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LC3-associated phagocytosis in microbial pathogenesis

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A B S T R A C T

Phagocytosis is essential for uptake and elimination of pathogenic microorganisms. Autophagy is a highly conserved mechanism for incorporation of cellular constituents to replenish nutrients by degradation. Recently, parts of the autophagy machinery – above all microtubule-associated protein 1 light chain 3 (LC3) – were found to be specifically recruited to phagosomal membranes resulting in phagosome-lysosome fusion and efficient degradation of internalized cargo in a process termed LC3-associated phagocytosis (LAP). Many pathogenic bacterial, fungal and parasitic microorganisms reside within LAP-targeted single-membrane phagosomes or vacuoles after infection of host cells. In this minireview we describe the state of knowledge on the interaction of pathogens with LAP or LAP-like pathways and report on various pathogens that have evolved strategies to circumvent degradation in LAP compartments.

1. Introduction

Phagocytosis is defined as the internalization of particles bigger than 0.5 µm in size. It is one of the major mechanisms in development and cell homeostasis. Uptake of dying or expendable cells is an anti-inflammatory form of phagocytosis termed efferocytosis. The release of 'find-me'-signals attracts phagocytes towards the dying cells. 'Eat-me'-signals such as phosphatidylserine and calreticulin are recognized by specific engulfment receptors leading to incorporation of the apoptotic cells. Subsequent release of mainly anti-inflammatory cytokines tempers the local immune response and protects neighboring cells (reviewed in Arandjelovic and Ravichandran, 2015). Phagocytosis of foreign particles or pathogenic microbes usually is an inflammatory process essential for immune defense and resolution of infection. In this context, emerging phagosomes serve the spatial delimitation of the cargo from the cytosol and hence limitation of nutrition, restriction of multiplication and finally degradation. Metchnikoff already described phagocytotic uptake of *Bacillus anthracis* in 1884. Since then many more pathogenic bacteria, fungi and parasites were shown to be taken up by receptor-mediated phagocytosis (Christophers, 1904; Metchnikoff, 1884; Wright, 1903). In principal, uptake is facilitated by phagocytic receptors such as Fc (fragment crystallizable)-receptors or complement-receptors (CRs) following either recognition of microbe-associated opsonins such as antibodies or complement factors or direct recognition of pathogen-specific surface antigens. Additionally, specific pathogen-associated molecular patterns (PAMPs) can be sensed by pattern recognition receptors (PRRs) triggering intracellular pro-inflammatory signaling pathways (Pauwels et al., 2017).

In efferocytosis as well as in phagocytosis of foreign particles, receptor-recognition is accompanied by actin-rearrangement and the formation of a phagocytic cup by pseudopods that surround the cargo (Swanson, 2008). Upon closure of the compartment the phagosome matures by fusion events with vesicular structures that are mainly facilitated by G-proteins of the Rab-family (Yeo et al., 2016). This includes the acquisition of V-ATPases from multiple cellular sources which results in the formation of progressively acidified early and late endosomes (Kissing et al., 2017; Yates et al., 2005). Finally, the internalized cargo becomes degraded. Processing of the cargo either results from production of reactive oxygen species (ROS) by phagocytic NADPH oxidase that is recruited early in phagosome-biogenesis or by fusion of the phagosome with lysosomes. The latter are acidic organelles that supply the phagosome with hydrolytic enzymes such as cathepsins, glycosidases, DNases or lipases (Nunes et al., 2013; Yates et al., 2005). The resulting phagolysosome is positive for the marker proteins LAMP1/2. Proton influx into the phagolysosome and concommitant acidification of the compartment lumen gives the optimal pH needed for enzymatic degradation of the cargo (Yates et al., 2005).

