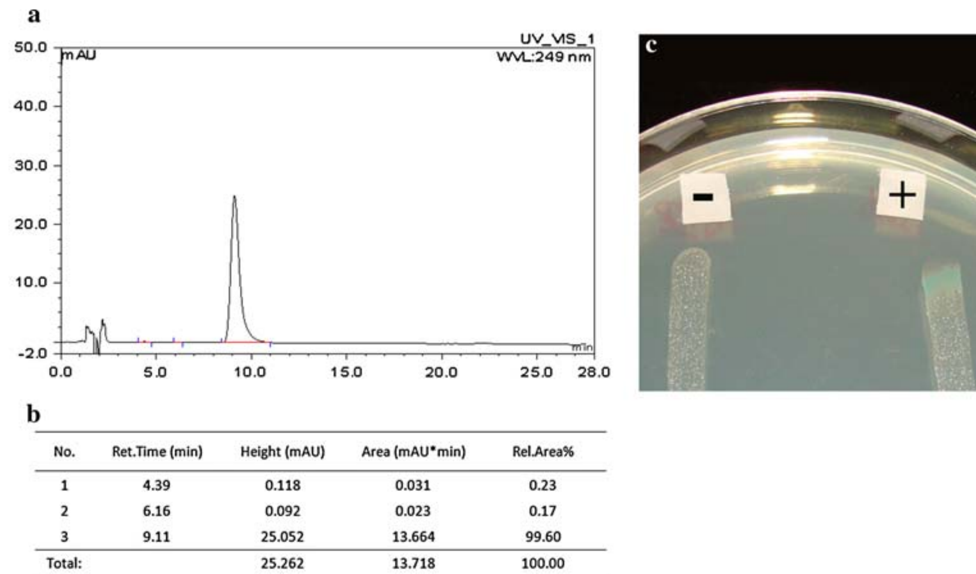
There are special structural requirements for $3OC_{12}$-HSL to remain active to induce apoptosis [24]. Thus, the structure of synthesized $3OC_{12}$-HSL was confirmed by NMR (data not shown). The purity was 99.6% as determined by HPLC (Fig. 1a, b). We checked the biological activity of synthesized $3OC_{12}$-HSL by using a sensor strain *E. coli* MG4 (pKDT17), the strain turned blue on agar containing X-gal and synthetic butyrylhomoserine lactone when $3OC_{12}$-HSL diffused (Fig. 1c).

Cytotoxicity of $3OC_{12}$-HSL to mast cells

$3OC_{12}$-HSL induces cell death in macrophage/monocyte and breast cancer cell lines but not in epithelial cell lines [25, 26]. Thus, we first examined the possible cytotoxic effects of AHL by measuring cellular dehydrogenase activity through WST-1 metabolization. Cells treated with 100 μM $3OC_{12}$-HSL, drastically lost viability (Fig. 2a). Cell viability decreased to less than 50% at 2 h and to nearly 30% at 24 h after addition of $3OC_{12}$-HSL (Fig. 2a). At 12 h of incubation, a slight loss of viability was observed from 25 μM $3OC_{12}$-HSL (Fig. 2b), but significant decreases were observed at the 50–100 μM $3OC_{12}$-HSL range (Fig. 2b). Typical morphological changes, such as cell shrinkage, were also observed in cells treated with 50 and 100 μM $3OC_{12}$-HSL compared to untreated cells (Fig. 2c). These data shows that $3OC_{12}$-HSL induces cell viability loss in P815 cells in a concentration-dependent manner.

Apoptosis analysis

$3OC_{12}$-HSL has been observed to specifically induce apoptosis in neutrophils and macrophages, which share similarities with mast cells in morphology and function [10, 20, 25]. Since P815 cells exhibit loss of cell viability when exposed to $3OC_{12}$-HSL, we speculated that apoptosis would play a role in reduction of viability. Therefore, we investigated the apoptotic effect of $3OC_{12}$-HSL on P815 cells. After incubation with different concentrations of $3OC_{12}$-HSL for 4 h, about 20 and 40% of apoptotic cells were observed in response to 50 and 100 μM $3OC_{12}$-HSL, respectively (Fig. 3). However, no significant apoptotic effects were observed when P815 cells were treated with a low concentration of $3OC_{12}$-HSL (data not shown).



b

No.	Ret.Time (min)	Height (mAU)	Area (mAU*min)	Rel.Area%
1	4.39	0.118	0.031	0.23
2	6.16	0.092	0.023	0.17
3	9.11	25.052	13.664	99.60
Total:		25.262	13.718	100.00

Fig. 1 Purity and biological activity of 3OC12-HSL. **a**, **b** HPLC analysis report shows $3OC_{12}$-HSL purity is 99.6%. **c** Biological activity assay of $3OC_{12}$-HSL. *E. coli* MG4 (pKDT17) were streaked for sensing $3OC_{12}$-HSL. − for DMSO; + for DMSO dissolved $3OC_{12}$-HSL

