Chapter

Host-Pathogen Interaction in the Lung of Patients Infected with *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is an opportunistic bacterium that can proliferate in the soil, water, and even humans if they are immunologically depressed. During lung infections, *P. aeruginosa* goes through significant morphological changes turning into the mucoid form after which its eradication becomes almost impossible. Within this chapter, we explore the bioenergetics changes produced within *P. aeruginosa* during infections in humans and the metabolic pathways that are involved in those changes that lead to chronic infection.

Keywords: *P. aeruginosa*, host, bioenergetics, phosphate, choline

1. Introduction

There are many lung pathogens but one of the most studied is *Pseudomonas aeruginosa* because it cannot be eradicated under certain conditions. As an opportunistic pathogen, its interaction with the host has some particularities that we will explore in this chapter.

The *Pseudomonadaceae* comprise Gram-negative microorganism, nonsporulated, aerobic strict of wide distribution in the environment from the soil, water, and plants to humans; this is due to their nutritional versatility. Of this vast group, only *Pseudomonas cepacia*, *mallei*, and *aeruginosa* infect humans, of which *aeruginosa* is the more relevant because it is the most frequent cause of nosocomial infections [1].

It is often said that *Pseudomonas aeruginosa* does not infect healthy individuals but there are reports on the contrary, as swimmers otitis [2]. Because it is an opportunist pathogen, it does not need the host for its survival, and it may be lethal after becoming a chronic infection in susceptible patients with cystic fibrosis (CF) [3–5], cancer [6–8], hepatic cirrhosis [9], keratitis [10–13], or spondylodiscitis [14]. This bacterium is most feared by pulmonologist because when acquired by nosocomial patients [15, 16], it complicates any existing conditions, and when it invades immune-compromised patients, its eradication may become impossible.

Colonization with *P. aeruginosa* is observed in all stages of chronic obstructive pulmonary disease (COPD), but the prevalence significantly increases with disease severity from 0.7%, in stage 1 of the Global Initiative for Obstructive Lung Disease, to 1.5% for stages 2 and 3 up to 2.6% for stage 4 [17, 18]. This prevalence rises to 8–13% in acute exacerbations of COPD [19–21]. But still, the main susceptibility for the infection and death by *P. aeruginosa* [22, 23] are the mutations of the CF

transmembrane conductance regulator (CFTR) identified as F508, G542X, G551D, W1282X, R1162X, and N1303K [24, 25]. CF also has co-morbidity such as liver cirrhosis [26] with 18% prevalence [27, 28] of *P. aeruginosa* infection in this subset.

2. Host-bacteria interaction in acute infection

2.1 Lung changes upon bacterial invasion

The flagella and lipopolysaccharide (LPS) from *P. aeruginosa* are the first to contact the ciliated epithelial cells [29]. In the airways, these cells are covered by the surfactants containing 45% less NaCl and 600 more K^+ than in plasma [30], while the alveolar epithelial cells are covered by a surfactant layer that contains mostly phosphatidylcholine (80%) [31] and surfactant proteins A, B, C, and D [32, 33] that bind LPS in a calcium-dependent manner [34]. After the surfactant layer is crossed, the flagellum binds to the epithelial cells through toll-like receptors (TLR) 2, 3, 4 and 5 [35–40] that are quickly endocytosed to be degraded in the proteasome. The activated TLR5 induces the macrophages chemoattractants CXCL1, CXCL2, and neutrophil chemokine CCL20, which are inhibited by TLR5 inhibitors [41]. The peptides digested are then presented to macrophages and dendritic cells.

When LPS binds to the host cells, where CFTR is also a receptor [42], it upregulates NF-κB at the gene level (**Table 1**), promoting inflammation [43] by secretion of IL1, IL6, IL8, ICAM-1, and also CXCL1 [44–47], although in different degrees of regulation. For example, CXCL1 expression is orchestrated by a fatty acid-binding protein (FABP4) that delivers fatty acids from the cytoplasm to the nuclear receptor PPAR. These prompt macrophage signaling through the myeloid differentiation protein-88 (MyD88) to induce cytokine production following engagement of TLRs with LPS [48–51]. Macrophages require MyD88 to produce CXCL1 but also eicosapentaenoic acid and docosahexaenoic acid, both substrates of FABP4. This demonstrates the importance of fatty acid metabolism to promote host resistance to *P. aeruginosa*, facilitating macrophage-neutrophil cross-talk during the infection [52, 53].

The T cells also play an important role in acute infection. IL17 producing T cells are expanded [54], via expression of STAT3 and retinoid orphan receptor [55]; these steps are crucial for B cell activation and immunoglobulin release for bacterial clearance [56]. On the contrary, excess of T regulatory cells (Treg) are associated with secondary *P. aeruginosa* infections, because depletion of Tregs decreases IL-10 levels and elevates IL-17A, IL-1β, and IL-6 [57, 58]. Therefore, the underlying immune suppression, by Treg accumulation, and Th17 depletion are the cause of chronic infection [57]. This may be reversed by treatment with IL7 or ethyl pyruvate increasing IL17, INFγ, and $CD8^+$ T cells [59, 60].

