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Listeria exploits damage and death to spread bad news

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Abstract

Pathogenic *Listeria monocytogenes* replicates within the host cytosol; little is known about how it transits from cell to cell, spreading infection. A recent study implicates infection-induced membrane damage as a trigger for efferocytosis, the recognition and uptake of dead cells, thereby tricking neighboring cells into taking up the invader.

Keywords

Listeria; dissemination; efferocytosis; membrane damage

Macrophages play a pivotal role in maintaining tissue homeostasis by efferocytosis, the process of apoptotic cell clearance. In a recent report in *Nature*, Czuczman *et al.* demonstrate that the bacterial pathogen, *Listeria monocytogenes*, exploits efferocytosis to facilitate cell-to-cell spread, thereby disseminating the infection [1].

L. monocytogenes is a Gram-positive foodborne pathogen that can cause gastrointestinal inflammation, meningitis or spontaneous miscarriage. The bacterium crosses the intestinal barrier where it is captured by phagocytes [2]. Upon entry into a host cell, *Listeria* must escape into the cytosol through the activity of its major virulence factor, the pore-forming toxin, listeriolysin O (LLO). Host factors, including the cystic fibrosis transmembrane conductance regulator (CFTR) and the gamma-interferon-inducible lysosomal thiol reductase (GILT), are exploited by *L. monocytogenes* to enhance LLO function and phagosomal escape [2]. Within the cytosol, the bacteria replicate robustly, protected from neutrophils and mediators of humoral immunity. *L. monocytogenes* can also spread from the cytosol of one infected cell to an adjacent cell without exposure to extracellular host defenses. The process of cell-to-cell spread is critical for pathogenesis and depends on actin-based motility. *L. monocytogenes* nucleates actin polymerization at the bacterial surface by expression of the ActA virulence factor. Actin-based motility enables the bacterium to exert force on the plasma membrane causing membrane protrusions, or pseudopods, that extend toward neighboring cells [3]. These protrusions can be taken up by recipient cells, resulting

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in a bacterium encapsulated in a double membrane-bound vacuole that requires LLO and two phospholipase C enzymes for escape and repetition of the intracellular life cycle [4].

Pseudopod resolution also involves host factors, including serine/threonine kinases, such as CSNK1A1 [5]. Although cell-to-cell spread by *L. monocytogenes* has been amply demonstrated by transmission electron and immunofluorescence microscopy [6], molecular mechanisms that govern the process of cell-to-cell spread itself are not well understood, particularly how spread is initiated at the plasma membrane, followed by uptake of the bacteria-containing pseudopod by a neighboring cell.

Czuczman and colleagues proposed that LLO-mediated plasma membrane damage could modulate cell-to-cell spread by *L. monocytogenes* [1]. LLO can transiently damage the host plasma membrane, relying on host membrane repair mechanisms to maintain the integrity of the intracellular niche [7]. *L. monocytogenes* infection induced plasma membrane damage in an LLO-dependent manner. Notably, ActA, which nucleates actin-based motility, also contributed to the ability of *L. monocytogenes* to cause plasma membrane damage, suggesting that ActA itself or the process of motility increased association with the plasma membrane. When host membrane repair was inhibited by performing infection in the absence of extracellular Ca^{2+} , membrane damage was exacerbated. Silencing host factors that promoted membrane repair also increased bacterially-induced plasma membrane damage. These results revealed new aspects of cell-to-cell spread by *L. monocytogenes* and highlighted additional functions for the virulence determinants ActA and LLO in initiating the spreading process.

Formation of bacteria-containing pseudopods is a hallmark of cell-to-cell spread by *L. monocytogenes*. The authors found that phosphatidylserine (PS), which is exposed on the outer leaflet of the plasma membrane upon membrane damage, was often co-localized with *L. monocytogenes* in round vesicles on the cell surface. These PS-positive structures were induced in an LLO-dependent manner, and co-localization with the bacteria required ActA. Although ActA is presumably required for actin-based motility for the bacteria to reach the plasma membrane, recent studies show that disassembly of actin tails is also a key part of the process of dissemination [8]. In real time, the authors observed that bacterial membrane protrusions recruited PS, before rounding into a cell surface vesicle. Notably, formation of these PS-positive structures was not accompanied by known characteristics of apoptosis, such as global membrane blebbing or nuclear condensation. The *L. monocytogenes*-containing vesicles were observed on the infected cell surface or were released into the surrounding environment. Collectively, these data show that plasma membrane damage by *L. monocytogenes* is a precursor to the formation of bacteria-containing membrane pseudopods or vesicles that display PS, a molecular flag of distress.

Phosphatidylserine is exposed on the outer leaflet of the plasma membrane as a result of damage or cell death and can be recognized by specific efferocytosis receptors, such as TIM4 [9]. Macrophages deficient in TIM4 were more resistant to cell-to-cell spread by *L. monocytogenes* compared to wild-type macrophages, and antibody blockade of TIM4 or PS also reduced cell-to-cell spread in wild-type but not in TIM4-deficient macrophages. Although TIM4 is also known to suppress cytokine production, which could potentially

contribute to *L. monocytogenes* cell-to cell spread, cytokine analysis revealed similar profiles in wild-type and TIM4-deficient macrophages. Thus, the *in vitro* data indicate that *L. monocytogenes* executes cell-to-cell spread by triggering PS exposure on associated host membrane structures to promote uptake through efferocytosis receptors.

Efferocytosis has been implicated in anti-microbial defense for some pathogens but can mediate immune evasion for others. Dying *Mycobacterium tuberculosis*-infected macrophages can be efferocytosed by uninfected macrophages killing the bacteria, whereas, the parasite *Leishmania major* gains access to uninfected macrophages that efferocytose apoptotic infected neutrophils [10]. To determine if efferocytosis was more important for *L. monocytogenes* pathogenesis or innate host defense in this infection, *Timd4*^{-/-} mice were infected with *L. monocytogenes* [1]. The TIM4-deficient mice supported lower bacterial burden in liver and spleen compared to controls, establishing a role for TIM4 in promoting wild-type *L. monocytogenes* infection. When *Timd4*^{-/-} and wild-type mice were infected with the *actA* mutant, however, the bacterial burden in the liver was similar. These data support the model proposed by Czuczman and colleagues that TIM4-mediated efferocytosis can augment *L. monocytogenes* infection through cell-to-cell spread. However, the spleen of *actA* infected *Timd4*^{-/-} mice exhibited a lower bacterial burden than control mice, indicating that in this secondary lymphoid organ, other functions of TIM4 may come into play. Taken together, the data reported by Czuczman *et al.* [1] uncover a new strategy for dissemination by *L. monocytogenes* that highlights efferocytosis in the arms race between host and pathogen.

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