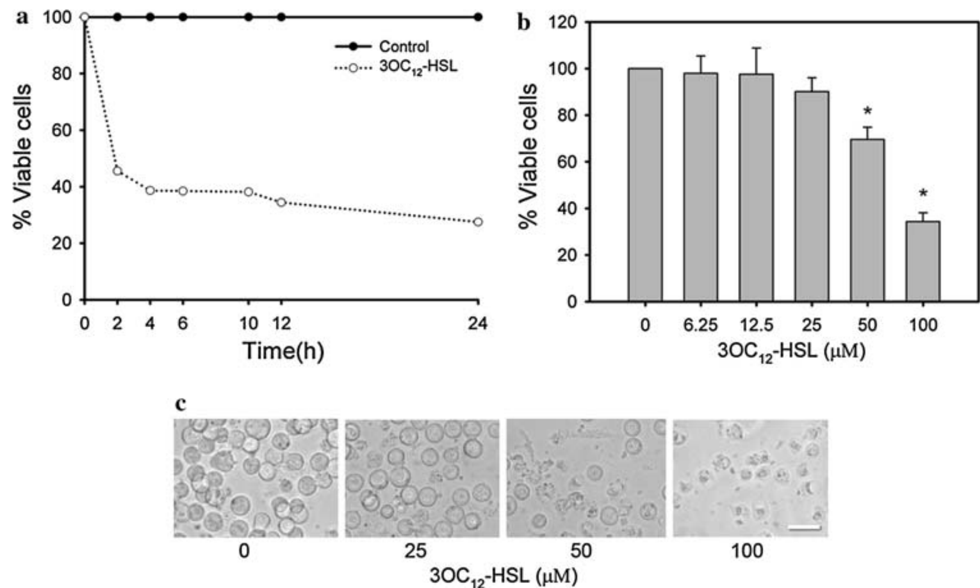


Fig. 2 Cytotoxic effect of $3OC_{12}$-HSL on P815 cells is concentration-dependent. **a** Cell viability of P815 cells treated with 100 μM $3OC_{12}$-HSL for 2–24 h. **b** Cell viability of P815 cells treated with different concentrations of $3OC_{12}$-HSL for 12 h. **c** Microscopic images (400×) of P815 cells after treatment with different concentrations of $3OC_{12}$-HSL for 12 h. Cell viability were measured by WST-1 assay. Data are expressed as means ± SD for three independent experiments performed in duplicated. *$P < 0.05$ compared with untreated control group. *White bar* indicates 25 μm

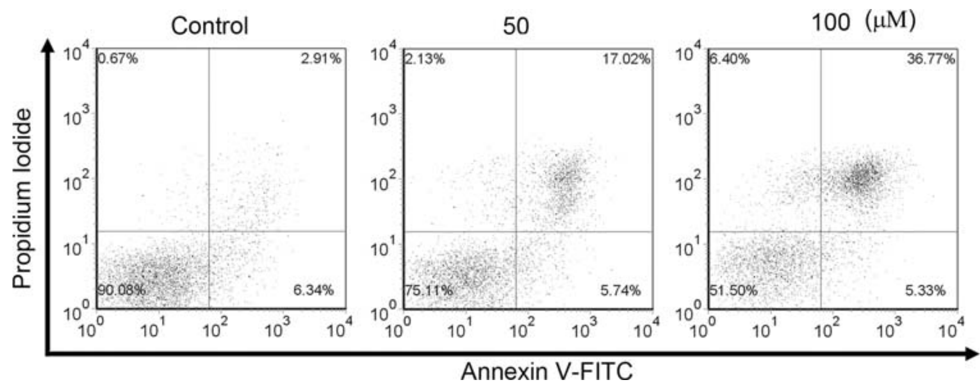


Fig. 3 $3OC_{12}$-HSL induces apoptosis in P815 cells. P815 cells were treated with or without 50 or 100 μM $3OC_{12}$-HSL for 4 h. Cells were stained with FITC-conjugated annexin V and propidium iodide (PI), and induction of apoptosis was measured by flow cytometry. Representative are shown for three independent experiments

