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Hindawi Publishing Corporation
Journal of Biomedicine and Biotechnology
Volume 2011, Article ID 852513, 10 pages
doi:10.1155/2011/852513

Review Article

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

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Received 15 December 2010; Accepted 9 May 2011

Academic Editor: Masao Kimoto

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 immucompromised, particularly transplant recipients, burn patients, patients with cancer, neutropenia,

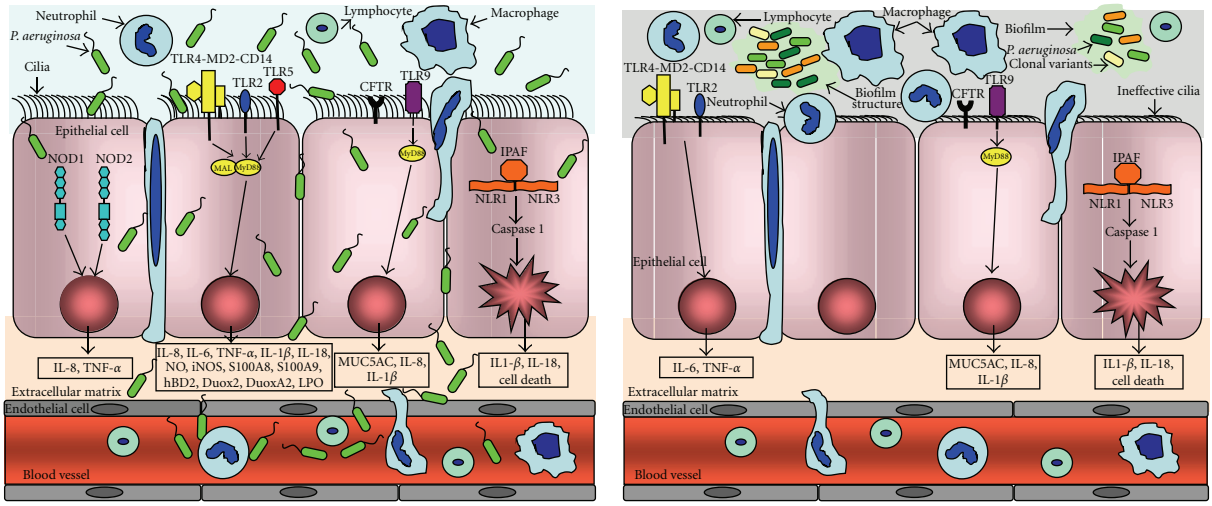

Figure 1: *Pseudomonas aeruginosa* recognition by PRRs during acute and chronic lung infection. (a) During acute lung infection, *P. aeruginosa* can invade, disseminate and lead to extensive tissue damage by means of potent array of extracellular and cell bound virulence factors. However the immunocompetent 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 cavitary 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 glycocalix 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 nomain-(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 leucin-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 κB (NF-κB) 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-1β. 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-1β [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	*P. aeruginosa* PAMP	References
Toll-like receptors		
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]
NLR receptors		
Nod 1	PGN	[30]
NLRC4/Ipaf	flagellin	[34]
	T3SS	[34]
Other receptors		
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-κB and MAPK. *P. aeruginosa* MR activation synergizes TLR2 activity to trigger maximum NF-κB 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-kB 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-kB 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-1β.

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 aeruginosaf* 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-κB 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-1β. 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 "Vorrei 2009" Rome and Potenza, Associazione Amici di Cortina, Furla Spa, GVS Spa Zola Predosa (BO), and Delegazione FFC Bologna.

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