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Autophagy and inflammasomes

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ABSTRACT

Autophagy is a ubiquitous cellular mechanism for the targeted lysosomal degradation of various cytosolic constituents, from proteins to organelles. As an essential homeostatic mechanism, autophagy is upregulated in response to numerous environmental and pharmacological stimuli, including starvation, where it facilitates the recycling of essential amino acids. In addition, autophagy plays specific roles within the immune system; it serves as a source of peptides for antigen presentation, a mechanism for the engulfment and degradation of intracellular pathogens and as a key regulator of inflammatory cytokines. In particular, autophagy has been shown to play a number of roles in regulating inflammasome activation, from the removal of inflammasome-activating endogenous signals, to the sequestration and degradation of inflammasome components. Autophagy also plays a role in determining the fate of IL-1β, which is concentrated in autophagosomes. This review discusses a growing body of literature that suggests autophagy is a critical regulator of inflammasome activation and the subsequent release of IL-1 family cytokines.

1. Introduction

Autophagy, specifically macroautophagy, is an intracellular homeostatic mechanism for the delivery of cytosolic constituents, including organelles, to lysosomes for degradation and amino acid recycling. Autophagy begins with the formation of a phagophore, a membranous structure that elongates to engulf cytoplasmic cargo, formation of an autophagosome with a double membrane. The autophagosome, similar to many other endocytic compartments, can then fuse with lysosomes resulting in degradation of the cargo (Mizushima et al., 2011) (Fig. 1). Autophagy is regulated by many proteins involved in endosome/phagosome biogenesis, and also by a wide range of proteins that are the products of autophagy-related genes (Atg), including Atg8 (LC3), which is commonly used as a marker for visualizing and quantitating autophagosomes (Klionsky et al., 2016). As an important homeostatic mechanism for the sequestration and degradation/recycling of cytosolic components, autophagy is up-regulated in response to amino acid starvation and more specifically in response to damaged organelles, including mitochondria (mitophagy) (Lazarou, 2015).

2. Autophagy and immunity

Interest in autophagy in immunity was largely ignited by a number of studies that demonstrated roles for autophagy in the response of phagocytes to intracellular pathogens, including *Legionella pneumophila* (Swanson and Isberg, 1995), *Porphyromonas gingivalis* (Dorn et al., 2001), *Coxiella burnetti* (Beron et al., 2002), *Chlamydia trachomatis* (Al-Younes et al., 2004), *Mycobacterium tuberculosis* (Gutierrez et al., 2004), Epstein Barr virus (Paludan et al., 2005), *Shigella flexneri* (Ogawa et al., 2005), *Salmonella typhimurium* (Birmingham et al., 2006) and *Toxoplasma gondii* (Ling et al., 2006). In many instances, autophagy appears to act as a direct intracellular killing mechanism, directing pathogen-containing phagosomes to lysosomes for degradation, while in others autophagosomes may be targeted by pathogens for intracellular survival. In addition, autophagosomes can connect with the MHC Class I and II pathways for presentation of endogenous and exogenous antigens (Munz, 2016). Importantly, autophagy regulates – and is regulated by – a wide range of cytokines (Harris, 2011). Moreover, an ever-growing number of studies have demon-

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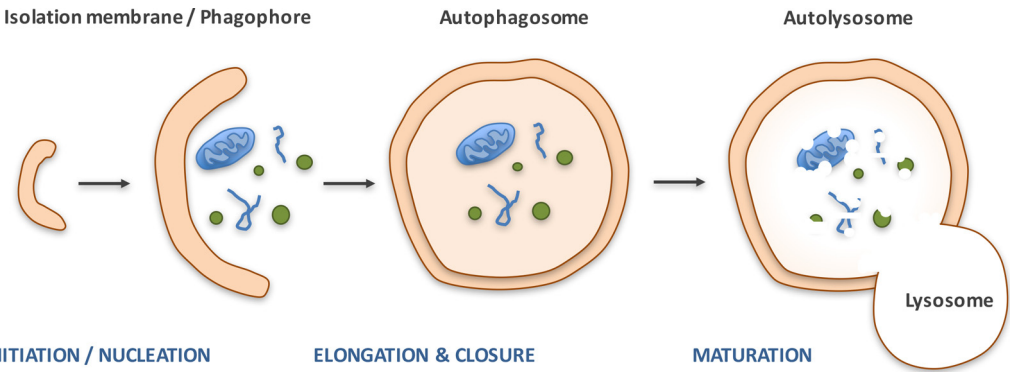

Fig. 1. Autophagosome biogenesis. Autophagy begins with the formation of a phagophore, or isolation membrane, (nucleation), which elongates and fuses with itself to capture cytosolic cargo in an autophagosome, in either a non-specific or targeted manner. The autophagosome can then fuse with lysosomes to form an autolysosome, or autophagolysosome (often referred to as maturation), in which the sequestered material is degraded and ultimately recycled.

strated multiple roles for autophagy in regulating inflammasome activation and controlling the production, processing and secretion of IL-1 family cytokines (Harris, 2013).