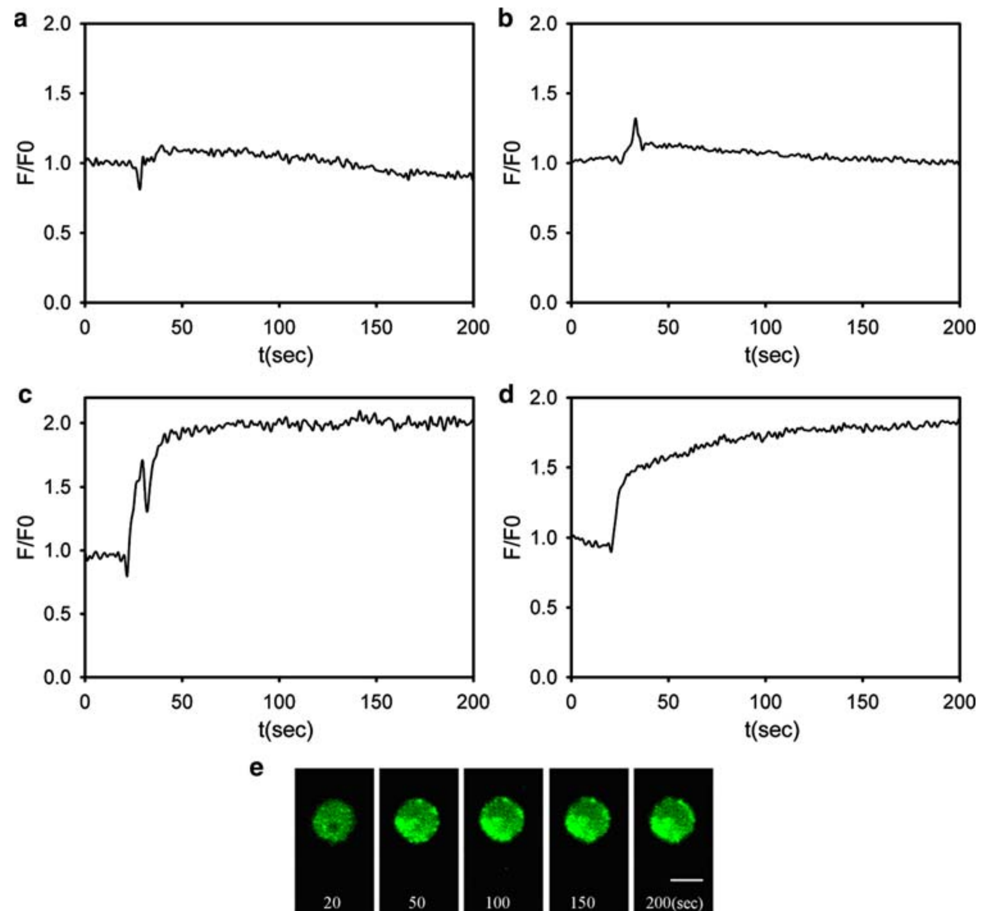


Fig. 4 Time-dependent changes of intracellular calcium in P815 cells in response to $3OC_{12}$-HSL. (**a**–**d**) Representative calcium traces are graphed over time after cells were differently treated. **a** Cells treated without DMSO or $3OC_{12}$-HSL. **b** Cells treated with DMSO. **c** P815 cells treated with DMSO dissolved $3OC_{12}$-HSL. **d** HMC-1 treated with DMSO dissolved $3OC_{12}$-HSL. **e** Representative confocal images (400×) of time-dependent changes of Fluo-3 signal in P815 cells in response to 100 μM $3OC_{12}$-HSL. *F* fluorescence at 515 nm, *F/F0* average fluorescence divided by background fluorescence. *White bar* indicates 10 μm

Effect of $3OC_{12}$-HSL on calcium signaling in mast cells

Calcium has long been recognized as a significant participant in cell apoptosis, and it is also an important second messenger associated with activation of mast cells [27, 28]. Moreover, $3OC_{12}$-HSL recently has been shown to induce calcium signaling linked apoptosis in a mouse embryonic fibroblast cell line named NIH3T3 [23]. We supposed a similar response may occur in mast cells. To address this, we investigated the effects of $3OC_{12}$-HSL on calcium signaling in P815 cells using a similar method described by Shiner et al. [23]. We observed a relatively milder calcium release manner, compare to those seen by Shiner's et al. [23], when P815 cells were treated with 100 μM $3OC_{12}$-HSL (Fig. 4c). Similar results were observed when HMC-1 were treated $3OC_{12}$-HSL (Fig. 4d).

Degranulation assay

Degranulation is an important cellular response that depends on calcium mobilization [27]. The rapid increase of intracellular calcium in P815 cells upon $3OC_{12}$-HSL stimulation led us to investigate whether $3OC_{12}$-HSL could have caused degranulation in mast cells. To explore this, we employed a human mast cell line HMC-1, which shows more similarities to classical mast cells than P815 cells does [29]. Interestingly, after 30-min incubation, we found that 100 μM $3OC_{12}$-HSL significantly increased histamine release in HMC-1 cells, but not in P815 cells (Fig. 5a). These different responses between two cell lines are probably because HMC-1 and P815 cells are derived from different species. However, the presence of relatively fewer degranulation in P815 cell than HMC-1 cells might explain these differences (Fig. 5b–d).

Cytokines release

Mast cell-derived IL-4 is a key cytokine that has been implicated in the development of Th2 cellular responses [30], whereas IL-6 is a multifunctional cytokine that is produced by activated mast cells, and it is a major proinflammatory cytokine that implicated in *P. aeruginosa* infections [31]. For further investigation, we selected IL-4 and IL-6 as markers of mast cell activation. We found IL-4 release neither induced nor inhibited release after $3OC_{12}$-HSL stimulation at both 12 and 24 h (Fig. 6a, b). No significant changes in IL-6 release were observed after $3OC_{12}$-HSL stimulation at 12 h (Fig. 6c). However, a significant

