Current concepts on Pseudomonas aeruginosa interaction with human airway epithelium

Anaëlle Muggeo, Christelle Coraux, Thomas Guillard

HAL Id: hal-04065670

https://hal.univ-reims.fr/hal-04065670

Submitted on 12 Apr 2023

REVIEW

Current concepts on *Pseudomonas aeruginosa* interaction with human airway epithelium
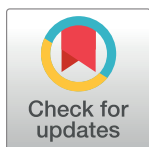
Anaëlle Muggeo[1], **Christelle Coraux**[2], **Thomas Guillard**[1]*

1 Université de Reims Champagne-Ardenne, INSERM, CHU de Reims, Laboratoire de bactériologie-Virologie-Hygiène hospitalière-Parasitologie-Mycologie, P3Cell, U 1250, Reims, France, **2** Université de Reims Champagne-Ardenne, INSERM, P3Cell, U 1250, Reims, France

* tguillard@chu-reims.fr

Abstract

Pseudomonas aeruginosa is a major, but opportunistic, respiratory pathogen, which rarely infects healthy individuals, mainly due to the barrier effect of the human airway epithelium (HAE). This review explores the interaction of *P. aeruginosa* with HAE and the progression of the infection. The basolateral part of the epithelium, which includes the basolateral membrane of the epithelial cells and the basement membrane, is inaccessible in normal tight epithelia with intact junctions. We highlight how *P. aeruginosa* exploits weaknesses in the HAE barrier to gain access to the basolateral part of the epithelium. This access is crucial to initiate respiratory infection and is mainly observed in the injured epithelium, in repairing or chronically remodeled epithelium, and during extrusion of senescent cells or cell multiplication during normal epithelium renewal. The subsequent adhesion of the bacteria and cytotoxic action of virulence factors, including the toxins delivered by the type 3 secretion system (T3SS), lead to retractions and cell death. Eventually, *P. aeruginosa* progressively reaches the basement membrane and propagates radially through the basal part of the epithelium to disseminate using twitching and flagellar motility.

Competing interests: The authors have declared that no competing interests exist.

Introduction

Pseudomonas aeruginosa is a ubiquitous gram-negative bacterium mainly found in aqueous environments and surfaces [1–4]. This opportunistic pathogen is responsible for various infections, particularly those involving the respiratory tract [5–7]. Owing to intact airway epithelial barrier [8–11] and optimal lung defenses, *P. aeruginosa* is rarely pathogenic in healthy individuals [12], and the development of infections depends on epithelial injuries [13–16], mucociliary clearance dysfunctions [17], or immune system impairment [18–21]. Acute or chronic pneumonia is limited to immunocompromised patients or those with defective pulmonary function, such as mechanically ventilated patients in intensive care unit [5,22,23], and those with cystic fibrosis (CF) [24,25] or chronic obstructive pulmonary disease (COPD) [7,26–28].

The intact human airway epithelium (HAE) is a physical and functional barrier against pathogens [29,30]. The pseudostratified and differentiated mucociliary bronchial epithelium is usually composed of several different types of cells, supported by the underlying basement membrane [31] (Fig 1). The ciliated and secretory cells, which are tall, functionally

differentiated, and highly polarized, reach the apical surface. The basal cells, which are located at the basal pole under the columnar cells, are multipotent progenitors, which generate the other cells during the differentiation process [31–33]. In intact healthy HAE, only apical membranes are exposed to the environment [20,34]. Injuries, varying from junction disruptions to partial shedding of the epithelium, or even to complete denudation of the basement membrane, induce the HAE to initiate a repair process to regenerate and restore its function [35–37]. In chronic inflammatory respiratory diseases such as CF or COPD, the dysregulated inflammation can alter the respiratory epithelial repairing process [14,15,35,38–42].

The airway epithelium protects the lungs from various external assaults (pollutants, pathogens, allergens, etc.) in three ways. First, the airway epithelium is a physical barrier. Its impermeability is ensured by strongly connected cells by tight junctions, adherens junctions, and desmosomes [43,44]. The tight junctions, located near the apical border, delimit the apical plasma membrane from the basolateral plasma membrane of the differentiated cells [45–47]. In healthy epithelium, the basolateral part, which is composed of the basolateral cell membrane and the underlying basement membrane, is not exposed to external agents [29,43]. Second, the airway epithelium can expel inhaled particles from the airways using the mucociliary clearance mechanism. The mucociliary clearance is carried out by the ciliated and secretory cells. It is the main process for removing inhaled foreign particles from the airways [44]. The airway surfaces are lined by epithelial cells and covered with an airway surface layer (ASL) composed of two parts: a mucus layer and a low-viscosity periciliary layer (PCL). Thus, the ASL lubricates airway surfaces and facilitates ciliary beating for efficient mucus clearance [17]. The mucus produced by secretory cells forms a continuous layer on the epithelial surface, and this three-dimensional matrix acts as a physical barrier protecting the underlying epithelia and as a trap for inhaled particles and exogenous microorganisms [48–50]. It is composed of mucins (MUC5ac and MUC5b), which are heavily glycosylated proteins within the mucus to which the pathogens attach [51–53]. Thereby, the coordinated beating of the ciliated cells' cilia sweeps the trapped bacteria out of the lungs toward the oropharynx where they are swallowed. Third, the airway epithelium is capable of defending itself against infectious agents. The airway epithelium can produce antimicrobial peptides and inflammatory cytokines, which participate in the innate immune response [20,29].

This review discusses the interaction of *P. aeruginosa* with HAE as it is an integral part of its virulence in the respiratory tract. We will first highlight that the essential step to initiate airway infection is access to the basolateral part of the HAE by *P. aeruginosa*. Then, we will identify the different steps in the HAE infection by *P. aeruginosa* and discuss some specific issues, such as how the bacteria adhere to the HAE cells, what is the role of pathogen internalization by HAE cells in infection, and how *P. aeruginosa* crosses and invades the epithelium.

Access to the basolateral part of HAE is crucial for *P. aeruginosa* to initiate respiratory infections

1) Breach of the epithelium is a hallmark of *P. aeruginosa* infections. The HAE plays a key role in defense against *P. aeruginosa* by being a functional physical barrier thanks to intercellular junctions, mainly tight junctions, leading to epithelial polarity and impermeability. The ability to exploit epithelial breaches to reach the binding receptors in deeper tissues is a hallmark of *P. aeruginosa* infections. Most, if not all, pathologies caused by this bacterium illustrate this point. Cutaneous infections mostly occur after skin injuries such as extended burns or chronic cutaneous wounds, the latter being quite common among diabetic patients [54,55]. Keratitis or corneal infections usually follow disruption of the corneal epithelium occurring after ocular trauma, surgery, or lesions caused by contact lenses [56–59]. The risk factors of
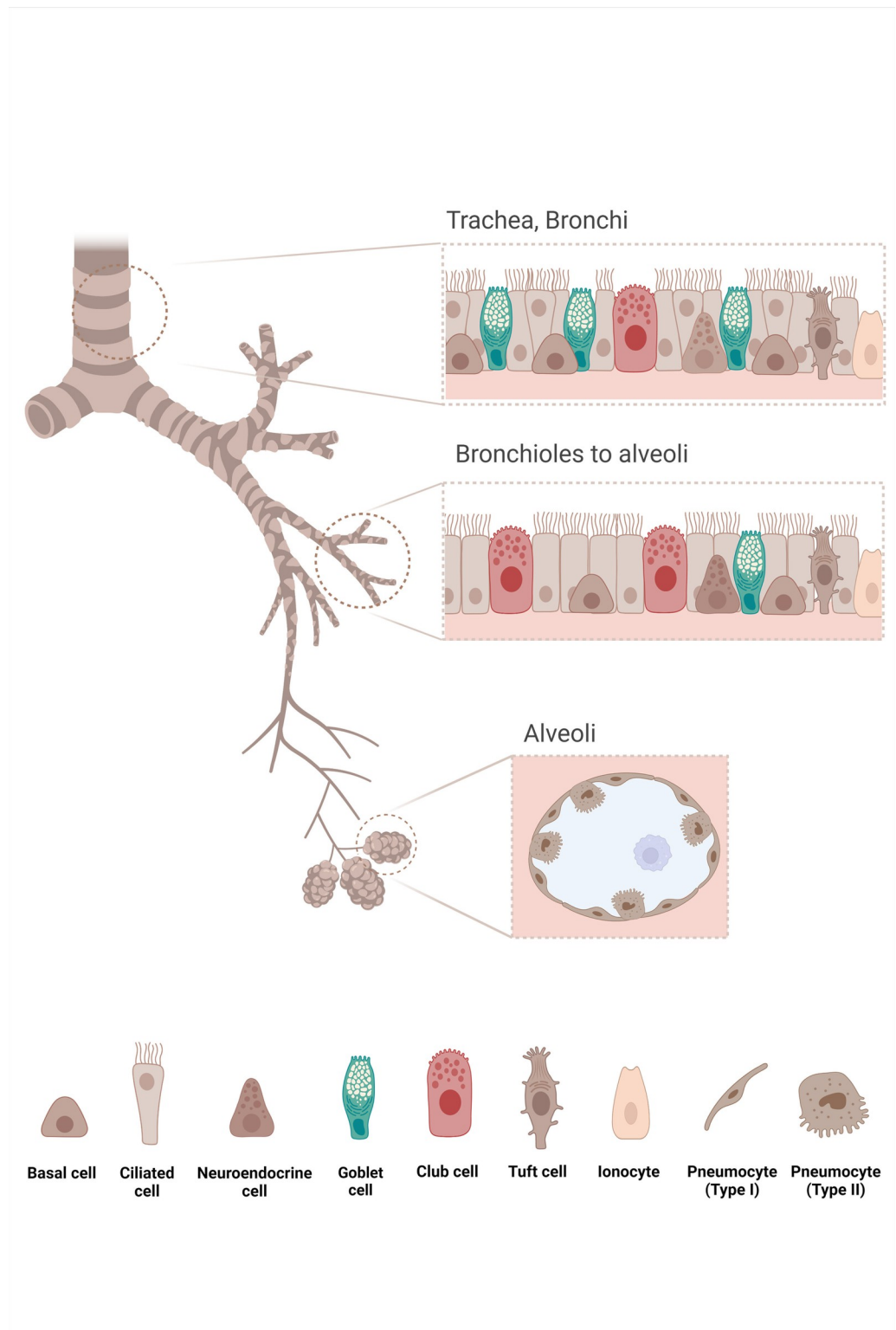
Fig 1. Lung epithelia structure. The lung is a complex organ composed of conducting airways and gas exchange zones. The conducting airways branch from the trachea to terminal bronchioles that end up in the alveoli. In airways, the lining epithelium provides a physical barrier between the external environment and the underlying parenchyma, whose integrity is maintained by intercellular junctions. The airway epithelium ensures the protection of the lung against inhaled particles, toxins, and pathogens through the mucociliary clearance and secretion of molecules with antibacterial,

antioxidant, and antiprotease activity that act in an orchestrated way to protect the epithelium from lesion factors. The diversity of cells constituting the epithelial barrier is adapted to the epithelial functions. In the pseudostratified epithelium lining the trachea and bronchi, different specific cell types such as basal, goblet, and ciliated cells are found. Basal cells are progenitor cells involved in the epithelial renewal and the anchor of the epithelium to the basement membrane through hemidesmosomes. Goblet cells, within subepithelial glands, produce the respiratory mucus that entraps noxious particles and is moved towards the oropharyngeal junction by the coordinated beating of ciliated cells. Some rare secretory Club cells are also described. In the distal bronchioles, the number of basal cells decreases, and the goblet cells are progressively replaced by Club cells, mainly involved in the production of anti-inflammatory factors and surfactant proteins. In addition, other cell types were found more rarely: neuroendocrine cells, which serve as communicators between the immune and nervous system by secreting neuropeptides; Tuft cells, which have chemosensory, neuronal, and immunological functions; and pulmonary ionocytes. The alveoli are lined by type I and type II pneumocytes whose functions are completely different: Type I pneumocytes are involved in the O_2/CO_2 exchange through their thin cytoplasm, whereas type II pneumocytes secrete surfactant proteins and act as progenitor cells of the alveolar epithelium. Created with BioRender.com.

acute otitis externa, which is mainly observed in pools and hot tub users [60,61], include ear canal epithelium disruptions, such as abrasion (scratching, clearing ear canals), maceration, psoriasis, or eczema [60,61]. The likelihood of *P. aeruginosa* catheter-related urinary tract infections is increased following damage to the epithelium structure during catheter insertion or manipulation [62]. For respiratory infections, the significance of epithelial breaches is also observed, in addition to biofilm formation and impaired mucociliary clearance. HAE lesions are described in CF and COPD patients, but also in those undergoing intubation or mechanical ventilation [13,63–65]. (See below.)

2) HAE breach allows *P. aeruginosa* access to the basolateral part of the epithelium. Epithelial breaches expose the basolateral part of the epithelium (i.e., the basolateral membrane of HAE cells and/or the basement membrane), which is normally inaccessible in healthy epithelium owing to intact tight junctions [34]. Access to the basolateral part is the main element of *P. aeruginosa* interaction and is crucial for initiating *P. aeruginosa* respiratory infections.

Many studies demonstrated that *P. aeruginosa* poorly interacts with an intact, healthy, and well-polarized HAE. Conversely, numerous reports demonstrated preferential interactions with injured or repairing epithelia, or with nonpolarized cells in the basolateral part of HAE. The first such report showed that in rat tracheal surface, *P. aeruginosa* interacts in vivo more easily with brush-injured sites than with those that are not injured [66]. Subsequent studies have also shown infrequent adhesion to the normal epithelium but increased adhesion to areas of epithelial damage and the basement membrane in airway culture models [9,67]. Lee and colleagues confirmed the crucial role of epithelial tight junctions in interaction with *P. aeruginosa*. They showed that bacterial adhesion and toxic effects are more frequently found near the free edges of epithelial wounds, where the basolateral cell plasma membrane is accessible [68]. HAE with intact tight junctions is entirely resistant to *P. aeruginosa*-induced cell apoptosis, as opposed to nonjunctional epithelia [69]. Although the mucociliary clearance decreases bacterial access to the epithelium, the Puchelle team reported that *P. aeruginosa* is unable to adhere to normal and uninjured cells because the functional tight junctions play a key role in masking the basolateral main receptors for bacterial attachment (see below). They described the preferential sites of interaction with *P. aeruginosa* on the exposed basolateral plasma membranes, the denuded basement membrane after injury, as well as the flattened migrating and spreading cells during repair [11,14,15,70,71]. Other studies confirmed that basolateral plasma membranes exposed after injury are more likely to bind *P. aeruginosa* and that the level of the polarity of the cells is also an important factor, given that nonpolarized migrating cells or incompletely polarized cells are more susceptible to *P. aeruginosa* binding and intoxication [72,73].

3) Main settings wherein *P. aeruginosa* could exploit opportunities to gain access to the basolateral part of HAE.   Injured epithelium. Following an injury to the epithelium, the

basolateral part becomes accessible to *P. aeruginosa* because the basolateral membrane of adjacent cells and basement membrane are exposed (Fig 2A and left panel).

First, this can result from endotracheal tube insertion and manipulation that, not infrequently, induce mechanical damage in ventilator-associated pneumonia (VAP). The endotracheal tube can cause scratching and abrasion of the HAE, removing fragments of the epithelium and leading to denudation of the basement membrane [13,63,74]. In addition, biofilm formation on the plastic surface and mechanical impairment of the mucociliary clearance by the tube facilitate infection [63].

Second, this can result from induced HAE cell death. Dying cells undergo retraction and tight junction disruptions, leading to subsequent detachment from the adjacent cells and the basement membrane. The causes of cell injury are extremely diverse and can be induced by pathogens (viruses, bacteria, etc.), chemical injury (pollutants, toxic substances, allergens, etc.), or hyperactive inflammation (excess of cytokines, proteases, oxidant stress, etc.) [14,35]. Injuries could be part of a potentially vicious circle since HAE injury by itself will attract immune cells. For instance, degranulation of neutrophils contributes to inflammation and tissue damage [19,25,75,76], which could increase the number of breaches and thereby provide an opportunity for *P. aeruginosa* to reach the basolateral part of HAE. But, a fine-tuned homeostasis has been described in peritoneal serosa, which is likely transposable to other tissues including the lung tissue. Indeed, Uderhardt and colleagues reported that such homeostasis is maintained to prevent the excess of deleterious inflammation in the case of microlesion. Resident tissue macrophages sense rapidly the death of individual cells and sequester the damage owing to extending membrane processes (pseudopods), thus preventing neutrophil activation [77]. In case of too many lesions, such as exemplified below for CF and COPD, the cloaking by resident tissue macrophages is overwhelmed and would not prevent neutrophil-driven inflammation and subsequent tissue damage.

