Role of quorum sensing by *Pseudomonas aeruginosa* in microbial keratitis and cystic fibrosis

M. D. P. Willcox, H. Zhu, T. C. R. Conibear, E. B. H. Hume, M. Givskov, S. Kjelleberg, and S. A. Rice

**Correspondence**
S. A. Rice
Scott.Rice@unsw.edu.au

1School of Optometry and Vision Science, University of New South Wales, Sydney, NSW 2052, Australia
2Institute for Eye Research, Sydney, Australia
3Vision CRC, Sydney, Australia
4BioScience and Technology, The Technical University of Denmark, DK-2800 Lyngby, Denmark
5School of Biotechnology and Biomolecular Sciences and The Centre for Marine Bio-Innovation, University of New South Wales, Sydney, NSW 2052, Australia

*Pseudomonas aeruginosa* is a ubiquitous bacterium that causes opportunistic infections in a range of host tissues and organs. Infections by *P. aeruginosa* are difficult to treat and hence there is interest in the development of effective therapeutics. One of the key mechanisms that *P. aeruginosa* uses to control the expression of many virulence factors is the N-acylated homoserine lactone (AHL) regulatory system. Hence, there is considerable interest in targeting this regulatory pathway to develop novel therapeutics for infection control. *P. aeruginosa* is the principal cause of microbial keratitis and of infections in cystic fibrosis (CF) sufferers, and AHL-dependent cell-to-cell signalling has been shown to be important for both infection types. However, keratitis tends to be an acute infection whereas infection of CF patients develops into a chronic, lifelong infection. Thus, it is unclear whether AHL-regulated virulence plays the same role during these infections. This review presents a comparison of the role of AHL signalling in *P. aeruginosa*-mediated microbial keratitis and chronic lung infections of CF patients.

**Introduction**

All evidence to date indicates that we are losing the race to combat what have been for the last 40 years simple bacterial infections. This is primarily due to the emergence of, and strong selection for, antibiotic-resistant bacteria, which has led to the current situation where many bacteria are so antibiotic resistant that patients can only be treated with what are considered to be drugs of last defence. A contributing factor in this steady failure of current drugs has been the strategy of making simple modifications of existing classes of antimicrobials to maintain a drug’s activity over the short term. This process is no longer working, and what needs to be done is to identify and develop new drug targets. This will take significant effort and may require a paradigm shift in thinking about how we deal with pathogenic bacteria, and the development of therapeutics that are highly specific for bacterial groups or even individual organisms. However, such outcomes should not be seen as limitations, but as benefits, reducing the likelihood of general resistance developing. Furthermore, bacterial control does not necessarily require bactericidal activity; it may be sufficient to target genetic pathways that are essential for virulence or the infection process, but that are otherwise non-essential systems. One such system is the acylated homoserine lactone (AHL) quorum sensing (QS) system found in a range of pathogenic Gram-negative bacteria, e.g. *Pseudomonas aeruginosa*, *Burkholderia cepacia* and *Serratia marcescens*. *P. aeruginosa* is of particular interest because it forms biofilms that contribute to the infection process and many of its virulence factors, including biofilm development, are QS regulated. Furthermore, it causes infections in wounds, eyes and lungs (both chronic and acute). In fact, *P. aeruginosa* infection is the most frequent cause of mortality in cystic fibrosis sufferers and is the principal aetiological agent of microbial keratitis. Thus, if the QS system of *P. aeruginosa* can be manipulated, it may be possible to control the virulence of and infection by this aggressive pathogen.
This review presents a comparison of the role of QS in two infection types, microbial keratitis and chronic lung infections. Although these infection types appear to be distinct, one being characterized as an acute infection and the other typically more chronic in nature, they share some common features. Both sites of infection are mucosal tissues that have limited microbial flora in the absence of infection. *P. aeruginosa* is one of the most important infecting bacteria for each tissue, and QS plays a role in the infection process in both cases. The QS system of *P. aeruginosa*, QS-regulated virulence factors and the effect of QS signals on the host are presented as background to the subsequent sections that specifically relate to QS in microbial keratitis and chronic lung infections.

**QS as a global regulator of gene expression**

Control over gene transcription is crucial to efficient cell function: the regulation of genes prevents unnecessary transcription and therefore conserves resources. QS is a global regulatory system that controls the expression of numerous genes and phenotypes, and is governed by the secretion and detection of signal molecules. When the QS signals reach a threshold concentration they bind to specific receptor proteins, which then initiate transcription of the QS-controlled genes. In this way, the majority of the bacterial population is able to simultaneously express a specific phenotype. Both Gram-positive and Gram-negative species utilize various signal molecules to mediate cell-to-cell communication (Kleerebezem et al., 1997; Pearson et al., 1994; Preston et al., 1997). As noted above, the AHL QS system is present in many Gram-negative proteobacteria and has been identified in a range of human, animal and plant pathogens. Of these organisms, *P. aeruginosa* is one of the most intensively studied due to its significant impacts on human health and inherent difficulty to treat. DNA microarray studies have demonstrated that QS controls the expression of somewhere between 3 and 10 % of the genome of *P. aeruginosa* (Hentzer et al., 2003; Schuster et al., 2003; Wagner et al., 2003); many of these genes either directly mediate the pathogenicity of *P. aeruginosa*, or play significant roles in the persistence of the organism during infection, mainly by contributing to the formation of biofilms that are able to withstand the action of the immune system.

There are two hierarchically arranged QS circuits within *P. aeruginosa* that are able to respond to AHL signals. The primary system is the Las system, which encodes the proteins LasI and LasR (Gambello & Iglewski, 1991). The LasI protein catalyses the production of the AHL molecule N-3-oxododecanoyl-3-Homoserine lactone (3O-C12-HSL) (Pearson et al., 1994). The 3O-C12-HSL molecule docks with the DNA-binding transcription regulator LasR, which allows LasR to bind to the promoters of QS-regulated genes to control virulence factor production, including such genes as lasB (elastase), lasA (staphylolysin), aprA (alkaline protease), toxA (exotoxin A), hcnABC (hydrogen-cyanide synthase) and lasI itself (Chapon-Herve et al., 1997; Gambello & Iglewski, 1991; Gambello et al., 1993; Passador et al., 1993; Pessi & Haas, 2000; Seed et al., 1995; Storey et al., 1998; Toder et al., 1991, 1994). The Las circuit induces a positive feedback loop to produce more AHL and also induces a secondary QS circuit, the Rhl system (Pearson et al., 1995; Seed et al., 1995).

The Rhl system consists of RhlI, which synthesizes N-butyryl-3-Homoserine lactone (C4-HSL) (Brint & Ohman, 1995; Pearson et al., 1997) and the receptor, RhlR (Ochsner et al., 1994; Pearson et al., 1995). As with the Las system, C4-HSL accumulates to a sufficient concentration and binds to RhlR. The Rhl system induces expression of rhlAB (rhamnolipid synthesis genes), rhlI, lasB, rpoS (the stationary-phase sigma factor), lecA (type-I lecin), lecB (type-II lecin), hcnABC and genes involved in pyocyanin production (Brint & Ohman, 1995; Latifi et al., 1995; Ochsner et al., 1994; Pearson et al., 1997; Pessi & Haas, 2000; Winzer et al., 2000).

