Review

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Ceramide and sphingosine in pulmonary infections

Abstract: Acid sphingomyelinase and ceramide have previously been shown to play a central role in infections with *Neisseria gonorrhoeae, Staphylococcus aureus, Listeria monocytogenes, Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli,* and *Mycobacterium avium*. Recent studies have extended the role of sphingolipids in bacterial infections and have demonstrated that ceramide and sphingosine are central to the defense of lungs against bacterial pathogens. Ceramide accumulates in the airway epithelium of cystic fibrosis and ceramide synthase 2 (CerS2)-deficient mice, which respond to the lack of very long chain (C22-C24-) ceramides with a profound compensatory increase of long chain (mainly C16-) ceramides. In contrast, sphingosine is present in healthy airways and is almost completely absent from diseased or deficient epithelial cells. Both sphingolipids are crucially involved in the high susceptibility to infection of cystic fibrosis and CerS2-deficient mice, as indicated by findings showing that the normalization of ceramide and sphingosine levels rescue these mice from acute infection with *P. aeruginosa*.

Keywords: ceramide; cystic fibrosis; pneumonia; *Pseudomonas aeruginosa*; sphingosine.

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Introduction

Pulmonary infections remain a significant global health concern. Efficacious prevention and treatment have both become increasingly challenged as infectious organisms have adapted. The injudicious use of prescribed antibiotics has also exerted unnecessary Darwinian pressure, accelerating this evolution of bacterial antibiotic resistance. As a result we are now witnessing the exhaustion of prophylactic and therapeutic efficacy for many of our existing antimicrobial compounds. The progressive emergence of resistant strains, an ongoing and growing global public health issue, is a currently referenced topic of concern in both the lay and medical press. National governments have proclaimed both the alarming current public health impact and growing threat, and budgeted as a priority the quest for novel antimicrobial agents as a urgent priority.

Sphingolipids play an important role in innate immunity and have considerable potential for this evolution of novel antimicrobial therapeutics. Ceramide in particular has been shown to be involved in the infection of mammalian cells with a variety of bacterial and viral pathogens, including *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Salmonella typhimurium*, *Escherichia coli*, mycobacteria, measles virus, rhinovirus, and sindbis virus (Grassmé et al., 1997, 2003a, 2005; Hauck et al., 2000; Jan et al., 2000; Esen et al., 2001; Pfeiffer et al., 2001; Utermöhlen et al., 2003; Falcone et al., 2004; McCollister et al., 2007; Utermöhlen et al., 2008; Gassert et al., 2009; Avota et al., 2011; Simonis et al., 2014). The important role of ceramide in the structure of such a variety of infections is explained by its biophysics: sphingolipids, cholesterol, and glycerolipids are the main components of mammalian cell membranes. Sphingomyelin, the most abundant sphingolipid, associates with other sphingomyelin molecules and with cholesterol by means of relatively tight hydrophilic-hydrophobic van der Waals interactions; these interactions result in the spontaneous separation of these lipids from other (phospho)lipids in the plasma membrane and the formation of very small distinct

domains in cellular membranes, called rafts (Simons and Ikonen, 1997). The generation of ceramide within membranes results in a marked change in the chemical properties of these membranes (Brown and London, 1998; Kolesnick et al., 2000; Gulbins and Kolesnick, 2003). The hydrophobic ceramide molecules bind strongly to each other by hydrophobic interactions and thereby spontaneously separate from other lipids in membranes. This self-association of ceramide molecules results in the formation of small ceramide-enriched membrane domains, which have the tendency to fuse spontaneously to form large ceramide-enriched membrane domains, called platforms (Grassmé et al., 2001; Nurminen et al., 2002). These platforms form a unique microenvironment with biophysical properties, such as hydrophobicity and membrane thickness that are very different from the properties of other parts of the plasma membrane. The change in biophysical membrane properties after ceramide is generated enables these ceramide-enriched membrane platforms to trap and cluster (activated) receptor molecules and intracellular signaling molecules (Grassmé et al., 2001, 2003a, b). This trapping of receptors and signaling molecules in ceramide-enriched membrane domains is very likely mediated by the preferential interaction of these (activated) receptors with ceramide or ceramide-enriched membrane domains, as determined for instance by the length of the hydrophobic transmembranous domain, whereas the presence of these molecules in other domains of the plasma membrane is energetically unfavorable (Bock and Gulbins, 2002). The high density of activated receptors and signaling molecules enables them to function as a specific 'signalosome', which strongly facilitates the transmission of the signal via the plasma membrane. The system seems to be similar to many other biological systems, such as blood coagulation, that are controlled and amplified by the proximity of the participating factors. It has been demonstrated that ceramide-mediated clustering serves to spatially and temporally organize a given signalosome and thereby amplifies initial signaling by more than 100-fold (Grassmé et al., 2003b). Thus, ceramide acts by re-organizing molecules in cells and can control many receptor-mediated and non-receptor-mediated cellular activation processes by changing the biophysics of the membrane. In accordance, ceramide-enriched membrane platforms have been shown to be formed in cells after the application of a variety of stimuli, including CD95 (Grassmé et al., 2001), CD40 (Grassmé et al., 2002), DR5 (Dumitru and Gulbins, 2006), FcγRII (Abdel Shakor et al., 2004), and the PAF-receptor (Göggel et al., 2004); infection with *P. aeruginosa* (Grassmé et al., 2003a), *Neisseriae gonorrhoeae* (Grassmé et al., 1997), *Neisseria meningitides* (Simonis et al., 2014), rhinovirus (Grassmé et al., 2005), or measles virus (Gassert et al., 2009; Avota et al., 2011); or stress stimuli such as UV-light (Charruyer et al., 2005), gamma-irradiation (Rotolo et al., 2005), or treatment with cisplatin (Lacour et al., 2004) or Cu^{2+} (Lang et al., 2007).

