Review Article

Dampening Host Sensing and Avoiding Recognition in *Pseudomonas aeruginosa* Pneumonia

Cristina Cigana,¹ Nicola Ivan Lorè,¹ Maria Lina Bernardini,^{2,3} and Alessandra Bragonzi¹

¹ Infections and Cystic Fibrosis Unit, Division of Immunology, Transplantation and Infectious Diseases, San Raffaele Scientific Institute, 20132 Milano, Italy

² Dipartimento di Biologia Cellulare e dello Sviluppo, Sapienza-Università di Roma, 00185 Roma, Italy

³ Istituto Pasteur-Fondazione Cenci Bolognetti, 00185 Roma, Italy

Correspondence should be addressed to Alessandra Bragonzi, bragonzi.alessandra@hsr.it

Received 15 December 2010; Accepted 9 May 2011

Academic Editor: Masao Kimoto

Copyright © 2011 Cristina Cigana et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Pseudomonas aeruginosa is an opportunistic pathogen and causes a wide range of acute and chronic infections. *P. aeruginosa* infections are kept in check by an effective immune surveillance in the healthy host, while any imbalance or defect in the normal immune response can manifest in disease. Invasive acute infection in the immunocompromised patients is mediated by potent extracellular and cell bound bacterial virulence factors. Life-threatening chronic infection in cystic fibrosis patients is maintained by pathogenic variants that contribute to evade detection and clearance by the immune system. Here, we reviewed the molecular basis of receptor-mediated recognition of *P. aeruginosa* and their role in initiating inflammation and the colonization. In addition, the consequence of the *P. aeruginosa* genetic adaptation for the antibacterial defence and the maintaining of chronic infection are discussed.

1. Pathogenesis of *P. aeruginosa* Pneumonia

Pseudomonas aeruginosa rarely causes infection in healthy host although it is one of the most important agents of nosocomial infections in diverse clinical setting. The immunocompetent host usually offers effective immune surveillance against infection by *P. aeruginosa*; however, any imbalance or defect in the normal immune response to this opportunistic pathogen can lead to infection and manifest in disease. The spectrum of clinical diseases caused by *P. aeruginosa* in humans ranges from invasive acute infections as in patients who are mechanically ventilated, individuals who are immunocompromised, and patients with malignancies or HIV infection, to life-threatening chronic infections as in cystic fibrosis (CF) patients. *P. aeruginosa* adaptability to environments that are inhospitable to most other microorganisms, minimal nutritional requirements, and high resistance to antibiotics allow it to survive in different hosts [1].

The first step in mounting a protective immune response is the recognition of the bacterial pathogen by cell surface receptors, which are located on professional phagocytes (granulocytes and monocytes/macrophages) and dendritic cells as well as nonimmune cells (Figure 1(a)) [2]. This is followed by the activation of intracellular signalling pathways and stimulation of inflammatory mediators. Subsequently, effector immune mechanisms are triggered such as neutrophil and macrophage activation as well as initiation of adaptive immunity through T helper cell (Th)1 or Th2 responses. In most cases, the disease process begins with some alteration or circumvention of normal host defenses. In patients with damaged mucosal barriers from mechanical ventilation, trauma or antecedent viral infection, *P. aeruginosa* colonization of the respiratory tract is often followed by acute pneumonia, sepsis, and death. Loss of defence mechanism and an inappropriate immune-response in patients who are immunocompromised, particularly transplant recipients, burn patients, patients with cancer, neutropenia,

FIGURE 1: *Pseudomonas aeruginosa* recognition by PRRs during acute and chronic lung infection. (a) During acute lung infection, *P. aeruginosa* can invade, disseminate and lead to extensive tissue damage by means of potent array of extracellular and cell bound virulence factors. However the immunocompetent host mounts an effective immune response characterized of bacterial recognition by cell surface receptors, which are located on immune cells as well as epithelial cells. Plasma membrane-bound TLRs (TLR2, TLR4-MD2-CD14, TLR5, and TLR9) and cytosolic NLRs (NOD1, NOD2, and IPAF) recognise *P. aeruginosa* PAMPs and recruit adaptors to induce downstream signalling cascades, which result in transcription of pro-inflammatory mediators and mucins. These pro-inflammatory mediators, including chemokines, recruit immune cells to the lung in order to clear *P. aeruginosa* and resolve the infection. (b) During chronic lung infection, *P. aeruginosa*, enmeshed in biofilm structures, does not have direct contact to the airways epithelium and probably only immunogenic bacterial components can access to airway epithelium and immune cells in the lung. In addition, during long-term colonization, bacteria undergo a number of genetic changes and gain the ability to evade detection and clearance by the immune system, thus surviving in the host. The loss or modification of several PAMPs (flagellin, LPS, and PGN) lead to reduced recognition by TLRs and NLRs although components of the alginate capsule can still be recognized both by TLR2 and TLR4. The inadequate immune response may explain the chronic colonization of *P. aeruginosa* strains. NOD, Nucleotide-binding oligomerization domain; TLR, Toll-like receptor; NLR, Nod-like receptor; IPAF, ICE-protease activating factor; MyD88, myeloid differentiation primary response protein; CFTR, cystic fibrosis transmembrane conductance regulator.

and with HIV are important risk factors for *P. aeruginosa* infection.

In CF, generalized immune deficiency and specific abnormalities in acquired immunity are highly unlikely, since systemic infection is not characteristic of this disease (Figure 1(b)). In fact, sepsis due to *P. aeruginosa*, even after decades of lung infection, is rare, presumably due to effective humoral immunity. More likely, the disease represents a failure of local airway defense. It has been suggested that bacteria adhere more readily to CF airway epithelial cells due to enhanced expression of the cell surface ganglioside asialoGM1, promoting infection [3]. Paradoxically, it has also been proposed that cystic fibrosis transmembrane conductance regulator (CFTR) serves as a bacterial receptor and that its absence leads to failure to internalize and kill bacteria [4, 5]. Furthermore, an abnormal accumulation of ceramide in the lungs of CF mice and in epithelial cells from CF patients has been shown to result in an increased death rate of respiratory epithelial cells and DNA deposits on the respiratory epithelium, which facilitate bacterial adherence [6].

Defects in antimicrobial activity of airway fluid [7], in neutrophil phagocytosis [8] or excessive neutrophil extracellular traps (NETs) formation [9] likely occur in the inflamed CF airway environment but are unlikely to be the primary

defect. The respiratory tract pathophysiology in CF principally results from the inability to secrete Cl^- and regulate Na^+ absorption, which causes relative dehydration of the airway surface, depleting the periciliary layer and causing accumulation of hyperviscous mucus that cannot be cleared by mucociliary clearance or cough [10]. Mucus plaques and plugs serve as a nidus for intra-luminal infection [11]. Bacteria resident within the thickened luminal mucus may evade chemical antimicrobial factors and phagocytes [12]. Complex bacterial evolution and host adaptation occur in the chronically infected airway, which is likely unique in CF due to the constant and severe degree of mucus dehydration and impaired mucus clearance. Bacterial colonies exhibiting biofilm-like properties may develop, which are difficult or impossible to eradicate. The continuous presence of bacteria and the accompanying intense inflammation ultimately remodel the airway wall, causing the ubiquitous mucous secretory cell hyperplasia and metaplasia, submucosal gland enlargement, hypertrophy of the bronchial circulation, ectasis of bronchi and bronchioles, and variable parenchymal cyst formation, sometimes progressing to cavitory disease, with adjacent fibrosis and pleural involvement [13].