The *Pseudomonas* quinolone signal (PQS) is a third *P. aeruginosa* QS signal that is dependent on the balanced production of 3O-C12-HSL and C4-HSL (Pesci et al., 1999). The PQS molecule (2-heptyl-3-hydroxy-4-quino-lone) plays a significant role in the transcription of Rhl-dependent *P. aeruginosa* virulence genes such as those encoding production of pyocyanin and rhamnolipid (Deziel et al., 2004). PQS production is intimately linked to the QS hierarchy, with its production and bioactivity requiring both the las and rhl QS systems (McGrath et al., 2004). Additionally, PQS was recently shown to be solubilized by rhamnolipids, the production of which is controlled by the Rhl system (Wade et al., 2005), which may be important for the activity of PQS as an extracellular signal. Thus, it is clear that QS controls a significant proportion of the arsenal of virulence factors used by *P. aeruginosa* to mediate infection. This dependence on QS as a key regulator of virulence factor production may represent the Achilles heel of *P. aeruginosa*, which can be exploited to control infection.

Additional genes can influence the QS response. For example, the QS regulator (QscR) was discovered as a repressor of 3O-C12-HSL-regulated virulence factors; it ensures that the QS cascade is not activated prematurely within a host, or in environments where it is not required (Chugani et al., 2001). This inhibitory effect is controlled by the global activator protein GacA (Ledgham et al., 2003; Reimmann et al., 1997). RsaL, the product of a gene found between lasI and lasR in the *P. aeruginosa* PA01 genome, is a negative regulator of the Las QS circuit (de Kievet et al., 1999). The product of the qrs gene is a cAMP receptor homologue that is required for the transcription of lasR (Albus et al., 1997; West et al., 1994). RsmA has been shown to downregulate several QS-dependent phenotypes in *P. aeruginosa*, such as protease, elastase and staphylolytic activities along with the production of a cytotoxic lectin, hydrogen cyanide and pyocyanin. Overexpression of RsmA
also resulted in reduced expression of the AHL synthase genes lasI and rhlI (Pessi et al., 2001). Furthermore, it has been shown that RpoN controls the expression of rhlI (Heurlier et al., 2003; Thompson et al., 2003).

The role of QS in microbial keratitis

Microbial keratitis (MK) is a significant disease with annualized incidence rates during contact lens wear of up to 19/10,000 people per year (Stapleton et al., 2007). With 80–140 million contact lens wearers worldwide, this represents a significant risk to sight. MK is a frank infection of the cornea characterized by replicating microbes in the tissue and infiltration of the tissue by white blood cells, especially polymorphonuclear leukocytes (PMNs), and can lead to eventual loss of sight. Wearing contact lenses is a primary risk factor for MK, and *P. aeruginosa* remains the most common cause of infection, accounting for around 40–70 % of isolates (Willcox et al., 2002).

Identification of QS signals and regulated products from clinical specimens and isolates

All ocular isolates from cases of infectious MK contain lasI, lasR, rhlI and rhlR (Zhu et al., 2004) and the majority produce C4-HSL, 3O-C12-HSL and C6-HSL (Zhu et al., 2002). In contrast, of the *P. aeruginosa* isolates from non-infective events from the eye, just under half appeared to have lost one or more las or rhl genes, and two that had lost the rhlI gene had also lost the rhlAB gene (Zhu et al., 2004). Furthermore, invasive strains of *P. aeruginosa* (exoS-positive) isolated from keratitis patients produced more AHL signal molecules, in terms of types and concentrations, compared with the amount produced by cytotoxic strains (Zhu et al., 2002).

In MK, lasI or rhlI QS-deficient strains are not able to induce corneal infection in the mouse model (Fig. 1; Zhu et al., 2004), highlighting the importance of QS systems, particularly the possession of functional lasI or rhlI genes. The virulence of the mutants was fully restored by complementation with a functional lasI or rhlI gene, respectively (Fig. 1; Zhu et al., 2004). However, single mutations in either lasR or rhlR mutants did not show statistically significant reductions in virulence in the eye, despite there being a reduction in bacterial numbers in the eye with the lasR mutant (Zhu et al., 2004). These results are in agreement with a previous report where there was no significant difference reported between the 50 % infective doses of PAO1 and its lasR mutant during corneal infection (Preston et al., 1997). It is also possible that the AHL signals (especially 3O-C12-HSL) may make a significant contribution to virulence, e.g. through immune modulation.

QS-regulated virulence factors involved in MK

The *P. aeruginosa* virulence factors most often associated with ocular damage are exoenzymes S (encoded by exoS) and U (exoU; Fleiszig et al., 1997), elastase (lasB; Kessler & Blumberg, 1987), alkaline protease (*aprA*; Twining et al., 1993) and protease IV (*prpI*; Engel et al., 1997; O’Callaghan et al., 1996; Traidej et al., 2003). There is good evidence that QS is involved in the upregulation of the genes lasB, aprA and prpI and it may be that the Las system is particularly involved in this activation (Nouwens et al., 2003). The Rhl system has been shown to repress the production of exoS (Hogardt et al., 2004). There is currently no evidence for a direct role of QS in regulation of exoU, but the Rhl system can downregulate the type III secretion system in the absence of Ca^{2+} (Bleves et al., 2005). Therefore, a functional Rhl system would likely result in the reduced secretion of ExoU and other type III secretion system toxins such as ExoT and ExoS, and thus reduce pathogenicity associated with these factors. Perhaps initial infection by small numbers of cells is not QS dependent and so ExoU/ExoS/ExoT are active and the bacterial cells can disrupt host cells (either via invasion or a cytotoxic effect). The type III toxins are then silenced during later stages of infection when larger numbers of cells are present and action of proteases is perhaps more important for cell survival. While the role of QS and type III secretion remains to be elucidated, there is clear evidence that other QS-controlled virulence factors, such as lasB, are important for eye infections.