Death of CF patients chronically infected with *P. aeruginosa* occurs due to the depletion of neutrophils, IL6, and granulocyte-colony stimulating factor which causes dysfunctional neutrophil burst. This reduces the secretion of reactive oxygen species, which are essential for bacterial killing and clearance [61].

2.2 Bacterial metabolic changes for invasion

Simultaneously, the contact of *P. aeruginosa* with the lung upregulates in the bacteria genes involved mainly in biofilm synthesis [62] (**Table 1**). These changes in gene expression result in downregulation of proteins involved in LPS biosynthesis, antimicrobial resistance, and phenazine production concomitant

P. aeruginosa			**Lung**		
Gene ID	**FC**	**Name**	**Gene ID**	**FC**	**Name**
hemE	16.1[a]	Uroporphyrinogen decarboxylase		4502	Dioxin-inducible cytochrome P
pyrC	12.1	Dihydroorotase (biofilm development)		252	ppGpp
pyrH	6.4	Uridylate kinase (biofilm development)		206.7	Tumor necrosis factor-α-inducible DNA-binding protein A
adhA	5.5	Alcohol dehydrogenase		133.1	Proteasome subunit C
pelB, pelE	5.6, 3.7	Extracellular polysaccharide (competitive disruption of *S. aureus* biofilms)	hORC2L	13.4[b]	Human origin recognition complex protein 2
cls	7.0	Cardiolipin synthase	MCP-1	13.3	Monocyte chemotactic protein 1
pscD	3.2	T3SS export protein		3.5	c-Jun
plcN	3.2	Phospholipase C precursor		3	GTP-binding protein rhoB4.
algD,E,F,8,amrZ	1.9–10.7	Alginate biosynthesis		2.9	Urokinase-type plasminogen activator
ppiA	2.5	Peptidyl-prolyl cis-trans isomerase	PKC	2.8	Protein kinase C, ETA type
hmgA	–7.2	Homogentisate 1–2-dioxygenase		2.7	Folylpolyglutamate synthetase
algC	–9.3	Phosphomannomutase	TTP	5.7	Tristetraproline
hemE	16.1	Uroporphyrinogen decarboxylase		2.4	Anti-oncogene
pyrC	12.1	Dihydroorotase (biofilm development)	MAD3	5.1	IκB-α
pyrH	6.4	Uridylate kinase (biofilm development)	hENT1	4.2	Placental equilibrative nucleoside transporter 1
adhA	5.5	Alcohol dehydrogenase	TEL	2.8	Transcription factor
pelB, pelE	5.6, 3.7	Extracellular polysaccharide (competitive disruption of *S. aureus* biofilms)	DPH2L	2.6	Diphtheria toxin resistance protein
cls	7.0	Cardiolipin synthase	TFPI2	2.3	Tissue factor pathway inhibitor 2
				2.1	Ankyrin motif
			ESE-1	2.1	Epithelial-specific transcription factor
				–12.5	8IRF
				–11.9	JAK-1
			EPB49	–2.0	Erythrocyte membrane protein band 4.9. (Dematin)
				–2.3	*Alu* repeat-containing sequence

FC, fold changes; NC, no change.
[a]Change relative to P. aeruginosa acute infection/chronic contact to host cell [62].
[b]Relative change of lung cell gene profile after 3 h contact with P. aeruginosa [43].
Data reported by Naughton [62] and Ichikawa et al. [43].

Table 1.
Genetic changes due to host-pathogen interaction quantified by microarrays of mRNA.

with the upregulation of proteins involved in adherence, lysozyme resistance, and inhibition of the chloride ion channel, and CFTR [63]. *P. aeruginosa* releases choline from surfactants [81]. *In vitro* studies utilizing choline, as a carbon and nitrogen source, shows that it produces accumulation of polyphosphates (polyPi), carbohydrates, and LPS accompanied by depletion of phosphate (Pi) and phospholipids (PL); deeply modifying its energetic metabolism, the bacteria save 45% of energy in polyPi [64] (**Table 2**).

After the invasion, the bacteria attach to the lung epithelium producing profound metabolic changes, which correlates with morphological changes to the rugose small-colony variant (RSCV) [65–67]. The transition to the RSCV precedes inactivation of serine hydroxymethyltransferase; this produces accumulation of cyclic diguanylate [68] and nucleotide ppGpp that leads to polyPi accumulation [69] and to alginate production [68, 70–72].

Table 2 shows that the total content of phosphate is reduced 3 times in choline feed bacteria, although it accumulates Pi in polyPi. The polyPi may be thought as the energetic savings of the bacteria which is done at expenses of phospholipid biosynthesis. This is possible reducing the size of the bacterium [73] and increasing the area/volume ratio that facilitates O_2 exchange for which the bacteria have to compete with the host [74]. The overall bacterial changes save energy accumulating ppGpp, the substrate for polyPi synthesis by polyphosphate kinase, which is also increased [75]. Some of these polyPi are located in the outer membrane where this highly energetic polymer has Pi bonds similar to the ATP and a highly negative charge neutralized by cations such as Ca^{2+} and Mg^{2+}. Thus, polyPi function as an energy storage, buffer, and ion chelator that may shield the bacterium from environmental changes.