In CF disease, different factors contribute to *P. aeruginosa* infections. The most common and well-accepted paradigm is the "low volume hypothesis" stating that in the absence of a functional chloride channel CFTR, the airway mucus is dehydrated, hyperviscous, and thickened, thereby compromising the mucociliary clearance [78,79]. The mucus stagnates in the airways, and this aberrant accumulation of mucus provides a nidus for colonization and recurrent infections by opportunistic pathogens, like *P. aeruginosa* [78]. As CF patients become chronically infected, *P. aeruginosa* adapts to the specific lung environment by genotypic and phenotypic variations, such as loss of virulence and/or increased resistance to antimicrobials and host immunity. *P. aeruginosa* usually grows as a biofilm on host tissues/epithelial surfaces during these chronic infections [78,80]. Bacterial multiplication, following their entrapment within the mucus, induces a vigorous inflammatory response. Moreover, the deficit in the immune response due to CFTR dysfunction leads to persistent and hyperactive immunological stimulation, resulting in chronic lung inflammation [18,19,78]. For instance, the decreased CFTR functionality reduces bicarbonate secretion in the airways, then decreases the pH of the mucus in CF patients. It leads to the inactivation of cationic antimicrobial peptides secreted by the host and to mucus tethering and impaired mucus detachment from the lung epithelium [78]. Activated neutrophils and macrophages accumulated in the airways, and these cells release multiples products such as inflammatory cytokines (tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-8), reactive oxygen species, or protease (like neutrophil elastase), all contributing to the HAE destruction [19,25,75,76]. This excessive and ineffective inflammatory response, probably exacerbated by bacterial toxins, leads to the inadvertent destruction and damage of the HAE [14,18,19,25]. Various injuries to the HAE have been previously described in CF airways, including microlesions, shedding areas (few basal cells are still present or even
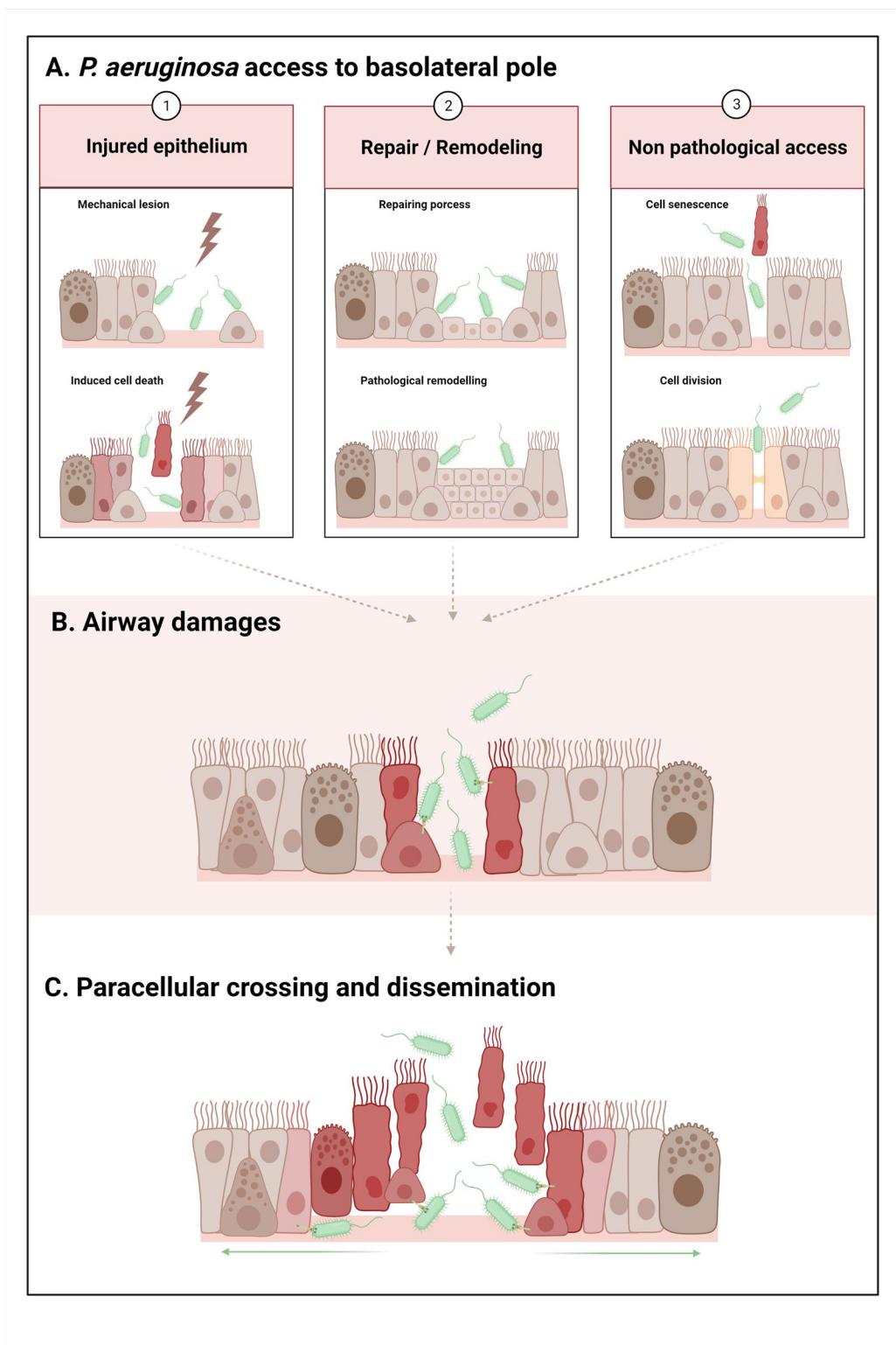
Fig 2. *P. aeruginosa* basolateral interaction and progression in the airway epithelium. A. In the first step of infection, *P. aeruginosa* gains access to the basolateral part, exploiting various opportunities: **1.** Injured epithelium: **a.** Mechanical lesion: removal of HAE cells and denudation of the basement membrane. Ex: endotracheal tube in VAP. **b.** Induced epithelial cell death: dying cells undergo retraction, tight junction disruption, and detachment from the adjacent cells and the basement membrane. The injury could be caused by pathogens (viruses, bacteria, etc.), chemical injury (pollutants,

toxic compounds, etc.), or excessive inflammatory processes (excess of cytokines, proteases, oxidant stress, etc.). Ex: excess inflammation injuring HAE in CF and COPD. **2.** Repair of the epithelium after injury/epithelium remodeling: **a.** After an injury, HAE undergoes a repair process: basal cell dedifferentiation, spread and migration, transitional squamous metaplasia or basal/mucous hyperplasia, and progressive differentiation. Cells display low differentiation levels, low polarity, and no functional tight junctions. Ex: repair in CF and COPD. **b.** Chronic and pathologically remodeled epithelium: squamous and goblet cells metaplasia, hyperplasia of surface goblet and basal cells. Cells display low differentiation levels, low polarity, and no functional tight junctions. Ex: remodeled epithelia in CF and COPD. **3.** Nonpathological access in differentiated epithelium: transient disruption of tight junctions during: **a.** Extrusion of a senescent cell. **b.** Cell division. **B.** *P. aeruginosa* virulence factors induce airway damage, notably by T3SS toxin injection on the basolateral membranes of the cells. T3SS effectors (ExoS, ExoT, and ExoU) induce cell retraction and death, facilitating the subsequent access of bacteria to the adjacent or underlying cells and the basement membrane. **C.** Bacteria cross the epithelium by this paracellular route, gain access to the basal part, and progressively propagate radially through the epithelium to disseminate, using pili and flagella. Created with BioRender.com.

https://doi.org/10.1371/journal.ppat.1011221.g002

total denudation of the basement membrane), epithelial sloughing and disorganization of the epithelial tight junctions, etc. [14,39,64,81,82].

Besides the mucociliary clearance elevator, the mucus composition could also play a role in bacterial virulence in the lung. In healthy epithelium, the mucus protects against pathogens' epithelial adhesion and cytotoxicity and appears to play an important role in suppressing bacterial virulence. It was shown that mucins prevented *P. aeruginosa* aggregation and adhesion to the underlying surface and that mucins triggered the dispersal of *P. aeruginosa* biofilm [83,84]. Mucins significantly enhanced twitching motility and decreased biofilm formation [85]. It was also described that mucins attenuated the virulence of *P. aeruginosa*, decreasing many virulence genes (such as type 3 and 6 secretion systems (T3SS/T6SS), siderophores, and quorum sensing), disintegrating biofilms, and reducing cytotoxicity on human epithelium colorectal cells and burn infections in a porcine model [86]. When mucus structure and/or properties are compromised, the mucus' protective abilities may be significantly decreased. It is exemplified in CF with a defect of mucin expression and mucus production associated with more susceptibilities to *P. aeruginosa* infection than in the healthy lung [50].

In COPD patients, the chronic and excessive inflammation due to the causative inhaled irritants (for instance, cigarette smoke) leads to an increased number of neutrophils and macrophages in the lungs, as well as to the activation of airway epithelial cells and mucus hypersecretion. Emphysema and luminal occlusion by aberrant mucus and inflammatory exudate is commonly observed [87]. A reduced mucociliary clearance is also described in COPD [88]. Cells produce pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-8, and release elastase. Combined with the products of oxidative stress, it contributes to the HAE breakdown [87,88]. Microbial colonization of the respiratory tract is often observed, which can be associated with acute exacerbation, enhancing the host inflammatory response [7]. This combined pathogen and host actions enhance HAE injuries [7]. Particularly, in COPD, the major alteration of the lung is parenchymal destruction [65]. Coinfections with viruses and bacteria have been described to cause more severe functional lung impairments in COPD patients [89]. Viruses, notably the respiratory syncytial virus, alter the expression of receptor molecules on respiratory epithelial cells and provoke cell death. In turn, an increase in the adhesion and invasion by *P. aeruginosa* leads to coinfections or superinfections [7,90].

Repair of the epithelium after injury/epithelium remodeling. To regenerate and restore its function following injury, the HAE has to repair itself. During this process, the surviving basal cells that are adjacent to the wound edge, spread, and migrate to cover the denuded basement membrane [35,91]. Basal cells then proliferate, forming a transitional squamous metaplasia [37]. Cells undergo a differentiation process, progressively regenerating their polarity and reform junctions, resulting in a complete and functional pseudostratified mucociliary epithelium. During this repairing process, the exposed cells display low or no differentiation levels,

low or no polarity, no functional tight junctions, and expressed alternative receptors, favoring interactions with *P. aeruginosa* [14,15,35,43,70,72] (see "Adhesion" section and Table 1). As for injuries described above, for CF and COPD, *P. aeruginosa* interactions with repairing HAE are facilitated (Fig 2A and middle panel).

In CF and COPD, chronic and pathological remodeling, such as squamous metaplasia or hyperplasia of goblet and basal cells, is typically observed [34,38–42,65,82]. As in the repairing process, cells in remodeling epithelia show reduced differentiation, low polarity, absence of functional tight junctions, and expression of alternative receptors, again enhancing their potential interaction with *P. aeruginosa* [14,15,70] (see "Adhesion" section and Table 1).

Access in nonpathological conditions. Bacteria can also gain access to the basolateral part of the HAE in differentiated epithelium without any injury or repair process, due to the normal epithelial renewal. Some studies have shown that after a transient disruption of the epithelial junctions, either following extrusion of a senescent cell or cell division during multiplication, the basolateral part becomes exposed and *P. aeruginosa* can interact with the HAE [92–94]. As previously described for the pathogenic bacteria *Listeria monocytogenes* in the MDCK cell line [95], Heiniger and colleagues described that *P. aeruginosa* adhesion to the apical membrane of ciliated cells from primary differentiated human epithelium is very low (see "Adhesion" section). They also showed that the interaction with the cells occurs after a transient disruption of the epithelial barrier during the extrusion of a senescent cell. The basolateral membrane of the ciliated cells is then exposed, and the bacteria can easily interact with it [94]. Disruptions of tight junctions occur during cell divisions or cell senescence and are both natural phenomena ensuring epithelial homeostasis [92,93]. In the MDCK cell line model, Golovkine and colleagues showed that very soon after HAE infection (approximately 3 hours), *P. aeruginosa* takes advantage of these brief and transient ruptures of the epithelial barrier to gain access to the basolateral part. In this model, cell deaths were not caused yet by *P. aeruginosa* but were natural/physiological deaths [93] (Fig 2A and right panel).

The clinical impact of the interaction of *P. aeruginosa* to the basolateral part in vivo in healthy humans might be low and insufficient to develop an infection, as *P. aeruginosa* is rarely described in community-acquired pneumonia [96]. The functional mucociliary clearance and competent immune defenses are likely to clear most of the *P. aeruginosa* present in the airways of healthy individuals. Moreover, airway cell divisions/senescence are infrequent, decreasing the probability of encountering *P. aeruginosa* to initiate infections in healthy individuals. Indeed, (i) the cell turnover rate of the HAE self-renewal is one of the lowest compared to other epithelia, more than 100 days for HAE [97,98] versus 3 to 5 days for intestinal epithelium, 10 days for corneal epithelium, or 20 days for cutaneous epithelium [99–102]; (ii) Golovkine and colleagues described that the opportune interaction of *P. aeruginosa* is not systematic in their model but is rather a rare event since many sites of cell divisions/deaths were not exploited by the bacteria [93]; (iii) the cloaking by resident tissue macrophages prevents excess tissue damages and preserves tissue homeostasis [77]; and (iv) the few cases of *P. aeruginosa* community-acquired pneumonia, described in healthy individuals, were often associated with prolonged or repeated exposures to contaminated whirlpools or hot tubs aerosols [61,103–105]. The number of *P. aeruginosa* observed in these contaminated waters, wherein the bacteria are proliferating, was high (up to 10^5 colony-forming units (CFU)/mL) [61,105–107]. We can speculate that the high infectious inoculum of *P. aeruginosa* can counter the strong barrier effect by increasing the probability of encountering the rare physiological breach event, thereby explaining the development of infection in a healthy individual.

Either way, it is noteworthy that the studies describing *P. aeruginosa* interaction with intact airway epithelium in ex vivo culture models used very high bacterial inocula (from 10^6 to 10^8 CFU [72,94,108–112]) or high concentration of purified virulence factors (such as elastase

[113–117], LPS [118], rhamnolipids [119,120], and 3OC12-HSL [121–123]). Although both are related to the bacterial load commonly detected in clinical samples [124,125], they correspond to thresholds commonly seen in clinical microbiology laboratories at the time of airway infection diagnosis. Considering the bacterial proliferation in the infected airways, they are likely far higher than the inoculum at the initiation of the epithelial breach. Additional studies are needed to address the relevance of the infectious inoculum in vivo, when *P. aeruginosa* pulmonary infections occur in CF, COPD, or VAP.

The steps of progression of *P. aeruginosa* during airway infection

1) Adhesion of *P. aeruginosa* to the airways. Respiratory infections by *P. aeruginosa* are usually initiated by the adhesion of the bacteria to the HAE cells or the basement membrane. Appendices of motility and adherence, pili, and flagella are virulence factors present on the bacterial surface, mainly responsible for movement and attachment to the cells [109].

Table 1. *P. aeruginosa* airway receptors.

	Airway receptors	Type of cells used to evidence airway receptors	Location in healthy airways	Pathological epithelia	PA adhesin	References
Glycosphingolipids (Lipid rafts components)	**Asialo GM1 (GalNAcβ1-4Gal disaccharides)**	Primary human nasal epithelial cells (differentiated and not differentiated)	Basal cell membrane	Specific apical membrane expression by regenerating epithelium or CF epithelium	- Type IV pili (C-terminal part) - Flagella	[16,127–131]
	Globotriaosylceramide Gb3 (CD77, αGal–βGal–βGlc–Cer saccharides)	Cell lines (H1299 kc, A549)	Unknown	-	LecA	[132–137]
N-glycoproteins	**N-glycans**	Cell lines (Calu 3) Primary bronchial cell	Apical membrane mainly (expressed on basal cell membrane, but few interactions with PA)	Enhanced expression in damaged epithelium	Type IV pili	[109,138,139]*
	CFTR	Cell lines (CFT-1, CFBE41o-, A549, WI-38) Primary human bronchial epithelial cells (differentiated)	Apical membrane	Decreased expression in dedifferentiated and remodeled epithelium	LPS	[140–145]
Integrins	**α5β1 integrin–Fibronectin**	Cell lines (16HBE) Primary nasal cells (differentiated)	Absent in normal airways	Apically exposed and overexpressed in repairing epithelium	OprQ[a]	[70,71,146–148]
	αvβ5 integrin–Vitronectin	Bronchial tissue	Unknown	Increased expression in repairing epithelium or inflamed epithelium	OprD	[149–152]
Proteoglycans	**HSPG (Heparan sulfate proteoglycans) (HS chain)**	Cell lines (Calu 3, 16HBE) Primary nasal cell	Basal cell membrane mainly	Apically increased during epithelial injury and dedifferentiation	Flagella	[109,138,153,154]*
ECM	**Laminin Type I and IV collagens**	Not applicable	Basement membrane (ECM beneath the epithelium)	Accessible only in injured epithelium	OprD, OprG, EstA, PA3923[b]	[14,155,156]

CFTR, cystic fibrosis transmembrane regulator; ECM, extracellular matrix; LPS, lipopolysaccharides; OMP, outer membrane protein; PA, *P. aeruginosa*.

[a]Roger and colleagues described a 50-kDa OMP, probably corresponding to the OprQ porin described later by Arhin and colleagues.

[b]de Bentzmann and colleagues described 60 kDa OMPs, probably corresponding to the OprD, OprG, EstA, PA3923a porin described later by Paulsson and colleagues.

*References [139,154], cited for the localization of the airway receptor, were performed in another context than *P. aeruginosa* infection.

https://doi.org/10.1371/journal.ppat.1011221.t001

Utilizing different mechanisms of motility (swarming, swimming, and twitching), the bacteria sense the optimal surface for initiating cell surface contact and properly attached to the surface [126]. Several molecules, present on the host epithelial cell and extracellular matrix components and containing long carbon chains, bind the different adhesins of *P. aeruginosa* (Fig 3 and Table 1).

It is noteworthy that some of these receptors, listed in Table 1, are expressed at different locations on the epithelium, depending on specific situations. At the apical side of healthy airway epithelium, very few receptors are present, which strongly limit interaction with *P. aeruginosa*. For instance, integrins or N-glycans are hardly or not at all present at the apical part of healthy epithelium, but they are overexpressed during the repair process specifically [138]. Asialo GM1 and HSPG are mainly expressed at the basolateral part but are specifically recovered at the apical part in regenerating or CF epithelia [16,128,138]. Migrating cells actively synthesize cellular fibronectin/vitronectin, and their receptors, the integrins, are up-regulated and apically exposed during migration in the repairing process [70,148,151]. As fully detailed in Part I, the adhesion of *P. aeruginosa* to receptors at the basolateral part of the epithelium can only occur in case of
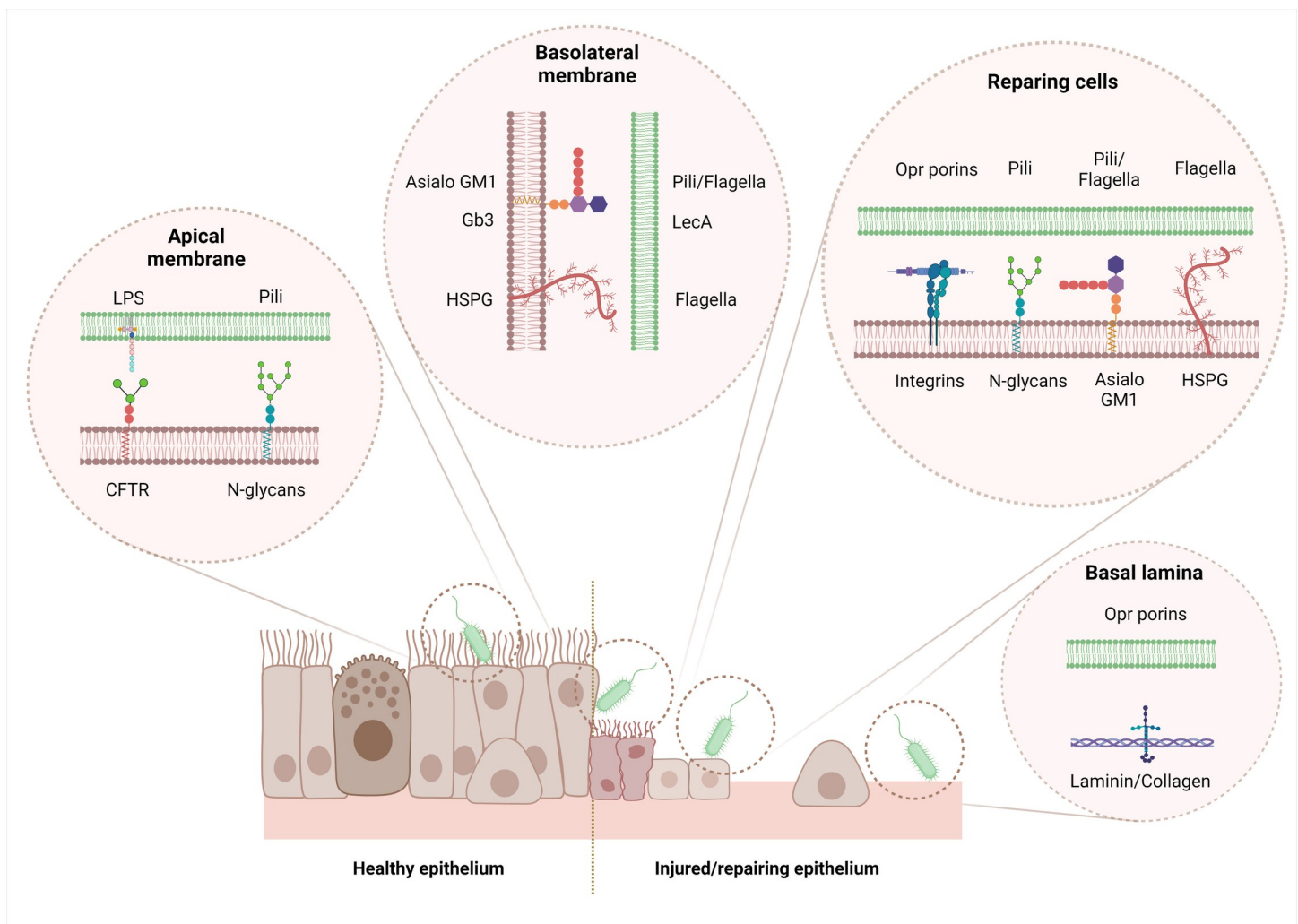

Fig 3. Airway receptors of *P. aeruginosa*. Green membrane: *P. aeruginosa*; brown membrane: host cells. Created with BioRender.com.

https://doi.org/10.1371/journal.ppat.1011221.g003

disruption or lack of tight junctions or when cells lose their polarization, which is mainly observed within injured or repairing/remodeled epithelium [9,14–16,67,68,94].