Ceramide in bacterial infections

The first studies to identify the role of ceramide and acid sphingomyelinase in bacterial infections investigated the infection of human epithelial cells with *N. gonorrhoeae* (Grassmé et al., 1997), a common pathogen that causes a sexually transmitted infection. *Neisseria gonorrhoeae* interact with human mucosal epithelial cells by binding their opacity-associated (Opa) proteins to heparan sulfate proteoglycan (HSPG) receptors on epithelial cells, resulting in uptake of the pathogen (Makino et al., 1991). The infection of epithelial cells activates acid sphingomyelinase and increases the total cellular ceramide concentration. Pharmacological and genetic inhibition of acid sphingomyelinase by imipramine or by acid sphingomyelinase-deficient fibroblasts obtained from patients with Niemann-Pick disease type A demonstrated that acid sphingomyelinase activity is required for gonococcal entry into epithelial cells and fibroblasts (Grassmé et al., 1997). These studies demonstrated for the first time that acid sphingomyelinase and ceramide play a crucial role in the infection of human epithelial cells with bacterial pathogens. Additional studies of human neutrophil infection with *N. gonorrhoeae* revealed that acid sphingomyelinase activity is also involved in the opsonin-independent uptake of the pathogen into human phagocytes via carcinoembryonic antigen-related cellular adhesion molecule (CEACAM) receptors. Uptake into JOSK-M cells, a human myelomonocytic cell line JOSK-M (Ohta et al., 1986; Hauck et al., 1997), is prevented by inhibition of acid sphingomyelinase and is restored after pretreatment with exogenous ceramide (Hauck et al., 2000).

During disseminated gonococcal infections, the outer membrane protein PorB of serotype A (PorBIA) binds to the scavenger receptor expressed on endothelial cells (SREC-I) and mediates bacterial uptake (Faulstich et al., 2014). Recent studies investigating the role of sphingomyelinases and ceramide in this process found that expression and activation of neutral sphingomyelinase and the concomitant increase in ceramide are necessary for PorBIA-mediated invasion into host cells (Faulstich et al., 2014).

Finally, studies of *N. meningitidis* reiterated the role of sphingomyelinases and ceramide in this clinically

important genus of pathogens. *Neisseria meningitidis* binds to brain endothelial cells during the initial phase of meningoencephalitis. This process is mediated by the transient activation of acid sphingomyelinase and the resultant increase in ceramide concentrations in brain endothelial cells (Simonis et al., 2014). Infecting endothelial cells with *N. meningitidis* results in surface exposure of acid sphingomyelinase, release of ceramide in the outer leaflet of the plasma membrane, and subsequent formation of large ceramide-enriched membrane platforms. Like the infection process of *N. gonorrhoeae*, this process requires the expression of the outer membrane protein Opc proteins, because absence of Opc in deficient mutants reduced acid sphingomyelinase activation, while ectopic expression of Opc in *E. coli* was sufficient to trigger acid sphingomyelinase activation (Simonis et al., 2014). Pharmacologic inhibition or genetic deficiency of acid sphingomyelinase prevents the internalization of *N. meningitidis* into endothelial cells, a finding that emphasizes the central significance of acid sphingomyelinase and ceramide in the infection of host endothelial cells and the development of meningoencephalitis (Simonis et al., 2014).