The pathogenesis of *P. aeruginosa* infections appears to be complex and multifactorial. The conditions present in acute infections force pathogens to injure or kill the host, with

multiorgan failure sometimes occurring in hours or days. In contrast, chronic infections occur without acute injury and in the presence of biofilm structures—a population of microorganisms that aggregates on a matrix—that develop over days or weeks, and bacterial genetic variants may grow in the biofilms. A potent array of *P. aeruginosa* extracellular and cell bound virulence factors are critical for the initial colonization phase of infection and then invasion, dissemination, and extensive tissue damage [14]. Flagella and pili, the motile surface appendages of *P. aeruginosa*, are responsible for bacterial motility, progression towards epithelial contact and dissemination throughout the host organism. These appendages also act as initial tethers in facilitating bacteria to epithelial cell contact by binding to the cell surface receptors. Additionally, lipopolysaccharide (LPS) plays a similar role in bacterial adhesion [15]. Upon cell contact, the type III secretion system (T3SS), a major virulence determinant, is activated [16, 17]. The T3SS allows *P. aeruginosa* to inject secreted toxins through a syringe-like apparatus directly into the eukaryotic cytoplasm. Four effector proteins are known: ExoY, ExoS, ExoT, and ExoU and all participate, at varying levels, in the cytotoxicity of *P. aeruginosa* leading to invasion and dissemination [18]. Other virulence factors secreted via type II secretion system into the extracellular space such as elastase, alkaline phosphatase, exotoxin A, and phospholipase C are also liable for invasion and dissemination by destroying the protective glycocalyx of the respiratory epithelium and exposing epithelial ligands to *P. aeruginosa* and participate in cytotoxicity [19]. A similar role has also been reported for pyoverdine and pyocyanin. Most of these *P. aeruginosa* invasive functions are selected against in CF chronic infection leading to less virulent but more persistent phenotypes [20–22]. The steric shielding or modification of these exposed molecules is the most effective strategy for avoiding host's innate recognition and establishing persistent chronic infection [23]. In addition, in the lung of CF patients, *P. aeruginosa* forms microcolonies encapsulated by mucoid exopolysaccharide. The emergence of mucoid variants is believed to mark the transition to the fatal, chronic stage of the infection [24].

2. Sensing and Defence against *Pseudomonas aeruginosa*

The innate immune system is the first line of defence against pathogens. Innate immune responses depend on a vast array of nonclonally expressed receptors, named pattern recognition receptors (PRRs), aimed at pathogen recognition. PRR bind to highly conserved invariant molecular complexes called pathogens (microbe-) associated molecular Patterns (PAMP or MAMPs), which are widespread and conserved among microorganisms [36]. PRR binding with PAMPs elicits a signalling response which results in a rapid response against any encountered microorganisms, including potential pathogens. This complexity of bindings allows the immune system not only to tailor its response to a specific pathogen but also to discriminate the site of infection or the microbial burden.

The best studied PRRs are Toll-like receptors (TLRs), which are transmembrane proteins present at the cell surface or on the membrane of endocytic vesicles or other intracellular organelles [37]. The extracellular domain of TLRs is characterized by leucine-rich repeats (LRRs) that are involved in ligand binding. Ligand recognition induces homodimerization or heterodimerization of the ectodomains, allowing the intracellular domains to initiate signalling. The cytoplasmic domain contains the highly conserved Toll/interleukin-1 (IL-1) receptor (TIR) domain, which interacts with various adaptor molecules such as myeloid differentiation primary response protein (MyD88) to elicit signalling. There are currently 12 known mammalian TLRs; they recognize and bind a wide variety of bacterial PAMPs, including LPS (typically recognized by TLR4 although some LPS species can be recognized by TLR2), lipopeptides (TLR1, TLR2, TLR6), lipoarabinomannan and lipoteichoic acid (TLR2 and other TLRs), flagellin (TLR5), and bacterial DNA (TLR9) [38].

Although the TLRs is the family of PRRs best studied another PRR family identified through homology to plant R proteins has emerged as playing a crucial role in host response. The PRR family of nucleotide-binding oligomerization domain-(NOD-) like receptor (NLR) proteins includes 23 members in humans, and it is divided in 5 subfamilies according to their effector domains. Several studies highlighted that NLR NOD1 and NOD2 are key cytoplasmic PRR [39, 40]. These proteins are characterized by a N-terminal effector domain, a centrally located nucleotide-binding domain and multiple leucine-rich repeats in their C-terminal end. While Nod1 is ubiquitous, Nod2 seems to be more restricted to myelomonocytic and epithelial cells. Both Nod1 and Nod2 sense peptidoglycan (PGN) motifs. Nod1 exhibits specificity for a diaminopimelate containing GlcNAc-MurNAc tripeptide (GM-Tri_{Dap}) fragment that is almost exclusively found in Gram-negative bacterial PGN, while Nod2 binds muramyl dipeptide (MDP) motif that is common to Gram-positive and Gram-negative bacteria [41, 42]. After recognition of bacterial ligands through the LRRs domain Nod1 and Nod2 activate the Nuclear Factor κ B (NF- κ B) and elicit the production of pro-inflammatory cytokines [43]. Other NLR members, such as NLR1, NLR3 and Ipaf, are involved in building a multisubunit protein complex called inflammasome following PAMP and DAMP (danger-associated molecular Patterns) detection [44]. This is a tightly controlled process leading to the proteolytic processing of procaspase-1, which in turns activate the interleukin-1 β . Inflammasome activation also accounts for a peculiar type of cell death called pyroptosis characterized by membrane cell lesions leading to the release of mature IL-1 β [45].

P. aeruginosa stimulates pro-inflammatory cytokine production [46]. *P. aeruginosa* expresses numerous PAMPs (Table 1), among which LPS and flagellin have been reported to play a special role in signalling bacterial presence in host. LPS is a glycolipid that constitutes the major portion of the outermost membrane of Gram-negative bacteria. LPS consists of three distinct regions: O-antigen, core, and lipid A. Both O-antigen and core consist of polysaccharide chains, whereas lipid A consists of fatty acid and phosphate

TABLE 1: PRRs sensing *P. aeruginosa*-associated molecular patterns.

