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Human and murine paraoxonase 1 are host modulators of *Pseudomonas aeruginosa* quorum-sensing

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Abstract

The pathogenic bacterium *Pseudomonas aeruginosa* uses acyl-HSL quorum-sensing signals to regulate genes controlling virulence and biofilm formation. We found that paraoxonase 1 (PON1), a mammalian lactonase with an unknown natural substrate, hydrolyzed the *P. aeruginosa* acyl-HSL 3OC12-HSL. In in vitro assays, mouse serum-PON1 was required and sufficient to degrade 3OC12-HSL. Furthermore, PON2 and PON3 also degraded 3OC12-HSL effectively. Serum-PON1 prevented *P. aeruginosa* quorum-sensing and biofilm formation in vitro by inactivating the quorum-sensing signal. Although 3OC12-HSL production by *P. aeruginosa* was important for virulence in a mouse sepsis model, *Pon1*-knock-out mice were paradoxically protected. These mice showed increased levels of PON2 and PON3 mRNA in epithelial tissues suggesting a possible compensatory mechanism. Thus, paraoxonase interruption of bacterial communication represents a novel mechanism to modulate quorum-sensing by bacteria. The consequences for host immunity are yet to be determined.

Keywords: *Pseudomonas aeruginosa*; Quorum-sensing; Paraoxonase; Innate immunity

1. Introduction

The pathogenic bacterium *Pseudomonas aeruginosa* uses acyl-homoserine lactone (acyl-HSL) quorum-sensing signals to regulate the expression of virulence genes implicated in infectivity, tissue damage, and the generation of a host inflammatory response [1–5]. Two hierarchically regulated acyl-HSL quorum-sensing molecules are produced and recognized: *N*-(3-oxododecanoyl)-L-homoserine lactone (3OC12-HSL), and *N*-butanoyl-L-homoserine lactone (C4-HSL) [6–8]. These same quorum-sensing signals also coordinate the establishment of biofilms [1–3,9]. One well-described example of the importance of quorum-sensing in establishing *P. aeruginosa* infection is found in the controlled expression

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of the rhamnolipid group of surfactants. Without coordinated expression of these surfactants by quorum-sensing, *P. aeruginosa* does not form structured, well-differentiated biofilms [10,11].

Other organisms have evolved to disrupt quorum-sensing signaling to compete with bacteria that use quorum-sensing to organize their behavior; some environmental strains of bacteria secrete lactonases or acylases to degrade acyl-HSL signals [12–16]. Bacteria expressing these anti-acyl-HSL factors have a competitive edge over their quorum-sensing neighbors [16,17]. Broad protection by anti-acyl-HSL factors has been demonstrated in transgenic experiments. AiiA (an acyl-HSL lactonase from a soil-dwelling species of *Bacillus*) was transgenically expressed in plants and protected them from soft-rot infection by *Erwinia carotovora*, a quorum-sensing plant pathogen [18]. Previously we reported that some acyl-HSL quorum-sensing signals lose biological activity when exposed to mammalian epithelial cells [19]. Therefore, we hypothesized that mammals, like bacteria, also degrade acyl-HSL quorum-sensing signals. Furthermore, we speculated that this innate activity would confer on the host a competitive advantage over pathogenic bacteria by reducing infection and biofilm formation.

The paraoxonases are a family of mammalian lactone hydrolases with high sequence similarity but distinct substrate specificities and expression patterns [20–22]. PON1 is expressed primarily in the liver and secreted into the serum where it associates with high density lipoprotein (HDL) particles [22–24]. PON3 is also expressed in liver and serum but at a lower level than PON1 [22,25], and PON2 is cell-associated and expressed in several tissues [26]. PON1 protects against organophosphate poisoning [27] and has anti-oxidative properties [28]; it is also associated with protection from atherosclerosis, presumably through its ability to hydrolyze oxidized phospholipids and protect serum lipids from oxidative modification [23,28]. Acyl-HSL molecules are substrates for the human paraoxonases (PON), PON1, PON2, and PON3 [29]. Here we report that human and mouse PON1 degrades 3OC12-HSL by hydrolyzing its lactone ring, thereby blocking *P. aeruginosa* quorum-sensing signaling. (A preliminary report of these findings has been presented [30]).

2. Materials and methods

2.1. Molecular analysis

Synthetic 3OC12-HSL was reconstituted in phosphate buffered saline containing magnesium and calcium (PBS) to 10 μM and incubated for 1 h on monolayers of A549 airway epithelial cells or in the absence of cells. 3OC12-HSL was extracted from the medium in acidified ethyl acetate, dried under sterile nitrogen gas, and reconstituted in deuterated chloroform. Proton-nuclear magnetic resonance (NMR) [6], high-pressure liquid chromatography (HPLC) column, and electrospray mass spectrometry (MS) methods are described in supplemental information.

2.2. Serum studies

Blood was collected from euthanized mice through cardiac puncture and serum was separated from red blood cells by centrifugation. Synthetic 3OC12-HSL (10 μM) was prepared in reaction buffer (100 mM HEPES, pH 7.4, 1 mM $CaCl_2$) and exposed to PBS or whole serum. EDTA (25 mM), oxindole (1 mM), isatin (1 mM), or normal saline was added to serum to evaluate inhibition of serum activity against 3OC12-HSL. 1% solutions of human serum, wild-type mouse serum, or PON1 knock-out (KO) serum in reaction buffer were compared for 3OC12-HSL degradation activity. All reactions were performed for 30 min at 37 °C.

2.3. Purified PON1 studies

Human PON1 was purified from serum as previously described [27,31]. Synthetic 3OC12-HSL (10 μM) was prepared in reaction buffer. Purified PON1 was added to the solution and incubated at 37 °C. Samples of the reaction solution were taken at 15 and 30 min, and 3OC12-HSL concentration determined by bioassay.

2.4. Transgene expression

Chinese hamster ovary (CHO) cells were cultured as monolayers in plastic dishes. Recombinant adenovirus serotype 5 (Ad5) expressing hPON1, hPON2, hPON3, or GFP was produced by the University of Iowa Vector and Gene Targeting core. CHO cells were infected with recombinant Ad5 by calcium phosphate transfection [32] and allowed to express for 48 h. Transfection efficiency was evaluated by fluorescence measurement in Ad5-GFP infected cells. Synthetic 3OC12-HSL (10 μM) in PBS was added to transgene expressing cells and incubated at 37 °C, 5.5% CO_2 for 30 min. Remaining 3OC12-HSL in the medium was measured by bioassay.

2.5. P. aeruginosa *quorum-sensing and biofilm formation*

The effect of PON1 in serum on *P. aeruginosa* biofilm formation was studied in vitro by means of a modified Calgary Biofilm Device assay [33]. Initially, a plastic peg was immersed in each well of a 96-well culture plate containing wild-type or *lasI* mutant PAO1 in 1% tryptic soy broth containing 1 mM $CaCl_2$. After 1 h, the pegs were removed from the PAO1 culture and transferred to wells containing 1% wild-type mouse serum, *Pon1*-KO mouse serum with or without purified human