International Journal of Medical Microbiology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

International Journal of Medical Microbiology

journal homepage: www.elsevier.com/locate/ijmm

Xenophagic pathways and their bacterial subversion in cellular self-defense – παντα ρει – everything is in flux

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ARTICLE INFO

Keywords:
Autophagy
Xenophagy
Bacterial pathogens
Mitochondria
Metabolic switch
Aggresome

ABSTRACT

Autophagy is an evolutionarily ancient and highly conserved eukaryotic mechanism that targets cytoplasmic material for degradation. Autophagic flux involves the formation of autophagosomes and their degradation by lysosomes. The process plays a crucial role in maintaining cellular homeostasis and responds to various environmental conditions. While autophagy had previously been thought to be a non-selective process, it is now clear that it can also selectively target cellular organelles, such as mitochondria (referred to as mitophagy) and/or invading pathogens (referred to as xenophagy). Selective autophagy is characterized by specific substrate recognition and requires distinct cellular adaptor proteins. Here we review xenophagic mechanisms involved in the recognition and autolysosomal or autophagolysosomal degradation of different intracellular bacteria. In this context, we also discuss a recently discovered cellular self-defense pathway, termed mito-xenophagy, which occurs during bacterial infection of dendritic cells and depends on a TNF-α-mediated metabolic switch from oxidative phosphorylation to glycolysis.

1. Introduction

Bacterial pathogens with an intracellular life cycle have evolved various mechanisms to control their fate and ensure their proliferation within infected host cells. Inside the host cell, they evade resistance mechanisms, thereby avoiding their delivery and subsequent degradation in lysosomes. According to their lifestyle, intracellular bacteria have been divided into vacuolar (i.e. those dwelling within a membrane-bound compartment) and cytosolic (those inhabiting the cytosol following phagosomal escape) pathogens. However, there is a blurred line between these groups since some classically vacuolar bacteria can become cytosolic (Knodler et al., 2010), while certain cytosolic bacteria exploit vacuolar compartments for trafficking (Checroun et al., 2006). Regardless of classification, intracellular pathogens have developed a variety of different strategies to circumvent bactericidal host defense in order to generate an environment suitable for replication. For instance, some vacuolar pathogens, such as *Mycobacterium tuberculosis* remodel their initial phagosome into a specialized compartment by inhibiting acidification and lysosomal fusion (Steele-Mortimer, 2008; Flannagan et al., 2009). In contrast, *Salmonella enterica* Typhimurium generates a salmonella-containing vacuole (SCV), which is highly enriched in late endosomal markers (Smith et al., 2005). Other intracellular bacteria, such as *Legionella pneumophila* and brucella ensure their survival within the host cell through prevention of lysosomal fusion and establishment of an endoplasmic reticulum (ER)-derived/decorated compartment (Roy et al., 2006). Chlamydia, employing a different strategy, escapes the endocytic pathway by exclusion of host components and builds an idiosyncratic vacuole (Cocchiaro and Valdivia, 2009). Moreover, there are intracellular pathogens like *Coxiella burnetii* that are not only resistant to the phagolysosomal environment, but rather benefit from it (Flannagan et al., 2009). In contrast, cytosolic bacteria including *Listeria monocytogenes*, *Shigella flexneri*, rickettsia species, *Mycobacterium marinarum*, burkholderia species, and *Francisella tularensis* lyse their initial phagosome immediately after uptake. This allows them to escape into the host cell cytosol where they replicate before subsequently infecting neighboring cells (Ray et al., 2009). In response to this diversity of manipulating strategies, host cells initiate immune defenses in order to restrict or clear intracellular infections (Tam and Jacques, 2014). One prominent immune effector mechanism counteracting intracellular pathogens is xenophagy, an autophagic flux that mediates the delivery of invading organisms to bactericidal lysosomes (Levine, 2005).