Additional studies showed that infecting mammalian cells with *S. aureus, L. monocytogenes, S. typhimurium, E. coli*, or pathogenic mycobacteria also activates the acid sphingomyelinase/ceramide system (Esen et al., 2001; Pfeiffer et al., 2001; Utermöhlen et al., 2003; Falcone et al., 2004; McCollister et al., 2007; Utermöhlen et al., 2008). The use of genetically altered cells or pharmacological inhibitors proved the importance of the acid sphingomyelinase/ceramide system in the infection of mammalian cells by these pathogens. Consistent with the general function of ceramide in the signaling processes described above, the acid sphingomyelinase/ceramide system has been shown to be involved in several effects induced by the pathogens, including uptake/invasion of the pathogen, induction of apoptosis in the infected mammalian host cell, and regulation of the cellular and humoral immune responses (Grassmé et al., 1997, 2003a, 2005; Hauck et al., 2000; Esen et al., 2001; Pfeiffer et al., 2001; Utermöhlen et al., 2003; Falcone et al., 2004; McCollister et al., 2007; Utermöhlen et al., 2008; Simonis et al., 2014).

Specifically, the acid sphingomyelinase/ceramide system mediates the induction of apoptosis in endothelial cells infected with *S. aureus* (Esen et al., 2001), a pathogen causing nosocomial infections such as pneumonia, wound infections, and sepsis. *S. aureus*-mediated apoptosis of endothelial cells is a result of a signaling cascade starting with the activation of acid sphingomyelinase, the release of ceramide, the activation of cellular caspases and Jun-N-terminal kinase, and the release of cytochrome c from mitochondria into the cytosol. A genetic deficiency of acid sphingomyelinase prevents *S. aureus*-triggered apoptosis (Esen et al., 2001).

Listeria monocytogenes causes listeriosis, a disease usually characterized by gastrointestinal symptoms, but it also causes sepsis, perinatal infections, meningoencephalitis, and spontaneous abortions in pregnant women. *Listeria monocytogenes* invades and kills host macrophages (Vázquez-Boland et al., 2001). However, intracellular lysosome-mediated killing of *L. monocytogenes* is a key mechanism of the host's defense against this pathogen (Cossart et al., 1989). A deficiency in acid sphingomyelinase impairs the intracellular killing of the pathogen and causes mice with this deficiency to be highly susceptible to infection with *L. monocytogenes* (Utermöhlen et al., 2003). In particular, the acid sphingomyelinase/ceramide system has been shown to be required for the fusion of *L. monocytogenes*-containing late phagosomes with lysosomes. The absence of this step in acid sphingomyelinase-deficient cells prevents killing of the pathogen and thereby mediates the high susceptibility of deficient mice to *L. monocytogenes*.

Infections with *S. typhimurium* result in severe gastroenteritis or even typhoid fever. The pathogen infects by invading macrophages in Peyer's patches. While *L. monocytogenes* rapidly escapes from the acidified phagosome into the cytoplasm, intracellular *S. typhimurium* prevents lysosomal maturation of the phagosomes and survives within the lysosomal compartment (Oh et al., 1996; McCollister et al., 2005). Expression of acid sphingomyelinase is required for the activation of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that catalyzes the release of the reactive oxygen species (ROS) that are necessary for macrophages to kill *S. typhimurium* (McCollister et al., 2007). This finding is consistent with those of Zhang et al. (2008) demonstrating that protein subunits of the NADPH oxidase gp91 cluster in ceramide-enriched membrane domains. This clustering of the NADPH oxidase within these domains is required for the release of ROS, as evidenced by the absence of ROS formation in neutrophils and macrophages that lack acid sphingomyelinase. The inability of cells that lack acid sphingomyelinase to kill *S. typhimurium in vitro* is corroborated by the *in vivo* finding that mice deficient in acid sphingomyelinase are highly susceptible to infection with *S. typhimurium* (Utermöhlen et al., 2003).