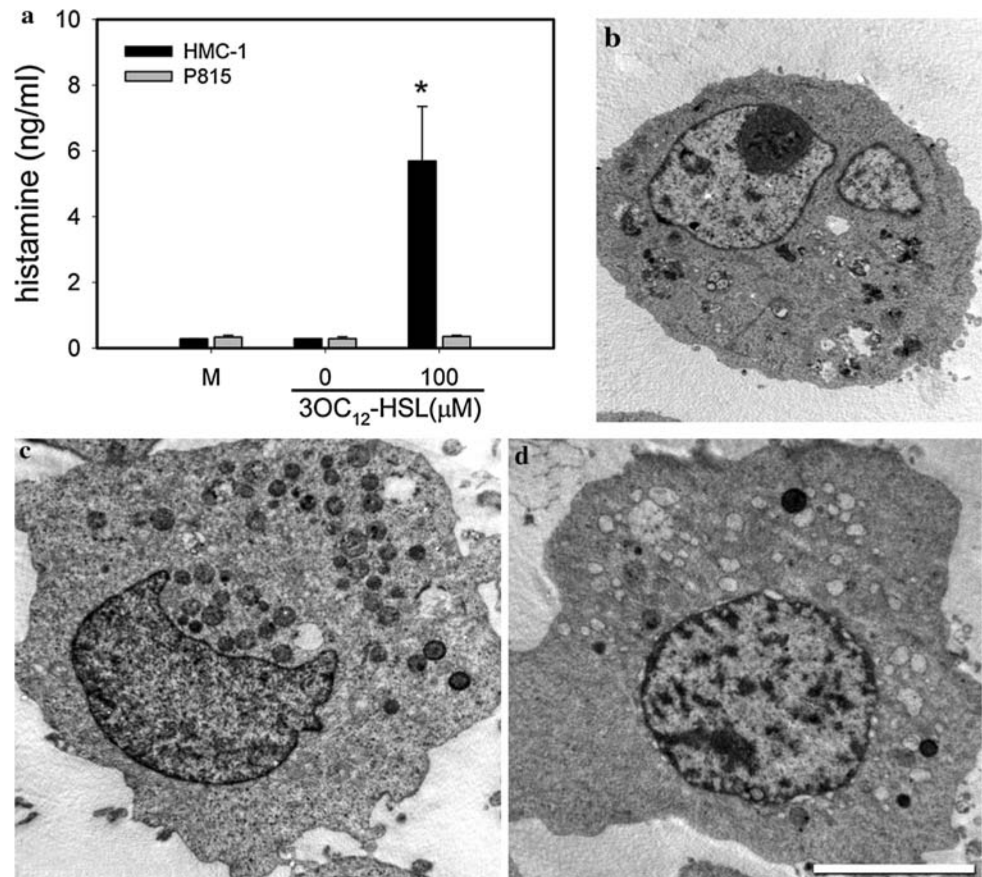


Fig. 5 Degranulation assay of MCs. **a** 100 μM $3OC_{12}$-HSL significantly increased histamine release by HMC-1 but not by P815 cells. Histamine was measured by ELISA kit. Data are expressed as means ± SD for three independent experiments performed in duplicates. *$P < 0.05$ compared with untreated control group. **b–d** Transmission electron micrograph of P815 and HMC-1 cells. **b**, **c** Relatively few granules were observed in P815 cells compared to HMC-1 cells. **d** Typical degranulation was observed in HMC-1 treated with $3OC_{12}$-HSL. *White bar* indicates 5 μm

increase of IL-6 release was found when P815 cells were treated with 6.25 and 12.5 μM $3OC_{12}$-HSL for 24 h (Fig. 6d). We found it interesting that IL-6 production steadily dropped when cells were treated with 25 μM $3OC_{12}$-HSL (Fig. 6d).

Discussion

Pseudomonas aeruginosa is a major cause of nosocomial infections frequently encountered in patients who are immunocompromised, mechanically ventilated, have malignancies or HIV infection, and it is also a leading cause of morbidity and mortality in cystic fibrosis (CF) patients [32, 33]. However, it rarely causes infection in the normal host [32, 33], strongly suggesting that the host immune status plays a critical role in *P. aeruginosa* infection.

Studies have shown that MCs provide important contributions to bacterial clearance and play a crucial role in *P. aeruginosa*-related infections [12, 13, 15, 34, 35]. Moreover, *P. aeruginosa* and *P. aeruginosa*-produced factors are proved to activate MCs and induce a variety of cytokines [36–39]. Therefore, it is reasonable and important to investigate the relationship between *P. aeruginosa* and MCs.

QS system is a cell-to-cell communication system between bacteria that has been extensively studied in *P. aeruginosa* [1, 2]. In vitro and in vivo, QS signals were required for virulence production because QS-mutant strains were nearly avirulent and induced relatively mild pneumonia compared to wild strain [40]. Recent evidence shows, however, that QS signals are not restricted to orchestrating the expression of virulence genes, but are also involved in the modulation of the host immune response [3, 25, 41–47]. The effects of the QS signal $3OC_{12}$-HSL to inhibit proliferation and induce apoptosis in macrophages and neutrophils strongly indicate that $3OC_{12}$-HSL may act as an independent pathogenic factor during infection [25, 48].