Autophagy (Greek for “to eat oneself”) is an evolutionarily highly conserved eukaryotic mechanism that maintains cellular homeostasis by removing protein aggregates and damaged organelles. The process also delivers essential anabolic nutrients through the degradation of proteins and other macromolecules in response to nutrient deprivation

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http://dx.doi.org/10.1016/j.ijmm.2017.10.012
Received 12 June 2017; Received in revised form 24 October 2017; Accepted 29 October 2017

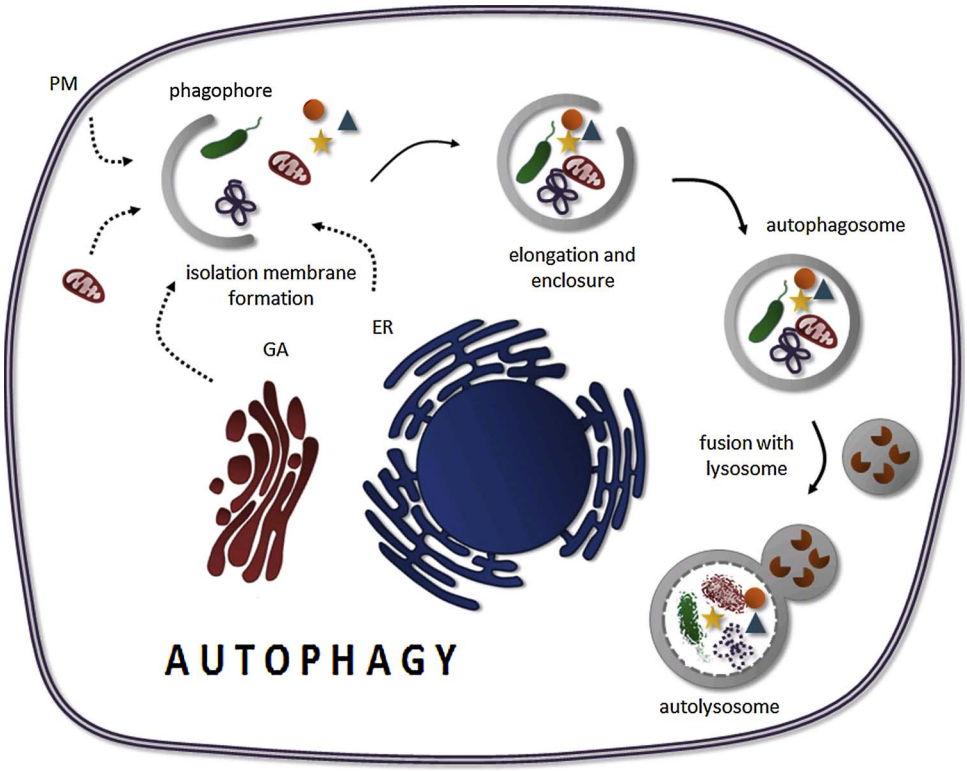

Fig. 1. General mechanism of autophagy and autophagic flux. Once autophagy is initiated an isolation membrane called the phagophore forms. The phagophore is typically derived from membranes of the endoplasmic reticulum (ER) or the Golgi apparatus (GA), but can also originate from membranes of mitochondria or the plasma membrane. Next, the phagophore elongates and enwraps the cargo, which is to be degraded. This cargo can include portions of cytoplasm, mitochondria or other organelles, invading pathogens, as well as misfolded or aggregated proteins. Finally, the mature autophagosome is built through closure of the double membrane. During autophagic flux the autophagosome fuses with lysosomes, resulting in the interior organellar content and the autophagosomal membrane are being degraded.