Fig. 1. Infection rate in mice challenged with wild-type and QS mutants of *P. aeruginosa*. The signal-deficient strain, JP-1, was complemented with a functional copy of the lasI gene carried on a plasmid (PAO-JP1+lasI). Bacterial cells (5×10^6 c.f.u.) were inoculated into the scratched surface of the eye in BALB/c mice. Twenty-four hours after challenge, mice (the number of mice for each group, n, is indicated along the x-axis) were examined for infection using a slit lamp biomicroscope. The percentage of infected mice in each group is presented on the y-axis. A two-sided Fisher’s Exact test was used to analyse the statistical difference in infection rate between groups. *P<0.05 compared to other test strains.
AHL effects on the ocular immune response

The lack of infection caused by lasI mutants could be due to direct effects of 3O-C12-HSL, which can stimulate an immune response (Smith et al., 2002) or disrupt epithelial cells' barrier function (Vikstrom et al., 2006). It is often the host immune response which results in severe corneal damage, including perforation of the globe and blindness (Hazlett et al., 1992; Hazlett, 2005; Laibson, 1990). Corneal epithelial cells with intact barrier function appear to be resistant to infection by P. aeruginosa (Hazlett et al., 1978, 1980). Therefore if 3O-C12-HSL is able to alter the barrier function of corneal epithelial cells, as has been demonstrated for colon cells, it may provide an entry into the cornea for the bacterium and thus the AHLs could represent virulence factors in their own right. This may partially explain the observation that strains lacking the lasI AHL synthase have reduced virulence, while lasR mutants are only minimally affected in virulence. However, the apparent lack of immune stimulation by C4-HSL (Shiner et al., 2006; Smith et al., 2002; Zimmermann et al., 2006) argues that the loss of virulence observed for the rhlI mutant is likely to be dependent on a mechanism other than direct effects of the C4-HSL on the immune system. Interestingly, it has been reported that interferon-γ (IFN-γ) can be sensed by the outer-membrane protein OprF of P. aeruginosa, which results in the expression of QS-controlled genes (Wu et al., 2005), and this links QS to the Th1/Th2 dichotomy involved in MK. The Th1 response is associated with high levels of IFN-γ and susceptibility of the cornea to perforation following infection with P. aeruginosa, whereas the Th2 response is associated with resistance to perforation (Hazlett et al., 2000; Hazlett, 2005). Thus the increased pathology seen in Th1/IFN-γ-producing mice might be caused not only directly by the immune response, but indirectly by the immune response upregulating virulence factors of P. aeruginosa by stimulating their production through the AHL pathway(s).

QS in chronic lung infections

Cystic fibrosis (CF) affects approximately 70 000 people per year worldwide, and although the disease affects multiple organs, the most common cause of mortality is bacterial infection of the lungs, caused by P. aeruginosa. Despite aggressive antibiotic intervention, clearance of P. aeruginosa infections from CF sufferers is rare and most patients are persistently colonized by this bacterium, suggesting that antibiotic treatments are not effective.

Identification of QS signals and regulated products from clinical specimens and isolates

It has been well established that AHL-mediated QS is active in the lungs of CF patients. For example, ARLs have been detected in sputum samples and lung extracts of CF patients (Erickson et al., 2002; Geisenberger et al., 2000) and it has been shown that QS-regulated genes are upregulated in CF samples (Storey et al., 1998). Analysis of a clonal lineage of P. aeruginosa from CF patients in Australia showed that all strains contained genes rhlI/rhlR and 42/43 strains retained genes lasI/lasR (Tingpej et al., 2007). QS has also been shown to affect biofilm tolerance to antibiotics (Davies et al., 1998; Hentzer et al., 2003), and the ratio of C4-HSL to 3O-C12-HSL detected in the lungs has been used to support the hypothesis that P. aeruginosa is present in the lungs in a biofilm (Singh et al., 2000). Given the high numbers of P. aeruginosa reported in sputum, ranging from 10^6 to 10^{10} c.f.u. ml^{-1} (Giwercman et al., 1990), it is not surprising that QS is active or that biofilms form in the lungs of CF patients. The quinolone signal, PQS, can also be detected in the lungs of CF patients (Collier et al., 2002), further supporting an active role of QS in chronic lung infections.

In vivo studies of acute and chronic lung infections using QS mutants have shown that QS signal synthase mutants (either mutations in lasI or rhlI or double mutants) were less virulent than the isogenic wild-type parental strains (Hoffmann et al., 2005; Pearson et al., 2000; Smith et al., 2002; Wu et al., 2001). It has also been demonstrated that loss of the HSL receptor genes (lasR/rhlR) results in strains that are less able to colonize and infect the lungs. As shown in Fig. 2, there was a significant reduction in the number of bacteria recovered from the lungs of mice infected with a QS mutant (ΔlasR-rhlR) of P. aeruginosa compared to the isogenic wild-type (Bjarnsholt et al., 2005). Furthermore, an infection model...
based on the implantation of silicone chips pre-colonized with *P. aeruginosa* into lungs of mice has also demonstrated that a functional QS system is critical for the establishment of infection (Christensen *et al.*, 2007). However, a number of studies have demonstrated that QS mutants can be isolated from CF samples. Frequencies of QS mutants ranged from 3% (6 out of 200 isolates) to 50% (3 of 6 isolates tested; reviewed by Heurlier *et al.*, 2006). Whole-genome sequencing of early- and late-stage isolates of *P. aeruginosa* from a CF patient showed that lasR was inactivated in the late-stage infection isolate (Smith *et al.*, 2006). In a follow-up study, comparing isolates from the same patient over an 8 year period, lasR mutants could be detected in isolates obtained after approximately 2 years of infection (D’Argenio *et al.*, 2007). However, wild-type strains could also be detected until approximately 5 years post-infection, after which time all of the isolates tested carried a single lasR mutation, suggesting that loss of LasR occurs over time (D’Argenio *et al.*, 2007). The clonal lineage of *P. aeruginosa* strains from Australia, whilst retaining QS genes, often do not produce the QS signals, at least in *vitro* (Tingpej *et al.*, 2007). One of the interesting observations from these studies has been that the majority of mutations leading to loss of QS have been found in lasR, with few mutations in the AHL synthase genes (*lasI* or *rhlI*). The explanation for the predominance of lasR mutants that are isolated from long-term infected CF patients remains elusive. It has been proposed that loss of LasR might confer an adaptive or growth advantage in the lung, especially when the bacteria are growing on amino acids as carbon sources (D’Argenio *et al.*, 2007). Alternatively, it is possible that the lasR mutants are over-represented in the dispersal population and hence are more likely to be observed. The lower frequency of isolation of rhl mutants may indicate that this part of the QS system plays an important role in long-term infection or colonization, which may be mediated through the role of the rhl system in regulating rhamnolipid production (see below). Thus, the loss of QS in chronic lung infections may reflect a change in strategy away from virulence and more towards persistence (Smith *et al.*, 2006), where an established strain, in a biofilm, may no longer require or benefit from the production of high levels of virulence factors. This latter concept may partly explain the difference in appearance of QS mutants between eye infections and lung infections. For MK (above), all clinical isolates tested, where pathology was noted, were QS competent, and QS mutants were only detected in isolates from eyes without infections. Thus, infection of the eye may represent a more acute infection, dependent on the expression of specific virulence factors, whilst lung infection selects for strains with a long-term maintenance strategy, where high levels of virulence factors are not produced after the initial acute phase and colonization.