Escherichia coli are found in the normal mammalian bacterial flora, but can also be opportunistic pathogens that cause a variety of infectious diseases, including urinary tract infections, meningitis, wound infections, and sepsis. *Escherichia coli* and many other gram-negative

bacteria contain and release lipopolysaccharide (LPS), a potent inflammatory glycolipid in the outer membrane of *E. coli*. Studies by Haimowitz-Friedman et al., (1997) and Falcone et al. (2004) demonstrated that LPS activates the acid sphingomyelinase/ceramide system in endothelial and dendritic cells, thereby causing the death of these cells. LPS activates cells via Toll-like receptor 4 (TLR4) (Poltorak et al., 1988), and the activation of the CD14/TLR4 complex by LPS results in clustering of CD14/TLR4 within ceramide-enriched membrane domains (Pfeiffer et al., 2001). This clustering may amplify the CD14/TLR4 signal, although this hypothesis has not been formally supported. At least in dendritic cells, acid sphingomyelinase-dependent apoptosis is inhibited by nitric oxide (NO), but our understanding of this process is not yet complete (Falcone et al., 2004).

The role of the acid sphingomyelinase/ceramide system in mycobacterial infections is still poorly characterized. It has been shown that acid sphingomyelinase–deficient mice are more resistant to lethal infections with *Mycobacterium avium* than are wild-type mice (Utermöhlen et al., 2008); however, the molecular mechanisms of this resistance are unknown. A very detailed study of the role of lipids in the fusion of phagosomes with lysosomes demonstrated that several lipids, including ceramide, are involved in actin nucleation on phagosomes and the subsequent phagosome-lysosome fusion that results in the killing of *M. avium* and *M. smegmatis* in macrophages (Utermöhlen et al., 2008). Additionally, Okino et al discovered a neutral ceramidase in *M. tuberculosis* that shares similarities with the neutral ceramidase of *P. aeruginosa* (Okino et al., 2010). The *in vivo* significance of these findings remains to be established.

The role of the acid sphingomyelinase/ceramide system and the function of this system in the described bacterial infections are summarized in Table 1.

Ceramide in pulmonary *P. aeruginosa* infections

The role of the acid sphingomyelinase/ceramide system in *P. aeruginosa* infections has been extensively studied. Several studies have demonstrated the activation of acid sphingomyelinase, the translocation of acid sphingomyelinase to the outer leaflet of the plasma membrane, and the release of ceramide after the infection of epithelial cells or macrophages with *P. aeruginosa* (Grassmé et al., 2003a; Zhang et al., 2008). Ceramide forms membrane platforms that promote the clustering of several molecules, including NADPH-oxidases, CD95, and the cystic fibrosis transmembrane conductance regulator (CFTR) (Grassmé et al., 2003a, Zhang et al., 2009). Upon infection of phagocytes with *P. aeruginosa*, NADPH-oxidases release ROS, an event that is necessary for killing and eliminating *P. aeruginosa* (Zhang et al., 2008). CD95 has been shown to be involved in the *P. aeruginosa*-mediated death of host epithelial cells, and CD95 deficiency reduces *P. aeruginosa*-mediated killing of host cells (Grassmé et al., 2000). Although this hypothesis is counterintuitive, host epithelial cell death may benefit the defense of the host. By removing infected cells that contain intracellular bacteria from the epithelial cell layer, the organism may be able to eliminate intracellular pathogens and thereby protect itself against further infection. The molecular mechanisms that mediate internalization of *P. aeruginosa* require better definition, and further investigation is needed to determine whether ceramide-mediated clustering of CFTR is involved in this process.

The crucial role of the acid sphingomyelinase/ceramide system in pulmonary *P. aeruginosa* infections has been demonstrated in acid sphingomyelinase-deficient mice and in cells treated with acid sphingomyelinase inhibitors (Grassmé et al., 2003a). These studies demonstrated

Table 1 Diferent roles and sites of action of ceramide.

Pathogen	Role and site of action
Neisseria gonorrhoeae	Formation of membrane platforms, internalization of pathogen
Neisseria meningitidis	Formation of membrane platforms, internalization of pathogen
Pseudomonas aeruginosa	Formation of membrane platforms, internalization of pathogen, clustering of CD95, Cftr and NADPH-oxidases, induction of cell death, control of cytokine release, oxidative burst
Staphylococcus aureus	Induction of cell death
Listeria monocytogenes	Fusion of phagosomes with lysosomes, intracellular killing, systemic resistance
Salmonella typhimurium	Systemic resistance
Escherichia coli	Formation of membrane platforms, clustering of CD14, induction of cell death
Pathogenic mycobacteria	Actin nucleation, systemic resistance

that acid sphingomyelinase-deficient mice are highly susceptible to pulmonary *P. aeruginosa* infections. The results of these studies are consistent with those of studies on membrane rafts, which found that pharmacological destruction of membrane rafts prevents the internalization of *P. aeruginosa* and the induction of apoptosis (Kowalski and Pier, 2004).