An important issue of phagocytosis and resulting degradation of antigens associated with foreign particles by macrophages and dendritic cells lies in the activation of the adaptive immune system. This is

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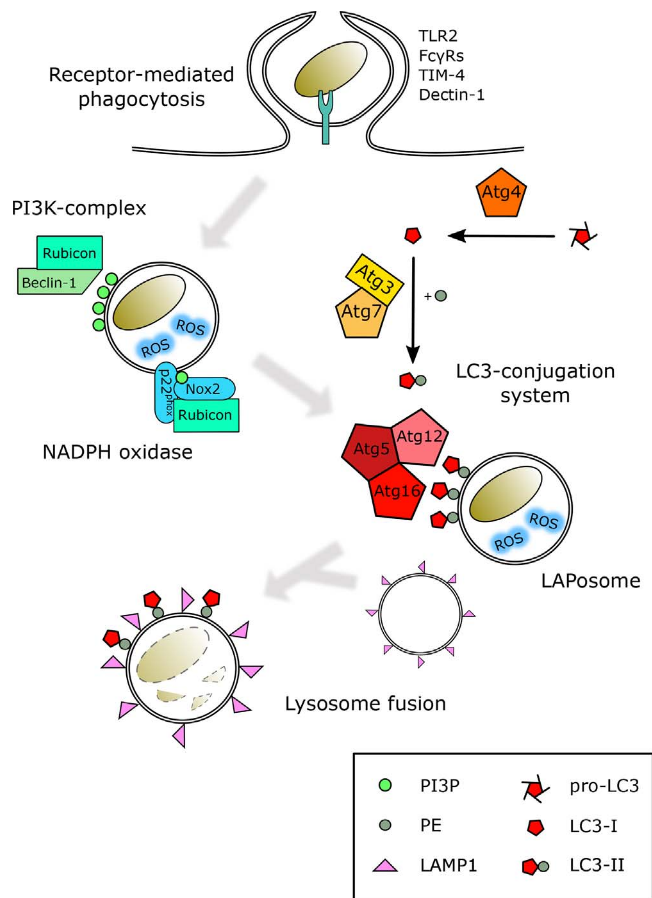

Fig. 1. Mechanism of LC3-associated phagocytosis. LAP is initiated by engagement of specific receptors such as TLR2, FcγRs (upon antibody opsonization), TIM-4 or Dectin-1 on the phagocyte. The cargo is internalized by receptor-mediated phagocytosis and engulfed within a single-membrane phagosome. Recruitment of NADPH oxidase initiates production of reactive oxygen species (ROS). ROS generation leads to rapid lipidation of LC3 with the phagosomal membrane. The emerging phagosome, now referred to as LAPosome, matures by fusion with lysosomes and the cargo is degraded.

mainly achieved through peptide presentation facilitated by the major histocompatibility complex (MHC) II on the phagocytes' surface. Antigen processing, MHCII presentation and hence T cell activation upon phagocytosis is a critical step of infection control. A decade ago, a novel form of phagocytosis was discovered that introduces the autophagy machinery to phagosome maturation. Upon stimulation of murine macrophages with TLR-agonists, recruitment of the autophagy protein microtubule-associated protein 1A/1 B light-chain 3 (LC3) towards emerging single membrane phagosomes was identified. This process, efficiently promoting phagosome maturation, was termed LC3-associated phagocytosis, short LAP (Sanjuan et al., 2009).