After adhering to the host ciliated epithelial cells, through mucin, the bacterium is enabled to form aggregates, secrete alginate, and modify its LPS [76]; this is a process regulated by 3,5-cyclic diguanylic acid [68]. The LPS is a macromolecule

Composition	**Succinate[a] + NH_4Cl**		**Choline[a]**			
	μg/mg of protein	**μmol/mg of protein**	**μg/mg of protein**	**μmol/mg of protein**	**%**	**p[b]**
Phosphate	1400 ± 100	14.7 ± 0.7	460 ± 90	4.8 ± 0.7	33	0.001
ATP	1650 ± 330	3.0 ± 0.6	1270 ± 165	2.3 ± 0.3	–23	0.32
Polyphosphates	4.0 ± 1.8	0.042 ± 0.01	6.3 ± 1.4	0.066 ± 0.008	57	0.004
Carbohydrates[c]	210 ± 40	1.2 ± 0.2	330 ± 50	1.8 ± 0.2	50	0.03
LPS[d]	19 ± 4	0.08 ± 0.02	41 ± 9	0.16 ± 0.03	100	0.02
Phospholipids[e]	114 ± 7	0.65 ± 0.04	71 ± 4	0.1 ± 0.02	–85	
Biosynthetic energy (ATP)[f]	—	1675	—	924	45	

[a]*Bacteria were grown in a high phosphate basal salt medium. All chemical determinations were done on 1.05 ± 0.16 and 1.00 ± 0.20 mg ml $^{-1}$ of culture from whole bacteria grown with 20 mM succinate plus 18.7 mM NH_4C1 or 20 mM choline chloride, respectively. Results are the average of four independent experiments ± SD.*
[b]*Values obtained by ANOVA analysis.*
[c]*Total carbohydrates were measured by the phenol method.*
[d]*Measured as the content of KDO according to the determination of formylpyruvic with thiobarbituric acid.*
[e]*Total phospholipids from bacteria grown with succinate/NH4Cl or choline.*
[f]*Value obtained by calculation of the biosynthetic cost of LPS 470 μmol ATP/gr of cells, 1 μmol ATP/g polyphosphate, 470 μmol ATP/g of glycoside, and 2578 μmol ATP/g of phospholipids. Table taken from Grumelli [64].*

Table 2.
Metabolic changes in the bacteria upon infection.

($C_{205}H_{366}N_3O_{117}P_5$) of 4899.956 g/mol that covers the outer membrane extending 40 nm outward. It is released with vesicle-containing enzymes and outer membrane (OM). Its extended formula was determined in 2003 (**Figure 1**); it is anchored to the OM through the lipid A which binds to the 3-deoxy-D-manno-2-octulosonic acid (KDO), the first glycoside of the core oligosaccharide, bound to the distal O antigen, a highly variable region [77, 78]. A metabolic crossroad between the LPS and alginate biosynthesis (**Figure 2**) is mannose-6-phosphate isomerization to mannose-1-phosphate by phosphomannomutase (Alg C). The glucose-6 phosphate (G6P) can be transformed to G1P to produce LPS or to isomerize mannose-6-phosphate to G1P. Similarly, fructose-6-phosphate (F6P) can be converted to mannose-6-phosphate and then isomerized to mannose-1-phosphate that becomes alginate by D-mannuronate linkage to L-guluronate via a P-1,4 glycosidic bond. Thus, isomerization of mannose 6-phosphate to mannose 1-phosphate by phosphomannomutase, encoded as algC, is common to the biosynthesis of LPS and alginate since mutants in this phosphomannomutase are hindered in their ability to infect *in vivo* [79].

2.3 Interaction between lung and bacteria

The host-pathogen interaction studied *in vivo* utilizing LPS in the lung of mice exposed to cigarette smoke model exacerbations of COPD in patients chronically infected with *P. aeruginosa*. **Figure 3** proposes that this extracellular pathogen releases to the medium phospholipase C (PLC) [80] and phosphoryl-choline phosphatase (PChP) [81] within vesicles [82]. These vesicles degrade the surfactant, from phosphatidylcholine [85] to phosphoryl-choline and diacylglycerol (DAG) [83], causing Ca^{2+} mediated vaso-constriction [84]. Choline and phosphate (Pi) released by PChP produce airway constriction and inflammation in the lung tissue.