Ceramide in cystic fibrosis

Cystic fibrosis is the most common autosomal recessive disorder in Western countries. It is caused by mutations of *CFTR*. *CFTR* deficiency results in severe pulmonary and gastrointestinal problems. Pulmonary problems are presently of greater clinical importance and include acute and chronic infections, in particular with *P. aeruginosa*, *Burkholderia cepacia*, *S. aureus*, and *Haemophilus influenzae*. More than 80% of adult cystic fibrosis patients are infected with *P. aeruginosa*. Furthermore, cystic fibrosis airways are characterized by chronic inflammation with an imbalance between proinflammatory and antiinflammatory cytokines (Inoue et al., 1994; Bonfield et al., 1995; Khan et al., 1995; Tirouvanziam et al., 2000; Venkatakrishnan et al., 2000; Tabary et al., 2001; Oceandy et al., 2002; Verhaeghe et al., 2007). An increase in the concentrations of inflammatory mediators was observed in lungs from aborted fetuses, a finding suggesting that aseptic inflammation occurs very early in cystic fibrosis. In addition, neutrophils accumulate in the lungs of cystic fibrosis patients (Goldstein and Döring 1986, Teichgräber et al., 2008), and clinical data indicate that a large number of dead neutrophils may contribute to the impairment of mucociliary clearance observed in these patients (Whitchurch et al., 2002; Worlitzsch et al., 2002; Matsui et al., 2005).

The reasons for cystic fibrosis patients' chronic airway inflammation and high sensitivity to pulmonary infections are unknown. A study using biochemical techniques, fluorescence microscopy, and mass spectrometry demonstrated that *CFTR* deficiency results in the accumulation of ceramide in bronchial, tracheal, and intestinal epithelial cells, in alveolar macrophages in cystic fibrosis mice, and in nasal epithelial cells from cystic fibrosis patients (Teichgräber et al., 2008; Zhang et al., 2009; Becker et al., 2010a,b) (Figure 1). The accumulation of ceramide in lung

