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General review

Targeting mechanisms of *Pseudomonas aeruginosa* pathogenesis

Thérapeutiques ciblant les mécanismes pathogéniques de *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen responsible for ventilator-acquired pneumonia, acute lower respiratory tract infections in immunocompromised patients and chronic respiratory infections in cystic fibrosis patients. High incidence, infection severity and increasing resistance characterize *P. aeruginosa* infections, highlighting the need for new therapeutic options. One such option is to target the many pathogenic mechanisms conferred to *P. aeruginosa* by its large genome encoding many different virulence factors. This article reviews the pathogenic mechanisms and potential therapies targeting these mechanisms in *P. aeruginosa* respiratory infections.

Résumé

Pseudomonas aeruginosa est un pathogène opportuniste responsable de pneumonies nosocomiales, infections des voies respiratoires basses chez les patients immunodéprimés et infections respiratoires chroniques lors de la mucoviscidose. L'incidence élevée, la sévérité et la résistance croissante sont caractéristiques des infections à *P. aeruginosa* et soulignent le besoin d'ouvrir de nouvelles voies thérapeutiques. Une voie thérapeutique possible est de cibler un des nombreux mécanismes pathogéniques dont est doté *P. aeruginosa* grâce à son génome de grande taille codant pour de nombreux facteurs de virulence. Nous exposons les différents mécanismes pathogéniques au cours des infections respiratoires à *P. aeruginosa* ainsi que les voies thérapeutiques potentielles qui les ciblent.

Keywords: Pseudomonas aeruginosa; Pathogenicity; Virulence factors

Mots clés : Pseudomonas aeruginosa ; Pathogénicité ; Facteurs de virulence

1. Introduction

Pseudomonas aeruginosa is the most common pathogen responsible for both acute respiratory infections in ventilated or immunocompromised patients and chronic respiratory infections in cystic fibrosis patients [1,2]. *P. aeruginosa* is also responsible for excessive mortality in VAP [1,3,4]. Adding to the problems of high incidence and infection severity, the resistance of *P. aeruginosa* to conventional antimicrobial treatment has increased over the past decade [5,6].

These three problems of high incidence, severity and resistance persist even though they are now broadly recognized and various strategies have been proposed addressing them [1,7–12].

Therefore, it is crucial that new therapeutic options for *P. aeruginosa* infections be explored. Such new options may come from specifically targeting the pathogenic mechanisms of *P. aeruginosa*. Indeed, *P. aeruginosa* is a remarkable pathogen in that it is endowed with a uniquely large genome containing genes for many different virulence factors and regulatory mechanisms allowing it to adapt to hostile environments.

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This article reviews the pathogenic mechanisms and potential therapies targeting these mechanisms in *P. aeruginosa* respiratory infections.

2. Schematic overview of *P. aeruginosa* pathogenesis in respiratory infections

P. aeruginosa is an opportunistic pathogen that, after being acquired from the environment, colonizes the respiratory epithelium in patients with predisposing conditions such as cystic fibrosis, mechanical ventilation, immunodeficiency or preexisting respiratory disease. Flagella and pili, the motile surface appendages of *P. aeruginosa* are responsible for bacterial motility and progression towards epithelial contact. These appendages also act as initial tethers in facilitating bacteria to epithelial cell contact by binding to the epithelial surface glycolipid asialo-GM1. Additionally, lipopolysaccharide (LPS) also plays a similar role in bacterial adhesion through asialo-GM1 binding. These appendages then play a major role in the irreversible adhesion to epithelial cells, which is the initial critical step in colonization of the respiratory epithelium. Upon cell contact, the type III secretion system, a major virulence determinant, is activated. The type III secretion system allows *P. aeruginosa* to inject secreted toxins through a syringe-like apparatus directly into the eukaryotic cytoplasm. Four effector proteins are known: ExoY, ExoS, ExoT, and ExoU and all participate, at varying levels, in the cytotoxicity of *P. aeruginosa* leading to invasion and dissemination of *P. aeruginosa*. Other virulence factors secreted via type II secretion system into the extracellular space such as elastase, alkaline phosphatase, exotoxin A, and phospholipase C also participate in invasion by destroying the protective glycocalix of the respiratory epithelium and exposing epithelial ligands to *P. aeruginosa*. These secretins also participate in cytotoxicity. A similar role also exists for pyoverdine and pyocyanin.

