Review

Listeriolysin O: the Swiss army knife of *Listeria*

Mélanie Anne Hamon[1,2,3*], David Ribet[1,2,3*], Fabrizia Stavru[1,2,3*] and Pascale Cossart[1,2,3]

[1] Institut Pasteur, Unité des Interactions Bactéries-Cellules, Département de Biologie Cellulaire et Infection, F-75015 Paris, France
[2] INSERM, U604, F-75015 Paris, France
[3] INRA, USC2020, F-75015 Paris, France

Listeriolysin O (LLO) is a toxin produced by *Listeria monocytogenes*, an opportunistic bacterial pathogen responsible for the disease listeriosis. This disease starts with the ingestion of contaminated foods and mainly affects immunocompromised individuals, newborns, and pregnant women. In the laboratory, *L. monocytogenes* is used as a model organism to study processes such as cell invasion, intracellular survival, and cell-to-cell spreading, as this Gram-positive bacterium has evolved elaborate molecular strategies to subvert host cell functions. LLO is a major virulence factor originally shown to be crucial for bacterial escape from the internalization vacuole after entry into cells. However, recent studies are revisiting the role of LLO during infection and are revealing new insights into the action of LLO, in particular before bacterial entry. These latest findings along with their impact on the infectious process will be discussed.

Introduction to pore formation by LLO

Listeria monocytogenes is a Gram-positive bacterium that causes gastroenteritis, meningitis, encephalitis, and mother-to-fetus infections. This versatile pathogen has the remarkable ability to cross three tight human barriers: the intestinal barrier, the blood–brain barrier, and the feto-placental barrier. At the cellular level, *L. monocytogenes* invades host cells, in which it is able to survive and replicate within the cytoplasm. Many reviews have been written on several aspects of *Listeria*–host interactions, ranging from bacterial entry into cells to adaptation to the intracellular milieu [1–3]. Reviews on listeriolysin O (LLO) have also been written [4–6]; here we will focus on the new striking findings regarding LLO and its roles during infection.

LLO is a pore-forming toxin belonging to the family of cholesterol-dependent cytolysins (CDCs), which contains more than 20 pore-forming toxins produced by different bacterial species (Table 1). All CDCs are secreted as soluble monomers by their cognate bacteria and are characterized by their ability to bind to the cholesterol of host membranes, oligomerize, and form large pores of up to 35 nm in diameter. Comparison of the crystal structure of water-soluble monomers of two CDCs, perfringolysin O (PFO) and intermedilysin [7,8], as well as sequence homologies between different members of the CDC family (between 40% and 70%), suggest that these toxins share a common tertiary structure and use similar mechanisms for pore formation (Figure 1). LLO distinguishes itself from other CDC family members by being, to our knowledge, the only toxin whose activity is regulated by pH. This is due to the presence of an acidic triad in the transmembrane domain, that acts as a pH sensor and triggers a premature unfurling of LLO at neutral pH, thereby allowing pore formation to occur mainly at acidic pH [9,10]. The role of LLO during infection was first described as being a virulence factor required for bacterial escape from the primary internalization vacuole [11–14] or from the secondary vacuole formed upon bacterial spreading to neighboring cells [15,16] (Figure 1e). The low pH requirement for LLO inside the vacuole (which has an estimated pH of 5.5 [17]) explains how the pore formation activity is restricted to avoid disruption of the host cell by uncontrolled LLO insertion into the endomembrane system of the host cell.

In the past 5 years, studies have revealed new aspects of LLO pore formation and new roles for LLO both before and after bacterial internalization. We will review these new findings and discuss their implications on infection.

Novel insights into the mechanism of action of LLO on membranes

Although it is clear that LLO is necessary for allowing *Listeria* to escape the host vacuole, the exact role LLO exerts during phagosomal membrane disruption remains unclear. Indeed, the difference in the osmotic pressure between the inside of the vacuole and the host cytoplasm excludes a 'lysis'-like mechanism for vacuolar disruption. The studies described below contribute to our understanding of this process by addressing both the structure of the pore formed by LLO and the recently described cofactors that are important for vacuole disruption.

LLO pore formation and repair

As their name indicates, CDCs first bind to cholesterol present in the host cell membrane. Of note, bacterial membranes, unlike eukaryotic ones, lack cholesterol and thus are protected from the cytolytic activity of CDCs. Early studies suggested that a conserved undecapeptide

Corresponding author: Cossart, P. (pascale.cossart@pasteur.fr)
Keywords: *Listeria*; membrane damage; virulence factor; pore-forming toxin; pathogenesis
[*] These authors contributed equally.