Receptors	<i>P. aeruginosa</i> PAMP	References
<i>Toll-like receptors</i>		
TLR2	PGN	[25]
	LPS	[26]
	ExoS	[25]
	mannuronic acid polymers	[27]
	flagellin	[28]
	Slime-GLP	[29]
TLR4-MD2-CD14	LPS	[30]
	ExoS	[25]
	mannuronic acid polymers	[27]
TLR5	Flagellin	[31]
TLR9	DNA	[32] [33]
<i>NLR receptors</i>		
Nod 1	PGN	[30]
NLRC4/Ipaf	flagellin	[34]
	T3SS	[34]
<i>Other receptors</i>		
CFTR	LPS	[35]

moieties bonded to a central glucosamine dimer [47]. This last portion of LPS molecule is recognized by TLR4, in association with CD14 and the adaptor molecule MD2 [48, 49]. It has been reported that *P. aeruginosa* can vary LPS acylation (number and structure of fatty acids) during its biosynthesis [50, 51]. This variation results on a modified sensing of this PAMP from the cognate receptor TLR4, which classically recognizes hexa-acylated LPS, and/or recognition from TLR2, which can bind tetra- and penta-acylated pseudomonal LPS. The role of TLR2 in *P. aeruginosa* recognition is widely treated in several studies. TLR2 is a “promiscuous” member of the TLR family as it recognizes several ligands. TLR2 has been reported to participate in recognition of multiple *P. aeruginosa* components, including lipoproteins [52], alginate [27], flagellin [53], and exoenzyme S [25].

In addition to direct TLR2 activation it has been proposed that *P. aeruginosa* triggers tumor necrosis factor TNF- α production by human monocytes through slime glycolipoprotein (slime-GLP) [29], an extracellular glycolipid component, which is present at the bacterial surface and produced during *in vivo* infection by mucoid and nonmucoid strains. *P. aeruginosa* slime-GLP is rich in mannose, a sugar which is absent from the homologous LPS. Slime-GLP can activate innate immune responses through interaction with MR (Mannose receptors) which synergize and complement the activity of TLRs. Following interaction with *P. aeruginosa* MR activation elicits proinflammatory cytokine production by stimulating NF- κ B and MAPK. *P. aeruginosa* MR activation synergizes TLR2 activity to trigger maximum NF- κ B stimulation and proinflammatory cytokine production [54]. Likewise, studies in TLR9 deficient mice demonstrated that signalling induced by this TLR plays a role in cornea inflammation experimentally provoked by *P. aeruginosa* [55]. However, a more recent report identified a

new-based CpG motif (which is the natural agonist of TLR9), CpG-ODNc41, in *P. aeruginosa* genome [56]. In human and murine monocytes CpG-ODNc41 was nonstimulatory and noncytotoxic and acted as an antagonist by inhibiting the stimulatory activity of conventional CpG-DNAs.

Modification in host sensing may switch the immune response from an appropriate reaction to the presence of the pathogen to a vicious circle in which inapt signalling leads to excessive inflammation which exacerbates the effect of *P. aeruginosa* presence in the organism. Flagellin is a protein that arranges itself in a hollow cylinder to form the filament bacterial flagellum. Indeed, *P. aeruginosa* is motile via a single polar flagellum that has structural properties very similar to those of enteric Gram-negative bacteria, with the added structural feature of being glycosylated [57]. A specific motif [58–60] in flagellin monomer is a ligand for TLR5 and induces the expression of proinflammatory mediators in monocytes, macrophages, intestinal, airway, and corneal epithelial cells, resulting from the activation of the transcription factor NF- κ B and production of pro-inflammatory cytokines [61]. The majority of studies focused on the interaction of TLR activity and *P. aeruginosa* have been carried out *in vitro*, on cellular models. However, up to now no single TLR deficiency in transgenic mice seems to affect the host response to *P. aeruginosa* in lung infection, which is one of the more common and serious aspect of *P. aeruginosa* pathogenesis. Though controversial results have been obtained by different groups, common opinion emerged that mice lacking individual TLR4, TLR2, or TLR5 are equally sensitive to lung *P. aeruginosa* infection [62–64]. Likewise, the combination of TLR4 and TLR2 absence does not impair the ability of *P. aeruginosa* to establish infection albeit the defects in cytokine responses have been recorded. Multitransgenic mice lacking TLR5 and TLR4 appear to be more sensitive to *P. aeruginosa* [62]. Most of TLR signalling following bacterial sensing depends on the MyD88 adaptor protein that leads the activation of NF- κ B and the production of pro-inflammatory cytokines. Therefore, mice lacking MyD88 infected intranasally with *P. aeruginosa* displayed elevated bacterial counts in lung [65] along with reduced levels of cytokines such as TNF- α and IL-1 β .

Moreover, several studies report that *P. aeruginosa* is sensed by the NLR family members. Notably, *P. aeruginosa* is recognized by the Nod1 PRR following infection in HEK293 cells overexpressing this receptor [66]. Likewise by exploiting various cell model systems, it was demonstrated that Nod1, but not Nod2, recognizes in a different way *P. aeruginosa* PGN of clonal clinical strains isolated by airways of a patient of CF at the initial and chronic stages of infection [30].

In contrast to TLR5, which senses extracellular flagellin, the Ipaf inflammasome is generally activated by cytosolic flagellin, which is sufficient for IPAF-dependent caspase-1 activation [67, 68]. In accordance with this issue, during infection, *P. aeruginosa* is recognized by Ipaf through flagellin sensing [34, 69]. However, parallel studies reported that Ipaf-mediated inflammasome is also mounted by the presence of a T3SS carried by several pathogens, including *P. aeruginosa* [70]. More recently, a basal body rod component of the

TABLE 2: Frequent mutations in *P. aeruginosa* virulence factors of CF airways isolates.

Virulence factors	Mutation*	References
LPS	pagL	[30]
Flagella	rpoN, fleQ	[72] [20]
Alginate	mucA	[73] [74]
Quorum sensing	LasR	[75] [76]
T3SS	exsA	[20]

* Only most common mutations in *P. aeruginosa* clinical strains are reported.

T3SS apparatus has been identified as the T3SS-dependent responsible for Ipaf activation. This component has been found in *Salmonella typhimurium* (PrgJ), *Burkholderia pseudomallei* (BsaK), *Escherichia coli* (EprJ and EscI), *Shigella flexneri* (MxiI), and *P. aeruginosa* (PscI), inducing caspase-1 activation [71]. These rod proteins share a sequence motif that is essential for detection by Ipaf, and that is similar to the flagellin motif recognized by the same receptor.

3. Avoiding Host Recognition in *Pseudomonas aeruginosa* and Establishing Chronic Infection

Although the process of recognition of PAMPs is rapid and efficient, it is now clear that bacteria are able to alter their structures in order to avoid or modulate this immune recognition. This bacterial behaviour is classed as an “immune evasion strategy”. Most CF patients acquire chronic *P. aeruginosa* infections by their teenage years and these respiratory infections are responsible for much of the morbidity and mortality. It has been established that the majority of *P. aeruginosa* strains infecting the lungs of CF patients are acquired independently, presumably from diverse environmental reservoirs. However, the long-term colonization of the CF host is maintained by *P. aeruginosa* pathoadaptive lineages, which are clonal with the initially acquired strain and carried phenotypic variants [77]. A variety of host-derived inflammatory product and environmental factors contribute to select clonal variants in *P. aeruginosa*. As a result of inflammation, *P. aeruginosa* is exposed to high levels of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which are generated primarily by neutrophils as part of the host’s innate immune response. ROS and RNS contribute to mutations that confer an adaptive advantage to *P. aeruginosa* in the airway [78, 79]. Furthermore, within the highly viscous mucus, a microaerobic/anaerobic milieu prevails due to oxygen consumption by bacterial pathogens or invading neutrophils. *P. aeruginosa* growth in oxygen restricted environments leads to changes in the bacterial phenotypes that facilitate chronic infection [11, 80].