In acute infections, invasion, dissemination and extensive tissue damage predominate. However, in chronic infections, particularly in cystic fibrosis patients, *P. aeruginosa* may also adapt, by losing its most immunogenic features such as pili and flagella to avoid clearance, and by isolating itself from host defenses and adhering to the respiratory epithelium by forming biofilms. In chronic infections, a persistent inflammatory state is maintained by extracellular secreted virulence factors.

Whether in acute or chronic infections, *P. aeruginosa* possesses a multiplicity of regulating systems allowing it to adapt to its environment and notably to host defenses. Among these systems, quorum-sensing (QS) showcases *P. aeruginosa* adaptability. Quorum sensing systems are complex bacterial cell-to-cell signaling systems that allow the bacteria to sense their own cell density and to communicate with each other resulting in coordinated production of virulence factors depending on bacterial density. QS has been shown to be critical to maintaining airway inflammation through virulence factor production and to the formation of biofilm in chronic infections.

This schematic overview shows that there are many levels in the pathogenesis of *P. aeruginosa* infections that may represent therapeutic targets, which we will now describe in detail.

3. Bacterial cell surface virulence factors (Fig. 1)

3.1. Flagella

Flagella are complex proteic structures forming a filamentous polar appendage at the surface of *P. aeruginosa*. Flagella are the main motile appendage of gram negative bacteria and allow the swimming movement of *P. aeruginosa* through a propeller or screw-like motion. Flagella have a critical role in pathogenesis by tethering and adhering to epithelial cells through binding with a common membrane component, asialoGM1 [13]. They also participate in virulence and elicit an NFkB dependent inflammatory response through interactions with Toll-receptors TLR5 and TLR2 and a calcium entry dependent activation of the extracellular regulated kinase pathway leading to IL-8 production [14,15]. However, flagella are also very immunogenic, rendering their presence a liability for *P. aeruginosa* after successful colonization. This is why *P. aeruginosa* is capable of adapting by selecting aflagellar mutants to evade host response in chronic infections [16]. It is therefore not surprising that flagella have been considered interesting targets for immunotherapy. *P. aeruginosa* pneumonia was attenuated in rats receiving human antiflagellar monoclonal antibodies [17]. Similar findings led to the development of a *P. aeruginosa* flagella vaccine for cystic fibrosis patients that successfully completed phase I and II trials and is undergoing phase III evaluation [18,19].

3.2. Pili

Pili or fimbriae are smaller filamentous surface appendages of *P. aeruginosa*. Multiple pili are usually present on the surface. *P. aeruginosa* pili are among the rare prokaryotic pili involved in bacterial motility. This motility, called twitching is due to the retractile properties of *P. aeruginosa* pili and allows *P. aeruginosa* to "spread" along hydrated surfaces rather than "swim" [20]. This feature facilitates the rapid colonization of the airway [20]. Like flagella, pili are crucial to the adhesion phase of colonization through binding to asialoGM1 of the epithelial cell membrane [21,22]. Furthermore, studies have shown that both pili-mediated adherence and twitching motility are critical to *P. aeruginosa* virulence [23,24]. In an infant mouse model of lung infection, piliated strains of *P. aeruginosa* caused more severe and diffuse pneumonia than corresponding non-piliated mutants [25]. Therefore, pili, like flagella seem to be legitimate targets in developing antipseudomonal immunotherapy [26–28]. Among such strategies, a chimeric vaccine incorporating both pilin and non-toxic modified exotoxin A successfully reduced bacterial adherence [29]. However, there are issues linked to the lack of cross reactivity of antigens targeting pili across different *P. aeruginosa* strains that need to be resolved [26,28].

3.3. LPS

Although the inner face of the outer membrane resembles a typical phospholipid bilayer, the outer face of the outer mem-