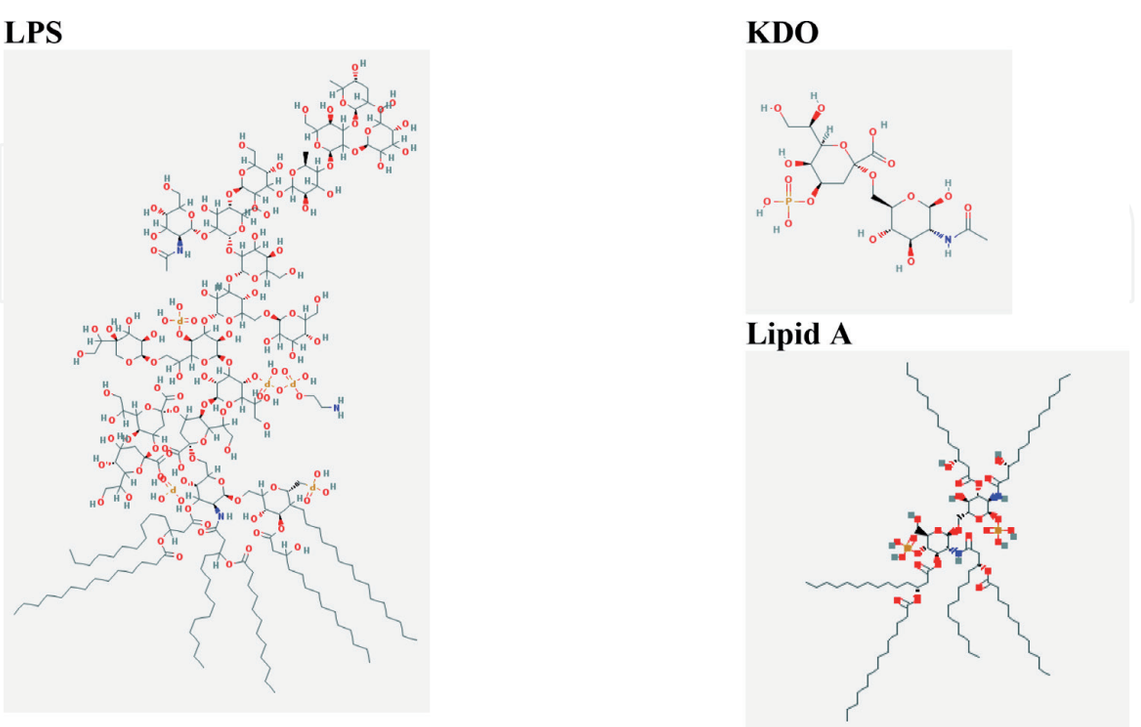

Figure 1.
LPS formula and structure set forth in PubChem (CID 11970143); and its parts KDO, (CID 49792052); and Lipid A (CID 9877306).

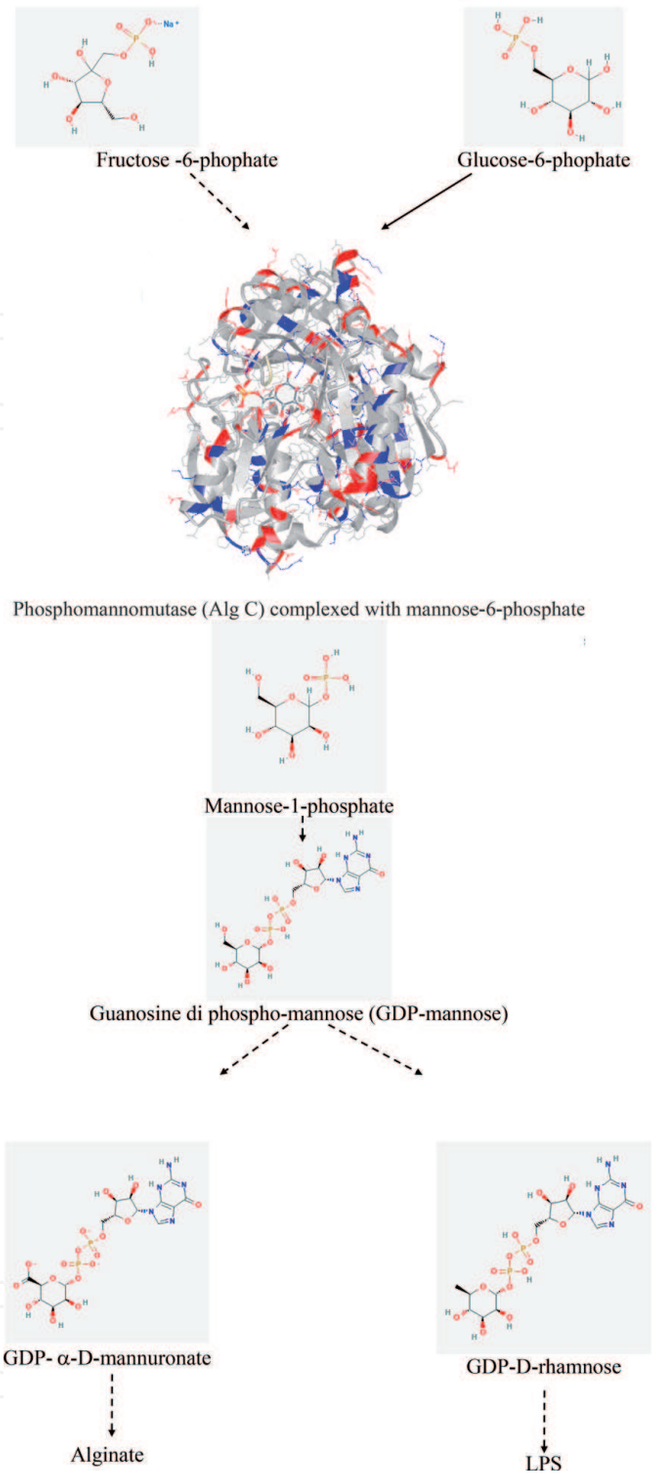

Figure 2.
The metabolic fork that derives glucose-6 phosphate (G6P) from biosynthesis of LPS to alginate. Tridimensional structure of phosphomannomutase; red and blue represent oppositely charged regions.