Pili-mediated adhesion, combined with their retractive ability, creates close contact between bacteria and host cells and induces the action of various export systems. In particular, the T3SS is an important contact-dependent virulence factor that injects toxins through the membrane of the host cell [157,158]. Pili mechanical retraction induces the chemosensory system phosphorelay (Chp), leading to stimulation of adenylate cyclase CyaB and up-regulating the signaling molecule adenosine-cyclic monophosphate (cAMP) production, which allosterically activates the virulence factor regulator (Vfr)-dependent virulence system [158,159]. Vfr regulates multiple virulence factors in *P. aeruginosa*, including the T3SS, but also the T2SS and the quorum sensing [160,161].

2) Airway damage induced by *P. aeruginosa*. *P. aeruginosa* virulence factors involved in respiratory infections. *P. aeruginosa* is an environmental bacterium adapted to the soil and aqueous habitat. It is an opportunistic pathogen found mainly in immunocompromised patients. Human airway pathogenicity of *P. aeruginosa* is explained by the production of many virulence factors, displaying varied and complementary properties [162,163]. Some factors are involved in motility and adhesion, persistence in the host, iron uptake, or resistance to oxidative stress. The toxins are mostly involved in the destruction of the host cells [163].

The main virulence factors of *P. aeruginosa* involved in respiratory infections are illustrated in Fig 4 and their functions are detailed in Table 2.

Most of these virulence factors are not synthesized constitutively but are regulated depending on the needs of the bacteria in different types of settings and collaterally during infection. The production is fine-tuned by quorum sensing, which is a communication system based on the detection of bacterial density in the environment [164]. The communication is mediated by the secretion of small signaling molecules, such as homoserines lactones or quinolones, used as autoinducer messengers. Each bacterium produces these messengers, thereby their concentration depends on the growing bacterial population [164]. When the critical threshold is reached (i.e., *quorum*), four regulatory systems are activated to coordinate the response of the bacterial population: Las, Rhl, PQS, and IQS systems [164–167]. Around 10% of the *P. aeruginosa* genome is estimated to be regulated by the quorum sensing system [168].

In some conditions, and notably in CF disease, *P. aeruginosa* is known to form structured aggregates known as biofilms. After adhesion to a surface, the bacteria congregate in highly organized communities, surrounded by an extracellular matrix composed of exopolysaccharides (alginate, PEL, and PSL), extracellular DNA, lipids, and proteins [169]. This matrix, representing between 50% and 90% of the total volume of the biofilm, strengthens its structure and protects bacteria from effectors of the host's immune response (such as phagocytosis or antibody actions) and antibiotics, making their eradication very difficult [18,167,169]. In addition, bacteria within the biofilm are exposed to oxygen gradients and nutrient limitations leading to modification of bacterial metabolism. One clinically relevant phenotype that occurred in such a situation is the small colony variant phenotype, which is characterized by slow growth and a loss of cytotoxicity [25]. Biofilm formation is regulated by the quorum sensing system [18,165]. The development of the mucoid phenotype by overproduction of alginate found in biofilms is a strategy for the bacteria to survive in a hostile environment and is mainly observed in *P. aeruginosa* chronic respiratory infections (such as in CF patients) and biomaterial-associated infections (endotracheal tubes, urinary catheters, etc.) [25,170]. The inert surfaces of the biomaterial are used as a base that facilitates the development of biofilms [18,171].

During the progression of chronic *P. aeruginosa* infection in CF patients, phenotypic and genotypic changes occur in *P. aeruginosa* to adapt to the specific mucus-plugging environment. Strains established in this long-term colonization show less inflammation and less
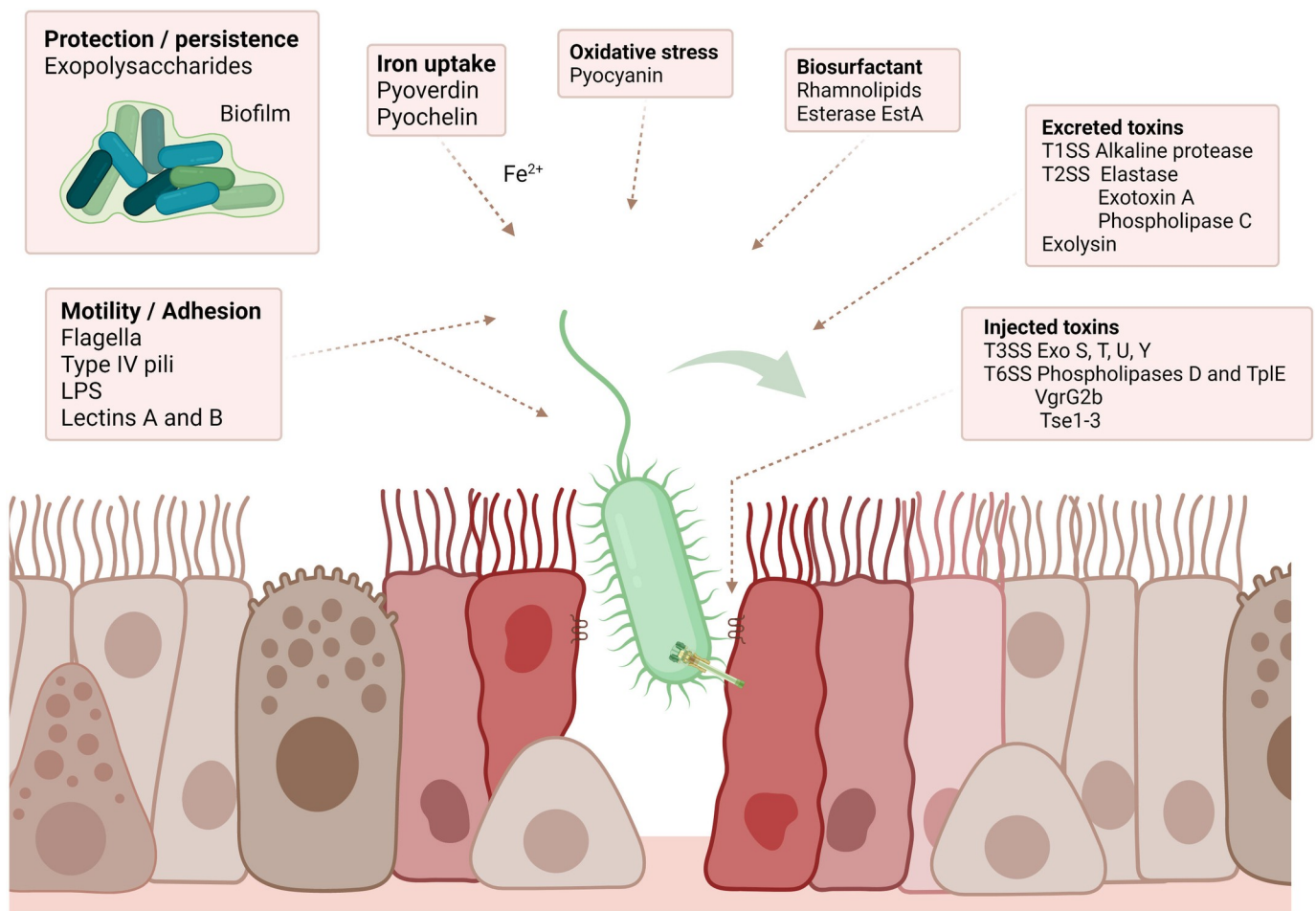
Fig 4. *aeruginosa* **main virulence factors in respiratory infections.** *P.* Created with BioRender.com.

cytotoxicity against HAE than the first strains years earlier in the same patient [18]. A switch from acute to chronic phenotype occurred. It has been described to be characterized by down-regulation of some virulence factors (motility with loss of flagellum and pili, protease, rhamno-lipids, pigments, . . .), increased biofilm formation, the apparition of mucoid or small colony variant phenotype, change in LPS, and alteration of quorum sensing system [18,78].

From the first stages of *P. aeruginosa* interaction with the airway epithelium, the first line of defense of the innate immune system in the lung recognizes *P. aeruginosa* components and virulence factors, activating an immune reaction to prevent or resolve the infection. Various immunity actors are then recruited, and inflammation processes are observed [19].

First, specific *P. aeruginosa* patterns are recognized thanks to pattern recognition receptors (PRRs) [19,221]. Three types are described: (i) Secreted PRR, such as Complement C1q, collectins, and surfactant proteins SP-A and D, which bind *P. aeruginosa* cell wall and mark it for clearance by macrophages and neutrophils. (ii) Transmembrane PRR, the most important actors, represented by the Toll-like receptors (TLRs) present on immune and epithelial cell membranes. They recognize different components of *P. aeruginosa* call the PAMPs (pathogen-associated molecular patterns). For example, TLR2 recognizes the pili, TLR4 and CFTR the LPS, and TLR5 the flagella. (iii) Cytosolic PRRs, such as TLR9 and NOD-1, can recognize bacterial DNA or cell wall components of the internalized/phagocytosed bacteria.

Table 2. Virulence factors of *P. aeruginosa* in respiratory infections.

Class	Virulence factor	Gene(s)	General function(s)	HAE-specific function(s)	Type of cells used to evidence HAE-specific function(s)	Molecular host target	SS	Reference
Motility/ Adhesion	Flagella	*fliC*	Swimming and swarming motility Host cell adhesion and internalization	Motility and basal adhesion to HAE cells HAE infection progression	Primary nasal cells, cell lines (Calu 3, 1HAEo-), mice pneumonia	Heparan sulfate, proteoglycans, AsialoGM1	-	[127,130,138,172]
	Type IV pili	*pilA*	Twitching motility Host cell adhesion, biofilm formation	Motility and apical adhesion to HAE cells Allow T3SS injection. HAE infection progression	Cell lines (Calu 3)	N-glycans	-	[138,173,174]
	LPS	*waa*, *wzy*, *wzz*, *wbp*, *wzx*	Resistance to serum killing and phagocytosis, inflammatory response Binding to CFTR Cell junctions' disruptions and apoptosis	Apical adhesion to HAE cells Disruption of HAE tight junctions HAE cell apoptosis	Cell lines (A549, NCL-H292, Beas-2b)	CFTR, Tight junctions (ZO-1)	-	[54,118,175–177]
	Lectin A Lectin B	*lecA* *lecB*	Host cell adhesion: binding to galactose (LecA) and fucose (LecB) Internalization (LecA) Cytotoxicity (LecA and LecB)	Apical and basal adhesion to HAE cells HAE cell internalization HAE cell cytotoxicity	Primary nasal cells, cell lines (H1299, A549), mice pneumonia	Globotriaosylceramide Gb3 (CD77)	-	[132,134,178]
Protection/ Persistence	Exopolysaccharides (Alginate, Psl, Pel)	*alg*, *muc*, *psl, pel*	Biofilm main component: polymeric matrix Persistence, bacteria protection (from IS and antibiotics)	Biofilm persistence in chronic HAE infection	-	-	-	[167,179–181]
Iron uptake	Siderophores: - Pyoverdin - Pyochelin	*pvd* *pch*	Iron siderophore, green pigment (pyoverdine) Cytotoxicity (ROS production)	-	-	DNA, lipid membrane, proteins	-	[182–185]
	Heme uptake systems: - *Pseudomonas* heme uptake - Heme assimilation systems	*phu* *has*	Extracellular heme acquisition (uptake in the cytoplasm)	-	-	-	-	[185–187]
Oxidative stress	Pyocyanin	*phz*	Cytotoxicity (ROS production: O_2^-, H_2O_2) IS regulation (apoptosis), blue-green pigment	Inhibition of ciliated HAE function	Primary sheep cells	DNA, lipid membrane, proteins	-	[188–190]

(*Continued*)

Table 2. (Continued)

Class	Virulence factor	Gene(s)	General function(s)	HAE-specific function(s)	Type of cells used to evidence HAE-specific function(s)	Molecular host target	SS	Reference
Biosurfactant	Rhamnolipid	*rhl*	Amphiphilic: detergent and solubilizing properties Disruption cell junctions IS regulation (neutrophils lysis) Biofilm formation, motility	Solubilization of airway surfactant Disruption of HAE tight junctions	Primary nasal cells	Tight junctions (ZO-1, occludin, JAM-A)	-	[120,191–193]
	Esterase EstA	*estA*	Autotransporter enzyme: Hydrolyze glycerol esters Rhamnolipids production	Disruption of HAE tight junctions	See Rhamnolipid	See Rhamnolipid	-	[194]
Excreted toxins	Alkaline protease	*aprA*	Protease activity: IS inactivation (antibodies, neutrophils, complement, cytokines) and laminin	Degradation of HAE extracellular matrix: (basement membrane: laminin)	-	Laminin	T1SS	[195,196]
	Elastase	*lasB*, *(lasA)*	Protease activity: elastin, collagen, transferrin, antibodies, complement → Tissue damage Disruption of cell junctions	Degradation of HAE extracellular matrix (basement membrane and alveolar septum: elastin and collagen) Disruption of HAE tight junctions	Primary nasal cells, cell lines (Calu 3)	Elastin, Collagen, tight junction (ZO-1, claudin, occludin)	T2SS	[113,114,117,197]
	Exotoxin A	*toxA*	Host cells protein synthesis inhibition: eEF2 inhibition by ADP-ribosylation → Cell death	HAE cell death	Cell lines (CuFi-1)	eEF2	T2SS	[198,199]
	Phospholipase C	*plcH*	Hydrolysis of phospholipids (erythrocyte and leukocyte cytolysis), surfactant degradation	Pulmonary surfactant degradation	-	Phosphatidylcholine, Sphingomyelin	T2SS	[200–203]
	Exolysin	*exlA*	Pore-forming toxin: cell membrane disruptions (erythrocytes, leukocytes, epithelial cells)	HAE cell death and retraction	Cell lines (A549), mice pneumonia	Phophoslipid bilayers	-	[108,204,205]
	CFTR inhibitory factor	*cif*	-	TAP-1 mediated-MHC class 1 antigen presentation and CFTR-mediated chloride secretion inhibition	Cell lines (CFBE41o-, A549)	CFTR and TAP-1	OMV	[206,207]

(*Continued*)

Table 2. (Continued)

Class	Virulence factor	Gene(s)	General function(s)	HAE-specific function(s)	Type of cells used to evidence HAE-specific function(s)	Molecular host target	SS	Reference
Injected toxins	Exo S (exoenzyme S)	*exoS*	GTPase-activating protein activity and ADP ribosyltransferase activity: inhibition of several host cell functions (cell apoptosis, cell division and cell migration inhibitions, junctions and actin cytoskeleton disruptions)	HAE cell death and retraction	Mice pneumonia	Rho family of GTPases, Ras superfamily GTPases	T3SS	[163,208,209]
	Exo T (exoenzyme T)	*exoT*						
	Exo U (exoenzyme U)	*exoU*	Phospholipase A2 activity: membrane phospholipids hydrolysis (rapid cell necrosis)	Rapid HAE cell death and retraction	Mice pneumonia, cell lines (Beas-2b)	Phospholipids	T3SS	[163,208,210]
	Exo Y (exoenzyme Y)	*exoY*	Adenylate cyclase activity: actin cytoskeleton disruptions	HAE cell death and retraction	Mice pneumonia, cell lines (PMVECR1)	Tau protein (microtubule)	T3SS	[211–214]
	Phospholipase D	*pldA*, *pldB*	- Host cell internalization - Bacterial competition	HAE cell internalization	Cell lines (Calu 3)	Akt kinase	H2 (*pldA*) H3 (*pldB*) T6SS	[110,215,216]
	VgrG2b	*vgrG*	- Host cell internalization - Bacterial competition	HAE cell internalization	Cell lines (Calu 3	γ-tubulin ring complex (γTuRCn microtubule component)	H2 T6SS	[110,217]
	Phospholipase TplE	*TplE*	- Host cells endoplasmic reticulum disruption - Bacterial competition	-	-	Endoplasmic reticulum apparatus	H2 T6SS	[218]
	Tse1-3	*Tse1-3*	Bacterial competition (peptidoglycan degradation)	-	-	-	H1 T6SS	[215]
Short RNA	sRNA52320	-	-	Decrease of IL-8 secretion	Bronchial primary cells	Kinases of the LPS-stimulated MAPK pathway	OMV	[219]
Global regulatory system	Quorum sensing	*las*, *rhl*, *pqs*, *iqs*	Autoinducer peptides (for instance, homoserines lactones 3O–C12–HS) detect critical density → activation of 4 regulation systems: impact on virulence factors production and biofilm formation	Disruption of tight junctions[a]	-	Tight junctions (ZO-1, ZO-3, JAM-A, occludin)	-	[122,123,164,165,220]

[a]Disruption of cells' tight junction described in the intestinal epithelial model (Caco2 cells), not yet in HAE.

CFTR, cystic fibrosis transmembrane regulator; eEF2, eukaryotic elongation factor 2; HAE, human airway epithelium; H_2O_2, hydrogen peroxide; LPS, lipopolysaccharide; MHC, major histocompatibility complex; OMV, outer membrane vesicle; O^2, superoxide; IS, immune system; ROS, reactive oxygen species; SS, secretion system; TAP, transporter associated with antigen processing; TxSS, type x secretion system.

