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COMMENTARY

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LC3-associated phagocytosis initiated by integrin ITGAM-ITGB2/Mac-1 enhances immunity to *Listeria monocytogenes*

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ABSTRACT

The macroautophagic/autophagic machinery cannot only target cell-endogenous components but also intracellular pathogenic bacteria such as *Listeria monocytogenes*. *Listeria* are targeted both by canonical autophagy and by a noncanonical form of autophagy referred to as LC3-associated phagocytosis (LAP). The molecular mechanisms involved and whether these processes contribute to anti-listerial immunity or rather provide *Listeria* with a replicative niche for persistent infection, however, remained unknown. Recently, using an *in vivo* mouse infection model, we have been able to demonstrate that *Listeria* in tissue macrophages are targeted exclusively by LAP. Furthermore, our data show that LAP is required for killing of *Listeria* by macrophages and thereby contributes to anti-listerial immunity of mice, whereas canonical autophagy is completely dispensable. Moreover, we have elucidated the molecular mechanisms that trigger LAP of *Listeria* and identified the integrin ITGAM-ITGB2/Mac-1/CR3/integrin $\alpha_M\beta_2$ as the receptor that initiates LAP in response to *Listeria* infection.

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Macrophages are white blood cells that are crucial for immunity as they patrol the tissues to detect, phagocytose and kill invading pathogens. Some pathogens such as *Listeria*, however, specialize in escaping killing by macrophages. To this end, *Listeria* use their pore-forming toxin, listeriolysin O (LLO), and the phospholipases PlcA and PlcB to destroy the membrane of the phagosome and then colonize the cytosol (Figure 1). Cytosolic *Listeria* can be targeted by canonical autophagy resulting in killing in autolysosomes. However, virulent *Listeria* largely avoid this fate by surrounding themselves, in a process mediated by the virulence factor ActA, with a halo of host actin and stalling phagophore assembly via PlcA and PlcB.

Indeed, we were recently able to show that canonical autophagy is completely dispensable for killing of *Listeria* by tissue macrophages *in vivo* and that *Listeria*, in fact, are not targeted by canonical autophagy at all [1]. Nonetheless, *Listeria* are targeted by the autophagic machinery as they colocalize with LC3. Strikingly, this targeting is completely independent of the capability of *Listeria* to damage the phagosome, as avirulent or even dead *Listeria* also colocalize with LC3. Therefore, we investigated the molecular mechanisms underlying LC3 recruitment to *Listeria in vivo* in more detail. LC3 is recruited i) exclusively to *Listeria* in single-membrane phagosomes, ii) independently of the ULK complex components ULK1/2 and RB1CC1/FIP200 and iii) only upon generation of reactive oxygen species (ROS) by the phagocyte NADPH oxidase CYBB/Nox2. These all are hallmarks of LAP, a noncanonical form of autophagy that results in attachment of LC3 to the single membrane of phagosomes, which requires some components of the autophagic machinery (e.g., ATG5, ATG7 and BECN1/Beclin-1) but not others (e.g., ULK1/2, RB1CC1 and ATG14) and depends on ROS production by CYBB. Thus, our data demonstrate that *Listeria* in tissue macrophages *in vivo* are targeted exclusively by LAP, but not canonical autophagy.

We next addressed the question of whether LAP contributes to anti-listerial immunity or, as has been suggested, rather provides *Listeria* with a replicative niche for persistent infection. Our data show that deficiency for LAP markedly reduces anti-listerial activity of tissue macrophages. Furthermore, deficiency for LAP in macrophages, but not that for canonical autophagy, increases susceptibility of mice to infection with *Listeria*. Thus, our data show that LAP *in vivo* substantially contributes to anti-listerial activity of macrophages and immunity of mice.

We also elucidated the molecular mechanisms underlying the anti-listerial function of LAP. LC3-positive *Listeria*-containing phagosomes fuse more often with lysosomes than conventional LC3-negative phagosomes, and deficiency for LAP markedly impairs fusion of *Listeria*-containing phagosomes with lysosomes. Consequently, *Listeria* in LC3-positive phagosomes are exposed to higher levels of lysosomal acid hydrolases than *Listeria* in conventional phagosomes or in LAP-deficient macrophages. Because lysosomal acid hydrolases are of particular importance for killing of *Listeria* by macrophages, our data indicate that LAP enhances the anti-listerial activity of macrophages by promoting fusion of *Listeria*-containing phagosomes with lysosomes.

Concerning the pathway triggering LAP in response to *Listeria* infection, we were able to show that previously reported LAP-initiating receptors such as Toll-like receptors recognizing pathogen-associated molecular patterns or