**QS-regulated virulence factors involved in lung infections**

Interestingly, some of the same QS-regulated virulence factors that are important for infection in the eye are similarly important for chronic infection in the lung. The Australian clonal strains of *P. aeruginosa* all possess the genes *vfr, lasA, lasB, aprA, rhlAB* and *exoS* (Tingpej *et al.*, 2007). Shaver & Hauser (2004) demonstrated that for acute lung infections, mutants deficient in the production of ExoS or ExoU were less virulent than their isogenic wild-type, and the *exoU* mutant was the least virulent of the two. Strains isolated from patients carried either *exoU* or *exoS*, but not both, and CF isolates were more frequently *exoS* positive (Feltman *et al.*, 2001; Tingpej *et al.*, 2007). This latter finding contrasts with isolates from MK, where there is an approximately equal likelihood of isolating either *exoS-* or *exoU*-containing strains (Zhu *et al.*, 2006). As noted above, the QS system has been shown to regulate the production of the type III secretion system (Bleves *et al.*, 2005; Hogardt *et al.*, 2004), but does not appear to directly control expression of the effector proteins, such as ExoS or ExoU. Thus, the role of QS in the secretion of these toxins is indirect. The frequency of strains that are capable of type III mediated secretion appears to decrease as the length of time of CF infection increases: in one study approximately 49% of isolates from recently infected patients were positive compared to 18% for isolates from chronically infected patients (Jain *et al.*, 2004). Corech *et al.* (2005) reported that type III secretion components could be detected during early infection in children, suggesting a role for type III secretion during the initial colonization stages of infection. Antibodies directed at proteins involved in the type III secretion pathway were protective to the host (Sawa *et al.*, 1999), and hence it may be to the long-term benefit of colonizing strains to reduce expression of these virulence factors; this again supports the hypothesis that chronic lung infection may reflect a shift away from overt virulence towards one of quiet persistence. In contrast, Moss *et al.* (2001) detected antibodies for ExoS and the type III secretion system in chronically infected patients (Moss *et al.*, 2001). Thus, it remains to be determined whether QS regulation of the type III secretion system is an important factor determining chronic infection in CF patients. It should be noted, however, that there is evidence that QS-regulated genes are important in lung infection. Erickson *et al.* (2002) demonstrated that the QS-regulated gene *lasB*, encoding the virulence factor elastase (LasB), was expressed in the lungs of chronically infected patients. This observation is supported by data showing that elastase activity is commonly detected in the lungs of CF patients and that elastase mutants were less virulent (Azghani *et al.*, 2000). The QS-regulated virulence factor pyocyanin is present in relatively high concentrations in the sputum of infected patients, contributing to the greenish colour of such sputa, and acts to inhibit the activity of cilia in the respiratory mucosa (Wilson *et al.*, 1988). Mutants defective in pyocyanin production were more effectively cleared from the lungs of infected mice (2–3 log reduction) than the wild-type (1.4 log increase) (Lau *et al.*, 2004). In addition to directly causing tissue destruction, elastase also protects *P. aeruginosa* during infection by degrading immunoglobulins and complement proteins (Bainbridge
& Fick, 1989; Cripps et al., 1995). Another class of QS-controlled product that affects the immune system is rhamnolipids, bioactive molecules with surfactant properties, synthesized by RhlAB, with the most abundant being L-rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate (rhamnolipid 1) and 2- O-α-L-rhamnopyranosyl-α-L-rhamnopyranosyl-β-hydroxydecanoyl-β-hydroxydecanoic acid (rhamnolipid 2, also referred to as rhamnolipid B by Jensen et al., 2007; Lang & Wullbrandt, 1999; Maier & Soberon-Chavez, 2000). Although rhamnolipids were initially proposed to play a role in maintaining void spaces between microcolonies or for biofilm dispersal in vitro, we have subsequently shown that PMNs are unable to eradicate bacterial cells within P. aeruginosa biofilms in vitro (Bjarnsholt et al., 2005). This is associated with a QS-regulated phenotype, most notably with the production of rhamnolipid 2 (Jensen et al., 2007) and we have recently suggested that rhamnolipid 2 forms a protective biofilm shield against incoming PMN neutrophils in vivo (Jensen et al., 2007). This model is corroborated by recent data showing that a rhlA mutant is more easily cleared from a pulmonary infections (M. V. Gennip and others, unpublished). In contrast, rhlA/B appear to play no role during MK infection (Zhu et al., 2004).

**Immune effects of QS signals and regulated virulence factors**

3O-C12-HSL appears to induce apoptosis in neutrophils and macrophages, highlighting that this signal may have dual roles as a global regulator of gene expression in P. aeruginosa and as a virulence factor, directed at the host immune system. It is particularly interesting to note that 3O-C12-HSL, but not HSL signals with shorter side chains such as C4-HSL, acts as a chemoattractant for PMNs (Zimmermann et al., 2006). The selective advantage of luring the PMNs to the infection site and their subsequent killing is not clear, but it is possible that the lysed PMNs are used as a nutrient source or that their release of oxidative radicals or degradative enzymes might cause local tissue damage which could aid in the subsequent dissemination of P. aeruginosa. This possibility is supported by the observation that infected CF patients have been reported to have elevated levels of H2O2 in their breath, which has been linked to high levels of oxidative burst due to cell-mediated defences (Jobsis et al., 2000). Thus it is clear that a significant component of this aggressive pathogen’s arsenal of virulence factors is directed at the host immune response. In this regard, effects on the host defence systems may be the dominant mechanism behind its ability to persist. Thus, the normally well-prepared host is subverted by the invader, and either the host’s defensive protocols are manipulated in favour of the pathogen, by modifying the Th1-Th2 response, or the immune response may be blocked by degradation of antibodies (elastase) or the killing of PMNs (rhamnolipid), or the cells within the biofilm may simply not be accessible to immune attack. In this respect, it has been suggested that most of the damage to the lung is due to the host’s frustrated immune system attack on the biofilm and not virulence factor production per se (Hoiby et al., 2001). This may be particularly relevant to both eye and lung infections, where 3O-C12-HSL has been shown to attract PMNs. Both infections are characterized by marked infiltration of PMNs, and these have been linked to localized tissue destruction.

**QS inhibition for infection control**

The link between QS and virulence factor production in P. aeruginosa strongly argues that the inhibition of QS should control infection by this pathogen. There are a number of strategies for targeting QS control, which include sequestering or degrading the AHL signal, inhibiting the AHL synthase, or blocking the receptor–ligand interaction. In vitro studies using antibodies directed against AHLs reduced the activation of bioassay strains, but it remains to be determined whether this strategy will work in vivo to control P. aeruginosa infection (Smith & Iglewski, 2003). Similarly, Wang et al. (2007) have shown in vitro that enzymes which degrade AHLs can reduce biofilm and virulence factor production, but this also remains to be demonstrated in vivo (Wang et al., 2007). It is of interest to note that antibodies directed at AHLs can provide some protection in vivo (Miyairi et al., 2006). Although at least part of that effect was attributed to a reduction in 3O-C12-HSL-mediated apoptosis of neutrophils, such antibodies may also mediate a protective effect by preventing the more general changes to the host immune response (described above) or by silencing the QS response. A strategy that uses QS antagonists to block the native signal from interacting with the receptor has also been shown in vitro to control biofilm development (Hentzer et al., 2002), to inhibit virulence factor expression (Hentzer et al., 2002; Rasmussen et al., 2005), to reduce biofilm toxicity and remove the protective shield to PMNs (Bjarnsholt et al., 2005) and to make the biofilm more sensitive to antibiotic treatment (Bjarnsholt et al., 2005; Hentzer et al., 2003). Furthermore, this strategy has been shown to work in animal models of lung infections, demonstrating the utility of targeting the QS system to control these chronic infections (Bjarnsholt et al., 2005; Hentzer et al., 2003; Wu et al., 2001, 2004).

