The different autophagic roads by which phagosomes travel to lysosomes

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Autophagy delivers cytoplasmic constituents, like damaged mitochondria, to lysosomes. Recently, it was noted that the same molecular machinery also regulates phagosome delivery for degradation. In this issue of *The EMBO Journal*, Brooks and colleagues demonstrate that KIM-1/TIM-1 receptor-mediated phagocytosis in epithelial cells does not seem to cause modification of the phagosomal membrane itself by the autophagic machinery, but engulfment of phagosomes by autophagosomes for delivery to lysosomes and MHC restricted antigen presentation. This study suggests that the autophagic machinery can regulate phagocytosis via two pathways, modification of phagosomes during LC3-associated phagocytosis (LAP) and macroautophagy of phagosomes.

See also: **CR Brooks *et al*** (October 2015)

Autophagy constitutes a number of pathways, including chaperone-mediated, micro- and macroautophagy, that allow delivery of cytoplasmic material, like damaged mitochondria, to lysosomes for degradation (Mizushima *et al*, 2011). Macroautophagy, the pathway most frequently studied, employs more than 30 autophagy-related gene (atg) products to form double-membrane vesicles around portions of the cytoplasm and then fuses these vesicles to lysosomes for the hydrolysis of the inner autophagosomal membranes and their cargo. In addition to the degradation of cellular constituents, which gave the pathway its name (autophagy for self-eating), it has been recently appreciated that the Atg machinery also influences the degradation of extracellular material after

endocytosis (Münz, 2015). For this degradation, two pathways have been proposed (Fig 1), namely macroautophagic degradation of damaged endosomes and phagocytosis associated with the essential autophagic membrane-coupled protein LC3 (LC3-associated phagocytosis or LAP).

During macroautophagic degradation of damaged endosomes, luminal glycosylations get exposed to the cytosol. These are detected by cytosolic galectins 3 and 8 and lead to the recruitment of the macroautophagic machinery for damaged endosome degradation (Thurston *et al*, 2012; Chen *et al*, 2014). In addition, proteins in these damaged endosomes get ubiquitinated and then recruit the macroautophagic machinery via the ubiquitin and LC3-binding proteins p62 and NDP52 (Thurston *et al*, 2012; Fujita *et al*, 2013). Both mechanisms lead to the engulfment of the damaged endosomes by autophagosomes and delivery to lysosomes. In contrast, during LAP, LC3 gets directly coupled to the phagosomal membrane. This does not require early events of autophagosome generation like the serine/threonine protein kinase ULK1, but is dependent on reactive oxygen species (ROS) production via NADPH oxidase (NOX) at the phagosomal membrane (Romao *et al*, 2013; Martinez *et al*, 2015). Dependent on the cellular background, LAP accelerates or delays phagosome fusion with lysosomes.

In the study by Brooks and colleagues in this issue of *The EMBO Journal*, the authors studied phagocytosis that is induced in epithelial cells after expression of the kidney injury molecule 1/T-cell immunoglobulin and mucin domain 1 (KIM-1/TIM-1) molecule (Brooks *et al*, 2015). They found that KIM-1/TIM-1-mediated phagocytosis of

phosphatidylserine exposing apoptotic cells requires Atgs for efficient degradation in lysosomes. Similarly to what has been previously published for LAP (Romao *et al*, 2013), this pathway allowed MHC presentation of the phagocytosed cargo, leading, however, on epithelial cells to the suppression of T-cell proliferation and the induction of regulatory T-cells. Despite these functional similarities, all cell biological aspects point towards macroautophagic degradation of KIM-1/TIM-1-induced phagosomes. Along these lines, the authors observed multilamellar vesicles accumulating after KIM-1/TIM-1-mediated phagocytosis. This is consistent with the respective phagosomes getting engulfed by autophagosomes. Furthermore, known autophagosome cargo, specifically p62 and ubiquitin, co-localized with KIM-1/TIM-1-positive phagosomes. These phagosomes acquired LC3 also with slow kinetics of more than 30 min, which could indicate macroautophagy of formed phagosomes. The LC3 association did not depend on NOX and ROS production, but required ULK1 and Atg5. Furthermore, while LC3 is not degraded during LAP, because it remains at the cytosolic side of the phagosomal membrane, it is degraded with the inner autophagosomal membrane during macroautophagy of endosomes. The lysosomal low pH-sensitive GFP fluorescence of a GFP-RFP-LC3 reporter construct got indeed exposed to the lysosomal lumen during KIM-1/TIM-1-mediated phagocytosis, further arguing for macroautophagy of KIM-1/TIM-1-positive phagosomes in epithelial cells.

How this particular uptake mechanism, however, stimulates engulfment by autophagosomes remains somewhat enigmatic. The cytosolic domain of KIM-1/TIM-1 seems

Figure 1. The macroautophagy machinery in phagosome maturation.

During LC3-associated phagocytosis (LAP; right side), ligands of Toll-like receptors (TLRs) are endocytosed into vesicles that recruit NADPH oxidase (NOX). Reactive oxygen species (ROS) production by NOX is required for LC3 association with the phagosomal membrane. These LAP vesicles fuse with MHC class II-containing compartments (MIICs) after LC3 uncoupling for prolonged antigen presentation on MHC class II molecules to CD4⁺ T cells. In this issue of *The EMBO Journal*, Brooks and colleagues demonstrate that KIM-1/TIM-1-mediated phagocytosis of apoptotic bodies (left side) leads to their engulfment by autophagic isolation membranes. This results in multilamellar vesicles, of which at least the outer two membranes are derived from autophagosomes. These are then getting targeted to MIICs for cargo presentation on MHC class II molecules to CD4⁺ T cells.

required and its phosphorylation recruits the PI3 kinase regulatory subunit p85. Which of these events recruits the macroautophagic machinery versus facilitates phagocytosis needs to be further investigated. Nevertheless, the provided data suggest that KIM-1/TIM-1-induced phagosomes are processed via canonical macroautophagy for tolerogenic MHC presentation, as has been previously demonstrated for cytosolic and mitochondrial antigens (Schmid *et al*, 2007; Aichinger *et al*, 2013). This mechanism might be designed to maintain tissue tolerance during kidney injury.

Several intriguing questions are raised by the presented findings. Firstly, the authors observed both MHC class I- and MHC II-restricted antigen presentation of KIM-1/TIM-1 phagocytosed cargo. Since it is difficult to envision that the respective phagocytosed antigen could have escaped into the cytosol from the observed multilamellar vesicles, this might indicate vesicular MHC class I loading for cross-presentation in epithelial cells. In addition, the pathway that was thought to contain pathogens trying to escape from damaged endosomes to the cytosol after phagocytosis might be

specifically stimulated via some endocytic receptors. The detailed mechanisms behind these novel cell biological aspects are worth further investigations.

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