Pathogenicity-adaptive mutations represent a genetic mechanism for enhancing bacterial virulence without horizontal transfer of specific virulence factors [81]. Common mutations are consistently acquired by most CF patients as those in regulators of alginate biosynthesis (*mucA* and *algT*) [74] and virulence genes including in the LPS (*pagL*)

and muropeptide modifications [30], motility (*rpoN*) [72], in the quorum-sensing regulator (*lasR*) [75, 76], in the T3SS [82], in the multidrug-efflux pump (*mexA*) and in mutator phenotypes (*mutS*) [83] (Table 2). Whole-genome comparison between an isogenic early and late *P. aeruginosa* pair recovered from one patient 90 months apart showed that the late isolate accumulated 68 mutations [20]. Interestingly, virulence factors required for the initiation of acute infections were selected against during chronic infection. This indicates reduced virulence of the late strains with regard to their ability to provoke acute infection and host recognition [23, 84]. High adaptive genetic diversification underlines the immune evasion strategies, resulting in increasing chances of bacterial survival in their niche, that is, in the airway environment of CF patients. This evolutionary scenario is similar to that of the genomes of pathogens which establish chronic infection as *Escherichia coli* [85], *Haemophilus influenzae* [86], or *Helicobacter pylori* [87].

Recent reports have showed that *P. aeruginosa* exploits PAMPs modification as a strategy to hijack genes involved in innate immune responses and to favor survival in CF patients [28, 30]. Loss of flagellar expression enables immune evasion by the bacteria due to loss of engagement by phagocytic receptors that recognize flagellar components and loss of immune activation through flagellin-mediated TLR signaling. Using a variety of *in vitro*, *ex vivo*, and *in vivo* infection models, Amiel et al. [28], showed that loss of *P. aeruginosa* motility dramatically alters immune responses to these bacteria compared to those for motile isogenic bacterial strains and that it is the loss of flagellum-mediated motility, but not flagellum expression itself, that results in dramatic bacterial resistance to phagocytosis by murine and human phagocytes. Likewise, studies in the agar beads murine model by using the *P. aeruginosa* isolates from patients with CF demonstrated that the risk of chronic infection is increased by the absence of pili and flagella [21]. These studies provide an explanation for the clinical observation that *P. aeruginosa* isolates obtained from CF hosts often exhibit a nonmotile phenotype [88] and explain how this phenotype can confer a survival advantage for bacteria that modulate or lose their motility during chronic infection.

Chemical structure of LPS and PGN were determined for three *P. aeruginosa* clones isolated from airways of a CF patient during a period of 7.5 years [30]. Lipid A, that is variably penta-, hexa-, or hepta-acylated, was temporally associated with different stages of CF infection. Among the three strains LPS lipid A diversity was observed in the number and location of fatty-acid side chains. Early and late mucoid *P. aeruginosa* strains synthesized a LPS blend essentially composed by tetra-, penta-, and hexa-acylated species. In contrast, the late nonmucoid strain was constituted by homologue lipid A species which carried hexa-acylated and hepta-acylated moieties. These findings are in accordance with previous observations [50, 89, 90], that *P. aeruginosa* synthesizes more highly acylated (hexa- and hepta-acylated) LPS structures during adaptation to the CF airways. Characterization of the bacterial genes that modify lipid A revealed that the *pagL* gene was mutated in

the strain obtained at the later stage of CF. As for the PGN, diversity in early and late *P. aeruginosa* strains consisted in different distribution of canonical monomeric and dimeric species. When tested in human cells including those of CF origin, the strong inflammatory response induced by *P. aeruginosa* LPS and PGN isolated at early stage of infection was attenuated at late stage. Significantly higher NF- κ B activation, IL-8 expression and production were detected after direct activation of TLR4/MD2-CD14 by LPS and Nod1 by PGN of early strain when compared to late strains [30].

Lipid A structures of *P. aeruginosa* affected also the inflammatory response in mice [30]. Leukocyte recruitment in the bronchoalveolar lavage fluid (BALF) of mice exposed to different LPS structures of clinical strains showed striking differences in total differential cell counts. Significant higher recruitment of neutrophils was observed in mice exposed to LPS from early strain in comparison to those treated with late strains. Cytokine levels, tested in murine lung homogenates, showed higher MIP-2 levels for mice treated with early LPS than late LPS. Similar trends were obtained with KC and IL-1 β . The impaired ability of the host to mount an adequate immune response could explain the ineffective eradication of the infection and the resulting persistent infection of late strains in comparison to the clonal early strain in the agar beads mouse model [21].

A prominent feature of *P. aeruginosa* strains infecting CF patients is the conversion to a mucoid, exopolysaccharide alginate-overproducing phenotype. Mannuronic acid polymers, the main components of the alginate capsule, induce immunostimulation via TLR2 and TLR4 pathways [27]. However, the overproduction of alginate by *P. aeruginosa* may be advantageous for the bacteria by impeding phagocytosis, and providing protection against reactive oxygen species and antibiotics [91–93]. The response of airway epithelia to the stimuli presented by mucoid *P. aeruginosa* is not pro-inflammatory and, hence, may not be conducive to the effective elimination of the pathogen [94]. Indeed, *in vivo* studies suggest that clearance of mucoid strains from murine lungs is diminished compared with nonmucoid strains, indicating improved survival of alginate-producing strains in the respiratory tract [95, 96]. Alginate enhances mucin secretion by tracheal epithelial cells and may inhibit neutrophil migration to the sites of infection. Interestingly, the production of flagellin and alginate by *P. aeruginosa* are inversely regulated by the alternative sigma factor AlgT, which is a positive regulator of mucoidy and a negative regulator of flagella mediated motility.

Among the four effector proteins secreted by the T3SS, exogenous ExoS has been demonstrated to activate TLR2 and TLR4 [25], thus contributing to inflammatory responses. However, it has been proposed that, following the infection of CF patient airways, *P. aeruginosa* strains evolve to reduce T3SS expression [18], or that populations of cells gradually change from a type III protein secretion-positive phenotype to a secretion-negative phenotype [97]. In addition, QS-related and T3SS genes were downregulated in a modified artificial-sputum medium, more closely resembling

CF sputum [98]. It has also been reported that the cyclic AMP/Vfr-dependent signaling (CVS) pathway, responsible for the regulation of the expression of multiple invasive virulence factors, including T3SS, exotoxin A, protease IV, and TFP, is defective in *mucA* mutants [99]. All these findings suggest that mucoid conversion and inhibition of invasive virulence determinants may both confer a selective advantage to *P. aeruginosa* strains in the CF lung.

