

Legionella pneumophila restrains autophagy by modulating the host's sphingolipid metabolism

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1 ***Legionella pneumophila* restrains autophagy by modulating the host's**
2 **sphingolipid metabolism**

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38 **Sphingolipids are bioactive molecules playing a key role as membrane components, but**
39 **they are also central regulators of many intracellular processes including**
40 **macroautophagy/autophagy. In particular, sphingosine-1-phosphate (S1P) is a critical**
41 **mediator that controls the balance between sphingolipid-induced autophagy and cell death.**
42 **S1P levels are adjusted *via* S1P synthesis, dephosphorylation or degradation, catalyzed by**
43 **SGPL1 (sphingosine-1-phosphate lyase 1). Intracellular pathogens are able to modulate**
44 **many different host cell pathways to allow their replication. We have found that infection of**
45 **eukaryotic cells with the human pathogen *Legionella pneumophila* triggers a change in the**
46 **host cell sphingolipid metabolism and specifically affects the levels of sphingosine. Indeed,**
47 ***L. pneumophila* secretes a protein highly homologous to eukaryotic SGPL1 (named *LpSPL*).**
48 **We solved the crystal structure of *LpSPL*, showed that it encodes lyase activity, targets the**
49 **host's sphingolipid metabolism and plays a role in starvation-induced autophagy during**
50 ***L. pneumophila* infection to promote intracellular survival.**

51
52 Xenophagy is a cellular mechanism allowing an infected cell to rapidly degrade the
53 invading bacteria by relying on the core autophagy machinery. The important role of autophagy
54 in restricting the replication of pathogens has been demonstrated for several intracellular bacteria:
55 the invaders are surrounded by autophagosome-like structures that allow fusion with lysosomes
56 and consequently the degradation of the pathogen. Furthermore, it has been shown that the
57 induction of autophagy suppresses the survival of intracellular bacteria. Thus the subversion of
58 autophagy is a key strategy employed by pathogens to block the host response and to promote
59 their survival.

60 *Legionella pneumophila* is one of those intracellular pathogens that interferes with the host
61 autophagy machinery. *L. pneumophila* is a Gram-negative bacterium that is naturally found in
62 aquatic environments where it replicates in protists, but that can also cause a severe pneumonia in
63 humans called Legionnaires' disease. The high conservation of many signaling pathways in
64 human macrophages and protists allows *L. pneumophila* to invade and to replicate in human
65 cells. Once the host cell is infected, *L. pneumophila* is able to delay its delivery to lysosomes and
66 to build up a specialized vacuole where it replicates until nutrients are depleted. The ability of *L.*
67 *pneumophila* to subvert host defenses to set up its intracellular cycle relies on its uniqueness to
68 encode over 300 secreted effector proteins that interfere with diverse cellular pathways. Our

69 analyses of *L. pneumophila* genomes showed for the first time that many of these effectors share
70 high similarity with eukaryotic proteins, and are thus proteins never or only rarely found in
71 prokaryotic genomes, a finding that led to the hypothesis that these proteins had been acquired
72 through horizontal gene transfer from its hosts.

73 One example is the *lpp2128/spl* gene that encodes a protein highly similar to eukaryotic SGPL1,
74 named *LpSPL* for *Legionella pneumophila* SGPL1. Phylogenetic analyses of the *LpSPL* sequence
75 show that it branches within the eukaryotic clade of SGPL1, closest to the one of *Acanthamoeba*
76 *castellanii*, further supporting the idea that the *LpSPL*-encoding gene had been acquired from a
77 protist host. In order to characterize its function and role in host-pathogen interaction, we
78 resolved its crystal structure. This showed that *LpSPL* exhibits a dimeric multidomain
79 architecture like eukaryotic SGPL1 and revealed a high level of structural conservation in its
80 active site composition with its eukaryotic counterparts. Eukaryotic SGPL1 catalyzes the
81 irreversible cleavage of S1P, a product of the sphingolipid degradation pathway. By using a
82 fluorogenic homolog of S1P we showed that *LpSPL* exhibits lyase activity in both transfected
83 and infected cells. Furthermore, mutations of the residues reported to be involved in eukaryotic
84 SGPL1 activity also abrogated the lyase activity of *LpSPL*.