**Concluding remarks**

Certain QS factors or QS-controlled virulence factors are involved in both ocular and lung infections, yet others appear to be important in only one condition (Fig. 3). For example, alkaline protease, elastase and exoenzyme S are virulence factors that play roles in the infection of both eyes and lungs. In contrast, while there is strong evidence that rhamnolipids and pyocyanin play roles in chronic lung infection, there is no similar evidence that they play roles during infection of the eye. Loss of las and/or rhl QS genes
renders *P. aeruginosa* strains non-infective in the eye, yet when it occurs after initial infection of the lung, appears to allow a state of chronic infection to occur. QS mutants are rarely isolated from MK, whilst *lasR* and *rhlR* mutants are commonly isolated from the lungs of infected patients. This may reflect different strategies for infection in the two diseases, where MK is favoured by an acute, aggressive infection, whilst chronic lung infections are geared for persistence and hence are characterized by lower expression levels of virulence factors and an increased dependence on biofilm formation and maintenance. Single mutations in signal synthase genes lead to reduced virulence in animal models, while at least for the eye, both signal receptors must be lost to affect virulence. Chronic lung isolates are commonly *lasR* mutants. Thus it is possible that the receptors are required for initial infection, but are dispensable for long-term infection. Immune modulation makes a significant contribution to infection (Fig. 3), where *P. aeruginosa*, via the secreted HSL signal, can alter the host response to favour an inflammatory response. In addition, much of the damage to the host appears to be the result of an inflammatory response. It will be intriguing to further evaluate the role of QS in these infection types to elucidate how QS manifests itself to result in these distinct infections. One possibility may be related to the role of accessory regulators, noted above, that modulate the QS response. Thus, tissue-specific cues, such as local nutrients or oxygen levels, may play important roles in QS modulation. Despite the differences noted, it is likely that ways of controlling QS would be effective in controlling/treating both types of infections. There have been several suggestions for achieving QS control as a therapeutic. For example, it has been proposed to remove the signal, either through the application of AHL-degrading enzymes, acylases or lactonases (Dong et al., 2001; Xu et al., 2003), or by using monoclonal antibodies to bind to and sequester the signal (Smith & Iglewski, 2003). Alternatively, it may be possible to develop inhibitors that block the signal–receptor interaction and in this way, control the QS response (Rice et al., 2005; Smith et al., 2003). There may yet be many more unidentified strategies for controlling the QS response with a view to controlling infections that are QS dependent.

**Acknowledgements**

This work was supported by a project grant (350900) from the Australian National Health and Medical Research Council to M. D. P. W., E. B. H. H., S. A. R. and S. K., and by a grant from the Danish Strategic Research Council to M. G.

**References**

Albus, A. M., Pesci, E. C., Runyen-Janecky, L. J., West, S. E. H. & Iglewski, B. H. (1997). Vfr controls quorum sensing in *Pseudomonas aeruginosa*. J Bacteriol 179, 3928–3935.

Azghani, A. O., Bedinghaus, T. & Klein, R. (2000). Detection of elastase from *Pseudomonas aeruginosa* in sputum and its potential role in epithelial cell permeability. Lung 178, 181–189.

---

**Fig. 3.** Model for QS involvement in acute or chronic infection of the eye or lung, respectively. QS-regulated virulence factors that are important for infection of either eyes or lungs are shown in green. Many of the virulence factors are the same for both infections, although to date, biofilm formation, rhamnolipid production and pyocyanin production appear to be exclusively involved in lung infections. Shown in red are the effects of the QS AHL signal on the host response. Both infections show significant infiltration by PMNs, which contribute to host destruction by lysis and subsequent release of reactive oxygen species.
Bainbridge, T. & Fick, R. D. (1989). Functional importance of cystic fibrosis immunoglobulin fragments generated by Pseudomonas aeruginosa elastase. *J Lab Clin Med* **114**, 728–733.

Bjarnsholt, T., Jensen, P. O., Burmolle, M., Hentzer, M., Haagensen, J. A., Hougen, H. P., Calum, H., Madsen, K. G., Moser, C. & other authors (2005). *Pseudomonas aeruginosa* tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leukocytes is quorum-sensing dependent. *Microbiology* **151**, 373–383.

Bleves, S., Soscia, C., Nogueira-Oriandi, P., Lazdunski, A. & Filloux, A. (2005). Quorum sensing negatively controls type III secretion regulon expression in *Pseudomonas aeruginosa* PAO1. *J Bacteriol* **187**, 3898–3902.

Brint, J. M. & Ohman, D. E. (1995). Synthesis of multiple exoproducts in *Pseudomonas aeruginosa* is under the control of RhlR-RhlI, another set of regulators in strain PAO1 with homology to the autoinducer-responsive LuxR-Luxl family. *J Bacteriol* **177**, 7155–7163.

Chapon-Herve, V., Akrim, M., Latifi, A., Williams, P., Lazdunski, A. & Filloux, A. (2005). Quorum sensing dependent bacterial *quorum* synthesis and virulence in *Pseudomonas aeruginosa*. *FEMS Microbiol Lett* **24**, 1169–1178.

Christensen, L. D., Moser, C., Jensen, P. O., Rasmussen, T. B., Christophersen, L., Kjelleberg, S., Kumar, N., Heiby, N., Givskov, M. & Bjarnsholt, T. (2007). Impact of *Pseudomonas aeruginosa* quorum sensing on biofilm persistence in an in vivo intraperitoneal foreign-body infection model. *Microbiology* **153**, 2312–2320.

Chugani, S. A., Whiteley, M., Lee, K. M., D’Argenio, D., Manoil, C. & Greenberg, E. P. (2001). QscR, a modulator of quorum sensing signal synthesis and virulence in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci USA* **98**, 2752–2757.

Collier, D. N., Anderson, L., McKnight, S. L., Noah, T. L., Knowles, M., Boucher, R., Schwab, U., Gilligan, P. & Peschi, E. C. (2002). A bacterial cell to cell signal in the lungs of cystic fibrosis patients. *FEMS Microbiol Lett* **215**, 41–46.