2. LC3-associated phagocytosis

Like with phagocytosis, LAP is initiated by receptor-dependent recognition of either apoptotic cells or pathogenic microbes. Fig. 1 gives a schematic representation of the molecular mechanisms behind LAP. In addition to phagocytotic Fc-receptors, already known to induce conventional phagocytosis, signaling of toll-like receptors (TLRs), the phosphatidylserine-receptor TIM4 or the ß-glucan receptor Dectin-1 can trigger LAP (Henault et al., 2013; Ma et al., 2012; Martinez et al., 2011; Sanjuan et al., 2007). Receptor recognition is followed by phagocytosis and the internalized cargo is engulfed within a single-membrane phagosome. This compartment is rapidly decorated with phosphatidylinositol-3-phosphate (PI3P) generated by the class III PI3-Kinase complex consisting of VPS15, VPS34, Beclin-1, UVRAG and Rubicon. The latter is essential and specific for LAP (Martinez et al., 2015; Matsunaga et al., 2009; Sanjuan et al., 2007). PI3P is bound by the p40^{phox} subunit of the phagocytic NADPH oxidase complex. After recruitment of the cytosolic NADPH oxidase factors gp91^{phox} (isoform Nox2 in phagocytes), p22^{phox}, p47^{phox}, p67^{phox} and the GTPase Rac, ROS are generated in the phagosomal lumen (Huang et al., 2009; Lam et al., 2010; Martinez et al., 2015). Subsequently, the cytosolic autophagy marker (pro-) LC3 is modified by the autophagy-related protein (Atg) 4 to LC3-I. ROS-production recruits the autophagic conjugation systems Atg7-Atg3 and Atg12-Atg5-Atg16 that covalently links LC3-I to phosphatidylethanolamine (PE) on the phagosomal surface (LC3-II). The presence of LC3 on the phagosome (now termed LAPosome) can be detected as early as 10 min after phagocytosis (Henault et al., 2013; Martinez et al., 2011; Sanjuan et al., 2007; Tanida et al., 2004). Finally, LC3-II decoration mediates a rapid fusion of LAPosomes with lysosomes. Intriguingly, compared to conventional phagocytosis, LAP seems to have a dual function in antigen presentation. Depending on the cell type, MHCII loading and presentation is either accelerated or delayed. In murine macrophages, an accelerated cargo processing can be found (Sanjuan et al., 2007). Contrary to that, in human cells the maturation of LAPosomes was delayed (Romao et al., 2013).

LAP is indispensable in health as genetic defects in Atg proteins result in the development of autoimmune diseases: A genome-wide association study revealed that mutations in autophagy genes contribute to the establishment of chronic diseases (Harley et al., 2008). *E.g.* mutation of *Atg5* was linked to the pathogenesis of systemic lupus erythematosus (SLE). Mice deficient for LAP showed increased pro-inflammatory cytokine production upon injection of apoptotic cells, resulting in a SLE-like disease (Martinez et al., 2016).

During infection, many bacterial, fungal and parasitic pathogens are targeted by LAP or LAP-like pathways. Some of these microbes evolved strategies to circumvent degradation in LAPosomes or break out of the compartment. Instead they multiply within these compartments or the cytosol of the host cell. In contrast to the well-described role of conventional autophagy in immune defense, the underlying mechanisms which initiate LAP and lead to LC3 recruitment onto pathogen-containing phagosomes as well as the impact of LAP on infection are not well known. Here, we give an overview on the knowledge on the interplay between pathogenic microorganisms and LAP or LAP-like mechanisms and the pathogens' strategies that lead to evasion of the action of this novel microbicidal pathway (Fig. 2).

3. LAP and fungal pathogens

3.1. *Candida albicans*

C. albicans can be internalized by human macrophages and bone marrow-derived murine dendritic cells (BMDCs) in a Dectin-1-dependent manner that mediates a rapid Atg5-dependent recruitment of LC3 onto *C. albicans*-containing phagosomes. The examination of phagosomal ultrastructures revealed that phagosomes harboring *C. albicans* are surrounded by a single membrane. Upon stimulation of Dectin-1 signaling-deficient macrophages or BMDCs with β-glucan coated particles or *C. albicans*, LC3-II is not formed on phagosomes (Ma et al., 2012). ROS production by Nox2 is critical for LC3-decoration (Huang and Brumell, 2009). Inhibition of Nox2 with diphenyleneiodonium (DPI) or depletion of the Nox2 gene *cybb* in BMDCs and in BMDMs, resulted in diminished LC3-lipidation and co-localization with *C. albicans*-containing phagosomes (Ma et al., 2012; Tam et al., 2014). Co-incubation of eGFP-LC3-expressing macrophages with heat-killed *C. albicans*, which expose more β-glucan on their surface than viable *C. albicans*, showed the importance of β-glucan for infection. Fluorescence microscopy revealed an increase in LC3 recruitment towards heat-killed *C. albicans*-containing phagosomes. Pro-inflammatory cytokine production is increased in LC3-depleted murine macrophages in comparison to wild type cells (Tam et al., 2014). Similarly, supernatants of

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