85 Sphingolipids are a family of metabolites essential for membrane biogenesis, but they are also
86 important signaling molecules modulating numerous physiological processes. The central
87 component of the sphingolipid degradation pathway is ceramide that, hydrolyzed from
88 sphingomyelin or synthesized *de novo*, undergoes successive conversions to various other
89 sphingolipid intermediates like ceramide-1-phosphate, sphingosine and S1P. S1P levels are
90 tightly fine-tuned through synthesis and degradation because once S1P is present in the
91 intracellular milieu it functions as a cellular mediator (**Figure 1A**).

92 To understand the role *LpSPL* plays in the host cell, we measured host cell sphingolipid levels
93 during infection by using mass spectrometry techniques. We observed that infection of
94 macrophages with *L. pneumophila* leads to an overall reduction of bioactive sphingolipids such
95 as sphingomyelin and ceramide, showing for the first time that *L. pneumophila* infection affects
96 host cell sphingolipid metabolism. Interestingly, cells infected with a *L. pneumophila* strain that
97 does not express *LpSPL* reveal an accumulation of sphingosine compared to cells infected with
98 wild-type *L. pneumophila*. Thus, the bacterium specifically secretes *LpSPL* to prevent an increase
99 of sphingosine levels in the infected host cell. This observation correlates with the fact that

100 ectopically expressed *LpSPL* localizes at the endoplasmic reticulum, like eukaryotic SGPL1.
101 Given the role of sphingolipids as regulators of autophagy, we further explored whether secretion
102 of *LpSPL* into the host cell affects the autophagic response to infection.

103 Indeed, *L. pneumophila* is already known to interfere with the autophagy machinery: 2 secreted
104 effectors have been previously identified to target the autophagy machinery (RavZ and LegA9).
105 By using multiple assays, including SQSTM1/p62 and LC3 western blots we found that *LpSPL* is
106 a third effector secreted by *L. pneumophila* that modulates autophagy, as *LpSPL* restrains
107 starvation-induced autophagy by acting on autophagosome biogenesis (**Figure 1B**). Importantly,
108 this effect depends on its enzymatic activity as a catalytically inactive mutant fails to decrease
109 host autophagy. By using high-content image-based analyses we quantified the formation of
110 LC3 puncta in cells infected with wild type or a mutant strain deleted for the *spl* gene, showing
111 that *LpSPL* limits the autophagic response during *L. pneumophila* infection to counteract
112 antibacterial response by the host cell.

113 Taken together, our work discovered that the modulation of the sphingolipid metabolism of the
114 host cell is a sophisticated strategy used by *L. pneumophila* to exploit the host autophagy
115 machinery and to evade the host cell response.

116

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122 **Figure 1.** *Legionella pneumophila* modulates sphingolipid metabolism and autophagy during
123 infection. (A) Simplified cartoon of sphingolipid metabolism: *i*) sphingomyelin from plasma
124 membranes is turned into ceramide by the enzyme sphingomyelinase (SMase). *ii*) Ceramide is
125 then metabolized by ceramidase into sphingosine that *iii*) is phosphorylated by SPHK
126 (sphingosine kinase) into sphingosine-1-phosphate (S1P), *iv*) a reaction that can be reverted by
127 SGPP (sphingosine-1-phosphate phosphatase). *v*) S1P is secreted into the extracellular milieu,
128 where it might act in a paracrine or autocrine way, or *vi*) may be broken down to hexadecenal and
129 ethanolamine-1-phosphate (EA1P) by sphingosine-1-phosphate lyase (SGPL1). *L. pneumophila*
130 decreases sphingolipid levels during infection (red arrows). It encodes 3 eukaryotic-like proteins

131 that are homologs to enzymes that act in sphingolipid metabolism: Lpp2641 (putative
 132 sphingomyelinase), Lpp2295 (putative sphingosine kinase) and *LpSPL* (sphingosine-1 phosphate
 133 lyase) (blue arrows). *LpSPL* has been characterized functionally. **(B)** *L. pneumophila* restrains
 134 host autophagy during infection by secreting effectors that inhibit both autophagosome formation
 135 (*LpSPL*) and maturation (*RavZ* and *LegA9*).
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