4. Conclusions

In conclusion, the last decade has witnessed an increase in our understanding of the molecular mechanisms involved in the recognition of *P. aeruginosa* as invading and standing pathogen. It is expected that future efforts will try to elucidate in much more detail and precision the role of genetic variability in the PRRs for the susceptibility to *P. aeruginosa* infection and the mechanism of *P. aeruginosa* adaptation to host. Research in the field might lead to novel discoveries and new therapies for patients at risk factors of *P. aeruginosa* infection and might have an important impact on the quality of life for patients with CF.

Acknowledgments

Funded by European Commission (NABATIVI-223670, EU-FP7-HEALTH-2007-B) and Italian Cystic Fibrosis Research Foundation, Grants FFC#17/2009 and FFC#8/2007, with the contribution of Antonio Guadagnin e Figlio s.r.l., Delegazione FFC Roma 2 “Vorre 2009” Rome and Potenza, Associazione Amici di Cortina, Furla Spa, GVS Spa Zola Predosa (BO), and Delegazione FFC Bologna.

References

- [1] C. K. Stover, X. Q. Pham, A. L. Erwin et al., “Complete genome sequence of *Pseudomonas aeruginosa* PA01, an opportunistic pathogen,” *Nature*, vol. 406, no. 6799, pp. 959–964, 2000.
- [2] R. T. Sadikot, T. S. Blackwell, J. W. Christman, and A. S. Prince, “Pathogen-host interactions in *Pseudomonas aeruginosa* pneumonia,” *American Journal of Respiratory & Critical Care Medicine*, vol. 171, no. 11, pp. 1209–1223, 2005.
- [3] L. Saiman and A. Prince, “*Pseudomonas aeruginosa* pili bind to asialoGM1 which is increased on the surface of cystic fibrosis epithelial cells,” *Journal of Clinical Investigation*, vol. 92, no. 4, pp. 1875–1880, 1993.
- [4] G. B. Pier, M. Grout, T. S. Zaidi et al., “Role of mutant CFTR in hypersusceptibility of cystic fibrosis patients to lung infections,” *Science*, vol. 271, no. 5245, pp. 64–67, 1996.
- [5] G. B. Pier, M. Grout, and T. S. Zaidi, “Cystic fibrosis transmembrane conductance regulator is an epithelial cell receptor for clearance of *Pseudomonas aeruginosa* from the lung,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 22, pp. 12088–12093, 1997.
- [6] V. Teichgräber, M. Ulrich, N. Endlich et al., “Ceramide accumulation mediates inflammation, cell death and infection susceptibility in cystic fibrosis,” *Nature Medicine*, vol. 14, no. 4, pp. 382–391, 2008.
- [7] T. Ganz, “Antimicrobial polypeptides in host defense of the respiratory tract,” *Journal of Clinical Investigation*, vol. 109, no. 6, pp. 693–697, 2002.

- [8] M. Berger, R. U. Sorensen, M. F. Tosi, D. G. Dearborn, and G. Döring, "Complement receptor expression on neutrophils at an inflammatory site, the *Pseudomonas*-infected lung in cystic fibrosis," *Journal of Clinical Investigation*, vol. 84, no. 4, pp. 1302–1313, 1989.
- [9] V. Marcos, Z. Zhou, A. O. Yildirim et al., "CXCR2 mediates NADPH oxidase-independent neutrophil extracellular trap formation in cystic fibrosis airway inflammation," *Nature Medicine*, vol. 16, no. 9, pp. 1018–1023, 2010.
- [10] H. Matsui, B. R. Grubb, R. Tarran et al., "Evidence for periciliary liquid layer depletion, not abnormal ion composition, in the pathogenesis of cystic fibrosis airways disease," *Cell*, vol. 95, no. 7, pp. 1005–1015, 1998.
- [11] D. Worlitzsch, R. Tarran, M. Ulrich et al., "Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients," *Journal of Clinical Investigation*, vol. 109, no. 3, pp. 317–325, 2002.
- [12] H. Matsui, M. W. Verghese, M. Kesimer et al., "Reduced three-dimensional motility in dehydrated airway mucus prevents neutrophil capture and killing bacteria on airway epithelial surfaces," *Journal of Immunology*, vol. 175, no. 2, pp. 1090–1099, 2005.
- [13] A. Livraghi and S. H. Randell, "Cystic fibrosis and other respiratory diseases of impaired mucus clearance," *Toxicologic Pathology*, vol. 35, no. 1, pp. 116–129, 2007.
- [14] H. B. Tang, E. Dimango, R. Bryan et al., "Contribution of specific *Pseudomonas aeruginosa* virulence factors to pathogenesis of pneumonia in a neonatal mouse model of infection," *Infection & Immunity*, vol. 64, no. 1, pp. 37–43, 1996.
- [15] C. Madjdpour, B. Oertli, U. Ziegler, J. M. Bonvini, T. Pasch, and B. Beck-Schimmer, "Lipopolysaccharide induces functional ICAM-1 expression in rat alveolar epithelial cells in vitro," *American Journal of Physiology*, vol. 278, no. 3, pp. L572–L579, 2000.
- [16] A. Filloux, M. Bally, G. Ball, M. Akrim, J. Tommassen, and A. Lazdunski, "Protein secretion in gram-negative bacteria: transport across the outer membrane involves common mechanisms in different bacteria," *EMBO Journal*, vol. 9, no. 13, pp. 4323–4329, 1990.
- [17] T. L. Yahr, J. Goranson, and D. W. Frank, "Exoenzyme of *S* of *Pseudomonas aeruginosa* is secreted by a type III pathway," *Molecular Microbiology*, vol. 22, no. 5, pp. 991–1003, 1996.