Further validation of this host-pathogen interaction is verified by the metabolite variations in a mouse model that uses live bacteria, instead of LPS. **Figure 4A** shows that phosphatidylcholine and glycine are significantly reduced in the lung upon infection, due to their consumption, while succinate and lactate are significantly accumulated [85]. Variations of choline concentration in the lung are not significant although glycerophosphocholine and glycine are [86, 87], which are the degradation

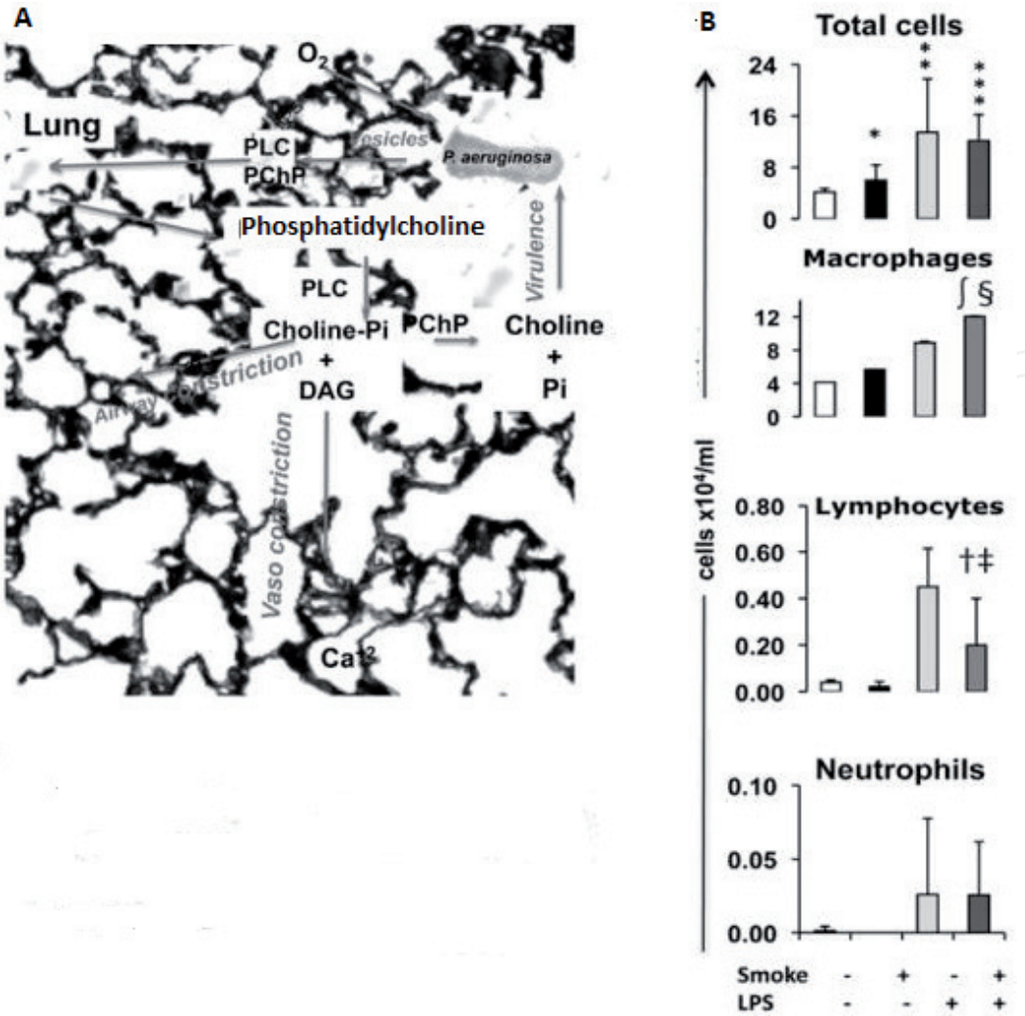

Figure 3.
*(A) Representative scheme of the host-pathogen interaction in mice lung during exacerbations of COPD. As an extracellular pathogen, P. aeruginosa releases to the medium phospholipase C (PLC) and phosphorylcholine phosphatase (PChP) within vesicles that degrades the membranes and surfactant of lung epithelial cells from phosphatidylcholine to phosphorylcholine and diacylglycerol (DAG) that cause Ca_2^+ mediated vaso-constriction. Choline and Pi released by PChP produces airway constriction in the lung tissue, and LPS and PolyPi accumulation in P. aeruginosa. (B) Representative experiment of inflammatory cells present in BAL of naïve mice (n = 5), mice treated with of LPS (n = 4), smoke exposed (n = 8) and smoke plus 100 ng/weekly of LPS (n = 3) from P. aeruginosa. *P = 0.01 relative to naïve mice, **P = 0.04 relative to naïve mice, ***P = 0.01 relative to smoke exposed, §P = 0.01 relative to naïve mice, †P = 0.05 relative to smoke exposed, ∫P = 0.05 relative to naïve mice, and ‡P = 0.01 relative to smoke exposed. The figure is taken from Grumelli et al. [64].*