 0966-842X/$ – see front matter http://dx.doi.org/10.1016/j.tim.2012.04.006 Trends in Microbiology, August 2012, Vol. 20, No. 8

Table 1. Diversity of pore-forming toxins produced by prokaryotes and eukaryotes involved in virulence[a]

Toxin type	Species	Toxin	Receptor[b]	Pore diameter (nm)	M_w (kDa)[c]
α-Pore-forming toxins					
Colicins					
	Escherichia coli	Colicin Ia	Cir	2	67
Actinoporins					
	Actinia equina	Equinatoxin II	lipids	2	24
β-Pore-forming toxins					
Cholesterol-dependent cytolysins[d]					
	Arcanobacterium pyogenes	Pyolysin	Cholesterol	250–350	62
	Bacillus anthracis	Anthrolysin O (ALO)	Cholesterol	250–350	57
	Clostridium botulinum	Botulinolysin	Cholesterol	250–350	58
	Clostridium perfringens	Perfringolysin O (PFO)	Cholesterol	250–350	56
	Clostridium tetani	Tetanolysin	Cholesterol	250–350	59
	Gardnerella vaginalis	Vaginolysin	CD59/cholesterol	250–350	57
	Listeria ivanovii	Ivanolysin	Cholesterol	250–350	59
	Listeria monocytogenes	Listeriolysin O (LLO)	Cholesterol	250–350	59
	Streptococcus intermedius	Intermedilysin (ILY)	CD59/cholesterol	250–350	58
	Streptococcus pneumoniae	Pneumolysin (PLY)	Cholesterol	250–350	53
	Streptococcus pyogenes	Streptolysin O (SLO)	Cholesterol	250–350	64
Aerolysin and related toxins					
	Aeromonas hydrophila	Aerolysin	GPI-anchored proteins	2–3	54
	Chlorohydra viridissima	Hydralysin-1	ND	1–2	26
Others					
	Bacillus anthracis	Anthrax toxin (PA moiety)	TEM8/CMG2	1–2	86
	Staphylococcus aureus	α-Hemolysin	ADAM-10	2–3	36
Others					
	Helicobacter pylori	Vacuolating cytotoxin (VacA)	Several	6–10	140

[a]Toxins are divided into two categories based on the mechanism of pore formation. α-toxins form pores by inserting hydrophobic α-helices into the membrane, whereas β-toxins insert β-strands. For each class of toxin, only one or few examples are indicated.

[b]Abbreviations: cir, colicin Ia receptor; GPI, glycosylphosphatidylinositol; TEM8, tumor endothelial marker 8; CMG2, capillary morphogenesis gene 2; ADAM-10, a disintegrin and metalloprotease 10; ND, not determined.

[c]Molecular weight of full-length toxins, including potential signal peptides or propeptides, for toxins requiring proteolytic processing.

[d]Only some examples of CDCs produced by pathogenic bacteria are listed in this table. For a complete overview of currently known CDCs, see [101].

was the cholesterol-binding domain of these toxins, as mutations in it abolished pore formation [18]. However, the mechanistic contribution of this domain was unclear. Recently, it was demonstrated that the undecapeptide was not responsible for cholesterol binding. Instead, a threonine–leucine pair in the C terminal part of the protein was important (Figure 1) [19,20]. In fact, the undecapeptide was found to only be a structural requirement, allowing the correct conformation of the cholesterol-binding motif. Although not formally demonstrated to be important in the case of LLO, the threonine–leucine pair is conserved among all CDC members, and the binding mechanism has been proposed as a paradigm for many protein–membrane interactions that depend on cholesterol [20].

The mechanism of CDC pore formation after membrane binding remains controversial. Two main models have been proposed. In the first model, monomers bind, oligomerize to form a prepore ring, which once assembled forms a pore traversing the membrane (reviewed in [21]). This model implies that pores will always be of the same size. In the second model, the conformational transition required for pore formation can occur even if the ring of monomers is not complete, leading to the formation of a pore delineated by an arc-shaped oligomer faced by a free edge of the lipid membrane [22]. In this second model, pore size could vary, as smaller channels can be formed (in contrast to full rings), and as progressive incorporation of new monomers was proposed to gradually increase the size of these pores [22]. Although the second model is more controversial, two recent studies suggest that smaller pores could be formed. The first study shows that inside the bacteria containing vacuole, LLO initially creates 'micropinosomes', that are only permeable to ions and small molecules, and persist several minutes before becoming permeable to larger molecules [23]. The second study showed that preincubation of LLO with cholesterol, which has been widely used to saturate cholesterol-binding sites of LLO and prevent pore formation without compromising membrane binding [24], only partially blocked pore formation, leaving cells incubated with cholesterol-treated LLO still permeable to ions, but not to macromolecules [25]. These two studies bring up the possibility that pores of different sizes could coexist and could lead to different responses during infection.

Interestingly, pore-dependent membrane damage is reversible. Indeed, cells efficiently repair membrane injury and surprisingly, large pores (such as those formed by CDCs) are repaired more efficiently than small pores (such as those formed by aerolysin) [26,27]. Pores formed by the CDC streptolysin O (SLO) are repaired by mechanisms involving c-jun N-terminal kinase (JNK) signaling, calcium influx followed by endocytosis of the pore, and