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# Interkingdom signaling and its consequences for human health

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Quorum sensing (QS) is a cell density-dependent system of bacterial communication used by several species for triggering a coordinated response when the population reaches a given cell density threshold.[1,2] Basically, the QS regulatory circuits are formed by two elements, one signal molecule that is constantly produced, and a transcriptional regulator, which activity depends on the presence of a given concentration of the signal molecule. This allows the coordinated expression by the bacterial population of specific sets of genes involved in processes relevant for microbial physiology as the control of the production of secondary metabolites, motility, biofilm formation or the production of virulence determinants among several others.

In the case of bacterial pathogens as *Pseudomonas aeruginosa*, the QS response is a relevant element for their infective process. *P. aeruginosa* presents two types of QS signaling molecules, the *N*-Acyl homoserine lactones (AHLs) and the *Pseudomonas* quinolone signal (PQS) 2-heptyl-3-hydroxy-4-quinolone. A large number of gram-negative bacteria produce AHLs,[3] which consist of fatty acids, with different lengths and residues, bound to a homoserine core. *P. aeruginosa* has two AHL-regulated circuits. One of them, the Las system, responds to *N*-oxo-dodecanoyl homoserine lactone (3-oxo-$C_{12}$-HSL), whereas the other, the Rhl system, responds to *N*-butanoyl homoserine lactone ($C_4$-HSL). The PQS signal forms part of another QS circuit in which the signal molecules are PQS and its metabolic precursor 2-heptyl-4(1H)-quinolone (HHQ). A hierarchical relationship exists among these systems that finally leads to the regulation of the expression of different virulence determinants.[4,5] This regulation is physiologically relevant since the deletion of genes encoding the components of the QS regulatory circuits reduces *P. aeruginosa* virulence in mice.[6,7]

In addition, to their role in regulating the expression of virulence determinants, different works, reviewed in references 8 and 9, have shown that the QS-signals themselves can induce specific responses of the human cell that can also be relevant for the infective process. These responses include modulation of the production of pro-inflammatory cytokines and induction of apoptosis. 3-oxo-$C_{12}$-HSL induces a potent inflammatory response in the absence of any bacterial pathogen and this inflammation lead to significant tissue destruction. In the presence of an antigen however, the effect might be different. It has been shown that 3-oxo-$C_{12}$-HSL inhibits the production of IL-12 and TNFα by LPs-activated macrophages. The immunomodulatory effect of 3-oxo-$C_{12}$-HSL seems to be concentration-dependent; inhibitory at low concentrations and stimulatory at high concentrations. In addition, 3-oxo-$C_{12}$-HSL induces dose-dependent apoptosis in different cell lines including macrophages and neutrophils, a feature not described for $C_4$-HSL. The immunomodulatory activity of QS signals is not restricted to AHLs; both PQS and HHQ seem to impair some aspects of the immune response including the inhibition of cytokine release, macrophage activation, or proliferation of T cells, monocytes, and dendritic cells.

The article in this issue of *Virulence* published by Holban et al.[10] is a continuation of the works on the effect of QS molecules on human cells. *P. aeruginosa* is the main cause of mortality and mobility of patients suffering cystic fibrosis, the main genetically inherited disease among Caucasians. In these patients, *P. aeruginosa* produces chronic infections in which the same bacterial clone can colonize the lungs of patients for decades. In addition, *P. aeruginosa* is a major cause of nosocomial acute infections in patients with underlying diseases. Most works on the role of QS signal molecules on the pathology of *P. aeruginosa* target these two types of patients. In their article, Holban et al.[10] go one step beyond and study whether the immunomodulatory and pro-apoptotic effect that QS signal molecules present may compromise the development of novel therapeutic procedures in which *P. aeruginosa* infection is an associated risk. One of these procedures is the use of mesenchymal stem cells in regenerative medicine for the treatment of patients suffering severe lung injuries, as those with cystic fibrosis. In their work, Holban et al.[10] show that 3-oxo-$C_{12}$-HSL, HHQ, and PQS present a potent pro-apoptotic activity over the studied mesenchymal stem cells, whereas the effect of $C_4$-HSL is minor in comparison. The pro-apoptotic effect was mediated by the induction of the expression of pro-apoptotic genes and the repression of anti-apoptotic ones. In addition, the studied QS signal molecules induced the production of cytokines as IL-8 (induced by all the signals) and IL-10 (induced by 3-oxo-$C_{12}$-HSL, $C_4$-HSL, and HHQ). Given that infections are common complications for stem cell-based therapies, the authors of the work indicate these results may be a concern for the

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development of the field of regenerative medicine.[10] Furthermore, since several different gram-negative bacteria produce AHLs,[3] the results presented in this article go beyond *P. aeruginosa* infections and may apply for infections by other microorganisms.

While this concern may be of relevance, one important issue that remains to be fully established concerns the effect of QS signaling molecules in vivo, during infection. Deciphering this effect is not an easy task, because when regular models of infections are used, it is difficult to discriminate between the effect of the QS signal in triggering bacterial virulence and its effect on the human cell independently on the presence of the pathogen. Most experiments using pure QS signals, including the one by Holban et al.,[10] have been performed using cell cultures; however some studies have also been performed injecting the QS signals in animal models, and similar response phenotypes as those observed in vitro have been detected.[11] This seems to validate the activity of QS signals during infection, independently of the presence of the bacterial producer. However one aspect still remains that must be taken with caution. Since the immunomodulatory activity of QS signals on human cells is concentration-dependent, the effects these molecules may have in human cells can be sharply different depending on the actual concentration in the human body of such signals. Estimations on the amount of free oxo-$C_{12}$-HSL (the most abundant AHL in human sputum) can vary by more than 50-fold (from 20 to >1000 nM) in the sputa of cystic fibrosis patients infected with *P. aeruginosa*.[12,13] Early stages of infections or body allocations were bacterial load is low may present small amounts of QS molecules, whereas the concentration of these signals in places with high bacterial densities (as biofilms) will be likely high. The consequences of each one of these situations will probably be different.

Interference with the QS response has been suggested to be a good approach for treating bacterial infections, either as an alternative to the use of antibiotics either as accessory therapeutic elements to be used in combination with antibiotics.[14] The use of homologs of the QS signals will inhibit the QS response; however they will not preclude the activity of the QS signals that will remain and may hence challenge the immune response. In addition, it is unclear at the moment whether or not the QS signals homologs may affect the immune response. A different approach to tackle this problem is the use of quorum-quenching compounds. It has been shown that anti-3-oxo-$C_{12}$-HSL antibodies protect macrophages from the cytotoxic effect of this signal molecule.[15,16] This type of compounds, targeting the signal molecule, not the bacterial cell, may serve to reduce bacterial virulence and also to avoid the effect that QS molecules themselves have over human cells.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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