products of choline. This is because *P. aeruginosa* is capable of releasing choline and converting it to betaine and then to glycine (**Figure 4B**) [88–91], for osmo-protection [92, 93] from the hyperosmolarity in the CF lung. Glycine also triggers chloride influx, inhibiting the Ca^{2+} mobilized by LPS [94]. This is a mechanism of self-preservation because macrophages are activated by LPS but suppressed by free glycine [95].

The succinate accumulated in the lung after infection [85], as Krebs cycle metabolite, inhibits histone demethylases, collagen hydrolases, α-ketoglutarate dioxygenases, and the 5-methylcytosine hydroxylase family [96]. *In vitro* succinate is the favorite carbon source for *P. aeruginosa*. Its consumption reduces the length of the LPS (**Table 3**), increasing the PL and Pi content and preventing the polyPi accumulation (**Table 2**), which is essential to the stress response [64]. The LPS and

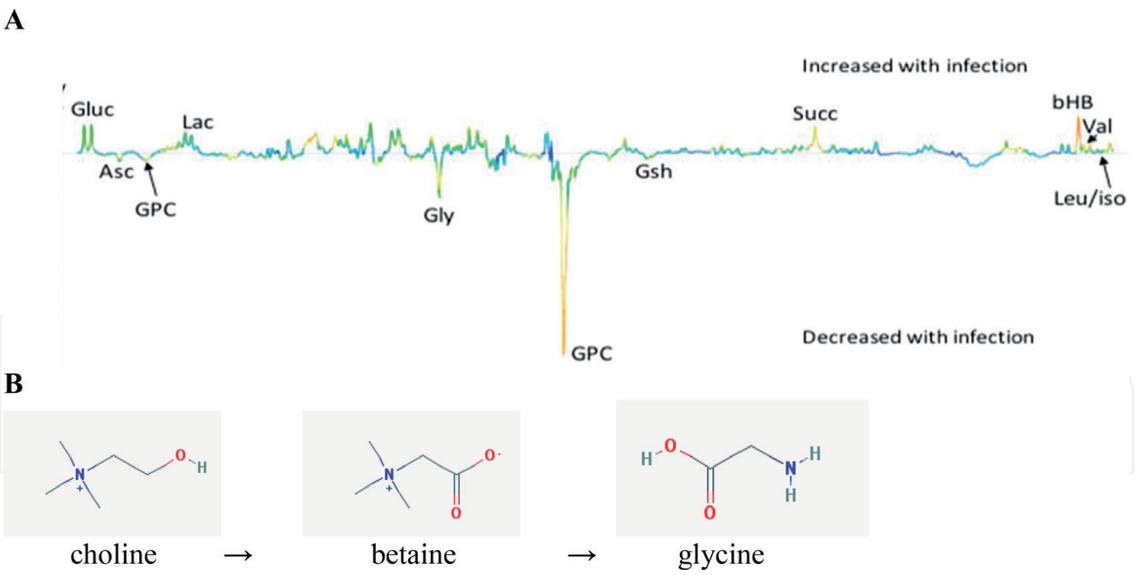

Figure 4.
(A) Lung alterations due to host-pathogen interaction upon infection. Gluc, glucose; Asc, ascorbate; GPC, glycerophosphocholine; Gly, glycine; Succ, succinate; bHB, beta-hydroxybutyrate; Val, valine; Leu/iso, leucine/isoleucine; Lac, lactate; and Gsh, glutathione reduced; figure taken from [85] and (B) choline conversion by P. aeruginosa.

Composition	Succinate[a] + NH_4 (μmol/μmol KDO)	Choline[a] (μmol/μmol KDO)	%	p
LPS[b]				
Total Pi	27 ± 5	33 ± 8	22	NS
Carbohydrates[c]	0.09 ± 0.01	0.15 ± 0.02	67	≤0.05
Lipid A				
Palmitic ac.[d]	34 ± 2	39 ± 5	15	NS[e]
12 carbon-hydroxyl ac.	32 ± 14	45 ± 20	41	NS

[a]Bacteria were grown in a high phosphate basal salt medium with 20 mM succinate plus 18.7 mM NH_4C1 or 20 mM choline chloride. All chemical determinations were carried out on LPS isolated with Triton X-100 from whole bacteria harvested at absorbance at 660 nm of 0.7. Total cellular contents were 1.05 + 0.16 and 1.00 + 0.20 mg/ml for succinate and choline, respectively. Results are the average of four independent experiments ± SD. P values were obtained by ANOVA analysis.
[b]KDO quantified.
[c]Carbohydrates quantified by the phenol method.
[d]Lipids were hydrolyzed from lipid A, identified by mass spectrometry. Results are expressed relative to stearic acid and averaged of three independent experiments ± SD.
[e]No significative. Data taken from Grumelli [64].