Corech, R., Rao, A., Laxova, A., Moss, J., Rock, M. J., Li, Z., Kosorok, M. R., Spaingard, M. L., Farrell, P. M. & Barbieri, J. T. (2005). Early immune response to the components of the type III system of *Pseudomonas aeruginosa* in children with cystic fibrosis. *J Clin Microbiol* **43**, 3956–3962.

Cripps, A. W., Dunkley, M. L., Clancy, R. L. & Kyd, J. (1995). Pulmonary immunity to *Pseudomonas aeruginosa*. *Immunol Cell Biol* **73**, 418–424.

D’Argenio, D. A., Wu, M., Hoffman, L. R., Kulasekara, H. D., Déziel, E., Smith, E. E., Nguyen, H., Ernst, R. K., Larson Freeman, T. J. & other authors (2007). Growth phenotypes of *Pseudomonas aeruginosa lasR* mutants adapted to the airways of cystic fibrosis patients. *Mol Microbiol* **64**, 512–533.

Davies, D. G., Parsek, M. R., Pearson, J. P., Iglewski, B. H., Costerton, J. W. & Greenberg, E. P. (1998). The involvement of cell-cell signals in the development of a bacterial biofilm. *Science* **280**, 295–298.

de Kievet, T., Seed, P. C., Nezeson, J., Passador, L. & Iglewski, B. H. (1999). RsaL, a novel repressor of virulence gene expression in *Pseudomonas aeruginosa*. *J Bacteriol* **181**, 2175–2184.

Déziel, E., Lepine, F., Milot, S., He, J., Mindrinos, M. N., Tompkins, R. G. & Rahme, L. G. (2004). Analysis of *Pseudomonas aeruginosa* 4-hydroxy-2-alkylquinolines (HAQs) reveals a role for 4-hydroxy-2-heptylquinoline in cell-to-cell communication. *Proc Natl Acad Sci USA* **101**, 1339–1344.

Dong, Y.-H., Wang, L.-H., Xu, J.-L., Zhang, H.-B., Zhang, X.-F. & Zhang, L.-H. (2001). Quenching quorum-sensing dependent bacterial infection by an N-acetyl homoserine lactone. *Nature* **411**, 813–817.

Engel, L. S., Hobden, J. A., Moreau, J. M., Callegan, M. C., Hill, J. M. & O’Callaghan, R. J. (1997). *Pseudomonas* deficient in protease IV has significantly reduced corneal virulence. *Invest Ophthalmol Vis Sci* **38**, 1535–1542.

Ericson, D. L., Endersby, R., Kirkham, A., Stubber, K., Vollman, D. D., Rabin, H. R., Mitchell, I. & Storey, D. G. (2002). *Pseudomonas aeruginosa* quorum-sensing systems may control virulence factor expression in the lungs of patients with cystic fibrosis. *Infect Immun* **70**, 1783–1790.

Feltman, H., Schulert, G., Khan, S., Jain, M., Peterson, L. & Hauser, A. R. (2001). Prevalence of type III secretion genes in clinical and environmental isolates of *Pseudomonas aeruginosa*. *Microbiology* **147**, 2659–2669.

Fleiszg, S. M. J., Wienerkronisch, J. P., Miyazaki, H., Vallas, V., Mostov, K. E., Kanada, D., Sawia, T., Yen, T. S. B. & Frank, D. W. (1997). *Pseudomonas aeruginosa*-mediated cytotoxicity and invasion correlate with distinct genotypes at the loci encoding exoenzyme S. *Infect Immun* **65**, 579–586.

Gambrullo, M. J. & Iglewski, B. H. (1991). Cloning and characterization of the *Pseudomonas aeruginosa lasR* gene, a transcriptional activator of elastase expression. *J Bacteriol* **173**, 3000–3009.

Gambrullo, M. J., Jaye, S. & Iglewski, B. H. (1993). LasR of *Pseudomonas aeruginosa* is a transcriptional activator of the alkaline protease gene (apr) and an enhancer of exotoxin A expression. *Infect Immun* **61**, 1180–1184.

Geisenberger, O., Givskov, M., Riedel, K., Hoiby, N., Tumbler, M. & Eberl, L. (2000). Production of N-acetyl-L-homoserine lactones by *Pseudomonas aeruginosa* isolates from chronic lung infections associated with cystic fibrosis. *FEMS Microbiol Lett* **184**, 273–278.

Giwersman, B., Lambert, P. A., Rosdahl, V. T., Shand, G. H. & Hoiby, N. (1990). Rapid emergence of resistance in *Pseudomonas aeruginosa* in cystic fibrosis patients due to in-vivo selection of stable partially derepressed beta-lactamase producing strains. *J Antimicrob Chemother* **26**, 247–259.

Hazlett, L. D. (2005). Role of innate and adaptive immunity in the pathogenesis of keratitis. *Ocul Immunol Inflamm* **13**, 133–138.

Hazlett, L. D., Rosen, D. D. & Berk, R. S. (1978). Age-related susceptibility to *Pseudomonas aeruginosa* ocular infections in mice. *Infect Immun* **20**, 25–29.

Hazlett, L. D., Wells, P., Spann, B. & Berk, R. S. (1980). Penetration of the unwounded immature mouse cornea and conjunctiva by *Pseudomonas* SEM-TEM analysis. *Invest Ophthalmol Vis Sci* **19**, 694–697.

Hazlett, L. D., Zucker, M. & Berk, R. S. (1992). Distribution and kinetics of the inflammatory cell response to ocular challenge with *Pseudomonas aeruginosa* in susceptible versus resistant mice. *Ophthalmic Res* **24**, 32–39.

Hazlett, L. D., McClellan, S., Kwon, B. & Barrett, R. (2000). Increased severity of *Pseudomonas aeruginosa* corneal infection in strains of mice designated as Th1 versus Th2 responsive. *Invest Ophthalmol Vis Sci* **41**, 805–810.

Hentzer, M., Riedel, K., Rasmussen, T. B., Heydorn, A., Andersen, J. B., Parsek, M. R., Rice, S. A., Eberl, L., Molin, S. & other authors (2002). Inhibition of quorum sensing in *Pseudomonas aeruginosa* biofilm bacteria by a halogenated furanone compound. *Microbiology* **148**, 87–102.

Hentzer, M., Wu, H., Andersen, J. B., Riedel, K., Rasmussen, T. B., Bagge, N., Kumar, N., Schembri, M. A., Song, Z. & other authors (2003). Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *EMBO J* **22**, 3803–3815.

Heurlier, K., De’nervaud, V., Pessi, G., Reimmann, C. & Haas, D. (2003). Negative control of quorum sensing by RpoN (*σ^74*) in *Pseudomonas aeruginosa* PAO1. *J Bacteriol* **185**, 2227–2235.
Immunization with 3-oxododecanoyl-l-homoserine lactone-protein conjugate protects mice from lethal Pseudomonas aeruginosa lung infection. *J Med Microbiol* 55, 1381–1387.