Various cells coordinate efforts to produce an appropriate innate immune response [18,19,78]. Epithelial cells of the HAE play an important role of alert of *P. aeruginosa* presence. After TLR-mediated activation, they produce a large panel of pro-inflammatory cytokines (TNF-α, IL-1β, IL-2, Il-6, . . .) and the chemokine IL-8, which initiate inflammation and recruit other more specialized immune cells such as neutrophils. They also produce antimicrobial peptides (defensin, cathelicidin LL-37) and reactive oxygen species (radical hydroxide, hypothiocyanite), acting directly against *P. aeruginosa*. Neutrophils play a critical role in *P. aeruginosa* destruction and the development of the inflammatory response. Not present in the uninfected lung, they are recruited by attractive chemokines released by sentinel cells during infection, such as macrophages or epithelial cells, mediated by PRR signals. They phagocyte and kill the bacteria and they generate a great number of antibacterial molecules, such as reactive oxygen species (superoxide anion, hydrogen peroxide, radical hydroxide, nitric oxide, responsible for an oxidative burst), proteases (elastase), and antimicrobial peptides (defensin, cathelicidin LL-37), lactoferrin and lysozyme. Alveolar macrophages are the resident leukocyte of the lung, acting as the main sentinel for infectious agents' detection. They recognize *P. aeruginosa* with their PRR, phagocytose it, and complete the immune cells activation of the HAE cells, through pro-inflammatory cytokines secretion.

After this review of all *P. aeruginosa* virulence factors involved in respiratory infection, we will focus on the most important damages described in airway infections, caused by the main virulence factors secreted by *P. aeruginosa*. Two types of degradation will be described: (i) HAE cell damages, wherein the key role of T3SS in host cell retraction and disruption of tight junctions facilitates the subsequent access of bacteria to the adjacent or underlying cells (ii) airway components degradation (basement membrane, surfactant), allowing the radial progression of *P. aeruginosa* in the epithelium (Fig 2B and 2C).

HAE cells damages: The key role of the T3SS cytotoxic activity Among all the virulence factors produced by *P. aeruginosa*, the T3SS and its effectors are commonly considered the major determinant of virulence during infection [157]. This extensively studied secretion system plays a key role in the *P. aeruginosa* pathogenesis by injecting effectors that include toxins directly into the cytoplasm of the host cells including HAE cells [157]. *P. aeruginosa* strains that do not express T3SS are less virulent in human clinical infections and animal infection models [157,222,223].

T3SS-mediated delivery of the exoenzymes requires the adhesion of the bacteria to the host cell. As previously described, attachment via pili and their retraction activate signaling pathways, mediated by the Chp system, and cAMP/Vfr circuit, which lead to the transcription of virulence-associated genes, notably T3SS effectors [158,160,161]. Four well-known T3SS effectors have been described (exoenzymes ExoS, ExoT, ExoU, and ExoY), but not all are produced by every *P. aeruginosa* strain. ExoT and ExoY are found in nearly all strains, but ExoS and ExoU are mutually exclusive [224,225] and rarely found together in the same strain [226,227]. Two types of *P. aeruginosa* strains are therefore observed, with distinct pathogenesis phenotypes: (i) the "ExoU profile," expressing ExoT, ExoU, and ExoY, is highly virulent, induces rapid cell death of the host cells, and shows a low rate of cell internalization; and (ii) the "ExoS profile," expressing the ExoS, ExoT, and ExoY, causes slower death and exhibits a higher rate of internalization. The phenotypes associated with the expression of "ExoU profile" are mainly encountered in acute infections (bacteremia, ocular infections, acute pneumonia, etc.), while the "ExoS profile" is predominant in pulmonary chronic infections (in CF, COPD, etc.) [163,208,228–230].

ExoU, which is considered the most potent of type 3 secreted toxins, has a phospholipase A2 activity that induces host cell membrane disruption by phospholipids hydrolysis. It leads to rapid necrotic death of both epithelial and immune cells. ExoU was mainly described in severe and acute infections, especially respiratory infections [163]. ExoS and ExoT are bifunctional

proteins both displaying two distinct enzymatic activities: an N-terminal GTPase-activating protein (GAP) activity and a C-terminal adenosine diphosphate ribosyl-transferase (ADPRT) activity. Inhibiting host cell GTPases, such as Rho-, Rac-, Ras-, Rap-, and Cdc42 GTPases, ExoS, and ExoT cause actin cytoskeleton depolymerization of the HAE cells. It eventually results in the detachment of HAE cells from the basement membrane due to cell retraction (disruption of the cell structure and subsequent tight junctions disruption), cell rounding and apoptosis [157,163,231].

It is worth noting that the studies describing preferential T3SS cytotoxicity towards the basolateral part rather than the apical surface of HAE used mainly differentiated epithelium models, such as human or bovine primary airway epithelial cells cultured at the air–liquid interface (from 7 days to 6 weeks) [68,72,94] (Fig 5 and S1 Table). Such models likely mimic more in vivo conditions than immortalized cell lines [232].

Altogether, T3SS effectors play a key role in HAE damaging by facilitating the subsequent access of bacteria to the basolateral surface of other HAE cells nearby, allowing *P. aeruginosa* to break through the epithelium (Fig 2B and 2C).

Other secreted toxins or membrane constituents of *P. aeruginosa* have been shown to act complementarily to T3SS leading to enhanced cytotoxicity on HAE cells. For instance, the T2SS-secreted Exotoxin A catalyzes the ADP-ribosylation of its host target protein, the eukaryotic elongation factor 2 (eEF2), resulting in the inhibition of protein synthesis and ultimately leading to cell death [198,199]. The LecA and LecB binding proteins have also been shown to participate, at least in part, in a cytotoxic effect on HAE cells and to lung injury in a murine model [134]. The LPS can also induce apoptosis in lung endothelial and epithelial cells [177]. ExlA exolysin, a pore-forming toxin recently discovered in some strains of *P. aeruginosa* lacking T3SS, forms pores in the host cell membrane, leading to a massive entry of Ca^{2+} that in

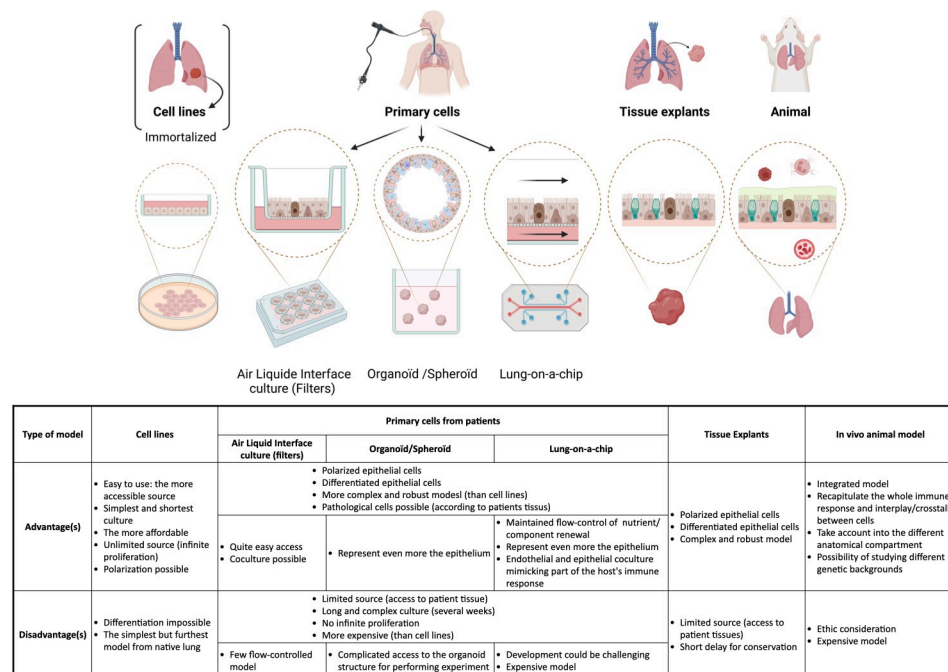| Type of model	Cell lines	Primary cells from patients			Tissue Explants	In vivo animal model
		Air Liquid Interface culture (filters)	Organoid/Spheroïd	Lung-on-a-chip		
Advantage(s)	• Easy to use: the more accessible source • Simplest and shortest culture • The more affordable • Unlimited source (infinite proliferation) • Polarization possible	• Quite easy access • Coculture possible	• Polarized epithelial cells • Differentiated epithelial cells • More complex and robust models! (than cell lines) • Pathological cells possible (according to patients tissus) • Represent even more the epithelium	• Maintained flow-control of nutrient/ component renewal • Represent even more the epithelium • Endothelial and epithelial coculture mimicking part of the host's immune response	• Polarized epithelial cells • Differentiated epithelial cells • Complex and robust model	• Integrated model • Recapitulate the whole immune response and interplay/crosstalk between cells • Take account into the different anatomical compartment • Possibility of studying different genetic backgrounds
Disadvantage(s)	• Differentiation impossible • The simplest but furthest model from native lung	• Few flow-controlled model	• Limited source (access to patient tissue) • Long and complex culture (several weeks) • No infinite proliferation • More expensive (than cell lines) • Complicated access to the organoid structure for performing experiment	• Development could be challenging • Expensive model	• Limited source (access to patient tissues) • Short delay for conservation	• Ethic consideration • Expensive model

Fig 5. Models of airway epithelia. It is of the utmost importance to use relevant experimental models to assess the host–pathogen interactions driving *P. aeruginosa* infection, depending on the physiological relevance needed: Various models exist, from cell lines to primary cells and tissue explants/animal models, each with their advantages and disadvantages. All the models used in the references of the review are listed in S1 Table. Created with BioRender.com.

https://doi.org/10.1371/journal.ppat.1011221.g005

turn activates the ADAM10 metalloprotease [233]. The subsequent cleavage of the adherens junctions constituent E cadherin leads to HAE cell membrane disruption and death [108,205].

Some virulence factors affect the cells' tight junctions and allow *P. aeruginosa* tissue progression. The proteolytic LasB elastase disrupts the tight junctions by acting on occludin, claudin, or ZO-1 proteins [113,114,116,117]. Rhamnolipids could also play a role thanks to their amphiphilic properties. They are incorporated into membranes and disrupt the architecture of tight junctions after several hours of infection in culture models [119,120,193]. In the same way, the LPS, a membrane component of *P. aeruginosa*, increases airway epithelium barrier paracellular permeability by disrupting the tight junctions, by affecting ZO-1 and ZO-2 [118,176]. Although studied only in an intestinal epithelium model, the homoserine lactone 3O–C12–HS of the *P. aeruginosa* quorum sensing has been shown to induce rupture of epithelium integrity [121]. It activates various cellular kinases implicated in tight junction's functions (p38 or p42/44), leading to a modification of ZO-1, ZO-3, JAM-A and occludin production, as well as a reorganization of the actin cytoskeleton [121–123].

However, the inoculum of *P. aeruginosa* in the healthy epithelium is likely to be very low and the produced quantity of these factors might probably not be sufficient to break down alone intact tight junctions. Their impact is more likely delayed in *P. aeruginosa* epithelial invasion, after access to the basolateral part and bacterial proliferation in the infected airways, resulting in an inoculum far higher (commonly detected from 10^5 to 10^7 CFU/mL in respiratory clinical samples [124,125]. The role of these components might be then complementary to the action of T3SS-secreted exoenzymes in tissue progression.

Degradation of other components of respiratory barriers In addition to the factors leading to the breakdown of the cells in respiratory epithelia, *P. aeruginosa* secretes several factors capable of destroying various components of the extra-epithelial components of the respiratory barriers, such as the basement membrane, the surfactant, and the mucins, which promote bacterial spread in the epithelium (Fig 2B and 2C).

P. aeruginosa produces proteases that play an important role in the degradation of the basement membrane and the mesenchyme. For instance, the LasB elastase, the most important protease of *P. aeruginosa*, degrades elastin and collagen and participates in tissue invasion and progression into the lung tissue [234]. The alkaline protease cleaves notably laminin, another important and biologically active component of the basement membrane [195].

Several other factors are involved in the degradation of the pulmonary surfactant: phospholipase C, which hydrolyses phospholipids [201,203,235], and the rhamnolipids, amphiphilic glycolipids having detergent and solubilizing properties [192].

It has been shown that *P. aeruginosa sdsA1* gene, encoding a secreted sulfatase, plays a central role in the degradation of mucin and that the *sdsA1* inhibition decreased the release of sulfate from mucin. *sdsA1* mutant showed a decreased mucin gel penetration and an attenuation of *P. aeruginosa* virulence in leukopenic mice intraperitoneally infected [236].

3) *P. aeruginosa* internalization in HAE cells.   Although *P. aeruginosa* is primarily an extracellular pathogen, internalization in nonphagocytic cells, such as airway epithelial cells, has been widely described for many years [71,72,217,237–240]. However, these descriptions were based on nonpolarized cells in the majority (S1 Table). Therefore, considering some of the recent findings, this mechanism might be not crucial, and, if observed, only a very small proportion of the bacteria is internalized, depending on bacterial clone and host cell types. The fraction of internalized bacteria in airway epithelium cultured from primary cells cultured at the air–liquid interface was estimated to be very low. From the primary airway cells model, Fleiszig and colleagues found an approximate rate of internalization of approximately 0.0001% within human nasal epithelium or approximately 0.001% in the bovine tracheal epithelium [72]. Conversely, internalization rates in cell lines were higher: approximately 1% to 2% within

MDCK or HeLa cells [72,110,138,241] (Fig 5). Furthermore, the host cell membrane composition, the intactness of tight junctions, and the level of cell polarization play a key role in the internalization process. It occurs more frequently on the basolateral cell surface, in cells with disrupted tight junctions or with low polarity levels, the latter being mainly observed on injured or repairing/remodeled epithelium [11,72,73,242–244].

Internalization process As with many other bacteria, *P. aeruginosa* internalization involves the formation of membrane lipid rafts [132,245], a signaling platform in the plasma membrane locally enriched in glycosphingolipids and cholesterol, which migrate within the phospholipid bilayer to form lipid aggregates [246]. No lysosomal fusion has been described after vacuole uptake and multiplying bacteria have been observed in the vacuoles [238]. Following bacterial binding, internalization is mediated by the rearrangement of the cellular actin cytoskeleton. The phosphatidylinositol 3-kinase (PI3K) coupled with the Akt pathway is involved in the entry into host cells through both apical and basolateral plasma membranes of epithelial cells [109]. The cytoskeleton microtubules have also been involved in the internalization of the bacteria, via the effectors delivered into cells by the T6SS. This mechanism promotes *P. aeruginosa* uptake into epithelial cells by interfering with the PI3K/Akt pathway, through the action of several effectors (H3-T6SS-dependent phospholipase D effector (PldB) and H2-T6SS VgrG2b effector), which interact with the γ-tubulin ring complex [110,216,217,247].

P. aeruginosa was shown to interfere with the epithelial polarity to enhance binding to the cells: It can induce a subversion of airway epithelial cell polarity to locally transform the apical into a basal plasma membrane and thus allow bacterial attachment and internalization into cells under. After remodeling, the membrane forms protrusions that internalize bacteria without disrupting the tight junctions [248–250].

Consequences of *P. aeruginosa* internalization on the pathogenicity The consequences of *P. aeruginosa* internalization in airway epithelial cells are still unclear. In an ALI airway epithelium model, which is highly representative of the conditions observed in the in vivo airway epithelium, the extremely low number of internalized bacteria found (around 0.0001%) is likely to indicate a probable accessory role of the internalization in the pathogenesis [72]. Different hypotheses have been proposed.

a. *The invasion of the epithelium*. Internalization, as a prerequisite to the invasion of the epithelium, is the most common hypothesis. Some authors argue that internalization, without any killing observed, represents a route for the bacteria to cross the cells, reaches the basal part and the bloodstream system, and allows dissemination to distant organs [109,238]. Thereby, the bacteria could multiply, cross cells to reach the basal part, and invade the surrounding tissues [244,250]. However, all these studies do not define precise mechanisms, and there is no evidence of this transcellular route as the mainstay of its pathogenicity towards the HAE.

b. *Persistence and establishment of chronic infections*. As there is no lysosomal killing after internalization, this mechanism has been hypothesized to allow *P. aeruginosa* to escape from the different effector cells of the host immune response, such as phagocytes, thereby favoring its persistence and establishment of chronic infections. [217,238,242,250]. Internalization could then induce the transition from an acute to a chronic phenotype in *P. aeruginosa* [110].

c. *P. aeruginosa clearance by airway cell desquamation*. This last and contradictory hypothesis proposes that internalization is more detrimental than beneficial for the bacteria. In healthy individuals, *P. aeruginosa* binding to CFTR channels leads to bacterial internalization and triggers host immune system activation, notably inflammation and neutrophil recruitment,

which causes the detachment of the infected cells and their removal by mucociliary clearance [251]. Conversely, in CF patients lacking functional CFTR channels, *P. aeruginosa* is not internalized, hampering its clearance. The bacteria persist in the dehydrated mucus protected from neutrophils and lead to chronic infections. In this pathology, chronically induced inflammation is ineffective and deleterious [144,237,251].

4) Crossing of the epithelium and dissemination. Recently, a new model has emerged to describe the crossing and the propagation of *P. aeruginosa* at the HAE. To gain access to the basement membrane, *P. aeruginosa* uses a paracellular route and then radial propagation through the basal compartment (Fig 2C). This new model excludes the suspected, but never demonstrated, theory of a transcellular route used by *P. aeruginosa* (by internalization) to cross the epithelium.

Zulianello and colleagues observed the absence of internalization but a paracellular invasion of *P. aeruginosa* in differentiated airway epithelia when the integrity of cellular junctions was compromised by rhamnolipids [120]. Subsequently, Heiniger and colleagues demonstrated the exact route used by the bacteria between cells to access the basement membrane during infection [94]. After *P. aeruginosa* adhesion to the basolateral surfaces of ciliated cells, the bacteria inject cytotoxic toxins using T3SS (ExoS and ExoT). The toxins lead to cell retraction and detachment, allowing access to other basolateral membranes of adjacent ciliated cells or the resting basal cells, and the progression of *P. aeruginosa* through the airway epithelium. Pili-mediated twitching mobility was necessary for this bacterial progression. Radial bacterial dissemination across the entire epithelium was then observed [94]. More recently, Golovkine and colleagues provided a detailed description of this phenomenon. Using a real-time microscopy technique, they described precisely the progression of the paracellular bacterial migration through the epithelium. During the epithelial infection, *P. aeruginosa* takes advantage of transient ruptures in the tight junctions to access the basolateral part and once the first bacterium has entered into the breach, a cohort of bacteria rapidly follows at the same entry point. A radial propagation from this entry point through the basal compartment followed, involving the injection of the T3SS-secreted toxins (ExoS and ExoT) into the cells. The pili-mediated twitching motility and the flagella were also necessary for this progression. Interestingly, no internalization of the bacteria was observed in this model, making transcellular migration highly unlikely [93].

Conclusions

P. aeruginosa is a major respiratory pathogen, expressing an impressive panel of complexes and complementary virulence factors that, in a well-coordinated manner, promote HAE invasion. However, *P. aeruginosa* is an opportunistic pathogen that rarely infects healthy individuals, and the barrier effect of the airway epithelium plays a key role in limiting its pathogenicity. *P. aeruginosa* exploits weaknesses in the HAE barrier to gain access to the basolateral part of the epithelium, which is normally inaccessible in healthy epithelium with intact tight junctions. This access is crucial for initiating infection and represents the common point of all the various clinical pathologies due to these bacteria, ranging from lung infections and corneal infections to catheter-related urinary tract infections.