Cell apoptosis plays a central role in the balance between host defense and the invading pathogen in the normal phase of inflammation [49]. A wide range of bacterial components have been proved to induce apoptosis in MC, Jenkins et al. [36] demonstrated that the live, but not killed *P. aeruginosa* induces apoptosis in MC, indicating that the specific components responsible for inducing MC apoptosis might be the toxic elements released by live *P. aeruginosa*. Since QS signals are diffusible molecules secreted by *P. aeruginosa*, associated with growth status, and induce apoptosis in host cells [6], we supposed that the *P. aeruginosa* QS signal

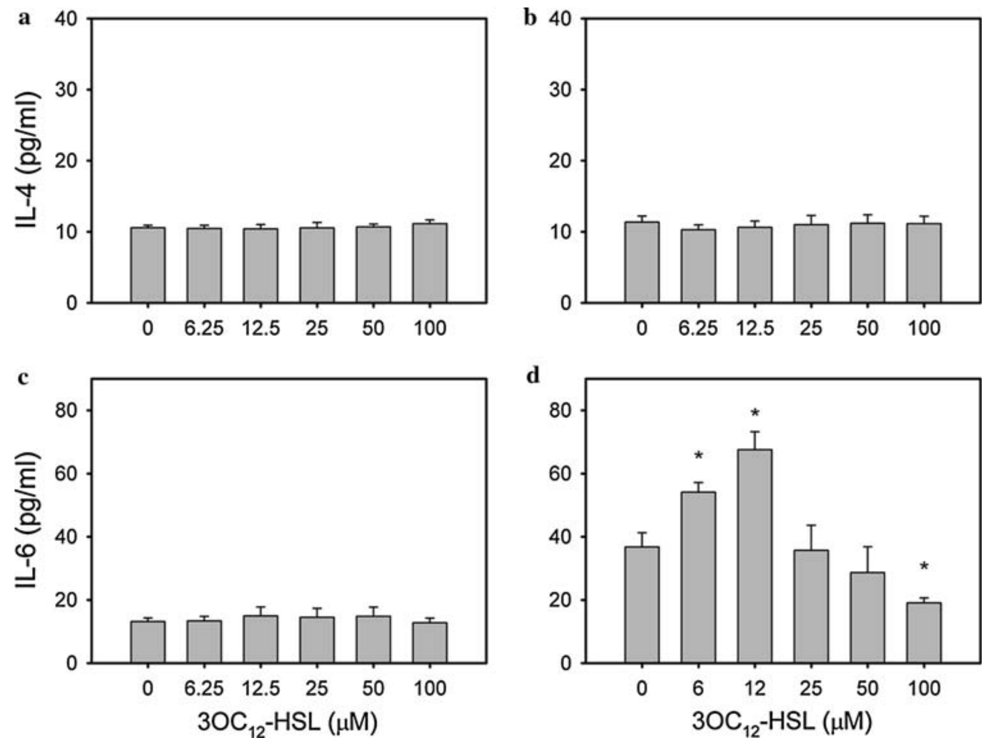


Fig. 6 $3OC_{12}$-HSL induces IL-4 and IL-6 release in P815 cells. **a**, **b** $3OC_{12}$-HSL did not induce nor inhibit IL-4 release after $3OC_{12}$-HSL stimulation at 12 and 24 h. **c**, **d** After 24 h incubation, $3OC_{12}$-HSL induces IL-6 release at low concentrations, but IL-6 release steadily drops at high concentration. Cytokines were measured by ELISA kit. Data are expressed as means ± SD for three independent experiments performed in duplicated. $*P < 0.05$ compared with untreated control group

$3OC_{12}$-HSL may also play a role in *P. aeruginosa*-induced apoptosis in mast cells. In our study, $3OC_{12}$-HSL at final concentration of 50 and 100 μM induced about 20 and 40% of apoptosis, respectively, in P815 cells (Fig. 3).

While the precise mechanism of $3OC_{12}$-HSL-induced mast cells apoptosis remained unknown, a line of studies indicated that $3OC_{12}$-HSL-mediated apoptosis occurred via at least two independent transduction pathways which included calcium signaling [23, 25]. In the present study, we discovered that $3OC_{12}$-HSL immediately induced an intracellular calcium increase of about 2-fold after $3OC_{12}$-HSL stimulation (Fig. 4c). However, direct evidence of a link between apoptosis and calcium mobilization in P815 cells stimulated with $3OC_{12}$-HSL still needs to be confirmed by further experiments. Since HMC-1 showed more representative characters than P815 (Fig. 5c), we preformed the same experiment in HMC-1. As expected, results were observed when HMC-1 were treated with $3OC_{12}$-HSL (Fig. 4d), suggesting that $3OC_{12}$-HSL has pro-apoptotic effects on HMC-1.

Several bacterial components are known to activate and cause degranulation in mast cells [11]. Since mast cell degranulation is dependent on calcium mobilization and we observed a rapid increase of intracellular calcium in $3OC_{12}$-HSL-stimulated mast cells, we tested the possible effect of degranulation in mast cells in response to $3OC_{12}$-HSL. In our experiments, we observed that P815 cells had relatively fewer granules than HMC-1 cells by transmission electron microscopy (Fig. 5b, c), which might help to explain why we found that $3OC_{12}$-HSL significantly increased the release of histamine in HMC-1 but not in P815 cells. In addition, P815 has showed lesser characteristic than classic mast cells, suggesting that P815 would not exhibit response as similar as matured one [50, 51]. However, we cannot exclude the possible explanation that these two cell lines differ because they are derived from different species.