Moss, J., Ehrmantraut, M. E., Banwart, B. D., Frank, D. W. & Barbieri, J. T. (2001). Sera from adult patients with cystic fibrosis contain antibodies to Pseudomonas aeruginosa type III apparatus. *Infect Immun* 69, 1185–1188.

Nouvens, A. S., Beatson, S. A., Whitchurch, C. B., Walsh, B. J., Schweizer, H. P., Mattick, J. S. & Cordwell, S. J. (2003). Proteome analysis of extracellular proteins regulated by the las and rhl quorum sensing systems in Pseudomonas aeruginosa PA01. *Microbiology* 149, 1311–1322.

O’Callaghan, R. J., Engel, L. S., Hobden, J. A., Callegan, M. C., Green, L. C. & Hill, J. M. (1996). *Pseudomonas aeruginosa* keratitis. The role of an uncharacterized exoprotein, protease IV, in corneal virulence. *Invest Ophthalmol Vis Sci* 37, 534–543.

Ochsner, U. A., Fiechter, A. & Reiser, J. (1994). Isolation, characterization, and expression in Escherichia coli of the *Pseudomonas aeruginosa rhlAB* genes encoding a rhamnosyltransferase involved in rhamnolipid biosurfactant synthesis. *J Biol Chem* 269, 19787–19795.

Passador, L., Cook, J. M., Gambello, M. J., Rust, L. & Iglewski, B. H. (1993). Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. *Science* 260, 1127–1130.

Pearson, J. P., Kendall, M. G., Passador, L., Tucker, K. D., Eberhard, A., Iglewski, B. H. & Greenberg, E. P. (1994). Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proc Natl Acad Sci U S A* 91, 197–201.

Pearson, J. P., Passador, L., Iglewski, B. H. & Greenberg, E. P. (1995). A second N-acetyl homoserine lactone signal produced by *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 92, 1490–1494.

Pearson, J. P., Pesci, E. C. & Iglewski, B. (1997). Roles of *Pseudomonas aeruginosa* las and rhl quorum-sensing systems in control of elastase and rhamnolipid biosynthesis genes. *J Bacteriol* 179, 5756–5767.

Pearson, J. P., Feldman, M., Iglewski, B. H. & Prince, A. (2000). *Pseudomonas aeruginosa* cell-to-cell signaling is required for virulence in a model of acute pulmonary infection. *Infect Immun* 68, 4331–4334.

Pesci, E. C., Milbank, J. B. J., Pearson, J. P., McKnight, S., Kende, A. S., Greenberg, E. P. & Iglewski, B. H. (1999). Quinolone signalling in the cell-to-cell communication system of *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 96, 11229–11234.

Pessi, G. & Haas, D. (2000). Transcriptional control of the hydrogen cyanide biosynthetic genes *hcnABC* by the anaerobic regulator ANR and the quorum-sensing regulators LasR and RhlR in *Pseudomonas aeruginosa*. *J Bacteriol* 182, 6940–6949.

Pessi, G., Williams, F., Hindle, Z., Heurlier, K., Holden, M. T. G., Camara, M., Haas, D. & Williams, P. (2001). The global posttranscriptional regulator RsmA modulates production of virulence determinants and N-acetylhomoserine lactones in *Pseudomonas aeruginosa*. *J Bacteriol* 183, 6676–6683.

Preson, M. J., Seed, P. C., Toder, D. S., Iglewski, B. H., Ohman, D. E., Jostin, K. J., Goldberg, J. B. & Pier, G. B. (1997). Contribution of protease and LasR to the virulence of *Pseudomonas aeruginosa* during corneal infections. *Infect Immun* 65, 3086–3090.

Rasmussen, T. B., Bjarnsholt, T., Skindersoe, M. E., Hentzer, M., Kristoffersen, P., Kote, M., Nielsen, J., Eberli, L. & Givskov, M. (2005). Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *J Bacteriol* 187, 1799–1814.

Reimmann, C., Beyeler, M., Latifi, A., Winteler, H., Foglino, M., Lazdunski, A. & Haas, D. (1997). The global activator GacA of...
Pseudomonas aeruginosa PAO1 positively controls the production of the autoinducer N-butyryl-homoserine lactone and the formation of the virulence factors pyocyanin, cyanide, and lipase. Mol Microbiol 24, 309–319.

Rice, S. A., McDougald, D., Kumar, N. & Kjelleberg, S. (2005). The use of quorum sensing blockers as therapeutic agents for the control of biofilm associated infections. Curr Opin Investig Drugs 6, 178–184.

Sawa, T., Yahr, T. L., Ohara, M., Kurahashi, K., Gropper, M. A., Wiener-Kronish, J. P. & Frank, D. W. (1999). Active and passive immunization with the Pseudomonas V antigen protects against type III intoxication and lung injury. Nat Med 5, 392–398.

Schuster, M., Lostroh, C. P., Ogi, T. & Greenberg, E. P. (2003). Identification, timing, and signal specificity of Pseudomonas aeruginosa quorum-controlled genes: a transcriptome analysis. J Bacteriol 185, 2066–2079.

Seed, P. C., Passador, L. & Iglewski, B. H. (1995). Activation of the Pseudomonas aeruginosa lasI gene by LasR and the Pseudomonas autoinducer PAI: an autoinduction regulatory hierarchy. J Bacteriol 177, 654–659.

Shaver, C. M. & Hauser, A. R. (2004). Relative contributions of Pseudomonas aeruginosa ExoU, ExoS, and ExoT to virulence in the lung. Infect Immun 72, 6969–6977.

Shiner, E. K., Terentyev, D., Bryan, A., Sennoune, S., Martinez-Zaguilan, R., Li, G., Gyorke, S., Williams, S. C. & Rumbaugh, K. P. (2006). Pseudomonas aeruginosa autoinducer modulates host cell responses through calcium signalling. Cell Microbiol 8, 1601–1610.

Singh, P. K., Schaefer, A. L., Parsek, M. R., Moninger, T. O., Welsh, M. J. & Greenberg, E. P. (2000). Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature 407, 762–764.

Smith, R. S. & Iglewski, B. H. (2003a). Pseudomonas aeruginosa quorum sensing as a potential antimicrobial target. J Clin Invest 112, 1460–1465.

Smith, R. S., Kelly, R., Iglewski, B. H. & Phipps, R. P. (2002). The Pseudomonas autoinducer N-(3-oxododecanoyl)-l-homoserine lactone induces cycloxygenase-2 and prostaglandin E2 production in human lung fibroblasts: implications for inflammation. J Immunol 169, 2636–2642.

Smith, K. M., Bu, Y. G. & Suga, H. (2003). Induction and inhibition of Pseudomonas aeruginosa quorum sensing by synthetic autoinducer analogs. Chem Biol 10, 81–89.

Smith, E. E., Buckley, D. G., Wu, Z., Saenphimmachak, C., Hoffman, L. R., D’Argenio, D. A., Miller, S. I., Ramsey, B. W., Speert, D. P. & other authors (2006). Genetic adaptation by Pseudomonas aeruginosa to the airways of cystic fibrosis patients. Proc Natl Acad Sci USA 103, 8487–8492.