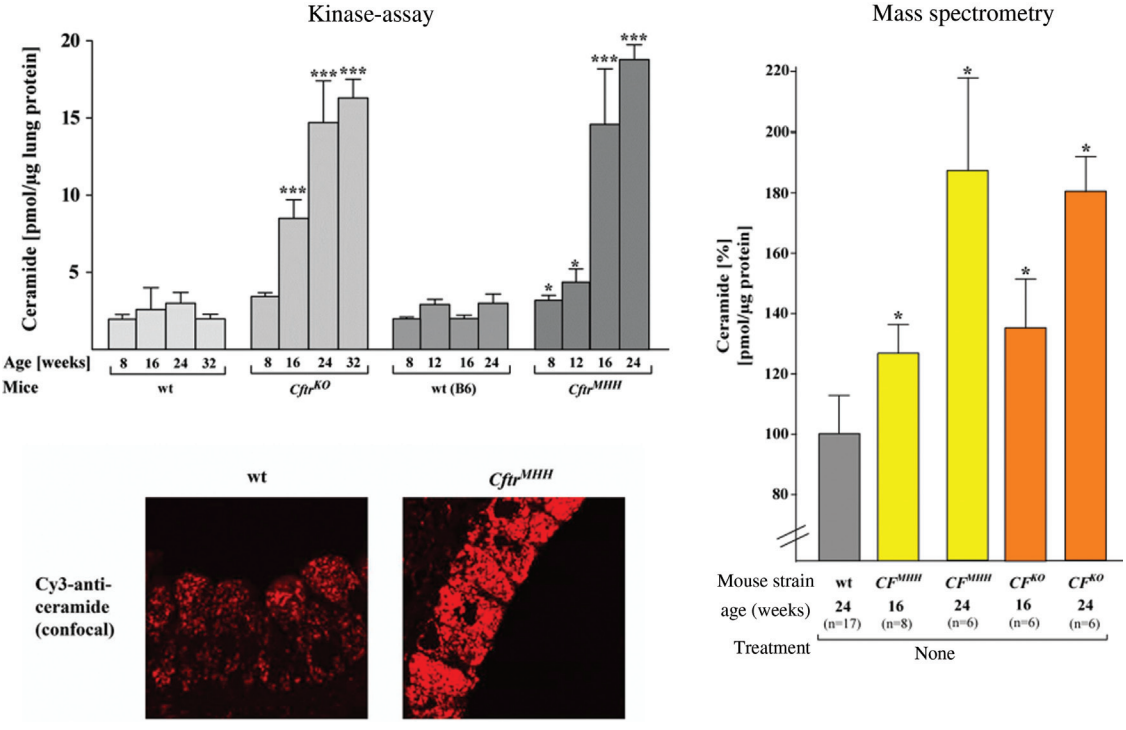

Figure 1 Ceramide accumulates in epithelial cells of cystic fibrosis mice.
The concentrations of ceramide in healthy lungs and epithelial cells are low. These concentrations are increased in the lungs and specifically in the bronchial epithelial cells of cystic fibrosis mice. Ceramide concentrations were determined by ceramide kinase assays (upper panel), mass spectrometry (right panel), and staining of lung sections with a ceramide-specific antibody followed by confocal microscopy analysis (lower panel). Printed from Teichgräber et al. (2008), and printed with permission of *Nature Medicine*.

tissues and cells from cystic fibrosis patients or mice has been confirmed by several studies using human transplant material, mouse lung tissue, and cultured cells (Brodlie et al., 2010; Ulrich et al., 2010; Bodas et al., 2011a,b; Caretti et al., 2014; Itokazu et al., 2014). Brodlie et al. (2010) provided detailed mass spectrometry data about the accumulation of various ceramide species in the lung and demonstrated the substantial accumulation of the ceramide species C16, C18, and C20 in the lungs of cystic fibrosis patients. However, at present it is unknown whether distinct ceramide species in the lung are associated with specific pathophysiological changes in CF patients.

Genetic or pharmacological normalization of ceramide concentrations in the lungs of cystic fibrosis mice, which is achieved either by crossing *Cftr*-deficient ($Cftr^{-/-}$) mice with acid sphingomyelinase-deficient ($Asm^{-/-}$) mice to obtain heterozygosity of Asm in mice lacking Cftr ($Cftr^{-/-}/Asm^{+/-}$) or by treating mice with functional acid sphingomyelinase inhibitors (acid sphingomyelinase-depleting drugs), normalizes all of the pathological changes and hallmarks associated with cystic fibrosis (Teichgräber et al., 2008; Zhang et al., 2009; Becker et al., 2010a,b). In particular, normalization of ceramide concentrations normalizes susceptibility to infection; (aseptic) pulmonary inflammation; the number of peribronchial neutrophils, macrophages, and dead cells; and the deposition of DNA in the airways of cystic fibrosis mice (Teichgräber et al., 2008; Zhang et al., 2009; Becker et al., 2010a,b). These findings demonstrate that ceramide plays a crucial role in the high susceptibility of cystic fibrosis patients to bacterial infection.

Functional inhibitors of acid sphingomyelinase/acid sphingomyelinase-depleting drugs that were used to inhibit infection with *P. aeruginosa* were amitriptyline, trimipramine, desipramine, chlorprothixene, fluoxetine, amlodipine, and sertraline (Becker et al., 2010a). These drugs are weak bases that diffuse into lysosomes and concentrate within lysosomes upon protonation (Hurwitz et al., 1994; Kornhuber et al., 2008). Their organic ring integrates into the inner lysosomal membrane, and the tertiary amino group displaces acid sphingomyelinase from the membrane; this displacement results in proteolytic degradation of acid sphingomyelinase in the lysosome (Hurwitz et al., 1994; Kornhuber et al., 2008). These drugs also induce degradation of the acid ceramidase *in vitro* (Elojeimy et al., 2006), although this effect seems to absent *in vivo* (Gulbins et al., 2013) Theoretically, the drugs should interfere with many proteins binding to the inner leaflet of the lysosomal membrane, but at present potential off-targets are not characterized yet.

The results of additional studies also support the role of distinct membrane domains in cystic fibrosis. The composition of rafts in cystic fibrosis cells has been shown to be altered compared to wild-type cells (Kowalski and Pier, 2004; Teichgräber et al., 2008). Most importantly, the destruction of rafts by the extraction of cholesterol impairs the internalization of *P. aeruginosa* into epithelial host cells and prevents apoptosis of these host cells after infection with *P. aeruginosa* (Kowalski and Pier, 2004; Grassmé et al., 2003a,b).