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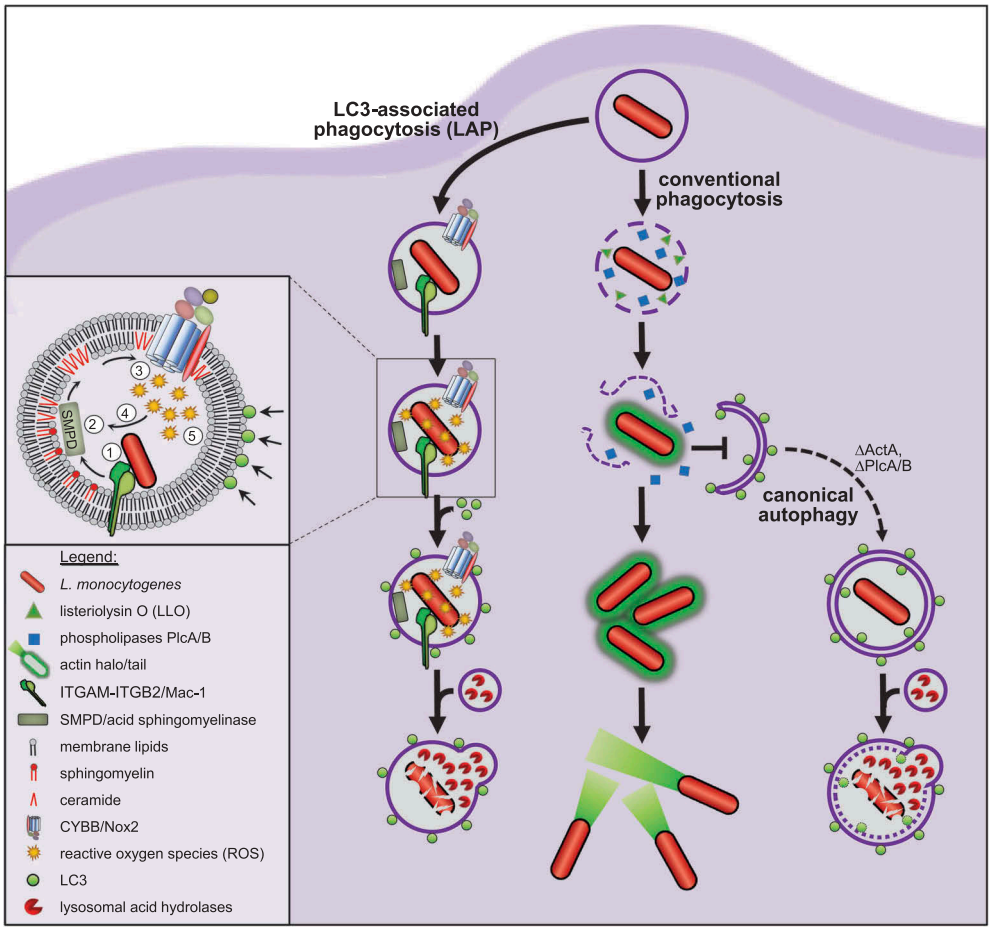

Figure 1. Model for LAP of *Listeria*. *Listeria* use their virulence factors LLO and PlcA/B to destroy the membrane of conventional phagosomes before these can fuse with lysosomes, and then colonize the cytosol. Cytosolic *Listeria* avoid targeting by canonical autophagy by surrounding themselves, in a process mediated by the virulence factor ActA, with a halo of host actin and by stalling phagophore assembly via PlcA/B. Consequently, canonical autophagy does not contribute to anti-listerial activity of macrophages. By contrast, recruitment of LC3 to *Listeria*-containing phagosomes by LAP markedly enhances anti-listerial activity of macrophages and immunity of mice. LAP of *Listeria* (1) is initiated by ITGAM-ITGB2 that induces (2) breakdown of the membrane lipid sphingomyelin into ceramide by SMPD. (3) The resulting ceramide-enriched membrane platforms facilitate CYBB assembly and activation. (4) CYBB-derived ROS amplify SMPD activity in a positive feedback loop and (5) induce recruitment of LC3 by LAP. LAP then promotes fusion of the phagosome with lysosomes, which increases exposure of *Listeria* to bactericidal lysosomal acid hydrolases and thus anti-listerial activity of macrophages.

FCGR/Fcγ receptor recognizing antibody-decorated pathogens are completely dispensable for LAP of *Listeria*. Furthermore, they also are completely dispensable for activation of the CYBB-mediated ROS production that is a prerequisite for LAP of *Listeria*. Instead, our data demonstrate that the pathway that initiates LAP of *Listeria* emanates from ITGAM-ITGB2, a receptor that can recognize a number of different ligands including diverse microbial molecules. Genetic deficiency for ITGAM or antibody-mediated blockade markedly impair infection-induced ROS production by CYBB and LAP. As a connecting link between ITGAM and CYBB activation and subsequent induction of LAP, we identified SMPD/acid sphingomyelinase, a lipid-converting enzyme hydrolyzing the major membrane lipid sphingomyelin into ceramide and phosphorylcholine. In response to *Listeria* infection, ITGAM induces SMPD activation resulting in an altered phagosomal membrane lipid composition that facilitates assembly and activation of CYBB. CYBB-derived ROS then induce LC3 recruitment to *Listeria*-containing phagosomes by LAP.

Interestingly, deficiency for ITGAM or SMPD does not completely impair LAP of *Listeria* indicating that LAP is not exclusively initiated via this pathway and that a, yet to be identified, ITGAM-SMPD-independent pathway of LAP initiation exists.

Taken together, we were able to demonstrate that LAP is an important anti-listerial mechanism of macrophages that contributes to immunity against *Listeria* infection. On the molecular level, LAP in response to *Listeria* infection is initiated by ITGAM. ITGAM induces SMPD-mediated membrane rearrangements that allow CYBB activation. CYBB-derived ROS then induce recruitment of LC3 to *Listeria*-containing phagosomes, which promotes phagosome-lysosome fusion. The resulting increased exposure of *Listeria* to lysosomal acid hydrolases underlies the anti-listerial function of LAP.

Our work highlights LAP as a particularly bactericidal form of phagocytosis. Nonetheless, only a subpopulation of approximately 20–25% of phagocytosed *Listeria* is targeted by LAP. Why not all phagocytosed *Listeria* are targeted by LAP

and how macrophages decide which *Listeria* are to be targeted by LAP and which not, remain open questions. Perspectively, our data indicate that increasing the relative contribution of LAP to phagocytosis may be a promising strategy to enhance immunity to bacterial infection.

Disclosure statement

No potential conflict of interest was reported by the authors.

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