Table 3.
Variation in LPS composition according to the lung environmental changes.

PL biosynthesis has a common metabolite, the *R*-3-hydroxyacyl-ACP that is the substrate for *R*-3-hydroxyacyl-ACP dehydrase (FabZ) [98], to synthesize PL, and for LpxA, for LPS synthesis. Thus, the increased content of PL is at the expense of Lipid A from LPS (**Figure 5**), as shown in **Table 2**.

The LPS of *P. aeruginosa* stimulates the O_2 uptake from mitochondria [97] producing decoupling of the oxidative phosphorylation, reducing the respiratory rate, which generates stress in the host lung triggering exacerbations [44, 64, 97]. Therefore, succinate accumulation signifies that choline consumption is increasing the adaptation of the bacteria to the lung environment and the transition to the RSVC form, for chronic infection.

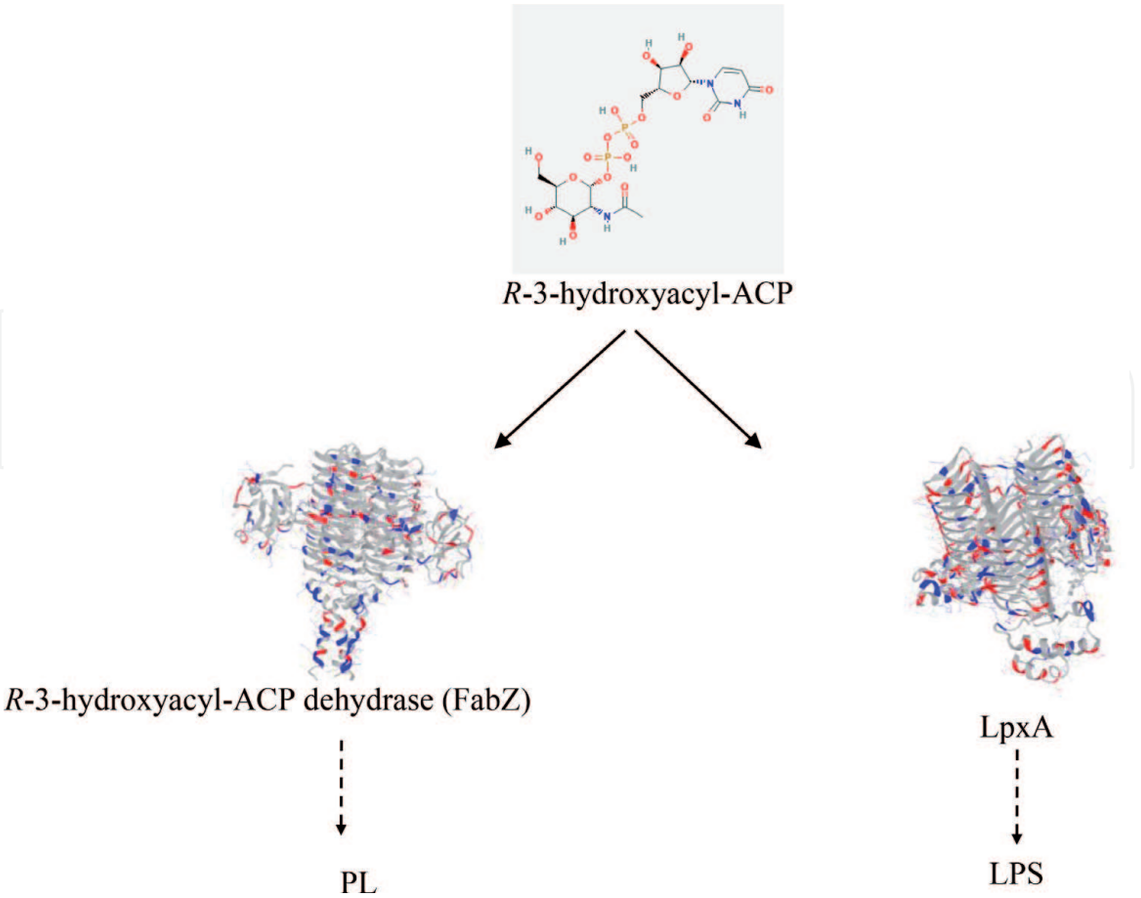

Figure 5.
R-3-hydroxyacyl-ACP, metabolite common to the biosynthesis of LPS and PL for which R-3-hydroxyacyl-ACP dehydrase (FabZ) and LpxA compete [98].