A new pathogenesis model has emerged, replacing a suspected, but incompletely supported, model, where *P. aeruginosa* invades through a transcellular route within the epithelium following its internalization. This new paradigm relates to a paracellular crossing of the HAE after access to the basolateral part of the HAE and the radial propagation of *P. aeruginosa* through the basal compartment. Therapeutic approaches, including the use of compounds that inhibit access of *P. aeruginosa* to the basolateral membrane, could allow alternative treatment

strategies, which have the potential to bypass the classic antibiotics, reducing the emergence of antimicrobial resistance.

Supporting information

S1 Table. Models of airway epithelia cells used in the references of the review. ECM, extracellular matrix; ND/NR, not determined/not relevant.
(DOCX)

Acknowledgments

We would like to deeply thank Prof. Stéphane Corvec and Prof. Steve Lory for critically revising the paper, which helped us in keeping with the important scientific content of this review. We are also indebted to Dr. Nicholas Judson for his comments to make this review of interest to a broad scientific community not specialized in microbiology. We thank Dr. Valérian Dormoy for his help in proofreading the manuscript.

References

1. Rutherford V, Yom K, Ozer EA, Pura O, Hughes A, Murphy KR, et al. Environmental reservoirs for exoS+ and exoU+ strains of Pseudomonas aeruginosa. Env Microbiol Rep. 2018; 10:485–492. https://doi.org/10.1111/1758-2229.12653 PMID: 29687624

2. Selezska K, Kazmierczak M, Musken M, Garbe J, Schobert M, Haussler S, et al. Pseudomonas aeruginosa population structure revisited under environmental focus: impact of water quality and phage pressure. Environ Microbiol. 2012; 14:1952–1967. https://doi.org/10.1111/j.1462-2920.2012.02719.x PMID: 22390474

3. Wiehlmann L, Wagner G, Cramer N, Siebert B, Gudowius P, Morales G, et al. Population structure of Pseudomonas aeruginosa. Proc Natl Acad Sci. 2007; 104:8101–8106. https://doi.org/10.1073/pnas.0609213104 PMID: 17468398

4. Rahme L, Stevens E, Wolfort S, Shao J, Tompkins R, Ausubel F. Common virulence factors for bacterial pathogenicity in plants and animals. Science. 1995; 268:1899–1902. https://doi.org/10.1126/science.7604262 PMID: 7604262

5. Walter J, Haller S, Quinten C, Kärki T, Zacher B, Eckmanns T, et al. Healthcare-associated pneumonia in acute care hospitals in European Union/European Economic Area countries: an analysis of data from a point prevalence survey, 2011 to 2012. Euro Surveill. 2018:23. https://doi.org/10.2807/1560-7917.ES.2018.23.32.1700843 PMID: 30107871

6. Hauser AR. Pseudomonas aeruginosa virulence and antimicrobial resistance: two sides of the same coin? Crit Care Med. 2014; 42:201–202. https://doi.org/10.1097/CCM.0b013e3182a120cd PMID: 24346525

7. Sethi S, Murphy TF. Infection in the pathogenesis and course of chronic obstructive pulmonary disease. N Engl J Med. 2008; 359:2355–2365. https://doi.org/10.1056/NEJMra0800353 PMID: 19038881

8. Philippon S, Streckert HJ, Morgenroth K. In vitro study of the bronchial mucosa during Pseudomonas aeruginosa infection. Virchows Archiv A Pathol Anat Histopathol. 1993; 423:39–43. https://doi.org/10.1007/BF01606430 PMID: 8212532

9. Tsang KWT, Rutman A, Tanaka E, Lund V, Dewar A, Cole PJ, et al. Interaction of Pseudomonas aeruginosa with human respiratory mucosa in vitro. Eur Respir J. 1994; 7:1746–1753. https://doi.org/10.1183/09031936.94.07101746 PMID: 7828679

10. Niederman MS, Rafferty TD, Sasaki CT, Merrill WW, Matthay RA, Reynolds HY. Comparison of Bacterial Adherence to Ciliated and Squamous Epithelial Cells Obtained from the Human Respiratory Tract1–3. Am Rev Respir Dis. 1983; 127:85–90. https://doi.org/10.1164/arrd.1983.127.1.85 PMID: 6401417

11. Plotkowski MC, Chevillard M, Pierrot D, Altemayer D, Zahm JM, Colliot G, et al. Differential adhesion of Pseudomonas aeruginosa to human respiratory epithelial cells in primary culture. J Clin Invest. 1991; 87:2018–2028. https://doi.org/10.1172/JCI115231 PMID: 1904070

12. Baltimore RS, Christie CDC, Smith GJW. Immunohistopathologic Localization of Pseudomonas aeruginosa in Lungs from Patients with Cystic Fibrosis: Implications for the Pathogenesis of Progressive

Lung Deterioration. Am Rev Respir Dis. 1989; 140:1650–1661. https://doi.org/10.1164/ajrccm/140.6.1650 PMID: 2513765

13. Ramphal R, Small PM, Shands JW, Fischlschweiger W, Small PA. Adherence of Pseudomonas aeruginosa to tracheal cells injured by influenza infection or by endotracheal intubation. Infect Immun. 1980; 27:614–619. https://doi.org/10.1128/iai.27.2.614-619.1980 PMID: 6769805

14. de Bentzmann S, Plotkowski C, Puchelle E. Receptors in the Pseudomonas aeruginosa adherence to injured and repairing airway epithelium. Am J Resp Crit Care. 1996; 154:S155–S162. https://doi.org/10.1164/ajrccm/154.4_Pt_2.S155 PMID: 8876535

15. de Bentzmann S, Roger P, Puchelle E. Pseudomonas aeruginosa adherence to remodelling respiratory epithelium. Eur Respir J. 1996; 9:2145–2150. https://doi.org/10.1183/09031936.96.09102145 PMID: 8902481

16. de Bentzmann S, Roger P, Dupuit F, Bajolet-Laudinat O, Fuchey C, Plotkowski MC, et al. Asialo GM1 is a receptor for Pseudomonas aeruginosa adherence to regenerating respiratory epithelial cells. Infect Immun. 1996; 64:1582–1588. https://doi.org/10.1128/iai.64.5.1582-1588.1996 PMID: 8613364

17. Bustamante-Marin XM, Ostrowski LE. Cilia and Mucociliary Clearance. Csh Perspect Biol. 2017; 9: a028241. https://doi.org/10.1101/cshperspect.a028241 PMID: 27864314

18. Gellatly SL, Hancock REW. Pseudomonas aeruginosa: new insights into pathogenesis and host defenses. Pathog Dis. 2013; 67:159–173. https://doi.org/10.1111/2049-632X.12033 PMID: 23620179

19. Williams BJ, Dehnbostel J, Blackwell TS. Pseudomonas aeruginosa: host defence in lung diseases. Respirology. 2010; 15:1037–1056. https://doi.org/10.1111/j.1440-1843.2010.01819.x PMID: 20723140

20. Hewitt RJ, Lloyd CM. Regulation of immune responses by the airway epithelial cell landscape. Nat Rev Immunol. 2021; 21:347–362. https://doi.org/10.1038/s41577-020-00477-9 PMID: 33442032

21. Xu-Chen X, Weinstock J, Rastogi D, Koumbourlis A, Nino G. The airway epithelium during infancy and childhood: a complex multicellular immune barrier. basic review for clinicians. Paediatr Respir Rev. 2021; 38:9–15. https://doi.org/10.1016/j.prrv.2021.04.002 PMID: 34030977

22. Fujitani S, Sun HY, Yu VL, Weingarten JA. Pneumonia due to Pseudomonas aeruginosa: part I: epidemiology, clinical diagnosis, and source. Chest. 2011; 139:909–919. https://doi.org/10.1378/chest.10-0166 PMID: 21467058

23. Rello J, Bodi M, Mariscal D, Navarro M, Diaz E, Gallego M, et al. Microbiological testing and outcome of patients with severe community-acquired pneumonia. Chest. 2003; 123:174–180. https://doi.org/10.1378/chest.123.1.174 PMID: 12527619

24. Sousa AM, Pereira MO. Pseudomonas aeruginosa Diversification during Infection Development in Cystic Fibrosis Lungs—A Review. Pathogens. 2014; 3:680–703. https://doi.org/10.3390/pathogens3030680 PMID: 25438018

25. Hauser AR, Jain M, Bar-Meir M, McColley SA. Clinical significance of microbial infection and adaptation in cystic fibrosis. Clin Microbiol Rev. 2011; 24:29–70. https://doi.org/10.1128/CMR.00036-10 PMID: 21233507

26. Murphy TF. The many faces of Pseudomonas aeruginosa in chronic obstructive pulmonary disease. Clin Infect Dis. 2008; 47:1534–1536. https://doi.org/10.1086/593187 PMID: 19025364

27. Martínez-Solano L, Macia MD, Fajardo A, Oliver A, Martinez JL. Chronic Pseudomonas aeruginosa infection in chronic obstructive pulmonary disease. Clin Infect Dis. 2008; 47:1526–1533. https://doi.org/10.1086/593186 PMID: 18990062

28. Parameswaran GI, Sethi S. Pseudomonas infection in chronic obstructive pulmonary disease. Future Microbiol. 2012; 7:1129–1132. https://doi.org/10.2217/fmb.12.88 PMID: 23030418

29. Vareille M, Kieninger E, Edwards MR, Regamey N. The Airway Epithelium: Soldier in the Fight against Respiratory Viruses. Clin Microbiol Rev. 2011; 24:210–229. https://doi.org/10.1128/CMR.00014-10 PMID: 21233513

30. Hiemstra PS, McCray PB, Bals R. The innate immune function of airway epithelial cells in inflammatory lung disease. Eur Respir J. 2015; 45:1150–1162. https://doi.org/10.1183/09031936.00141514 PMID: 25700381

31. Davis JD, Wypych TP. Cellular and functional heterogeneity of the airway epithelium. Mucosal Immunol. 2021:1–13. https://doi.org/10.1038/s41385-020-00370-7 PMID: 33608655

32. Rock JR, Randell SH, Hogan BLM. Airway basal stem cells: a perspective on their roles in epithelial homeostasis and remodeling. Dis Model Mech. 2010; 3:545–556. https://doi.org/10.1242/dmm.006031 PMID: 20699479

33. Hajj R, Baranek T, Naour RL, Lesimple P, Puchelle E, Coraux C. Basal cells of the human adult airway surface epithelium retain transit-amplifying cell properties. Stem Cells. 2007; 25:139–148. https://doi.org/10.1634/stemcells.2006-0288 PMID: 17008423

34. Carlier FM, de Fays C, Pilette C. Epithelial Barrier Dysfunction in Chronic Respiratory Diseases. Front Physiol. 2021; 12:691227. https://doi.org/10.3389/fphys.2021.691227 PMID: 34248677

35. Coraux C, Hajj R, Lesimple P, Puchelle E. In vivo models of human airway epithelium repair and regeneration. European Respir Rev. 2005; 14:131–136. https://doi.org/10.1183/09059180.05.00009702

36. Coraux C, Hajj R, Lesimple P, Puchelle E. Réparation et régénération de l'épithélium respiratoire. M/S: médecine sciences. 2005; 21:1063–1069.

37. Adam D, Perotin J-M, Lebargy F, Birembaut P, Deslée G, Coraux C. Regeneration of airway epithelium. Rev Mal Respir. 2014; 31:300–311. https://doi.org/10.1016/j.rmr.2013.11.001 PMID: 24750950

38. Adam D, Roux-Delrieu J, Luczka E, Bonnomet A, Lesage J, Mérol J-C, et al. Cystic fibrosis airway epithelium remodelling: involvement of inflammation. J Pathol. 2015; 235:408–419. https://doi.org/10.1002/path.4471 PMID: 25348090

39. Hubeau C, Lorenzato M, Couetil JP, Hubert D, Dusser D, Puchelle E, et al. Quantitative analysis of inflammatory cells infiltrating the cystic fibrosis airway mucosa. Clin Exp Immunol. 2001; 124:69–76. https://doi.org/10.1046/j.1365-2249.2001.01456.x PMID: 11359444

40. Jeffery PK. Comparison of the structural and inflammatory features of COPD and asthma. Giles F. Filley Lecture. Chest. 2000; 117:251S–260S. https://doi.org/10.1378/chest.117.5_suppl_1.251s PMID: 10843939

41. Cigana C, Lorè NI, Riva C, Fino ID, Spagnuolo L, Sipione B, et al. Tracking the immunopathological response to Pseudomonas aeruginosa during respiratory infections. Sci Rep. 2016; 6:21465. https://doi.org/10.1038/srep21465 PMID: 26883959

42. Kim V, Kelemen SE, Abuel-Haija M, Gaughan JP, Sharafkaneh A, Evans CM, et al. Small Airway Mucous Metaplasia and Inflammation in Chronic Obstructive Pulmonary Disease. Copd J Chronic Obstr Pulm Dis. 2009; 5:329–338. https://doi.org/10.1080/15412550802522445 PMID: 19353346

43. Herard AL, Zahm JM, Pierrot D, Hinnrasky J, Fuchey C, Puchelle E. Epithelial barrier integrity during in vitro wound repair of the airway epithelium. Am J Resp Cell Mol. 2012; 15:624–632. https://doi.org/10.1165/ajrcmb.15.5.8918369 PMID: 8918369

44. Jeffery PK. Morphologic features of airway surface epithelial cells and glands. Am Rev Respir Dis. 1983; 128:S14–S20. https://doi.org/10.1164/arrd.1983.128.2P2.S14 PMID: 6881701

45. Furuse M, Takai Y. Recent advances in understanding tight junctions. Fac Rev. 2021; 10:18. https://doi.org/10.12703/r/10-18 PMID: 33718935

46. Gumbiner B., Structure biochemistry, and assembly of epithelial tight junctions. Am J Physiol. 1987; 253:C749–C758. https://doi.org/10.1152/ajpcell.1987.253.6.c749 PMID: 3322036

47. Stevenson BR, Anderson JM, Bullivant S. The epithelial tight junction: Structure, function and preliminary biochemical characterization. Mol Cell Biochem. 1988; 83:129–145. https://doi.org/10.1007/BF00226141 PMID: 3059173

48. Gray T, Coakley R, Hirsh A, Thornton D, Kirkham S, Koo J-S, et al. Regulation of MUC5AC mucin secretion and airway surface liquid metabolism by IL-1beta in human bronchial epithelia. Am J Physiol Lung Cell Mol Physiol. 2004; 286:L320–L330. https://doi.org/10.1152/ajplung.00440.2002 PMID: 14527933

49. Webster MJ, Tarran R. Slippery When Wet: Airway Surface Liquid Homeostasis and Mucus Hydration. Curr Top Membr. 2018; 81:293–335. https://doi.org/10.1016/bs.ctm.2018.08.004 PMID: 30243435

50. Wagner CE, Wheeler KM, Ribbeck K. Mucins and Their Role in Shaping the Functions of Mucus Barriers. Annu Rev Cell Dev Bi. 2018; 34:189–215. https://doi.org/10.1146/annurev-cellbio-100617-062818 PMID: 30296390

51. Voynow JA, Rubin BK. Mucins, Mucus, and Sputum. Chest. 2009; 135:505–512. https://doi.org/10.1378/chest.08-0412 PMID: 19201713

52. Rose MC, Voynow JA. Respiratory Tract Mucin Genes and Mucin Glycoproteins in Health and Disease. Physiol Rev. 2006; 86:245–278. https://doi.org/10.1152/physrev.00010.2005 PMID: 16371599

53. Girod S, Zahm JM, Plotkowski C, Beck G, Puchelle E. Role of the physiochemical properties of mucus in the protection of the respiratory epithelium. Eur Respir J. 1992; 5:477–487. PMID: 1563506

54. Lyczak JB, Cannon CL, Pier GB. Establishment of Pseudomonas aeruginosa infection: lessons from a versatile opportunist. Microbes Infect. 2000; 2:1051–1060. https://doi.org/10.1016/s1286-4579(00)01259-4 PMID: 10967285

55. Mihai MM, Holban AM, Giurcăneanu C, Popa LG, Buzea M, Filipov M, et al. Identification and phenotypic characterization of the most frequent bacterial etiologies in chronic skin ulcers. Rom J Morphol Embryol. 2014; 55:1401–1408. PMID: 25611273

56. Green M, Apel A, Stapleton F. Risk factors and causative organisms in microbial keratitis. Cornea. 2008; 27:22–27. https://doi.org/10.1097/ICO.0b013e318156caf2 PMID: 18245962

57. Alarcon I, Kwan L, Yu C, Evans DJ, Fleiszig SMJ. Role of the Corneal Epithelial Basement Membrane in Ocular Defense against Pseudomonas aeruginosa. Infect Immun. 2009; 77:3264–3271. https://doi.org/10.1128/IAI.00111-09 PMID: 19506010

58. Keay L, Edwards K, Naduvilath T, Taylor HR, Snibson GR, Forde K, et al. Microbial Keratitis Predisposing Factors and Morbidity. Ophthalmology. 2006; 113:109–116. https://doi.org/10.1016/j.ophtha.2005.08.013 PMID: 16360210

59. Kam KW, Yung W, Li GKH, Chen LJ, Young AL. Infectious keratitis and orthokeratology lens use: a systematic review. Infection. 2017; 45:727–735. https://doi.org/10.1007/s15010-017-1023-2 PMID: 28534320

60. Roser DJ, Akker BVD, Boase S, Haas CN, Ashbolt NJ, Rice SA. Pseudomonas aeruginosa dose response and bathing water infection. Epidemiol Infect. 2014; 142:449–462. https://doi.org/10.1017/S0950268813002690 PMID: 24229610

61. Rice SA, van den Akker B, Pomati F, Roser D. A risk assessment of Pseudomonas aeruginosa in swimming pools: a review. J Water Health. 2012; 10:181–196. https://doi.org/10.2166/wh.2012.020 PMID: 22717744

62. Mittal R, Aggarwal S, Sharma S, Chhibber S, Harjai K. Urinary tract infections caused by Pseudomonas aeruginosa: A minireview. J Infect Public Heal. 2009; 2:101–111. https://doi.org/10.1016/j.jiph.2009.08.003 PMID: 20701869

63. Safdar N, Crnich CJ, Maki DG. The Pathogenesis of Ventilator-Associated Pneumonia: Its Relevance to Developing Effective Strategies for Prevention. Respir Care. 2005; 50:725–741. PMID: 15913465

64. Carson JL, Collier AM, Gambling TM, Knowles MR, Boucher RC. Ultrastructure of airway epithelial cell membranes among patients with cystic fibrosis. Hum Pathol. 1990; 21:640–647. https://doi.org/10.1016/s0046-8177(96)90011-8 PMID: 2351392

65. Jeffery PK. Remodeling and Inflammation of Bronchi in Asthma and Chronic Obstructive Pulmonary Disease. Proc Am Thorac Soc. 2004; 1:176–183. https://doi.org/10.1513/pats.200402-009MS PMID: 16113432

66. Yamaguchi T, Yamada H. Role of mechanical injury on airway surface in the pathogenesis of Pseudomonas aeruginosa. Am Rev Respir Dis. 1991; 144:1147–1152. https://doi.org/10.1164/ajrccm/144.5.1147 PMID: 1952446