- [18] V. T. Lee, R. S. Smith, B. Tümmler, and S. Lory, "Activities of *Pseudomonas aeruginosa* effectors secreted by the type III secretion system in vitro and during infection," *Infection & Immunity*, vol. 73, no. 3, pp. 1695–1705, 2005.
- [19] G. Ball, É. Durand, A. Lazdunski, and A. Filloux, "A novel type II secretion system in *Pseudomonas aeruginosa*," *Molecular Microbiology*, vol. 43, no. 2, pp. 475–485, 2002.
- [20] E. E. Smith, D. G. Buckley, Z. Wu et al., "Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 22, pp. 8487–8492, 2006.
- [21] A. Bragonzi, M. Paroni, A. Nonis et al., "*Pseudomonas aeruginosa* microevolution during cystic fibrosis lung infection establishes clones with adapted virulence," *American Journal of Respiratory & Critical Care Medicine*, vol. 180, no. 2, pp. 138–145, 2009.
- [22] I. Bianconi, A. Milani, C. Cigana et al., "Positive signature-tagged mutagenesis in *Pseudomonas aeruginosa*: tracking patho-adaptive mutations promoting airways chronic infection," *PLoS Pathogens*, vol. 7, no. 2, Article ID e1001270, 2011.
- [23] D. Nguyen and P. K. Singh, "Evolving stealth: genetic adaptation of *Pseudomonas aeruginosa* during cystic fibrosis infections," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 22, pp. 8305–8306, 2006.
- [24] N. Hoiby, "*Pseudomonas aeruginosa* infection in cystic fibrosis. Diagnostic and prognostic significance of *Pseudomonas aeruginosa* precipitins determined by means of crossed immunoelectrophoresis. A survey," *Acta Pathologica et Microbiologica Scandinavica Supplement*, no. 262, pp. 1–96, 1977.
- [25] S. Epelman, D. Stack, C. Bell et al., "Different domains of *Pseudomonas aeruginosa* exoenzyme S activate distinct TLRs," *Journal of Immunology*, vol. 173, no. 3, pp. 2031–2040, 2004.
- [26] C. Erridge, A. Pridmore, A. Eley, J. Stewart, and I. R. Poxton, "Lipopolysaccharides of *Bacteroides fragilis*, *Chlamydia trachomatis* and *Pseudomonas aeruginosa* signal via toll-like receptor 2," *Journal of Medical Microbiology*, vol. 53, no. 8, pp. 735–740, 2004.
- [27] T. Flo, L. Ryan, E. Latz et al., "Involvement of Toll-like receptor (TLR) 2 and TLR4 in cell activation by mannuronic acid polymers," *The Journal of Biological Chemistry*, vol. 277, no. 38, pp. 35489–35495, 2002.
- [28] E. Amiel, R. R. Lovewell, G. A. O'Toole, D. A. Hogan, and B. Berwin, "*Pseudomonas aeruginosa* evasion of phagocytosis is mediated by loss of swimming motility and is independent of flagellum expression," *Infection & Immunity*, vol. 78, no. 7, pp. 2937–2945, 2010.
- [29] G. Lagoumintzis, M. Christofidou, G. Dimitracopoulos, and F. Paliogianni, "*Pseudomonas aeruginosa* slime glycolipoprotein is a potent stimulant of tumor necrosis factor alpha gene expression and activation of transcription activators nuclear factor kappa B and activator protein 1 in human monocytes," *Infection & Immunity*, vol. 71, no. 8, pp. 4614–4622, 2003.
- [30] C. Cigana, L. Curcurù, M. R. Leone et al., "*Pseudomonas aeruginosa* exploits lipid A and mucopeptides modification as a strategy to lower innate immunity during cystic fibrosis lung infection," *PLoS one*, vol. 4, no. 12, p. e8439, 2009.
- [31] Z. Zhang, J. P. Louboutin, D. J. Weiner, J. B. Goldberg, and J. M. Wilson, "Human airway epithelial cells sense *Pseudomonas aeruginosa* infection via recognition of flagellin by toll-like receptor 5," *Infection & Immunity*, vol. 73, no. 11, pp. 7151–7160, 2005.
- [32] M. Magnusson, R. Tobes, J. Sancho, and E. Pareja, "Cutting edge: natural DNA repetitive extragenic sequences from gram-negative pathogens strongly stimulate TLR9," *Journal of Immunology*, vol. 179, no. 1, pp. 31–35, 2007.
- [33] H. Hemmi, O. Takeuchi, T. Kawai et al., "A toll-like receptor recognizes bacterial DNA," *Nature*, vol. 408, no. 6813, pp. 740–745, 2000.
- [34] L. Franchi, J. Stoolman, T. D. Kanneganti, A. Verma, R. Ramphal, and G. Núñez, "Critical role for Ipaf in *Pseudomonas aeruginosa*-induced caspase-1 activation," *European Journal of Immunology*, vol. 37, no. 11, pp. 3030–3039, 2007.
- [35] T. H. Schroeder, M. M. Lee, P. W. Yacono 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," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 10, pp. 6907–6912, 2002.
- [36] C. A. Janeway Jr., "Approaching the asymptote? Evolution and revolution in immunology," *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 54, no. 1, pp. 1–13, 1989.
- [37] S. Akira and K. Takeda, "Toll-like receptor signalling," *Nature Reviews Immunology*, vol. 4, no. 7, pp. 499–511, 2004.
- [38] S. Akira, S. Uematsu, and O. Takeuchi, "Pathogen recognition and innate immunity," *Cell*, vol. 124, no. 4, pp. 783–801, 2006.