In chronic *P. aeruginosa* infection, a Th1-dominated response was associated with a better lung function compared with that has a Th2-dominated response [52]. $3OC_{12}$-HSL has been speculated to shift the Th1 response to a Th2 response [41, 53]. Therefore, we investigated the influence of $3OC_{12}$-HSL on IL-4 production. In our experiments, we did not observe inducible IL-4 release in P815 cells stimulated with different concentrations of $3OC_{12}$-HSL at different time points. These results are consistent with Smith's work that demonstrated that $3OC_{12}$-HSL stimulation of T cells induces INF-γ (Th1 cell response) but not IL-4 (Th2 cell response) [43].

In a mouse *P. aeruginosa* keratitis model, IL-6-knockout mice showed more severe disease than wild-type mice, indicating that IL-6 is crucial to the host defense of *P. aeruginosa* [31]. Moreover, $3OC_{12}$-HSL was reported to induce IL-6 mRNA expression in mouse skin [3], where MCs are abundantly located. We discovered that 6-25 μM $3OC_{12}$-HSL significantly increased IL-6 release in MCs (Fig. 6d). Therefore, we speculate that MC-derived IL-6 may be involved in $3OC_{12}$-HSL-induced inflammation in mouse skin. However, up to 50 μM $3OC_{12}$-HSL result in a slight

drop of IL-6 release compared to that observed in the 25-μM group. The most likely explanation may be due to a cell viability suppression effect of $3OC_{12}$-HSL (Fig. 6d).

In conclusion, the present data demonstrate that the *P. aeruginosa* quorum sensing molecule $3OC_{12}$-HSL interacts with influences in MCs. It should be noted that the two cell lines we used in this study are immature mast cells, which may differ from mature MCs in their response to $3OC_{12}$-HSL [29, 51]. On the other hand, data reported by Shiner et al. showed that non-immortalized cells appear to be more sensitive to $3OC_{12}$-HSL than immortalized cell lines. According to these findings, we speculate that $3OC_{12}$-HSL might exhibit a relatively strong response in primary mature MCs than in the immature one used here. Therefore, further investigation of other mature MCs such as LAD2 or bone marrow derived MCs should be explored.

Acknowledgments This work was supported by National Natural Science Foundation of China (30571647), China National 973 project (MOST, 2007CB512900). We thank Professor Zhong, RQ (Changzheng Hospital, Shanghai, China) for kindly providing human mast cell line HMC-1.