Sphingosine in cystic fibrosis and pulmonary infections

The studies described above demonstrated that ceramide is a key molecule mediating important pathophysiological aspects of cystic fibrosis. However, the concentration of ceramide in healthy tracheal and bronchial epithelial cells is rather low, and only the pathological elevation of these concentrations results in increased susceptibility to bacterial pathogens. Recent studies demonstrated that sphingosine, a sphingoid long chain base and a degradation product of ceramide, is an important first-line defense of healthy airways against *P. aeruginosa*; this protective mechanism is absent from cystic fibrosis cells (Pewzner-Jung et al., 2014). These studies demonstrated that sphingosine levels are greatly reduced in the tracheal and bronchial epithelium of cystic fibrosis patients and mice (Figure 2). Therefore, in cystic fibrosis the distribution of sphingosine is the opposite of the distribution of ceramide. While cystic fibrosis cells accumulate ceramide, they lack sphingosine. The significance of the absence of sphingosine from cystic fibrosis cells has been demonstrated by infection experiments using cystic fibrosis mice, which are highly susceptible to *P. aeruginosa* infections. Having the mice inhale either sphingosine or acid ceramidase, which degrades ceramide to sphingosine, restores sphingosine levels on the surface of airway epithelial cells and, most importantly, prevents *P. aeruginosa* infections in cystic fibrosis mice. Cystic fibrosis mice are also protected from *P. aeruginosa* infection by inhaling sphingosine analogs such as FTY 720. These results have also been demonstrated in ceramide synthase 2 (CerS2)-deficient mice, which also lack sphingosine in their epithelial cells and that are highly susceptible to pulmonary bacterial infection (Pewzner-Jung et al., 2014).

These findings suggest that sphingosine is an important molecule that plays a role in antibacterial defense in healthy lungs. Our findings suggest a novel paradigm for the immediate innate defense mechanism of the (upper) airways (Figure 3): the constitutive presence of sphingosine in healthy airway epithelial cells serves to kill pathogens.

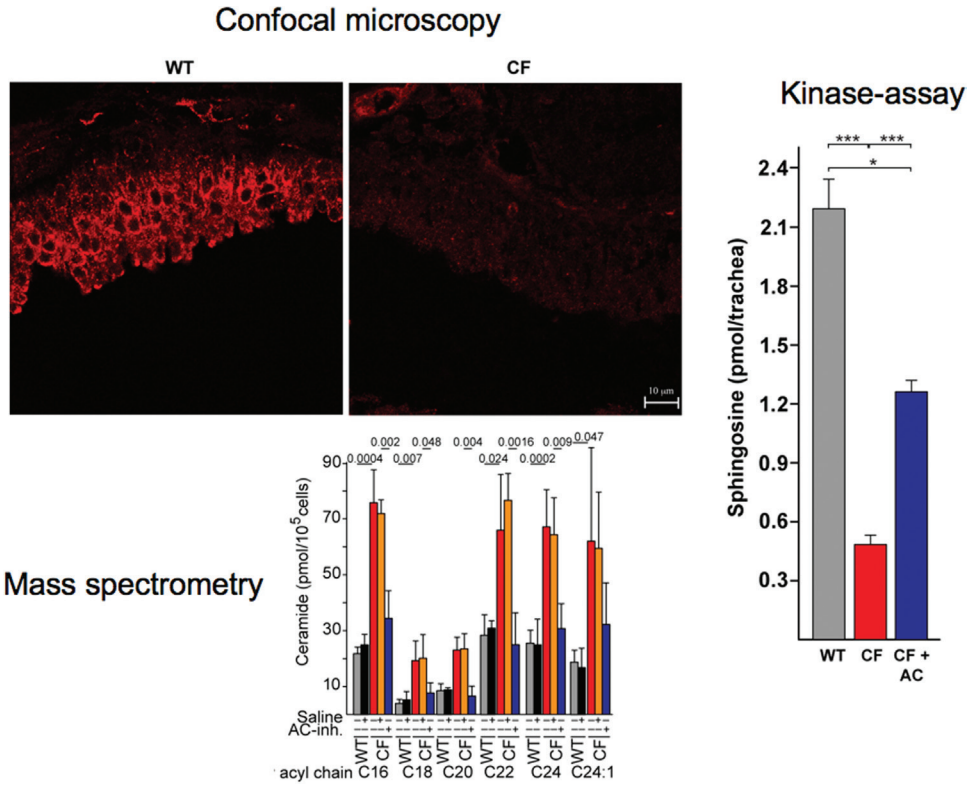

Figure 2 Sphingosine concentrations are reduced in cystic fibrosis epithelial cells.
Sphingosine concentrations in the trachea and specifically in tracheal epithelial cells of cystic fibrosis and wild-type mice were determined by staining lung sections with a Cy3-coupled anti-sphingosine antibody followed by confocal microscopy (upper panel), by a kinase assay (right panel), and by mass spectrometry (lower panel). Figures are from Pewzner-Jung et al. (2014), and printed with permission of *EMBO*.