Stapleton, F., Keay, L., Jabbert, I. & Cole, N. (2007). The epidemiology of contact lens related infections. Optom Vis Soc 84, 257–272.

Storey, D. G., Ujack, E. E., Rabin, H. R. & Mitchell, I. (1998). Pseudomonas aeruginosa lasR transcription correlates with the transcription of lasA, lasB, and toxA in chronic lung infections associated with cystic fibrosis. Infect Immun 66, 2521–2528.

Thompson, L. S., Webb, J. S., Rice, S. A. & Kjelleberg, S. (2003). The alternative sigma factor RpoN regulates the quorum sensing gene rhlI in Pseudomonas aeruginosa. FEMS Microbiol Lett 220, 187–195.

Tingpej, P., Smith, L., Rose, B., Zhu, H., Conibear, T., Al Nassafi, K., Manos, J., Elkins, M., Bye, P. & other authors (2007). Phenotypic characterization of clonal and nonclonal Pseudomonas aeruginosa strains isolated from lungs of adults with cystic fibrosis. J Clin Microbiol 45, 1697–1704.

Toder, D. S., Gambello, M. J. & Iglewski, B. H. (1991). Pseudomonas aeruginosa lasA: a second elastase under the transcriptional control of lasR. Mol Microbiol 5, 2003–2010.

Toder, D. S., Ferrell, S. J., Nezezon, J. L., Rust, L. & Iglewski, B. H. (1994). lasA and lasB genes of Pseudomonas aeruginosa: analysis of transcription and gene product activity. Infect Immun 62, 1320–1327.

Traidej, M., Caballero, A. R., Marquart, M. E., Thibodeaux, B. A. & O’Callaghan, R. J. (2003). Molecular analysis of Pseudomonas aeruginosa protease IV expressed in Pseudomonas putida. Invest Ophthalmol Vis Sci 44, 190–196.

Twinning, S. S., Kirschner, S. E., Mahnke, L. A. & Frank, D. W. (1993). Effect of Pseudomonas aeruginosa elastase, alkaline protease, and exotoxin A on corneal proteinases and proteins. Invest Ophthalmol Vis Sci 34, 2699–2712.

Vikstrom, E., Tafazoli, F. & Magnusson, K.-E. (2006). Pseudomonas aeruginosa quorum sensing molecule N-(3 oxododecanoyl)-l-homoserine lactone disrupts epithelial barrier integrity of Caco-2 cells. FEBS Lett 580, 6921–6928.

Wade, D. S., Cafee, M. W., Rocha, E. R., Ling, E. A., Engstrom, E., Coleman, J. P. & Pesci, E. C. (2005). Regulation of Pseudomonas quinolone signal synthesis in Pseudomonas aeruginosa. J Bacteriol 187, 4372–4380.

Wagner, V. E., Bushnell, D., Passador, L., Brooks, A. I. & Iglewski, B. H. (2003). Microarray analysis of Pseudomonas aeruginosa quorum-sensing regulons: effects of growth phase and environment. J Bacteriol 185, 2080–2095.

Wang, Y., Dai, Y., Zhang, Y., Hu, Y. B., Yang, B. Y. & Chen, S. Y. (2007). Effects of quorum sensing autoinducer degradation gene on virulence and biofilm formation of Pseudomonas aeruginosa. Sci China C Life Sci 50, 385–391.

West, S. E. H., Sample, A. K. & Runyen-janecky, L. J. (1994). The Vfr gene product, required for Pseudomonas aeruginosa exotoxin A and protease production, belongs to the cyclic AMP receptor protein family. J Bacteriol 176, 7532–7542.

Willcox, M. D. P., Sankardurg, P. R., Zhu, H., Hume, E. B. H., Cole, N., Conibear, T., Glasson, M., Harmis, N. & Stapleton, F. (2002). Inflammation and infection and effects of the closed eye. In Silicone Hydrogels: Continuous Wear Contact Lenses, pp. 90–125. Edited by D. Sweeney. Oxford: Butterworth-Heinemann.

Wilson, R., Sykes, D. A., Watson, D., Rutman, A., Taylor, G. W. & Cole, P. J. (1988). Measurement of Pseudomonas aeruginosa phanazine pigments in sputum and assessment of their contribution to sputum sol toxicity for respiratory epithelium. Infect Immun 56, 2515–2517.

Winzer, K., Falconer, C., Garber, N. C., Diggle, S. P., Camara, M. & Williams, P. (2000). The Pseudomonas aeruginosa lectins PA-IL and PA-III are controlled by quorum sensing and by RpoS. J Bacteriol 182, 6401–6411.

Wu, H., Song, Z., Givskov, M., Doring, G., Worlitzsch, D., Mathee, K., Rygaard, J. & Hoiby, N. (2001). Pseudomonas aeruginosa mutations in lasI and rhlI quorum sensing systems result in milder chronic lung infection. Microbiology 147, 1105–1113.

Wu, H., Song, Z., Hentzer, M., Andersen, J. B., Molin, S., Givskov, M. & Hoiby, N. (2004). Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in Pseudomonas aeruginosa lung infection in mice. J Antimicrob Chemother 53, 1054–1061.

Wu, L., Estrada, O., Zaborina, O., Bains, M., Shen, L., Kohler, J. E., Patel, N., Musch, M. W., Chang, E. B. & other authors (2005). Recognition of host immune activation by Pseudomonas aeruginosa. Science 309, 774–777.

Xu, F., Byun, T., Deussen, H. J. & Duke, K. R. (2003). Degradation of N-acylhomoserine lactones, the bacterial quorum-sensing molecules, by acylase. J Biotecnol 101, 89–96.
Zhu, H., Thuruthyil, S. J. & Willcox, M. D. P. (2002). Determination of quorum-sensing signal molecules and virulence factors of Pseudomonas aeruginosa isolates from contact lens-induced microbial keratitis. J Med Microbiol 51, 1063–1070.

Zhu, H., Bandara, R., Conibear, T., Thuruthyil, S. J., Rice, S. A., Kjelleberg, S., Givskov, M. & Willcox, M. D. P. (2004). Pseudomonas aeruginosa with lasI quorum-sensing deficiency is avirulent during corneal infection. Invest Ophthalmol Vis Sci 45, 1897–1903.

Zhu, H., Conibear, T., Bandara, R., Aliwarga, Y., Stapleton, F. & Willcox, M. (2006). Type III secretion system-associated toxins, proteases, serotypes, and antibiotic resistance of Pseudomonas aeruginosa isolates associated with keratitis. Curr Eye Res 31, 297–306.

Zimmermann, S., Wagner, C., Muller, W., Brenner-Weiss, G., Hug, F., Prior, B., Obst, U. & Hansch, G. M. (2006). Induction of neutrophil chemotaxis by the quorum-sensing molecule N-(3-oxododecanoyl)-l-homoserine lactone. Infect Immun 74, 5687–5692.