References

1. de Kievit TR, Iglewski BH (2000) Bacterial quorum sensing in pathogenic relationships. Infect Immun 68:4839–4849. doi:10.1128/IAI.68.9.4839-4849.2000
2. Fuqua C, Parsek MR, Greenberg EP (2001) Regulation of gene expression by cell-to-cell communication: acyl-homoserine lactone quorum sensing. Annu Rev Genet 35:439–468. doi:10.1146/annurev.genet.35.102401.090913
3. Smith RS, Harris SG, Phipps R et al (2002) The *Pseudomonas aeruginosa* quorum-sensing molecule *N*-(3-oxododecanoyl) homoserine lactone contributes to virulence and induces inflammation in vivo. J Bacteriol 184:1132–1139. doi:10.1128/jb.184.4.1132-1139.2002
4. Davies DG, Parsek MR, Pearson JP et al (1998) The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280:295–298. doi:10.1126/science.280.5361.295
5. Hentzer M, Wu H, Andersen JB et al (2003) Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. EMBO J 22:3803–3815. doi:10.1093/emboj/cdg366
6. Shiner EK, Rumbaugh KP, Williams SC (2005) Inter-kingdom signaling: deciphering the language of acyl homoserine lactones. FEMS Microbiol Rev 29:935–947. doi:10.1016/j.femsre.2005.03.001
7. Hughes DT, Sperandio V (2008) Inter-kingdom signalling: communication between bacteria and their hosts. Nat Rev Microbiol 6:111–120. doi:10.1038/nrmicro1836
8. Kravchenko VV, Kaufmann GF, Mathison JC et al (2008) Modulation of gene expression via disruption of NF-kappaB signaling by a bacterial small molecule. Science 321:259–263. doi:10.1126/science.1156499
9. Bradding P, Walls AF, Holgate ST (2006) The role of the mast cell in the pathophysiology of asthma. J Allergy Clin Immunol 117:1277–1284. doi:10.1016/j.jaci.2006.02.039
10. Bischoff SC (2007) Role of mast cells in allergic and non-allergic immune responses: comparison of human and murine data. Nat Rev Immunol 7:93–104. doi:10.1038/nri2018
11. Metz M, Maurer M (2007) Mast cells—key effector cells in immune responses. Trends Immunol 28:234–241. doi:10.1016/j.it.2007.03.003
12. Siebenhaar F, Syska W, Weller K et al (2007) Control of *Pseudomonas aeruginosa* skin infections in mice is mast cell-dependent. Am J Pathol 170:1910–1916. doi:10.2353/ajpath.2007.060770
13. Song Z, Johansen HK, Faber V et al (1997) Ginseng treatment reduces bacterial load and lung pathology in chronic *Pseudomonas aeruginosa* pneumonia in rats. Antimicrob Agents Chemother 41:961–964
14. Henderson WR Jr, Chi EY (1992) Degranulation of cystic fibrosis nasal polyp mast cells. J Pathol 166:395–404. doi:10.1002/path.1711660412
15. Wu H, Song Z, Givskov M et al (2001) *Pseudomonas aeruginosa* mutations in lasI and rhlI quorum sensing systems result in milder chronic lung infection. Microbiology 147:1105–1113
16. Metz M, Grimbaldeston MA, Nakae S et al (2007) Mast cells in the promotion and limitation of chronic inflammation. Immunol Rev 217:304–328. doi:10.1111/j.1600-065X.2007.00520.x
17. Erickson DL, Endersby R, Kirkham A et al (2002) *Pseudomonas aeruginosa* quorum-sensing systems may control virulence factor expression in the lungs of patients with cystic fibrosis. Infect Immun 70:1783–1790. doi:10.1128/IAI.70.4.1783-1790.2002
18. Singh PK, Schaefer AL, Parsek MR et al (2000) Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature 407:762–764. doi:10.1038/35037627
19. Charlton TS, de Nys R, Netting A et al (2000) A novel and sensitive method for the quantification of *N*-3-oxoacyl homoserine lactones using gas chromatography-mass spectrometry: application to a model bacterial biofilm. Environ Microbiol 2:530–541. doi:10.1046/j.1462-2920.2000.00136.x
20. Rumbaugh KP (2007) Convergence of hormones and autoinducers at the host/pathogen interface. Anal Bioanal Chem 387:425–435. doi:10.1007/s00216-006-0694-9
21. Chhabra SR, Harty C, Hooi DS et al (2003) Synthetic analogues of the bacterial signal (quorum sensing) molecule *N*-(3-oxododecanoyl)-L-homoserine lactone as immune modulators. J Med Chem 46:97–104. doi:10.1021/jm020909n
22. Chun CK, Ozer EA, Welsh MJ et al (2004) Inactivation of a *Pseudomonas aeruginosa* quorum-sensing signal by human airway epithelia. Proc Natl Acad Sci USA 101:3587–3590. doi:10.1073/pnas.0308750101
23. Shiner EK, Terentyev D, Bryan A et al (2006) *Pseudomonas aeruginosa* autoinducer modulates host cell responses through calcium signalling. Cell Microbiol 8:1601–1610. doi:10.1111/j.1462-5822.2006.00734.x
24. Horikawa M, Tateda K, Tuzuki E et al (2006) Synthesis of *Pseudomonas* quorum-sensing autoinducer analogs and structural entities required for induction of apoptosis in macrophages. Bioorg Med Chem Lett 16:2130–2133. doi:10.1016/j.bmcl.2006.01.054
25. Tateda K, Ishii Y, Horikawa M et al (2003) The *Pseudomonas aeruginosa* autoinducer *N*-3-oxododecanoyl homoserine lactone accelerates apoptosis in macrophages and neutrophils. Infect Immun 71:5785–5793. doi:10.1128/IAI.71.10.5785-5793.2003
26. Li L, Hooi D, Chhabra SR et al (2004) Bacterial *N*-acylhomoserine lactone-induced apoptosis in breast carcinoma cells correlated with down-modulation of STAT3. Oncogene 23:4894–4902
27. Pearce FL (1985) Calcium and mast cell activation. Br J Clin Pharmacol 20(Suppl 2):267S–274S
28. Giorgi C, Romagnoli A, Pinton P et al (2008) Ca^{2+} signaling, mitochondria and cell death. Curr Mol Med 8:119–130. doi:10.2174/156652408783769571
29. Butterfield JH, Weiler D, Dewald G et al (1988) Establishment of an immature mast cell line from a patient with mast cell leukemia. Leuk Res 12:345–355. doi:10.1016/0145-2126(88)90050-1