67. Schwarzer C, Fischer H, Machen TE. Chemotaxis and Binding of Pseudomonas aeruginosa to Scratch-Wounded Human Cystic Fibrosis Airway Epithelial Cells. PLos ONE. 2016; 11:e0150109. https://doi.org/10.1371/journal.pone.0150109 PMID: 27031335

68. Lee A, Chow D, Haus B, Tseng W, Evans D, Fleiszig S, et al. Airway epithelial tight junctions and binding and cytotoxicity of Pseudomonas aeruginosa. Am J Physiol. 1999; 277:L204–L217. https://doi.org/10.1152/ajplung.1999.277.1.L204 PMID: 10409249

69. Rajan S, Cacalano G, Bryan R, Ratner AJ, Sontich CU, van Heerckeren A, et al. Pseudomonas aeruginosa Induction of Apoptosis in Respiratory Epithelial Cells. Am J Resp Cell Mol. 2000; 23:304–312. https://doi.org/10.1165/ajrcmb.23.3.4098 PMID: 10970820

70. Roger P, Puchelle E, Bajolet-Laudinat O, Tournier JM, Debordeaux C, Plotkowski MC, et al. Fibronectin and alpha5beta1 integrin mediate binding of Pseudomonas aeruginosa to repairing airway epithelium. Eur Respir J. 1999; 13:1301–1309. https://doi.org/10.1034/j.1399-3003.1999.13f13.x PMID: 10445605

71. Plotkowski MC, de Bentzmann S, Pereira SHM, Zahm J-M, Bajolet-Laudinat O, Roger P, et al. Pseudomonas aeruginosa Internalization by Human Epithelial Respiratory Cells Depends on Cell Differentiation, Polarity, and Junctional Complex Integrity. Am J Resp Cell Mol. 1999; 20:880–890. https://doi.org/10.1165/ajrcmb.20.5.3408 PMID: 10226058

72. Fleiszig SM, Evans DJ, Do N, Vallas V, Shin S, Mostov KE. Epithelial cell polarity affects susceptibility to Pseudomonas aeruginosa invasion and cytotoxicity. Infect Immun. 1997; 65:2861–2867. https://doi.org/10.1128/iai.65.7.2861-2867.1997 PMID: 9199460

73. Kazmierczak BI, Mostov K, Engel JN. Epithelial cell polarity alters Rho-GTPase responses to Pseudomonas aeruginosa. Mol Biol Cell. 2004; 15:411–419. https://doi.org/10.1091/mbc.e03-08-0559 PMID: 14595106

74. Klainer AS, Turndorf H, Wu WH, Maewal H, Allender P. Surface alterations due to endotracheal intubation. Am J Medicine. 1975; 58:674–683. https://doi.org/10.1016/0002-9343(75)90504-5 PMID: 1130422

75. Plotkowski MC, Beck G, Tournier JM, Bernardo-Filho M, Marques EA, Puchelle E. Adherence of Pseudomonas aeruginosa to respiratory epithelium and the effect of leucocyte elastase. J Med Microbiol. 1989; 30:285–293. https://doi.org/10.1099/00222615-30-4-285 PMID: 2574749

76. Leroy A-G, Caillon J, Caroff N, Broquet A, Corvec S, Asehnoune K, et al. Could Azithromycin Be Part of Pseudomonas aeruginosa Acute Pneumonia Treatment? Front Microbiol. 2021; 12:642541. https://doi.org/10.3389/fmicb.2021.642541 PMID: 33796090

77. Uderhardt S, Martins AJ, Tsang JS, Lämmermann T, Germain RN. Resident Macrophages Cloak Tissue Microlesions to Prevent Neutrophil-Driven Inflammatory Damage. Cell. 2019; 177:541–555.e17. https://doi.org/10.1016/j.cell.2019.02.028 PMID: 30955887

78. Malhotra S, Hayes D, Wozniak DJ. Cystic Fibrosis and Pseudomonas aeruginosa: the Host-Microbe Interface. Clin Microbiol Rev. 2019:32. https://doi.org/10.1128/CMR.00138-18 PMID: 31142499

79. O'Sullivan BP, Freedman SD. Cystic fibrosis. Lancet. 2009; 373:1891–1904. https://doi.org/10.1016/S0140-6736(09)60327-5 PMID: 19403164

80. Singh PK, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. Nature. 2000; 407:762–764. https://doi.org/10.1038/35037627 PMID: 11048725

81. Voynow JA, Fischer BM, Roberts BC, Proia AD. Basal-like cells constitute the proliferating cell population in cystic fibrosis airways. Am J Resp Crit Care. 2005; 172:1013–1018. https://doi.org/10.1164/rccm.200410-1398OC PMID: 16020799

82. Dovey M, Wisseman CL, Roggli VL, Roomans GM, Shelburne JD, Spock A. Ultrastructural morphology of the lung in cystic fibrosis. J Submicr Cytol Path. 1989; 21:521–534. PMID: 2790733

83. Caldara M, Friedlander RS, Kavanaugh NL, Aizenberg J, Foster KR, Ribbeck K. Mucin Biopolymers Prevent Bacterial Aggregation by Retaining Cells in the Free-Swimming State. Curr Biol. 2012; 22:2325–2330. https://doi.org/10.1016/j.cub.2012.10.028 PMID: 23142047

84. Co JY, Cárcamo-Oyarce G, Billings N, Wheeler KM, Grindy SC, Holten-Andersen N, et al. Mucins trigger dispersal of Pseudomonas aeruginosa biofilms. npj Biofilms Microbiomes. 2018; 4:23. https://doi.org/10.1038/s41522-018-0067-0 PMID: 30323945

85. Haley CL, Kruczek C, Qaisar U, Colmer-Hamood JA, Hamood AN. Mucin inhibits Pseudomonas aeruginosa biofilm formation by significantly enhancing twitching motility. Can J Microbiol. 2014; 60:155–166. https://doi.org/10.1139/cjm-2013-0570 PMID: 24588389

86. Wheeler KM, Cárcamo-Oyarce G, Turner BS, Dellos-Nolan S, Co JY, Lehoux S, et al. Mucin glycans attenuate the virulence of Pseudomonas aeruginosa in infection. Nat Microbiol. 2019; 4:2146–2154. https://doi.org/10.1038/s41564-019-0581-8 PMID: 31611643

87. Barnes PJ, Burney PGJ, Silverman EK, Celli BR, Vestbo J, Wedzicha JA, et al. Chronic obstructive pulmonary disease. Nat Rev Dis Primers. 2015; 1:15076. https://doi.org/10.1038/nrdp.2015.76 PMID: 27189863

88. Gao W, Li L, Wang Y, Zhang S, Adcock IM, Barnes PJ, et al. Bronchial epithelial cells in COPD. Respirology. 2015; 20:722–729. https://doi.org/10.1111/resp.12542 PMID: 25868842

89. Papi A, Bellettato CM, Braccioni F, Romagnoli M, Casolari P, Caramori G, et al. Infections and Airway Inflammation in Chronic Obstructive Pulmonary Disease Severe Exacerbations. Am J Resp Crit Care. 2006; 173:1114–1121. https://doi.org/10.1164/rccm.200506-859OC PMID: 16484677

90. Jeannoël M, Lina G, Rasigade JP, Lina B, Morfin F, Casalegno JS. Microorganisms associated with respiratory syncytial virus pneumonia in the adult population. Eur J Clin Microbiol. 2019; 38:157–160. https://doi.org/10.1007/s10096-018-3407-3 PMID: 30353485

91. Erjefält JS, Erjefält I, Sundler F, Persson CGA. In vivo restitution of airway epithelium. Cell Tissue Res. 1995; 281:305–316. https://doi.org/10.1007/BF00583399 PMID: 7648624

92. Bojarski C, Weiske J, Schöneberg T, Schröder W, Mankertz J, Schulzke J-D, et al. The specific fates of tight junction proteins in apoptotic epithelial cells. J Cell Sci. 2004; 117:2097–2107. https://doi.org/10.1242/jcs.01071 PMID: 15054114

93. Golovkine G, Faudry E, Bouillot S, Elsen S, Attrée I, Huber P. Pseudomonas aeruginosa Transmigrates at Epithelial Cell-Cell Junctions, Exploiting Sites of Cell Division and Senescent Cell Extrusion. PLos Pathog. 2016; 12:e1005377. https://doi.org/10.1371/journal.ppat.1005377 PMID: 26727615

94. Heiniger RW, Winther-Larsen HC, Pickles RJ, Koomey M, Wolfgang MC. Infection of human mucosal tissue by Pseudomonas aeruginosa requires sequential and mutually dependent virulence factors and a novel pilus-associated adhesin. Cell Microbiol. 2010; 12:1158–1173. https://doi.org/10.1111/j.1462-5822.2010.01461.x PMID: 20331639

95. Pentecost M, Otto G, Theriot JA, Amieva MR. Listeria monocytogenes Invades the Epithelial Junctions at Sites of Cell Extrusion. PLos Pathog. 2006; 2:e3. https://doi.org/10.1371/journal.ppat.0020003 PMID: 16446782

96. Wang T, Hou Y, Wang R. A case report of community-acquired Pseudomonas aeruginosa pneumonia complicated with MODS in a previously healthy patient and related literature review. BMC Infect Dis. 2019; 19:130. https://doi.org/10.1186/s12879-019-3765-1 PMID: 30736735

97. Blenkinsopp WK. Proliferation of respiratory tract epithelium in the rat. Exp Cell Res. 1967; 46:144–154. https://doi.org/10.1016/0014-4827(67)90416-8 PMID: 6025276

98. Rawlins EL, Hogan BLM. Epithelial stem cells of the lung: privileged few or opportunities for many? Development. 2006; 133:2455–2465. https://doi.org/10.1242/dev.02407 PMID: 16735479

99. Sharma A, Coles WH. Kinetics of corneal epithelial maintenance and graft loss. A population balance model. Invest Ophth Vis Sci. 1989; 30:1962–1971.

100. Grove GL, Kligman AM. Age-associated changes in human epidermal cell renewal. J Gerontol. 1983; 38:137–142. https://doi.org/10.1093/geronj/38.2.137 PMID: 6827031

101. Barker N. Adult intestinal stem cells: critical drivers of epithelial homeostasis and regeneration. Nat Rev Mol Cell Bio. 2014; 15:19–33. https://doi.org/10.1038/nrm3721 PMID: 24326621

102. Darwich AS, Aslam U, Ashcroft DM, Rostami-Hodjegan A. Meta-Analysis of the Turnover of Intestinal Epithelia in Preclinical Animal Species and Humans. Drug Metab Dispos. 2014; 42:2016–2022. https://doi.org/10.1124/dmd.114.058404 PMID: 25233858

103. Christopher JC, Gordon B, Andes D. Hot Tub-Associated Necrotizing Pneumonia due to Pseudomonas aeruginosa. Clin Infect Dis. 2003; 36:e55–e57. https://doi.org/10.1086/345851 PMID: 12539092

104. Hatchette TF, Gupta R, Marrie TJ. Pseudomonas aeruginosa Community-Acquired Pneumonia in Previously Healthy Adults: Case Report and Review of the Literature. Clin Infect Dis. 2000; 31:1349–1356. https://doi.org/10.1086/317486 PMID: 11096001

105. Rose HD, Franson TR, Sheth NK, Chusid MJ, Macher AM, Zeirdt CH. Pseudomonas Pneumonia Associated With Use of a Home Whirlpool Spa. JAMA. 1983; 250:2027–2029. https://doi.org/10.1001/jama.1983.03340150069030 PMID: 6413704

106. Huhulescu S, Simon M, Lubnow M, Kaase M, Wewalka G, Pietzka AT, et al. Fatal Pseudomonas aeruginosa pneumonia in a previously healthy woman was most likely associated with a contaminated hot tub. Infection. 2011; 39:265–269. https://doi.org/10.1007/s15010-011-0096-6 PMID: 21455711

107. Mena KD, Gerba CP. Reviews of Environmental Contamination and Toxicology. Rev Environ Contam Toxicol. 2009; 201:71–115. https://doi.org/10.1007/978-1-4419-0032-6_3

108. Basso P, Ragno M, Elsen S, Reboud E, Golovkine G, Bouillot S, et al. Pseudomonas aeruginosa Pore-Forming Exolysin and Type IV Pili Cooperate To Induce Host Cell Lysis. Mbio. 2017; 8:e02250–e02216. https://doi.org/10.1128/mBio.02250-16 PMID: 28119472

109. Bucior I, Pielage JF, Engel JN. Pseudomonas aeruginosa pili and flagella mediate distinct binding and signaling events at the apical and basolateral surface of airway epithelium. PLos Pathog. 2012; 8: e1002616. https://doi.org/10.1371/journal.ppat.1002616 PMID: 22496644

110. Sana TG, Hachani A, Bucior I, Soscia C, Garvis S, Termine E, et al. The second type VI secretion system of Pseudomonas aeruginosa strain PAO1 is regulated by quorum sensing and Fur and modulates internalization in epithelial cells. J Biol Chem. 2012; 287:27095–27105. https://doi.org/10.1074/jbc.M112.376368 PMID: 22665491

111. Dosunmu EF, Emeh RO, Dixit S, Bakeer MK, Coats MT, Owen DR, et al. The anti-microbial peptide TP359 attenuates inflammation in human lung cells infected with Pseudomonas aeruginosa via TLR5 and MAPK pathways. PLoS ONE. 2017; 12:e0176640. https://doi.org/10.1371/journal.pone.0176640 PMID: 28467446

112. Hawdon NA, Aval PS, Barnes RJ, Gravelle SK, Rosengren J, Khan S, et al. Cellular responses of A549 alveolar epithelial cells to serially collected Pseudomonas aeruginosa from cystic fibrosis patients at different stages of pulmonary infection. Fems Immunol Medical Microbiol. 2010; 59:207–220. https://doi.org/10.1111/j.1574-695X.2010.00693.x PMID: 20528926

113. Azghani AO, Gray LD, Johnson AR. A bacterial protease perturbs the paracellular barrier function of transporting epithelial monolayers in culture. Infect Immun. 1993; 61:2681–2686. https://doi.org/10.1128/iai.61.6.2681-2686.1993 PMID: 8500907

114. Clark CA, Thomas LK, Azghani AO. Inhibition of Protein Kinase C Attenuates Pseudomonas aeruginosa Elastase–Induced Epithelial Barrier Disruption. Am J Resp Cell Mol. 2011; 45:1263–1271. https://doi.org/10.1165/rcmb.2010-0459OC PMID: 21757681

115. Azghani AO. Pseudomonas aeruginosa and epithelial permeability: role of virulence factors elastase and exotoxin A. Am J Resp Cell Mol. 1996; 15:132–140. https://doi.org/10.1165/ajrcmb.15.1.8679217 PMID: 8679217

116. Azghani AO, Miller EJ, Peterson BT. Virulence factors from Pseudomonas aeruginosa increase lung epithelial permeability. Lung. 2000; 178:261–269. https://doi.org/10.1007/s004080000031 PMID: 11147311

117. Nomura K, Obata K, Keira T, Miyata R, Hirakawa S, Takano K, et al. Pseudomonas aeruginosa elastase causes transient disruption of tight junctions and downregulation of PAR-2 in human nasal epithelial cells. Respir Res. 2014; 15:21. https://doi.org/10.1186/1465-9921-15-21 PMID: 24548792

118. Yi X, Wang Y, Yu FS. Corneal epithelial tight junctions and their response to lipopolysaccharide challenge. Invest Ophth Vis Sci. 2000; 41:4093–4100. PMID: 11095601

119. Wallace CJ, Medina SH, ElSayed MEH. Effect of Rhamnolipids on Permeability Across Caco-2 Cell Monolayers. Pharmaceut Res. 2014; 31:887–894. https://doi.org/10.1007/s11095-013-1210-5 PMID: 24065598

120. Zulianello L, Canard C, Köhler T, Caille D, Lacroix J-S, Meda P. Rhamnolipids are virulence factors that promote early infiltration of primary human airway epithelia by Pseudomonas aeruginosa. Infect Immun. 2006; 74:3134–3147. https://doi.org/10.1128/IAI.01772-05 PMID: 16714541

121. Vikström E, Tafazoli F, Magnusson K-E. Pseudomonas aeruginosa quorum sensing molecule N-(3 oxododecanoyl)-l-homoserine lactone disrupts epithelial barrier integrity of Caco-2 cells. FEBS Lett. 2006; 580:6921–6928. https://doi.org/10.1016/j.febslet.2006.11.057 PMID: 17157842

122. Vikström E, Bui L, Konradsson P, Magnusson K-E. Role of calcium signalling and phosphorylations in disruption of the epithelial junctions by Pseudomonas aeruginosa quorum sensing molecule. Eur J Cell Biol. 2010; 89:584–597. https://doi.org/10.1016/j.ejcb.2010.03.002 PMID: 20434232

123. Vikström E, Bui L, Konradsson P, Magnusson K-E. The junctional integrity of epithelial cells is modulated by Pseudomonas aeruginosa quorum sensing molecule through phosphorylation-dependent mechanisms. Exp Cell Res. 2009; 315:313–326. https://doi.org/10.1016/j.yexcr.2008.10.044 PMID: 19038248

124. Wilson MJB, Martin DE. Quantitative sputum culture as a means of excluding false positive reports in the routine microbiology laboratory. J Clin Pathol. 1972; 25:697. https://doi.org/10.1136/jcp.25.8.697 PMID: 4627746

125. Kalin M, Lindberg AA, Tunevall G. Etiological Diagnosis of Bacterial Pneumonia by Gram Stain and Quantitative Culture of Expectorates: Leukocytes or Alveolar Macrophages as Indicators of Sample Representativity. Scand J Infect Dis. 2015; 15:153–160. https://doi.org/10.3109/inf.1983.15.issue-2.05 PMID: 6192493

126. Khan F, Pham DTN, Oloketuyi SF, Kim Y-M. Regulation and controlling the motility properties of Pseudomonas aeruginosa. Appl Microbiol Biotechnol. 2020; 104:33–49. https://doi.org/10.1007/s00253-019-10201-w PMID: 31768614

127. Saiman L, Prince A. Pseudomonas aeruginosa pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells. J Clin Invest. 1993; 92:1875–1880. https://doi.org/10.1172/JCI116779 PMID: 8104958

128. Davies J, Dewar A, Bush A, Pitt T, Gruenert D, Geddes DM, et al. Reduction in the adherence of Pseudomonas aeruginosa to native cystic fibrosis epithelium with anti-asialoGM1 antibody and neuraminidase inhibition. Eur Respir J. 1999; 13:565–570. https://doi.org/10.1183/09031936.99.13356599 PMID: 10232427

129. Scharfman A, van Brussel E, Houdret N, Lamblin G, Roussel P. Interactions between Glycoconjugates from Human Respiratory Airways and Pseudomonas aeruginosa. Am J Resp Crit Care. 1996; 154: S163–S169. https://doi.org/10.1164/ajrccm/154.4_Pt_2.S163 PMID: 8876536

130. Adamo R, Sokol S, Soong G, Gomez MI, Prince A. Pseudomonas aeruginosa Flagella Activate Airway Epithelial Cells through asialoGM1 and Toll-Like Receptor 2 as well as Toll-Like Receptor 5. Am J Resp Cell Mol. 2004; 30:627–634. https://doi.org/10.1165/rcmb.2003-0260OC PMID: 14607814