- [39] N. Inohara, T. Koseki, L. Del Peso et al., "Nod1, an Apaf-1-like activator of caspase-9 and nuclear factor-kappaB," *The Journal of Biological Chemistry*, vol. 274, no. 21, pp. 14560–14567, 1999.
- [40] Y. Ogura, N. Inohara, A. Benito, F. F. Chen, S. Yamaoka, and G. Nunez, "Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB," *The Journal of Biological Chemistry*, vol. 276, no. 7, pp. 4812–4818, 2001.
- [41] S. E. Girardin, I. G. Boneca, L. A. Carneiro et al., "Nod1 detects a unique muropeptide from gram-negative bacterial peptidoglycan," *Science*, vol. 300, no. 5625, pp. 1584–1587, 2003.
- [42] N. Inohara, Y. Ogura, A. Fontalba et al., "Host recognition of bacterial muramyl dipeptide mediated through NOD2: implications for crohn's disease," *The Journal of Biological Chemistry*, vol. 278, no. 8, pp. 5509–5512, 2003.
- [43] N. Inohara, M. Chamaillard, C. McDonald, and G. Nunez, "NOD-LRR proteins: role in host-microbial interactions and inflammatory disease," *Annual Review of Biochemistry*, vol. 74, pp. 355–383, 2005.
- [44] J. H. Fritz, R. L. Ferrero, D. J. Philpott, and S. E. Girardin, "Nod-like proteins in immunity, inflammation and disease," *Nature Immunology*, vol. 7, no. 12, pp. 1250–1257, 2006.
- [45] B. T. Cookson and M. A. Brennan, "Pro-inflammatory programmed cell death," *Trends in Microbiology*, vol. 9, no. 3, pp. 113–114, 2001.
- [46] J. B. Lyczak, C. L. Cannon, and G. B. Pier, "Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist," *Microbes and Infection*, vol. 2, no. 9, pp. 1051–1060, 2000.
- [47] C. Alexander and E. T. Rietschel, "Bacterial lipopolysaccharides and innate immunity," *Journal of Endotoxin Research*, vol. 7, no. 3, pp. 167–202, 2001.
- [48] E. Lien, T. K. Means, H. Heine et al., "Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide," *Journal of Clinical Investigation*, vol. 105, no. 4, pp. 497–504, 2000.
- [49] R. Kitchens, "Role of CD14 in cellular recognition of bacterial lipopolysaccharides," *Chemical Immunology*, vol. 74, pp. 61–82, 1999.
- [50] R. K. Ernst, E. C. Yi, L. Guo et al., "Specific lipopolysaccharide found in cystic fibrosis airway *Pseudomonas aeruginosa*," *Science*, vol. 286, no. 5444, pp. 1561–1565, 1999.
- [51] R. Ernst, A. M. Hajjar, J. H. Tsai, S. M. Moskowitz, C. B. Wilson, and S. I. Miller, "*Pseudomonas aeruginosa* lipid A diversity and its recognition by Toll-like receptor 4," *Journal of Endotoxin Research*, vol. 9, no. 6, pp. 395–400, 2003.
- [52] A. M. Firoved, W. Ornatowski, and V. Deretic, "Microarray analysis reveals induction of lipoprotein genes in mucoid *Pseudomonas aeruginosa*: implications for inflammation in cystic fibrosis," *Infection & Immunity*, vol. 72, no. 9, pp. 5012–5018, 2004.
- [53] R. Adamo, S. Sokol, G. Soong, M. I. Gomez, and A. Prince, "*Pseudomonas aeruginosa* flagella activate airway epithelial cells through asialoGM1 and toll-like receptor 2 as well as toll-like receptor 5," *American Journal of Respiratory Cell & Molecular Biology*, vol. 30, no. 5, pp. 627–634, 2004.
- [54] P. Xaplanteri, G. Lagoumintzis, G. Dimitracopoulos, and F. Paliogianni, "Synergistic regulation of *Pseudomonas aeruginosa*-induced cytokine production in human monocytes by mannose receptor and TLR2," *European Journal of Immunology*, vol. 39, no. 3, pp. 730–740, 2009.
- [55] X. Huang, R. P. Barrett, S. A. McClellan, and L. D. Hazlett, "Silencing toll-like receptor-9 in *Pseudomonas aeruginosa* keratitis," *Investigative Ophthalmology & Visual Science*, vol. 46, no. 11, pp. 4209–4216, 2005.
- [56] X. Sun, H. Sui, J. T. Fisher et al., "Disease phenotype of a ferret CFTR-knockout model of cystic fibrosis," *The Journal of Clinical Investigation*, vol. 120, no. 9, pp. 3149–3160, 2010.
- [57] M. Schirm, S. K. Arora, A. Verma et al., "Structural and genetic characterization of glycosylation of type a flagellin in *Pseudomonas aeruginosa*," *Journal of Bacteriology*, vol. 186, no. 9, pp. 2523–2531, 2004.
- [58] M. A. Donnelly and T. S. Steiner, "Two nonadjacent regions in enteroaggregative *Escherichia coli* flagellin are required for activation of toll-like receptor 5," *The Journal of Biological Chemistry*, vol. 277, no. 43, pp. 40456–40461, 2002.
- [59] K. G. Murthy, A. Deb, S. Goonesekera, C. Szabó, and A. L. Salzman, "Identification of conserved domains in *Salmonella muenchen* flagellin that are essential for its ability to activate TLR5 and to induce an inflammatory response in vitro," *The Journal of Biological Chemistry*, vol. 279, no. 7, pp. 5667–5675, 2004.
- [60] A. Verma, S. K. Arora, S. K. Kuravi, and R. Ramphal, "Roles of specific amino acids in the N terminus of *Pseudomonas aeruginosa* flagellin and of flagellin glycosylation in the innate immune response," *Infection & Immunity*, vol. 73, no. 12, pp. 8237–8246, 2005.
- [61] T. Eaves-Pyles, K. Murthy, and L. Liaudet, "Flagellin, a novel mediator of *Salmonella*-induced epithelial activation and systemic inflammation: I kappa B alpha degradation, induction of nitric oxide synthase, induction of proinflammatory mediators, and cardiovascular dysfunction," *The Journal of Immunology*, vol. 166, no. 2, pp. 1248–1260, 2001.
- [62] V. Feuillet, S. Medjane, I. Mondor et al., "Involvement of toll-like receptor 5 in the recognition of flagellated bacteria," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 33, pp. 12487–12492, 2006.
- [63] M. R. Power, Y. Peng, E. Maydanski, J. S. Marshall, and T. J. Lin, "The development of early host response to *Pseudomonas aeruginosa* lung infection is critically dependent on myeloid differentiation factor 88 in mice," *The Journal of Biological Chemistry*, vol. 279, no. 47, pp. 49315–49322, 2004.
- [64] R. Ramphal, V. Balloy, M. Huerre, M. Si-Tahar, and M. Chignard, "TLRs 2 and 4 are not involved in hypersusceptibility to acute *Pseudomonas aeruginosa* lung infections," *Journal of Immunology*, vol. 175, no. 6, pp. 3927–3934, 2005.
- [65] S. J. Skerrett, H. D. Liggitt, A. M. Hajjar, and C. B. Wilson, "Cutting edge: myeloid differentiation factor 88 is essential for pulmonary host defense against *Pseudomonas aeruginosa* but not *Staphylococcus aureus*," *Journal of Immunology*, vol. 172, no. 6, pp. 3377–3381, 2004.
- [66] L. H. Travassos, L. A. Carneiro, S. E. Girardin et al., "Nod1 participates in the innate immune response to *Pseudomonas aeruginosa*," *The Journal of Biological Chemistry*, vol. 280, no. 44, pp. 36714–36718, 2005.
- [67] L. Franchi, A. Amer, M. Body-Malapel et al., "Cytosolic flagellin requires Ipaf for activation of caspase-1 and interleukin 1beta in *Salmonella*-infected macrophages," *Nature Immunology*, vol. 7, no. 6, pp. 576–582, 2006.
- [68] E. A. Miao, E. Andersen-Nissen, S. E. Warren, and A. Aderem, "TLR5 and Ipaf: dual sensors of bacterial flagellin in the innate immune system," *Seminars in Immunopathology*, vol. 29, no. 3, pp. 275–288, 2007.
- [69] E. A. Miao, R. K. Ernst, M. Dors, D. P. Mao, and A. Aderem, "*Pseudomonas aeruginosa* activates caspase 1 through Ipaf," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 7, pp. 2562–2567, 2008.