(Levine and Kroemer, 2008; Mizushima and Komatsu, 2011; Ravikumar et al., 2010) (Fig. 1). Besides starvation, it is highly induced under various conditions including hypoxia, oxidative stress, and radiation. Thus, autophagy plays a cytoprotective role during stress situations as it maintains cellular anabolic processes and ATP levels by degrading damaged cellular components that otherwise could be toxic to cells (Levine et al., 2011; Yang and Klionsky, 2010). Autophagy can be divided into three types: chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy. While macroautophagy involves the sequestration of intracellular components, such as polypeptides, organelles, intracellular aggregates, as well as pathogens, during microautophagy entire regions of cytosol are captured into vesicular compartments (Fig. 1). During macroautophagy, the cargo-carrying vesicles fuse with lysosomes for degradation, while microautophagy involves the direct uptake of cytoplasmic materials into the lysosome itself via invagination of lysosomal membranes (Mortimore et al., 1988) (Fig. 1). CMA differs from the other forms and drives the heat-shock cognate protein (HSC70)-mediated degradation of a selective subset of cytosolic proteins in lysosomes (Dice, 2007). Macroautophagy and microautophagy can happen as non-selective or selective processes. During non-selective autophagy, a portion of cytoplasm is engulfed randomly, e.g. in response to amino acid deprivation. In contrast, selective macroautophagy functions to specifically degrade organelles such as mitochondria and peroxisomes via mitophagy and pexophagy, respectively, and also involves the specific destruction of microorganisms via xenophagy.

However, regardless of selectivity the characteristic feature of macroautophagy (hereafter referred to as autophagy) is the formation of a double-membraned compartment, called the autophagosome, wherein cytoplasmic structures are sequestered for destruction. Autophagosome biogenesis can be divided into three steps: i) initiation, ii) membrane nucleation, and iii) vesicle elongation (Mizushima and Komatsu, 2011). This requires the coordinated involvement of proteins encoded by autophagy-related genes (ATGs).

The autophagic process initiates with the formation of an isolation membrane called the phagophore. This phagophore typically originates from the endoplasmic reticulum (ER), but can also derive from the Golgi apparatus, mitochondrial or plasma membrane (Lamb et al., 2013; Hayashi-Nishino et al., 2009; Hailey et al., 2010; Levine et al., 2011). The phagophore grows, enwraps portions of the cytoplasm, and subsequently builds the mature autophagosome through closure of the double membrane (Fig. 1). Finally, mature autophagosomes fuse with lysosomes to form autolysosomes. Inside these compartments, lysosomal enzymes such as hydrolases degrade the interior organellar content together with the inner autophagosomal membrane (Fujita et al., 2008; Mizushima et al., 2001). In contrast to non-selective autophagy, selective autophagy has as an additional step – cargo selection –, which is mediated by autophagy receptors and adaptor proteins.

For many years autophagy was thought to be a non-selective process, degrading bulk portions of cytoplasm including whole organelles during conditions of nutrient deprivation. However, recently several types of selective autophagy have been uncovered and today even the randomness of this process under starvation conditions is controversial (Gomes et al., 2011; Kristensen et al., 2008). Autophagy can selectively degrade aberrant proteins, dysfunctional organelles, lipids, and invading pathogens. According to the substrate target, the process is classified as aggrephagy (protein aggregates) (Bjorkoy et al., 2005; Pankiv et al., 2007), mitophagy (mitochondria) (Geisler et al., 2010; Novak et al., 2010), pexophagy (peroxisomes) (Iwata et al., 2006; Kim et al., 2008), reticulophagy (endoplasmic reticulum) (Bernales et al., 2006), ribophagy (ribosomes) (Kraft et al., 2008), glycophagy (glycogen) (Jiang et al., 2011), zymophagy (zymogen granules) (Grasso et al., 2011), lipophagy (lipid droplets) (Singh et al., 2009), or xenophagy (bacteria, viruses, and protozoa) (Levine, 2005). Because autophagy is an intracellular quality control mechanism, it must be able to distinguish between healthy and anomalous cellular components, such as damaged organelles, protein aggregates, or invading pathogens. For this selective process, the recognition of pathogen-associated molecular patterns (PAMPs) and damage signals by autophagy receptors is crucial. So far, five main cargo receptors have been identified including p62, also known as sequestosome-1 (SQSTM1), its paralog NBR1 (neighbor of BRCA1 gene 1), NDP52 (nuclear dot protein 52 kDa), its paralog