131. Bryan R, Kube D, Perez A, Davis P, Prince A. Overproduction of the CFTR R Domain Leads to Increased Levels of AsialoGM1 and Increased Pseudomonas aeruginosa Binding by Epithelial Cells. Am J Resp Cell Mol. 1998; 19:269–277. https://doi.org/10.1165/ajrcmb.19.2.2889 PMID: 9698599

132. Eierhoff T, Bastian B, Thuenauer R, Madl J, Audfray A, Aigal S, et al. A lipid zipper triggers bacterial invasion. Proc Natl Acad Sci. 2014; 111:12895–12900. https://doi.org/10.1073/pnas.1402637111 PMID: 25136128

133. Brandel A, Aigal S, Lagies S, Schlimpert M, Meléndez AV, Xu M, et al. The Gb3-enriched CD59/flotillin plasma membrane domain regulates host cell invasion by Pseudomonas aeruginosa. Cell Mol Life Sci. 2021; 78:3637–3656. https://doi.org/10.1007/s00018-021-03766-1 PMID: 33555391

134. Chemani C, Imberty A, de Bentzmann S, Pierre M, Wimmerová M, Guery BP, et al. Role of LecA and LecB lectins in Pseudomonas aeruginosa-induced lung injury and effect of carbohydrate ligands. Infect Immun. 2009; 77:2065–2075. https://doi.org/10.1128/IAI.01204-08 PMID: 19237519

135. Mewe M, Tielker D, Schönberg R, Schachner M, Jaeger K-E, Schumacher U. Pseudomonas aeruginosa lectins I and II and their interaction with human airway cilia. J Laryngol Otol. 2005; 119:595–599. https://doi.org/10.1258/0022215054516313 PMID: 16102212

136. Zheng S, Eierhoff T, Aigal S, Brandel A, Thuenauer R, de Bentzmann S, et al. The Pseudomonas aeruginosa lectin LecA triggers host cell signalling by glycosphingolipid-dependent phosphorylation of the adaptor protein CrkII. Biochim Biophys Acta Mol Cell Res. 2017; 1864:1236–1245. https://doi.org/10.1016/j.bbamcr.2017.04.005 PMID: 28428058

137. Mikolajczyk K, Sikora M, Hanus C, Kaczmarek R, Czerwinski M. One of the two N-glycans on the human Gb3/CD77 synthase is essential for its activity and allosterically regulates its function. Biochem Biophys Res Commun. 2022; 617:36–41. https://doi.org/10.1016/j.bbrc.2022.05.085 PMID: 35671609

138. Bucior I, Mostov K, Engel JN. Pseudomonas aeruginosa-Mediated Damage Requires Distinct Receptors at the Apical and Basolateral Surfaces of the Polarized Epithelium. Infect Immun. 2010; 78:939–953. https://doi.org/10.1128/IAI.01215-09 PMID: 20008530

139. Dorscheid DR, Wojcik KR, Yule K, White SR. Role of cell surface glycosylation in mediating repair of human airway epithelial cell monolayers. Am J Physiol Lung Cell Mol Physiol. 2001; 281:L982–L992. https://doi.org/10.1152/ajplung.2001.281.4.L982 PMID: 11557602

140. Schroeder TH, Lee MM, Yacono PW, Cannon CL, Gerçeker AA, Golan DE, et al. CFTR is a pattern recognition molecule that extracts Pseudomonas aeruginosa LPS from the outer membrane into epithelial cells and activates NF-kappa B translocation. Proc Natl Acad Sci. 2002; 99:6907–6912. https://doi.org/10.1073/pnas.092160899 PMID: 11997458

141. Brezillon S, Dupuit F, Hinnrasky J, Marchand V, Kälin N, Tümmler B, et al. Decreased expression of the CFTR protein in remodeled human nasal epithelium from non-cystic fibrosis patients. Lab Invest. 1995; 72:191–200. PMID: 7531792

142. Brézillon S, Hamm H, Heilmann M, Schafers H-J, Hinnrasky J, Wagner TOF, et al. Decreased expression of the cystic fibrosis transmembrane conductance regulator protein in remodeled airway epithelium from lung transplanted patients. Hum Pathol. 1997; 28:944–952. https://doi.org/10.1016/s0046-8177(97)90010-1 PMID: 9269831

143. Ruffin M, Bigot J, Calmel C, Mercier J, Givelet M, Oliva J, et al. Flagellin From Pseudomonas aeruginosa Modulates SARS-CoV-2 Infectivity in Cystic Fibrosis Airway Epithelial Cells by Increasing TMPRSS2 Expression. Front Immunol. 2021; 12:714027. https://doi.org/10.3389/fimmu.2021.714027 PMID: 34950129

144. Pier GB, Grout M, Zaidi TS. Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of Pseudomonas aeruginosa from the lung. Proc Natl Acad Sci. 1997; 94:12088–12093. https://doi.org/10.1073/pnas.94.22.12088 PMID: 9342367

145. Hampton TH, Ballok AE, Bomberger JM, Rutkowski MR, Barnaby R, Coutermarsh B, et al. Does the ΔF508-CFTR mutation induce a proinflammatory response in human airway epithelial cells? Am J Physiol Lung Cell Mol Physiol. 2012; 303:L509–L518. https://doi.org/10.1152/ajplung.00226.2011 PMID: 22821996

146. Arhin A, Boucher C. The outer membrane protein OprQ and adherence of Pseudomonas aeruginosa to human fibronectin. Microbiology+. 2010; 156:1415–1423. https://doi.org/10.1099/mic.0.033472-0 PMID: 20093292

147. Wang A, Yokosaki Y, Ferrando R, Balmes J, Sheppard D. Differential regulation of airway epithelial integrins by growth factors. Am J Resp Cell Mol. 1996; 15:664–672. https://doi.org/10.1165/ajrcmb.15.5.8918373 PMID: 8918373

148. Hérard AL, Pierrot D, Hinnrasky J, Kaplan H, Sheppard D, Puchelle E, et al. Fibronectin and its alpha 5 beta 1-integrin receptor are involved in the wound-repair process of airway epithelium. Am J Physiol. 1996; 271:L726–L733. https://doi.org/10.1152/ajplung.1996.271.5.L726 PMID: 8944715

149. Paulsson M, Singh B, Al-Jubair T, Su Y-C, Høiby N, Riesbeck K. Identification of outer membrane Porin D as a vitronectin-binding factor in cystic fibrosis clinical isolates of Pseudomonas aeruginosa. J Cyst Fibros. 2015; 14:600–607. https://doi.org/10.1016/j.jcf.2015.05.005 PMID: 26047937

150. Salazar-Peláez LM, Abraham T, Herrera AM, Correa MA, Ortega JE, Paré PD, et al. Vitronectin Expression in the Airways of Subjects with Asthma and Chronic Obstructive Pulmonary Disease. PLoS ONE. 2015; 10:e0119717. https://doi.org/10.1371/journal.pone.0119717 PMID: 25768308

151. Pilewski JM, Latoche JD, Arcasoy SM, Albelda SM. Expression of integrin cell adhesion receptors during human airway epithelial repair in vivo. Am J Physiol. 1997; 273:L256–L263. https://doi.org/10.1152/ajplung.1997.273.1.L256 PMID: 9252563

152. Smith JW, Vestal DJ, Irwin SV, Burke TA, Cheresh DA. Purification and functional characterization of integrin alpha v beta 5. An adhesion receptor for vitronectin. J Biol Chem. 1990; 265:11008–11013. PMID: 1694173

153. Plotkowski MC, Costa AO, Morandi V, Barbosa HS, Nader HB, Bentzmann SD, et al. Role of heparan sulphate proteoglycans as potential receptors for non-piliated Pseudomonas aeruginosa adherence to non-polarised airway epithelial cells. J Med Microbiol. 2001; 50:183–190. https://doi.org/10.1099/0022-1317-50-2-183 PMID: 11211227

154. Bermejo-Jambrina M, Eder J, Kaptein TM, Hamme JL, Helgers LC, Vlaming KE, et al. Infection and transmission of SARS-CoV-2 depend on heparan sulfate proteoglycans. EMBO J. 2021; 40:e106765. https://doi.org/10.15252/embj.2020106765 PMID: 34510494

155. Paulsson M, Su Y-C, Ringwood T, Uddén F, Riesbeck K. Pseudomonas aeruginosa uses multiple receptors for adherence to laminin during infection of the respiratory tract and skin wounds. Sci Rep. 2019; 9:1–10. https://doi.org/10.1038/s41598-019-54622-z PMID: 31796854

156. Plotkowski MC, Tournier JM, Puchelle E. Pseudomonas aeruginosa strains possess specific adhesins for laminin. Infect Immun. 1996; 64:600–605. https://doi.org/10.1128/iai.64.2.600-605.1996 PMID: 8550213

157. Hauser AR. The Type III Secretion System of Pseudomonas aeruginosa: Infection by Injection. Nat Rev Microbiol. 2009; 7:654–665. https://doi.org/10.1038/nrmicro2199 PMID: 19680249

158. Inclan YF, Persat A, Greninger A, Dollen JV, Johnson J, Krogan N, et al. A scaffold protein connects type IV pili with the Chp chemosensory system to mediate activation of virulence signaling in Pseudomonas aeruginosa. Mol Microbiol. 2016; 101:590–605. https://doi.org/10.1111/mmi.13410 PMID: 27145134

159. Persat A, Inclan YF, Engel JN, Stone HA, Gitai Z. Type IV pili mechanochemically regulate virulence factors in Pseudomonas aeruginosa. Proc Natl Acad Sci. 2015; 112:7563–7568. https://doi.org/10.1073/pnas.1502025112 PMID: 26041805

160. Wolfgang MC, Lee VT, Gilmore ME, Lory S. Coordinate Regulation of Bacterial Virulence Genes by a Novel Adenylate Cyclase-Dependent Signaling Pathway. Dev Cell. 2003; 4:253–263. https://doi.org/10.1016/s1534-5807(03)00019-4 PMID: 12586068

161. Fuchs EL, Brutinel ED, Jones AK, Fulcher NB, Urbanowski ML, Yahr TL, et al. The Pseudomonas aeruginosa Vfr Regulator Controls Global Virulence Factor Expression through Cyclic AMP-Dependent and -Independent Mechanisms. J Bacteriol. 2010; 192:3553–3564. https://doi.org/10.1128/JB.00363-10 PMID: 20494996

162. Klockgether J, Tümmler B. Recent advances in understanding Pseudomonas aeruginosa as a pathogen. F1000Res. 2017; 6:1261. https://doi.org/10.12688/f1000research.10506.1 PMID: 28794863

163. Engel J, Balachandran P. Role of Pseudomonas aeruginosa type III effectors in disease. Curr Opin Microbiol. 2009; 12:61–66. https://doi.org/10.1016/j.mib.2008.12.007 PMID: 19168385

164. Smith RS, Iglewski BH. P. aeruginosa quorum-sensing systems and virulence. Curr Opin Microbiol. 2003; 6: 56–60. https://doi.org/10.1016/s1369-5274(03)00008-0 PMID: 12615220

165. Lee J, Zhang L. The hierarchy quorum sensing network in Pseudomonas aeruginosa. Protein Cell. 2015; 6:26–41. https://doi.org/10.1007/s13238-014-0100-x PMID: 25249263

166. Turkina MV, Vikström E. Bacteria-Host Crosstalk: Sensing of the Quorum in the Context of Pseudomonas aeruginosa Infections. J Innate Immun. 2019; 11:263–279. https://doi.org/10.1159/000494069 PMID: 30428481

167. Thi MTT, Wibowo D, Rehm BHA. Pseudomonas aeruginosa Biofilms. Int J Mol Sci. 2020; 21:8671. https://doi.org/10.3390/ijms21228671 PMID: 33212950

168. Deep A, Chaudhary U, Gupta V. Quorum sensing and Bacterial Pathogenicity: From Molecules to Disease. J Lab Physicians. 2011; 3:4–11. https://doi.org/10.4103/0974-2727.78553 PMID: 21701655

169. Mann EE, Wozniak DJ. Pseudomonas biofilm matrix composition and niche biology. Fems Microbiol Rev. 2012; 36:893–916. https://doi.org/10.1111/j.1574-6976.2011.00322.x PMID: 22212072

170. Moreau-Marquis S, Stanton BA, O'Toole GA. Pseudomonas aeruginosa biofilm formation in the cystic fibrosis airway. Pulm Pharmacol Ther. 2008; 21:595–599. https://doi.org/10.1016/j.pupt.2007.12.001 PMID: 18234534

171. Adair CG, Gorman SP, Feron BM, Byers LM, Jones DS, Goldsmith CE, et al. Implications of endotracheal tube biofilm for ventilator-associated pneumonia. Intens Care Med. 1999; 25:1072–1076. https://doi.org/10.1007/s001340051014 PMID: 10551961

172. Feldman M, Bryan R, Rajan S, Scheffler L, Brunnert S, Tang H, et al. Role of flagella in pathogenesis of Pseudomonas aeruginosa pulmonary infection. Infect Immun. 1998; 66:43–51. https://doi.org/10.1128/IAI.66.1.43-51.1998 PMID: 9423837

173. Hahn HP. The type-4 pilus is the major virulence-associated adhesin of Pseudomonas aeruginosa—a review. Gene. 1997; 192:99–108. https://doi.org/10.1016/s0378-1119(97)00116-9 PMID: 9224879

174. Mattick JS. Type IV pili and twitching motility. Annu Rev Microbiol. 2002; 56:289–314. https://doi.org/10.1146/annurev.micro.56.012302.160938 PMID: 12142488

175. Rocchetta HL, Burrows LL, Lam JS. Genetics of O-antigen biosynthesis in Pseudomonas aeruginosa. Microbiol Mol Biol Rev. 1999; 63:523–553. https://doi.org/10.1128/MMBR.63.3.523-553.1999 PMID: 10477307

176. Eutamene H, Theodorou V, Schmidlin F, Tondereau V, Garcia-Villar R, Salvador-Cartier C, et al. LPS-induced lung inflammation is linked to increased epithelial permeability: role of MLCK. Eur Respir J. 2005; 25:789–796. https://doi.org/10.1183/09031936.05.00064704 PMID: 15863634

177. Cabrera-Benítez NE, Pérez-Roth E, Ramos-Nuez Á, Sologuren I, Padrón JM, Slutsky AS, et al. Inhibition of endotoxin-induced airway epithelial cell injury by a novel family of pyrrol derivates. Lab Invest. 2016; 96:632–640. https://doi.org/10.1038/labinvest.2016.46 PMID: 26999659

178. Bajolet-Laudinat O, Bentzmann SG, Tournier JM, Madoulet C, Plotkowski MC, Chippaux C, et al. Cytotoxicity of Pseudomonas aeruginosa internal lectin PA-I to respiratory epithelial cells in primary culture. Infect Immun. 1994; 62:4481–4487. https://doi.org/10.1128/iai.62.10.4481-4487.1994 PMID: 7927712

179. Nivens DE, Ohman DE, Williams J, Franklin MJ. Role of alginate and its O acetylation in formation of Pseudomonas aeruginosa microcolonies and biofilms. J Bacteriol. 2001; 183:1047–1057. https://doi.org/10.1128/JB.183.3.1047-1057.2001 PMID: 11208804

180. Pier GB, Coleman F, Grout M, Franklin M, Ohman DE. Role of alginate O acetylation in resistance of mucoid Pseudomonas aeruginosa to opsonic phagocytosis. Infect Immun. 2001; 69:1895–1901. https://doi.org/10.1128/IAI.69.3.1895-1901.2001 PMID: 11179370

181. Limoli DH, Jones CJ, Wozniak DJ. Bacterial Extracellular Polysaccharides in Biofilm Formation and Function. Microbiol Spectr. 2015:3. https://doi.org/10.1128/microbiolspec.MB-0011-2014 PMID: 26185074

182. Meyer JM, Neely A, Stintzi A, Georges C, Holder IA. Pyoverdin is essential for virulence of Pseudomonas aeruginosa. Infect Immun. 1996; 64:518–523. https://doi.org/10.1128/iai.64.2.518-523.1996 PMID: 8550201

183. Xiao R, Kisaalita WS. Iron acquisition from transferrin and lactoferrin by Pseudomonas aeruginosa pyoverdin. Microbiology+. 1997; 143 (Pt 7):2509–2515. https://doi.org/10.1099/00221287-143-7-2509 PMID: 9245831

184. Ankenbauer RG, Quan HN. FptA, the Fe(III)-pyochelin receptor of Pseudomonas aeruginosa: a phenolate siderophore receptor homologous to hydroxamate siderophore receptors. J Bacteriol. 1994; 176:307–319. https://doi.org/10.1128/jb.176.2.307-319.1994 PMID: 8288523

185. Minandri F, Imperi F, Frangipani E, Bonchi C, Visaggio D, Facchini M, et al. Role of Iron Uptake Systems in Pseudomonas aeruginosa Virulence and Airway Infection. Infect Immun. 2016; 84:2324–2335. https://doi.org/10.1128/IAI.00098-16 PMID: 27271740

186. Smith AD, Wilks A. Differential Contributions of the Outer Membrane Receptors PhuR and HasR to Heme Acquisition in Pseudomonas aeruginosa *. J Biol Chem. 2015; 290:7756–7766. https://doi.org/10.1074/jbc.M114.633495 PMID: 25616666

187. Ochsner UA, Johnson Z, Vasil ML. Genetics and regulation of two distinct haem-uptake systems, phu and has, in Pseudomonas aeruginosa. Microbiology+. 2000; 146:185–198. https://doi.org/10.1099/00221287-146-1-185 PMID: 10658665

188. Mavrodi DV, Bonsall RF, Delaney SM, Soule MJ, Phillips G, Thomashow LS. Functional analysis of genes for biosynthesis of pyocyanin and phenazine-1-carboxamide from Pseudomonas aeruginosa PAO1. J Bacteriol. 2001; 183:6454–6465. https://doi.org/10.1128/JB.183.21.6454-6465.2001 PMID: 11591691

189. Husson M-O, Harf-Monteil C, Monteil H. Genre Pseudomonas Pseudomonas aeruginosa La bacille pyocyanique. 2ème édition. Précis de Bactériologie Clinique; 2017.