This paradigm also explains the remarkable observation that although the upper airways are constantly exposed to pathogens, they usually eliminate pathogens such as *P. aeruginosa* and *S. aureus* without exhibiting any signs of inflammation. In cystic fibrosis lungs, the concentrations of both sphingosine and ceramide are altered, and these alterations result in high susceptibility to infection. Although sphingosine and ceramide are certainly not the only molecules controlling infection, their normalization is sufficient to prevent infection in cystic fibrosis mice, even if other defense mechanisms are still impaired (Pewzner-Jung et al., 2014). Our current hypothesis is that sphingosine kills invading bacteria in healthy epithelial cells. Cystic fibrosis epithelial cells, conversely, lack sphingosine, and enhanced ceramide concentrations promote lung infection by inducing chronic inflammation, reducing mucociliary clearance, and inducing the death of epithelial cells. These findings also suggest that acid ceramidase, sphingosine, or sphingosine analogs may be novel treatment options for patients susceptible to bacterial pneumonia (Pewzner-Jung et al., 2014).

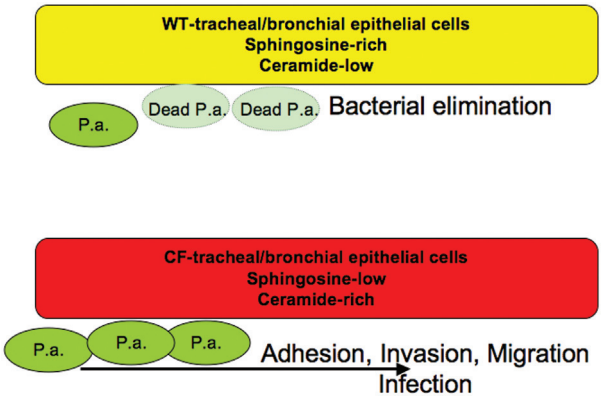

Figure 3 Model for the control of pulmonary infections by sphingosine and ceramide.
Healthy airways contain low concentrations of ceramide and high levels of sphingosine, whereas the concentrations of the two lipids are just the opposite in cystic fibrosis cells. The high concentrations of sphingosine in healthy cells kill invading bacterial pathogens, a defense mechanism that is absent from cystic fibrosis (CF) cells. The susceptibility of CF airways to infections is dramatically increased by direct promotion of the infection, i.e., bacterial adhesion, invasion and migration, by ceramide.

The role of sphingosine in the airways has not been previously identified; however, the antibacterial effect of sphingosine is consistent with previous findings demonstrating that human skin is protected from bacterial colonization (Bibel et al., 1992) with *S. aureus*. It is interesting to note that sphingosine does not only kill *P. aeruginosa*, but also *Moraxella catarrhalis*, *B. burgdorferi*, and *H. influenzae* (Pewzner-Jung et al., 2014). At present it is unknown how sphingosine kills pathogens, although recently published findings suggest that sphingosine causes ultrastructural damage in *E. coli* and *S. aureus* (Fischer et al., 2013). Many details about this activity of sphingosine remain to be identified.

We conclude that the balance between ceramide concentrations and sphingosine concentrations and, possibly, their sites of cellular accumulation in tracheal and bronchial epithelial cells determine the susceptibility of mice to *P. aeruginosa*. Interestingly, although *P. aeruginosa* lacks sphingolipids, it does harbor at least two genes that are involved in sphingolipid metabolism. One is a ceramidase, which can convert ceramide to sphingosine under neutral pH conditions (Okino et al., 1998). The other is a phospholipase that can convert sphingomyelin to ceramide, and vice versa (Luberto et al., 2003). Therefore, it is intriguing to speculate that *P. aeruginosa* subverts the host's defense by generating ceramide on the surface of the epithelial cells *in vivo*, an action that mimics the phenotype of cystic fibrosis mice. Additionally, a specific *P. aeruginosa* transcription factor, PA5324, which induces an uncharacterized protein, PA5325, has been found to directly bind sphingosine. Bacterial survival against sphingosine is reduced when this transcription factor is deleted (LaBauve and Wargo, 2014). Although we do not know the function of the protein encoded by PA5325, we may hypothesize that *P. aeruginosa* developed this resistance as a defense against an innate immune function of its host.

In summary, pulmonary infections remain a significant global health priority. Sphingolipids play a critical role in innate immunity, in inflammation, and in the pathophysiology of pulmonary infections. A better understanding of sphingolipid biology has the potential to advance the quest to develop novel antimicrobial agents and improve the prevention and treatment of pulmonary infections.

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