- [70] F. S. Sutterwala, L. A. Mijares, L. Li, Y. Ogura, B. I. Kazmierczak, and R. A. Flavell, "Immune recognition of *Pseudomonas aeruginosa* mediated by the IPAF/NLRC4 inflammasome," *Journal of Experimental Medicine*, vol. 204, no. 13, pp. 3235–3245, 2007.
- [71] E. A. Miao, D. P. Mao, N. Yudkovsky et al., "Innate immune detection of the type III secretion apparatus through the NLRC4 inflammasome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 7, pp. 3076–3080, 2010.
- [72] E. Mahenthiralingam, M. E. Campbell, and D. P. Speert, "Nonmotility and phagocytic resistance of *Pseudomonas aeruginosa* isolates from chronically colonized patients with cystic fibrosis," *Infection and Immunity*, vol. 62, no. 2, pp. 596–605, 1994.
- [73] D. W. Martin, M. J. Schurr, M. H. Mudd, J. R. W. Govan, B. W. Holloway, and V. Deretic, "Mechanism of conversion to mucoidy in *Pseudomonas aeruginosa* infecting cystic fibrosis patients," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, no. 18, pp. 8377–8381, 1993.
- [74] A. Bragonzi, L. Wiehlmann, J. Klockgether et al., "Sequence diversity of the mucABD locus in *Pseudomonas aeruginosa* isolates from patients with cystic fibrosis," *Microbiology*, vol. 152, no. 11, pp. 3261–3269, 2006.
- [75] D. A. D'Argenio, M. Wu, L. R. Hoffman et al., "Growth phenotypes of *Pseudomonas aeruginosa* lasR mutants adapted to the airways of cystic fibrosis patients," *Molecular Microbiology*, vol. 64, no. 2, pp. 512–533, 2007.
- [76] L. Hoffman, H. D. Kulasekara, J. Emerson et al., "*Pseudomonas aeruginosa* lasR mutants are associated with cystic fibrosis lung disease progression," *Journal of Cystic Fibrosis*, vol. 8, no. 1, pp. 66–70, 2009.
- [77] J. L. Burns, R. L. Gibson, S. McNamara et al., "Longitudinal assessment of *Pseudomonas aeruginosa* in young children with cystic fibrosis," *Journal of Infectious Diseases*, vol. 183, no. 3, pp. 444–452, 2001.
- [78] O. Ciofu, B. Riis, T. Pressler, H. E. Poulsen, and N. Høiby, "Occurrence of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis patients is associated with the oxidative stress caused by chronic lung inflammation," *Antimicrobial Agents & Chemotherapy*, vol. 49, no. 6, pp. 2276–2282, 2005.
- [79] K. Mathee, O. Ciofu, C. Sternberg et al., "Mucoid conversion of *Pseudomonas aeruginosa* by hydrogen peroxide: a mechanism for virulence activation in the cystic fibrosis lung," *Microbiology*, vol. 145, no. 6, pp. 1349–1357, 1999.
- [80] A. Bragonzi, D. Worlitzsch, G. B. Pier et al., "Nonmucoid *Pseudomonas aeruginosa* expresses alginate in the lungs of patients with cystic fibrosis and in a mouse model," *Journal of Infectious Diseases*, vol. 192, no. 3, pp. 410–419, 2005.
- [81] E. V. Sokurenko, D. L. Hasty, and D. E. Dykhuizen, "Pathoadaptive mutations: gene loss and variation in bacterial pathogens," *Trends in Microbiology*, vol. 7, no. 5, pp. 191–195, 1999.
- [82] M. Jain, D. Ramirez, R. Seshadri et al., "Type III secretion phenotypes of *Pseudomonas aeruginosa* strains change during infection of individuals with cystic fibrosis," *Journal of Clinical Microbiology*, vol. 42, no. 11, pp. 5229–5237, 2004.
- [83] A. Oliver, R. Cantón, P. Campo, F. Baquero, and J. Blázquez, "High frequency of hypermutable *Pseudomonas aeruginosa* in cystic fibrosis lung infection," *Science*, vol. 288, no. 5469, pp. 1251–1253, 2000.
- [84] D. Young, T. Hussell, and G. Dougan, "Chronic bacterial infections: living with unwanted guests," *Nature Immunology*, vol. 3, no. 11, pp. 1026–1032, 2002.
- [85] E. V. Sokurenko, V. Chesnokova, D. E. Dykhuizen et al., "Pathogenic adaptation of *Escherichia coli* by natural variation of the FimH adhesin," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 15, pp. 8922–8926, 1998.
- [86] E. R. Moxon and P. A. Murphy, "Haemophilus influenzae bacteremia and meningitis resulting from survival of a single organism," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 75, no. 3, pp. 1534–1536, 1978.
- [87] N. S. Akopyants, K. A. Eaton, and D. E. Berg, "Adaptive mutation and cocolonization during *Helicobacter pylori* infection of gnotobiotic piglets," *Infection & Immunity*, vol. 63, no. 1, pp. 116–121, 1995.
- [88] M. A. Luzar, M. J. Thomassen, and T. C. Montie, "Flagella and motility alterations in *Pseudomonas aeruginosa* strains from patients with cystic fibrosis: relationship to patient clinical condition," *Infection & Immunity*, vol. 50, no. 2, pp. 577–582, 1985.
- [89] R. K. Ernst, S. M. Moskowitz, J. C. Emerson et al., "Unique lipid A modifications in *Pseudomonas aeruginosa* isolated from the airways of patients with cystic fibrosis," *Journal of Infectious Diseases*, vol. 196, no. 7, pp. 1088–1092, 2007.
- [90] A. M. Hajjar, R. K. Ernst, J. H. Tsai, C. B. Wilson, and S. I. Miller, "Human toll-like receptor 4 recognizes host-specific LPS modifications," *Nature Immunology*, vol. 3, no. 4, pp. 354–359, 2002.
- [91] J. A. Simpson, S. E. Smith, and R. T. Dean, "Alginate inhibition of the uptake of *Pseudomonas aeruginosa* by macrophages," *Journal of General Microbiology*, vol. 134, no. 1, pp. 29–36, 1988.
- [92] J. A. Simpson, S. E. Smith, and R. T. Dean, "Alginate may accumulate in cystic fibrosis lung because the enzymatic and free radical capacities of phagocytic cells are inadequate for its degradation," *Biochemistry & Molecular Biology International*, vol. 30, no. 6, pp. 1021–1034, 1993.
- [93] N. Høiby, H. Krogh Johansen, C. Moser, Z. Song, O. Ciofu, and A. Kharazmi, "*Pseudomonas aeruginosa* and the in vitro and in vivo biofilm mode of growth," *Microbes & Infection*, vol. 3, no. 1, pp. 23–35, 2001.
- [94] L. M. Cobb, J. C. Mychaleckyj, D. J. Wozniak, and Y. S. López-Boado, "*Pseudomonas aeruginosa* flagellin and alginate elicit very distinct gene expression patterns in airway epithelial cells: implications for cystic fibrosis disease," *Journal of Immunology*, vol. 173, no. 9, pp. 5659–5670, 2004.
- [95] J. R. Govan, J. A. Fyfe, and N. R. Baker, "Heterogeneity and reduction in pulmonary clearance of mucoid *Pseudomonas aeruginosa*," *Reviews of Infectious Diseases*, vol. 5, pp. S874–S879, 1983.
- [96] J. C. Boucher, H. Yu, M. H. Mudd, and V. Deretic, "Mucoid *Pseudomonas aeruginosa* in cystic fibrosis: characterization of muc mutations in clinical isolates and analysis of clearance in a mouse model of respiratory infection," *Infection & Immunity*, vol. 65, no. 9, pp. 3838–3846, 1997.
- [97] M. Jain, D. Ramirez, R. Seshadri et al., "Type III secretion phenotypes of *Pseudomonas aeruginosa* strains change during infection of individuals with cystic fibrosis," *Journal of Clinical Microbiology*, vol. 42, no. 11, pp. 5229–5237, 2004.
- [98] C. Fung, S. Naughton, L. Turnbull et al., "Gene expression of *Pseudomonas aeruginosa* in a mucin-containing synthetic growth medium mimicking cystic fibrosis lung sputum," *Journal of Medical Microbiology*, vol. 59, no. 9, pp. 1089–1100, 2010.

- [99] A. K. Jones, N. B. Fulcher, and G. J. Balzer, "Activation of the *Pseudomonas aeruginosa* AlgU regulon through mucA mutation inhibits cyclic AMP/Vfr signaling," *The Journal of Bacteriology*, vol. 192, no. 21, pp. 5709–5717, 2010.