190. Jackowski JT, Szepfalusi Z, Wanner DA, Seybold Z, Sielczak MW, Lauredo IT, et al. Effects of P. aeruginosa-derived bacterial products on tracheal ciliary function: role of O2 radicals. Am J Physiol. 1991; 260:L61–L67. https://doi.org/10.1152/ajplung.1991.260.2.L61 PMID: 1899975

191. McClure CD, Schiller NL. Inhibition of macrophage phagocytosis by Pseudomonas aeruginosa rhamnolipids in vitro and in vivo. Curr Microbiol. 1996; 33:109–117. https://doi.org/10.1007/s002849900084 PMID: 8662182

192. Abdel-Mawgoud AM, Lépine F, Déziel E. Rhamnolipids: diversity of structures, microbial origins and roles. Appl Microbiol Biot. 2010; 86:1323–1336. https://doi.org/10.1007/s00253-010-2498-2 PMID: 20336292

193. Halldorsson S, Gudjonsson T, Gottfredsson M, Singh PK, Gudmundsson GH, Baldursson O. Azithromycin Maintains Airway Epithelial Integrity during Pseudomonas aeruginosa Infection. Am J Resp Cell Mol. 2010; 42:62–68. https://doi.org/10.1165/rcmb.2008-0357OC PMID: 19372247

194. Wilhelm S, Gdynia A, Tielen P, Rosenau F, Jaeger K-E. The Autotransporter Esterase EstA of Pseudomonas aeruginosa Is Required for Rhamnolipid Production, Cell Motility, and Biofilm Formation. J Bacteriol. 2007; 189:6695–6703. https://doi.org/10.1128/JB.00023-07 PMID: 17631636

195. Iiyama K, Takahashi E, Lee JM, Mon H, Morishita M, Kusakabe T, et al. Alkaline protease contributes to pyocyanin production in Pseudomonas aeruginosa. Fems Microbiol Lett. 2017;364:fnx051. https://doi.org/10.1093/femsle/fnx051 PMID: 28333255

196. Heck LW, Morihara K, Abrahamson DR. Degradation of soluble laminin and depletion of tissue-associated basement membrane laminin by Pseudomonas aeruginosa elastase and alkaline protease. Infect Immun. 1986; 54:149–153. https://doi.org/10.1128/iai.54.1.149-153.1986 PMID: 3093382

197. Kessler E, Safrin M, Gustin JK, Ohman DE. Elastase and the LasA protease of Pseudomonas aeruginosa are secreted with their propeptides. J Biol Chem. 1998; 273:30225–30231. https://doi.org/10.1074/jbc.273.46.30225 PMID: 9804780

198. Armstrong S, Yates SP, Merrill AR. Insight into the catalytic mechanism of Pseudomonas aeruginosa exotoxin A. Studies of toxin interaction with eukaryotic elongation factor-2. J Biol Chem. 2002; 277:46669–46675. https://doi.org/10.1074/jbc.M206916200 PMID: 12270928

199. Ferguson TEG, Reihill JA, Walker B, Hamilton RA, Martin SL. A Selective Irreversible Inhibitor of Furin Does Not Prevent Pseudomonas Aeruginosa Exotoxin A-Induced Airway Epithelial Cytotoxicity. PLoS ONE. 2016; 11:e0159868. https://doi.org/10.1371/journal.pone.0159868 PMID: 27459298

200. Songer JG. Bacterial phospholipases and their role in virulence. Trends Microbiol. 1997; 5:156–161. https://doi.org/10.1016/S0966-842X(97)01005-6 PMID: 9141190

201. Terada LS, Johansen KA, Nowbar S, Vasil AI, Vasil ML. Pseudomonas aeruginosa Hemolytic Phospholipase C Suppresses Neutrophil Respiratory Burst Activity. Infect Immun. 1999; 67:2371–2376. https://doi.org/10.1128/IAI.67.5.2371-2376.1999 PMID: 10225897

202. Ostroff RM, Vasil AI, Vasil ML. Molecular comparison of a nonhemolytic and a hemolytic phospholipase C from Pseudomonas aeruginosa. J Bacteriol. 1990; 172:5915–5923. https://doi.org/10.1128/jb.172.10.5915-5923.1990 PMID: 2120196

203. Wargo MJ, Gross MJ, Rajamani S, Allard JL, Lundblad LKA, Allen GB, et al. Hemolytic Phospholipase C Inhibition Protects Lung Function during Pseudomonas aeruginosa Infection. Am J Resp Crit Care. 2011; 184:345–354. https://doi.org/10.1164/rccm.201103-0374OC PMID: 21562128

204. Bouillot S, Munro P, Gallet B, Reboud E, Cretin F, Golovkine G, et al. Pseudomonas aeruginosa Exolysin promotes bacterial growth in lungs, alveolar damage and bacterial dissemination. Sci Rep. 2017; 7:2120. https://doi.org/10.1038/s41598-017-02349-0 PMID: 28522850

205. Reboud E, Elsen S, Bouillot S, Golovkine G, Basso P, Jeannot K, et al. Phenotype and toxicity of the recently discovered exlA-positive Pseudomonas aeruginosa strains collected worldwide. Environ Microbiol. 2016; 18:3425–3439. https://doi.org/10.1111/1462-2920.13262 PMID: 26914644

206. Bomberger JM, Ely KH, Bangia N, Ye S, Green KA, Green WR, et al. Pseudomonas aeruginosa Cif Protein Enhances the Ubiquitination and Proteasomal Degradation of the Transporter Associated with Antigen Processing (TAP) and Reduces Major Histocompatibility Complex (MHC) Class I Antigen Presentation*. J Biol Chem. 2014; 289:152–162. https://doi.org/10.1074/jbc.M113.459271 PMID: 24247241

207. Bomberger JM, Ye S, MacEachran DP, Koeppen K, Barnaby RL, O'Toole GA, et al. A Pseudomonas aeruginosa Toxin that Hijacks the Host Ubiquitin Proteolytic System. PLoS Pathog. 2011; 7:e1001325. https://doi.org/10.1371/journal.ppat.1001325 PMID: 21455491

208. Shaver CM, Hauser AR. Relative contributions of Pseudomonas aeruginosa ExoU, ExoS, and ExoT to virulence in the lung. Infect Immun. 2004; 72:6969–6977. https://doi.org/10.1128/IAI.72.12.6969-6977.2004 PMID: 15557619

209. Ganesan AK, Vincent TS, Olson JC, Barbieri JT. Pseudomonas aeruginosa exoenzyme S disrupts Ras-mediated signal transduction by inhibiting guanine nucleotide exchange factor-catalyzed nucleotide exchange. J Biol Chem. 1999; 274:21823–21829. https://doi.org/10.1074/jbc.274.31.21823 PMID: 10419499

210. Sato H, Frank DW, Hillard CJ, Feix JB, Pankhaniya RR, Moriyama K, et al. The mechanism of action of the Pseudomonas aeruginosa-encoded type III cytotoxin. ExoU. EMBO J. 2003; 22:2959–2969. https://doi.org/10.1093/emboj/cdg290 PMID: 12805211

211. Yahr TL, Vallis AJ, Hancock MK, Barbieri JT, Frank DW. ExoY, an adenylate cyclase secreted by the Pseudomonas aeruginosa type III system. Proc Natl Acad Sci. 1998; 95:13899–13904. https://doi.org/10.1073/pnas.95.23.13899 PMID: 9811898

212. Cowell BA, Evans DJ, Fleiszig SMJ. Actin cytoskeleton disruption by ExoY and its effects on Pseudomonas aeruginosa invasion. FEMS Microbiol Lett. 2005; 250:71–76. https://doi.org/10.1016/j.femsle.2005.06.044 PMID: 16039071

213. Ochoa CD, Alexeyev M, Pastukh V, Balczon R, Stevens T. Pseudomonas aeruginosa Exotoxin Y Is a Promiscuous Cyclase That Increases Endothelial Tau Phosphorylation and Permeability*. J Biol Chem. 2012; 287:25407–25418. https://doi.org/10.1074/jbc.M111.301440 PMID: 22637478

214. Kloth C, Schirmer B, Munder A, Stelzer T, Rothschuh J, Seifert R. The Role of Pseudomonas aeruginosa ExoY in an Acute Mouse Lung Infection Model. Toxins. 2018; 10:185. https://doi.org/10.3390/toxins10050185 PMID: 29734720

215. Chen L, Zou Y, She P, Wu Y. Composition, function, and regulation of T6SS in Pseudomonas aerugi-nosa. Microbiol Res. 2015; 172:19–25. https://doi.org/10.1016/j.micres.2015.01.004 PMID: 25721475

216. Bleves S, Sana TG, Voulhoux R. The target cell genus does not matter. Trends Microbiol. 2014; 22:304–306. https://doi.org/10.1016/j.tim.2014.04.011 PMID: 24836109

217. Sana TG, Baumann C, Merdes A, Soscia C, Rattei T, Hachani A, et al. Internalization of Pseudomonas aeruginosa Strain PAO1 into Epithelial Cells Is Promoted by Interaction of a T6SS Effector with the Microtubule Network. Mbio. 2015; 6:e00712–e00715. https://doi.org/10.1128/mBio.00712-15 PMID: 26037124

218. Jiang F, Wang X, Wang B, Chen L, Zhao Z, Waterfield NR, et al. The Pseudomonas aeruginosa Type VI Secretion PGAP1-like Effector Induces Host Autophagy by Activating Endoplasmic Reticulum Stress. Cell Rep. 2016; 16:1502–1509. https://doi.org/10.1016/j.celrep.2016.07.012 PMID: 27477276

219. Koeppen K, Hampton TH, Jarek M, Scharfe M, Gerber SA, Mielcarz DW, et al. A Novel Mechanism of Host-Pathogen Interaction through sRNA in Bacterial Outer Membrane Vesicles. PLoS Pathog. 2016; 12:e1005672. https://doi.org/10.1371/journal.ppat.1005672 PMID: 27295279

220. Smith RS, Harris SG, Phipps R, Iglewski B. The Pseudomonas aeruginosa quorum-sensing molecule N-(3-oxododecanoyl)homoserine lactone contributes to virulence and induces inflammation in vivo. J Bacteriol. 2002; 184:1132–1139. https://doi.org/10.1128/jb.184.4.1132-1139.2002 PMID: 11807074

221. Cigana C, Lorè NI, Bernardini ML, Bragonzi A. Dampening Host Sensing and Avoiding Recognition in Pseudomonas aeruginosa Pneumonia. J Biomed Biotechnol. 2011; 2011:852513. https://doi.org/10.1155/2011/852513 PMID: 21785567

222. Hauser AR, Cobb E, Bodi M, Mariscal D, Vallés J, Engel JN, et al. Type III protein secretion is associ-ated with poor clinical outcomes in patients with ventilator-associated pneumonia caused by Pseudo-monas aeruginosa. Crit Care Med. 2002; 30:521–528. https://doi.org/10.1097/00003246-200203000-00005 PMID: 11990909

223. El-Solh AA, Hattemer A, Hauser AR, Alhajhusain A, Vora H. Clinical outcomes of type III Pseudomo-nas aeruginosa bacteremia. Crit Care Med. 2012; 40:1157–1163. https://doi.org/10.1097/CCM.0b013e3182377906 PMID: 22080633

224. Ozer EA, Nnah E, Didelot X, Whitaker RJ, Hauser AR. The Population Structure of Pseudomonas aer-uginosa Is Characterized by Genetic Isolation of exoU+ and exoS+ Lineages. Genome Biol Evol. 2019; 11:1780–1796. https://doi.org/10.1093/gbe/evz119 PMID: 31173069

225. Pena C, Cabot G, Gomez-Zorrilla S, Zamorano L, Ocampo-Sosa A, Murillas J, et al. Influence of Viru-lence Genotype and Resistance Profile in the Mortality of Pseudomonas aeruginosa Bloodstream Infections. Clin Infect Dis. 2015; 60:539–548. https://doi.org/10.1093/cid/ciu866 PMID: 25378459

226. Horna G, Amaro C, Palacios A, Guerra H, Ruiz J. High frequency of the exoU+/exoS+ genotype asso-ciated with multidrug-resistant "high-risk clones" of Pseudomonas aeruginosa clinical isolates from Peruvian hospitals. Sci Rep. 2019; 9:10874. https://doi.org/10.1038/s41598-019-47303-4 PMID: 31350412

227. Yousefi-Avarvand A, Khashei R, Ebrahim-Saraie HS, Emami A, Zomorodian K, Motamedifar M. The Frequency of Exotoxin A and Exoenzymes S and U Genes Among Clinical Isolates of Pseudomonas aeruginosa in Shiraz. Iran. Int J Mol Cell Med. 2015; 4:167–173.

228. Wareham DW, Curtis MA. A genotypic and phenotypic comparison of type III secretion profiles of Pseudomonas aeruginosa cystic fibrosis and bacteremia isolates. Int J Med Microbiol. 2007; 297:227–234. https://doi.org/10.1016/j.ijmm.2007.02.004 PMID: 17412636

229. Hirakata Y, Finlay BB, Simpson DA, Kohno S, Kamihira S, Speert DP. Penetration of Clinical Isolates of Pseudomonas aeruginosa through MDCK Epithelial Cell Monolayers. J Infect Dis. 2000; 181:765–769. https://doi.org/10.1086/315276 PMID: 10669373

230. Feltman H, Schulert G, Khan S, Jain M, Peterson L, Hauser AR. Prevalence of type III secretion genes in clinical and environmental isolates of Pseudomonas aeruginosa. Microbiology+. 2001; 147:2659–2669. https://doi.org/10.1099/00221287-147-10-2659 PMID: 11577145

231. Soong G, Parker D, Magargee M, Prince AS. The Type III Toxins of Pseudomonas aeruginosa Disrupt Epithelial Barrier Function. J Bacteriol. 2008; 190:2814–2821. https://doi.org/10.1128/JB.01567-07 PMID: 18165298

232. Gingerich A, Pang L, Hanson J, Dlugolenski D, Streich R, Lafontaine ER, et al. Hypothiocyanite pro-duced by human and rat respiratory epithelial cells inactivates extracellular H1N2 influenza A virus. Inflamm Res. 2016; 65:71–80. https://doi.org/10.1007/s00011-015-0892-z PMID: 26608498

233. Reboud E, Bouillot S, Patot S, Béganton B, Attrée I, Huber P. Pseudomonas aeruginosa ExlA and Ser-ratia marcescens ShlA trigger cadherin cleavage by promoting calcium influx and ADAM10 activation. PLoS Pathog. 2017; 13:e1006579. https://doi.org/10.1371/journal.ppat.1006579 PMID: 28832671

234. Bleves S, Viarre V, Salacha R, Michel GPF, Filloux A, Voulhoux R. Protein secretion systems in Pseudomonas aeruginosa: A wealth of pathogenic weapons. Int J Med Microbiol. 2010; 300:534–543. https://doi.org/10.1016/j.ijmm.2010.08.005 PMID: 20947426

235. Wiener-Kronish JP, Sakuma T, Kudoh I, Pittet JF, Frank D, Dobbs L, et al. Alveolar epithelial injury and pleural empyema in acute P. aeruginosa pneumonia in anesthetized rabbits. J Appl Physiol. 1993; 75:1661–1669. https://doi.org/10.1152/jappl.1993.75.4.1661 PMID: 8282618

236. Kida Y, Yamamoto T, Kuwano K. SdsA1, a secreted sulfatase, contributes to the in vivo virulence of Pseudomonas aeruginosa in mice. Microbiol Immunol. 2020; 64:280–295. https://doi.org/10.1111/1348-0421.12772 PMID: 31907968

237. Pier GB, Grout M, Zaidi TS, Olsen JC, Johnson LG, Yankaskas JR, et al. Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections. Science. 1996; 271:64–67. https://doi.org/10.1126/science.271.5245.64 PMID: 8539601

238. Chi E, Mehl T, Nunn D, Lory S. Interaction of Pseudomonas aeruginosa with A549 pneumocyte cells. Infect Immun. 1991; 59:822–828. https://doi.org/10.1128/iai.59.3.822-828.1991 PMID: 1671777

239. Bucior I, Tran C, Engel J. Assessing Pseudomonas virulence using host cells. Methods Mol Biol. 2014; 1149:741–755. https://doi.org/10.1007/978-1-4939-0473-0_57 PMID: 24818947

240. Cappiello F, Grazia AD, Segev-Zarko L, Scali S, Ferrera L, Galietta L, et al. Esculentin-1a-Derived Peptides Promote Clearance of Pseudomonas aeruginosa Internalized in Bronchial Cells of Cystic Fibrosis Patients and Lung Cell Migration: Biochemical Properties and a Plausible Mode of Action. Antimicrob Agents Chemother. 2016; 60:7252–7262. https://doi.org/10.1128/AAC.00904-16 PMID: 27671059

241. Zaas DW, Duncan MJ, Li G, Wright JR, Abraham SN. Pseudomonas invasion of type I pneumocytes is dependent on the expression and phosphorylation of caveolin-2. J Biol Chem. 2005; 280:4864–4872. https://doi.org/10.1074/jbc.M411702200 PMID: 15545264

242. Pereira SH, Cervante MP, Bentzmann S, Plotkowski MC. Pseudomonas aeruginosa entry into Caco-2 cells is enhanced in repairing wounded monolayers. Microb Pathog. 1997; 23:249–255. https://doi.org/10.1006/mpat.1997.0153 PMID: 9344786

243. Kazmierczak BI, Jou TS, Mostov K, Engel JN. Rho GTPase activity modulates Pseudomonas aeruginosa internalization by epithelial cells. Cell Microbiol. 2001; 3:85–98. https://doi.org/10.1046/j.1462-5822.2001.00091.x PMID: 11207623

244. Lovewell RR, Patankar YR, Berwin B. Mechanisms of phagocytosis and host clearance of Pseudomonas aeruginosa. Am J Physiol Lung Cell Mol Physiol. 2014; 306:L591–L603. https://doi.org/10.1152/ajplung.00335.2013 PMID: 24464809

245. Yamamoto N, Yamamoto N, Petroll MW, Cavanagh HD, Jester JV. Internalization of Pseudomonas aeruginosa is mediated by lipid rafts in contact lens-wearing rabbit and cultured human corneal epithelial cells. Invest Ophth Vis Sci. 2005; 46:1348–1355. https://doi.org/10.1167/iovs.04-0542 PMID: 15790901

246. Lingwood D, Simons K. Lipid Rafts As a Membrane-Organizing Principle. Science. 2010; 327:46–50. https://doi.org/10.1126/science.1174621 PMID: 20044567

247. Jiang F, Waterfield NR, Yang J, Yang G, Jin Q. A Pseudomonas aeruginosa type VI secretion phospholipase D effector targets both prokaryotic and eukaryotic cells. Cell Host Microbe. 2014; 15:600–610. https://doi.org/10.1016/j.chom.2014.04.010 PMID: 24832454

248. Engel J, Eran Y. Subversion of mucosal barrier polarity by pseudomonas aeruginosa. Front Microbiol. 2011; 2:114. https://doi.org/10.3389/fmicb.2011.00114 PMID: 21747810

249. Kierbel A, Gassama-Diagne A, Rocha C, Radoshevich L, Olson J, Mostov K, et al. Pseudomonas aeruginosa exploits a PIP3-dependent pathway to transform apical into basolateral membrane. J Cell Biol. 2007; 177:21–27. https://doi.org/10.1083/jcb.200605142 PMID: 17403925

250. Lepanto P, Bryant DM, Rossello J, Datta A, Mostov KE, Kierbel A. Pseudomonas aeruginosa interacts with epithelial cells rapidly forming aggregates that are internalized by a Lyn-dependent mechanism. Cell Microbiol. 2011; 13:1212–1222. https://doi.org/10.1111/j.1462-5822.2011.01611.x PMID: 21615664

251. Pier GB. The challenges and promises of new therapies for cystic fibrosis. J Exp Med. 2012; 209:1235–1239. https://doi.org/10.1084/jem.20121248 PMID: 22753926