30. Brown JM, Wilson TM, Metcalfe DD (2008) The mast cell and allergic diseases: role in pathogenesis and implications for therapy. Clin Exp Allergy 38:4–18
31. Cole N, Bao S, Stapleton F et al (2003) *Pseudomonas aeruginosa* keratitis in IL-6-deficient mice. Int Arch Allergy Immunol 130:165–172. doi:10.1159/000069006
32. Sadikot RT, Blackwell TS, Christman JW et al (2005) Pathogen-host interactions in *Pseudomonas aeruginosa* pneumonia. Am J Respir Crit Care Med 171:1209–1223. doi:10.1164/rccm.200408-1044SO
33. Koch C, Hoiby N (1993) Pathogenesis of cystic fibrosis. Lancet 341:1065–1069. doi:10.1016/0140-6736(93)92422-P
34. Malaviya R, Ikeda T, Ross E et al (1996) Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF-alpha. Nature 381:77–80
35. Echtenacher B, Mannel DN, Hultner L (1996) Critical protective role of mast cells in a model of acute septic peritonitis. Nature 381:75–77. doi:10.1038/381075a0
36. Jenkins CE, Swiatoniowski A, Power MR et al (2006) *Pseudomonas aeruginosa*-induced human mast cell apoptosis is associated with up-regulation of endogenous Bcl-xS and down-regulation of Bcl-xL. J Immunol 177:8000–8007
37. Sun G, Liu F, Lin TJ (2005) Identification of *Pseudomonas aeruginosa*-induced genes in human mast cells using suppression subtractive hybridization: up-regulation of IL-8 and CCL4 production. Clin Exp Immunol 142:199–205. doi:10.1111/j.1365-2249.2005.02909.x
38. Lin TJ, Maher LH, Gomi K et al (2003) Selective early production of CCL20, or macrophage inflammatory protein 3alpha, by human mast cells in response to *Pseudomonas aeruginosa*. Infect Immun 71:365–373. doi:10.1128/IAI.71.1.365-373.2003
39. Lin TJ, Garduno R, Boudreau RT et al (2002) *Pseudomonas aeruginosa* activates human mast cells to induce neutrophil transendothelial migration via mast cell-derived IL-1 alpha and beta. J Immunol 169:4522–4530
40. Pearson JP, Feldman M, Iglewski BH et al (2000) *Pseudomonas aeruginosa* cell-to-cell signaling is required for virulence in a model of acute pulmonary infection. Infect Immun 68:4331–4334. doi:10.1128/IAI.68.7.4331-4334.2000
41. Telford G, Wheeler D, Williams P et al (1998) The *Pseudomonas aeruginosa* quorum-sensing signal molecule *N*-(3-oxododecanoyl)-L-homoserine lactone has immunomodulatory activity. Infect Immun 66:36–42
42. Saleh A, Figarella C, Kammouni W et al (1999) *Pseudomonas aeruginosa* quorum-sensing signal molecule *N*-(3-oxododecanoyl)-L-homoserine lactone inhibits expression of P2Y receptors in cystic fibrosis tracheal gland cells. Infect Immun 67:5076–5082
43. Smith RS, Kelly R, Iglewski BH et al (2002) The *Pseudomonas* autoinducer *N*-(3-oxododecanoyl) homoserine lactone induces cyclooxygenase-2 and prostaglandin E2 production in human lung fibroblasts: implications for inflammation. J Immunol 169:2636–2642
44. Ritchie AJ, Jansson A, Stallberg J et al (2005) The *Pseudomonas aeruginosa* quorum-sensing molecule *N*-3-(oxododecanoyl)-L-homoserine lactone inhibits T-cell differentiation and cytokine production by a mechanism involving an early step in T-cell activation. Infect Immun 73:1648–1655. doi:10.1128/IAI.73.3.1648-1655.2005
45. Wagner C, Zimmermann S, Brenner-Weiss G et al (2006) The quorum-sensing molecule *N*-3-oxododecanoyl homoserine lactone (3OC12-HSL) enhances the host defence by activating human polymorphonuclear neutrophils (PMN) Anal Bioanal Chem
46. Zimmermann S, Wagner C, Muller W et al (2006) Induction of neutrophil chemotaxis by the quorum-sensing molecule *N*-(3-oxododecanoyl)-L-homoserine lactone. Infect Immun 74:5687–5692. doi:10.1128/IAI.01940-05
47. Wagner C, Zimmermann S, Brenner-Weiss G et al (2007) The quorum-sensing molecule *N*-3-oxododecanoyl homoserine lactone (3OC12-HSL) enhances the host defence by activating human polymorphonuclear neutrophils (PMN). Anal Bioanal Chem 387:481–487. doi:10.1007/s00216-006-0698-5
48. Kravchenko VV, Kaufmann GF, Mathison JC et al (2006) *N*-(3-oxo-acyl) homoserine lactones signal cell activation through a mechanism distinct from the canonical pathogen-associated molecular pattern recognition receptor pathways. J Biol Chem 281:28822–28830. doi:10.1074/jbc.M606613200
49. Hotchkiss RS, Dunne WM, Swanson PE et al (2001) Role of apoptosis in *Pseudomonas aeruginosa* pneumonia. Science 294:1783. doi:10.1126/science.294.5548.1783a
50. Lunderius C, Xiang Z, Nilsson G et al (2000) Murine mast cell lines as indicators of early events in mast cell and basophil development. Eur J Immunol 30:3396–3402. doi:10.1002/1521-4141(2000012)30:12<3396::AID-IMMU3396>3.0.CO;2-O
51. Ohtsu H, Kuramasu A, Suzuki S et al (1996) Histidine decarboxylase expression in mouse mast cell line P815 is induced by mouse peritoneal cavity incubation. J Biol Chem 271:28439–28444. doi:10.1074/jbc.271.45.28439
52. Moser C, Jensen PO, Kobayashi O et al (2002) Improved outcome of chronic *Pseudomonas aeruginosa* lung infection is associated with induction of a Th1-dominated cytokine response. Clin Exp Immunol 127:206–213. doi:10.1046/j.1365-2249.2002.01731.x
53. Hooi DS, Bycroft BW, Chhabra SR et al (2004) Differential immune modulatory activity of *Pseudomonas aeruginosa* quorum-sensing signal molecules. Infect Immun 72:6463–6470. doi:10.1128/IAI.72.11.6463-6470.2004