3. Chronic infection of *P. aeruginosa*

Upon infection, the host decreases iron levels in the blood [99]; this iron deficiency regulates a great number of bacterial virulent genes like alginate, the most relevant virulence factor, for *P. aeruginosa* survival [100]. In the lung, iron deficiency turns on AlgQ, the bacterial biofilm production gene, also known as AlgR2 [101, 102], under the Pfr A regulation that assists to the formation of two kinds of cytoplasmic aggregates: large vacuole-like bodies and smaller granules containing iron in association with oxygen or phosphate, very likely polyPi [103]. This leads to the RSCV type of *P. aeruginosa.* Under these conditions, the bacteria secrete alginate, a linear polysaccharide of D-mannuronic acid linked to L-guluronic acid [104].

The first gene described for the biosynthesis of alginate was the phosphomannose isomerase and GDP-mannose dehydrogenase (AlgD) that catalyze the conversion of GDP-mannose to GDP-mannuronic acid [105]. Upon oxygen limitation, *P. aeruginosa* utilizes nitrate or arginine as electron acceptors, via the succinylarginine pathway [106, 107]. The AlgD expression is tightly regulated by several environmental sources including nitrogen, O_2, Pi, NaCl, etc. Although the regulation of AlgD has been extensively studied, it is not completely understood, and eradication of chronic infection greatly depends on control of alginate production.

Several authors have studied the AlgD regulation, **Figure 6** shows a 20-years breach in the finding of AlgD regulators. More positive regulators have been identified, such as AlgR that is upregulated by NaCl and also by the nitrogen source [108]. AlgD is also under the same promoter than PLC, which is sensitive to the nitrogen source [109] that regulates the anaerobiosis genes. These genes detect the ratio

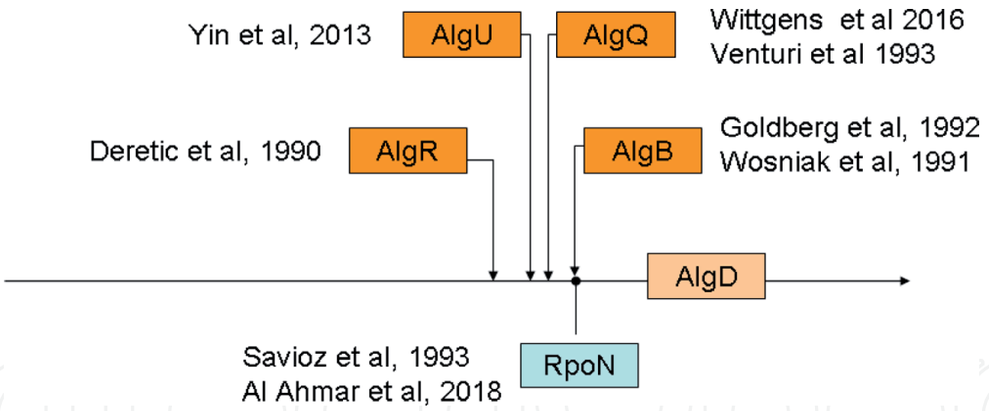

Figure 6.
Regulators of AlgD in alginate production. Negative and positive regulators found up-today.

of glutamine to 2-ketoglutarate, which is dependent on O_2 availability [108, 110]. Another positive regulator of AlgD is AlgU [111], but the only negative regulator known is the RpoN, a sigma factor, that regulates nitrogen metabolism. RpoN is increased by disruption of pyrimidine synthesis and decreased by the supplementation with uracil, showing that a high level of RpoN, in the RSCV form, may block the alginate biosynthesis [110, 112].

Studies on the biosynthetic pathway of biofilms show that chelation of iron by lactoferrin destabilizes the bacterial membrane [113], which combined with xylitol hinders the ability of the bacteria to respond to iron deficiency [101], showing some promise for CF treatment.

4. Conclusions

P. aeruginosa is a relevant pathogen given its widespread prevalence across different organs. The latent menace it poses for inpatients is a liability for institutions. For this, and the negative prognosis that *P. aeruginosa* infections in CF patients has, it is one of the subjects more researched for the last 40 years. The efforts have resulted in understanding the process of invasion, immune response, and bacterial tactics to achieve chronic infection. The complexity of the metabolic changes caused by the contact between the host and the bacteria is so extensive that the selection of variables for in vitro studies is difficult since the production of biofilm by *P. aeruginosa* seems to be regulated by everything, O_2, N_2, Fe^{2+}, Pi, and NaCl. This multiregulatory network is still a puzzle to be resolved.

Scientists agree that suppression of alginate production is vital to treat CF patients, but in 40 years of research, little has been achieved in suppressing its production *in vivo.*

5. Perspectives

The advancement of techniques with high output data like microarrays, proteomes, and mass spectrometry are closing the breach among the different approaches that have been used to tackle *P. aeruginosa* infections. For example, mass-spectrometry has verified through metabolite detection the metabolic pathways studied by molecular biologists and enzymologists. The integration of these studies with the physicians is needed to assess the areas that show more promises to control alginate production and *P. aeruginosa* eradication after it became a chronic infection.

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