3. Autophagy regulates inflammasome activation and IL-1 family cytokines

3.1. IL-1 family cytokines and inflammasomes

The IL-1 family includes IL-1α (IL-1F1), IL-1β (IL-1F2), IL-18 (IL-1F4), IL-33 (IL-1F11), IL-37 (IL-1F7), IL-38 (IL-1F10) and IL-1 receptor antagonist (IL-1Ra; IL-1F3). IL-1α, IL-1β and IL-1Ra all bind the same receptor (IL-1R1); both IL-1α and IL-1β share similar biological activities, while IL-1Ra, which does not activate downstream signaling, acts as a competitive inhibitor of IL-1α and IL-1β (Garlanda et al., 2013). The secretion of IL-1β and IL-18 is typically a two-stage process. First, transcription and translation of the inactive precursors is required, commonly induced following ligation of PRRs, particularly Toll-like receptors (TLRs). Pro-IL-1 and pro-IL-18 then require cleavage into the bioactive mature cytokines, a process usually reliant on activation of caspase-1 by an inflammasome.

Inflammasomes are multi-protein complexes that activate caspase-1 either directly (canonical inflammasome) or indirectly, via caspase-4/5 (caspase-11 in mice; non-canonical inflammasome) in response to pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) (Guo et al., 2015; Vigano et al., 2015). These complexes contain a pattern recognition receptor, including members of the nucleotide-binding domain, leucine-rich repeat containing proteins (NOD-like receptors, NLRs), such as NLRP1, NLRP3 and NLRC4, or absent in melanoma 2 (AIM2)-like receptors (ALRs). In most cases, the NLR or ALR engages apoptosis-associated speck-like protein containing a caspase activation and recruitment domain (ASC), leading to recruitment and activation of caspase-1. Caspase-1 then cleaves pro-IL-1β or pro-IL-18 into the mature bioactive cytokines.

IL-1 family cytokines are key regulators of innate and adaptive immune responses (Afonina et al., 2015; Garlanda et al., 2013). However, IL-1α, IL-1β and IL-18 are also potent pyrogens that play a pathological role in inflammation if released in an uncontrolled manner for extended periods of time. For example, NLRP3-dependent IL-1β release, driven by monosodium urate (MSU) crystals in the joints, is responsible for pathology in gout (Gonzalez, 2012). Similarly, gain-of-function mutations in the *NLRP3* (*CIAS1*) gene are associated with cryopyrin-associated periodic syndromes (CAPS), including familial cold autoinflammatory syndrome (FCAS), neonatal-onset multisystem inflammatory disease (NOMID) and Muckle-Wells syndrom (MWS) (Dode et al., 2002; Hoffman et al., 2001). Moreover, NLRP3 activation has been linked to many other diseases, including type II diabetes, obesity-induced insulin resistance and some cancers (Menu and Vince, 2011). Thus, balanced regulation of inflammasome activation and IL-1 family cytokine release is critical for maintaining healthy immune responses to many pathogenic stimuli, whilst avoiding inappropriate chronic inflammation that can lead to autoinflammatory pathologies.

3.2. Autophagy and inflammasome activation

Numerous studies have shown that loss or impairment of autophagy in macrophages and dendritic cells can lead to hyper-secretion of IL-1α, IL-1β and IL-18. The first of these studies to explicitly highlight the link between autophagy and IL-1 family cytokines demonstrated that loss of the autophagy-related protein ATG16L1 in mouse fetal liver macrophages resulted in hyper-secretion of IL-1β in response to TLR3/TLR4 stimulation (Saitoh et al., 2008). This effect was dependent on mitochondria-derived reactive oxygen species (ROS), potassium efflux, caspase-1 and Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF). A subsequent study confirmed this dependency on caspase-1 and TRIF, but also identified a significant role for NLRP3 and demonstrated that MyD88 signaling was not required (Harris et al., 2011).

Previous genome-wide association studies had identified *ATG16L1* as a candidate gene responsible for susceptibility to Crohn's disease (Hampe et al., 2007; Prescott et al., 2007; Rioux et al., 2007), so these links between autophagy and IL-1β secretion suggested a potential mechanism. In a model of dextran sulphate sodium-induced colitis, chimeric mice lacking *Atg16l1* in heamatopoeitic cells died by day 10, while all wild-type mice survived. The *Atg16l1* deficient mice also showed increased weight loss, greater gut inflammation and increased serum IL-1β and IL-6 (Saitoh et al., 2008). Moreover, injection of antibodies against both IL-1β and IL-18 conferred significant protection in the *Atg16L1* deficient mice.

In these studies, increased IL-1β secretion by autophagy-deficient cells occurs in response to TLR3 or TLR4 agonists, but in the absence of a secondary exogenous inflammasome activator. This suggests that autophagy regulates one or more endogenous inflammasome activators that are released (or recognised) in a TRIF-dependent manner (Fig. 2). It should be noted, however, that how TRIF is involved in this process is yet to be elucidated. Regardless, one such endogenous activator is ROS, released from damaged or dysfunctional mitochondria.

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