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Role of autophagy in IL-1 β export and release from cells

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Summary

The autophagy pathway known also as macroautophagy (herein referred to as autophagy) is characterized by the formation of double-membrane organelles that capture cytosolic material. Based on pathway termination alternatives, autophagy has been divided into degradative and secretory. During degradative autophagy, autophagosomes typically fuse with lysosomes upon which the sequestered material is degraded. During secretory autophagy, instead of degradation the sequestered cargo is subjected to active secretion or passive release. In this review, we focus on the mechanisms of secretion/passive release of the potent pro-inflammatory cytokine IL-1 β , as a prototypical leaderless cytosolic protein cargo studied in the context of secretory autophagy.

Keywords

Secretion; Autophagy; pyroptosis; IL-1; TRIM16; GSDMD

Introduction

Macroautophagy, hereafter referred as autophagy, is a complex membrane biogenesis and cargo vectorial-transport process regulated by autophagy-related (ATG) proteins. Typically, double-membrane organelles, called autophagosomes [reviewed in ref. (1)] fuse with lysosomes and degrade the captured material. The canonical autophagy process is inducible by nutritional conditions (e.g. amino acid and glucose starvation) and a variety of stress signals [reviewed in ref. (2)]. Its initiation rests upon activation of three systems: (i) the

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Disclosure of Potential Conflicts of Interest

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protein kinase ULK complexes including constituents such as FIP200; (ii) the lipid kinase VPS34 complexes including components such as BECLIN1 generating phosphatidylinositol 3-phosphate (PI3P) on membranes; and (iii), lipidation (lipid conjugation at the C-terminus) of mammalian ATG8 proteins (e.g. LC3) executed by the E3 ligase ATG12-ATG5-ATG16L1. The LC3 conjugation is a hallmark of autophagosome formation, and its conjugation machinery is linked to ULK complex via direct FIP200-ATG16L1 interactions (3–6), whereas PI3P generated by VPS34 is recognized by WIPI2, which in turn binds ATG16L1. Thus, the elements of the three complexes and their products are interconnected. Furthermore, the ATG12-ATG5-ATG16L1 E3 ligase promotes lipidation of the ATG8 family members, participating in production of closed double-membrane autophagosomes, which acquire syntaxins (STX) necessary for subsequent steps, e.g. STX17 for fusion with lysosomes and cargo degradation in autolysosomes (7, 8). Recent studies [reviewed in ref. (9)] have however shown that autophagy process may branch and avoid degradative end, by favoring cargo secretion.

Conventional protein secretion is the route used by proteins containing at their N terminus a leader peptide, allowing them to enter ER lumen and be transported from the endoplasmic reticulum (ER) to the Golgi apparatus and subsequently to the plasma membrane via secretory vesicles or granules. Nevertheless, cytosolic proteins that lack leader peptides can be secreted using one of several unconventional protein secretion (UPS) pathways (10). These include extracellular vesicle (EV) release (microvesicles and exosomes) (11, 12). Furthermore, three additional types have been recognized: Type I UPS of direct cargo translocation across the plasma membrane. Type II UPS occurs through ABC transporters. Type III UPS is a broad category of delivery to the lumen of membrane-bound organelles (secretory lysosomes, secretory autophagosomes, or other specialized compartments, such as “compartment for unconventional protein secretion/CUPS” described in yeast) that eventually fuse with the plasma membrane (13).

In mammalian cells, the pro-inflammatory cytokine interleukin (IL)-1 β is a well-known example of unconventionally secreted leaderless proteins with important extracellular functions. It has been reported in 1990 that despite not having a leader peptide, cytosolic IL-1 β is secreted from activated monocytes (14, 15). IL-1 β is synthesized as a pro-form (pro-IL-1 β). Upon inflammasome activation, pro-IL-1 β is cleaved by caspase-1 to produce a mature IL-1 β (mIL-1 β), ready to be secreted (16–18). Interestingly, IL-1 β has been shown to be secreted by at least two mechanisms. One is secretory autophagy (19–21) and the other one is Type I-like/gasdermin mediated pore formation (22–24).

In this review, we will focus on the above proposed mechanisms involved in secretion of IL-1 β , with the emphasis on secretory autophagy.

1. Evidence for IL-1 β autophagy based secretion

Starved macrophages during inflammasome activation have been reported to secrete IL-1 β in an autophagy-dependent manner (19), albeit it was not explored in that study how IL-1 β manages to be secreted instead of being degraded in autophagic organelles. Additional studies have extended this work, showing that IL-1 β secretion pathway is affected by the

autophagy process (20, 21). Autophagy induction by starvation or pharmacological drugs promotes inflammasome-dependent IL-1 β secretion as well as the secretion of IL-18 and HMGB1, which belong to the category of leaderless proteins that can be found in the cytosol (19). Stimulation of autophagy concomitantly with inflammasome agonists such as nigericin, alum or silica strongly enhanced IL-1 β secretion, in a process depending on Atg5. Furthermore, intracellular IL-1 β colocalize with LC3 puncta indicating the intersection between the autophagy process and secretion of IL-1 β .

Secretion of IL-1 β from cytoplasmic IL-1 β ⁺LC3B⁺ profiles (19) is controlled by Rab8A, a small GTPase that regulates transport of intracellular vesicles to plasma membrane for fusion, and GRASP55, a Golgi reassembly stacking protein. This is interesting because another Type III UPS cargo, the leaderless protein AcbA/1 from Dictyostelium and yeast, also depends on a GRASP family member (25, 26) even though the membrane compartment mediating Acb1 release is not a conventional autophagosome but has been referred to as CUPS [(27) (Cruz Garcia, this issue, 2018)]. Of note, several unconventionally trafficked integral membrane proteins are affected by GRASP family proteins: CFTR (28), Integrin PS1 (29), and Peripherin/rds (30). Of these, only CFTR trafficking to the plasma membrane has been linked to the process of autophagy (28).

However, these findings that autophagy genes are required for IL-1 β release raise two questions: The first is how IL-1 β could be released in a soluble form in the extracellular medium. The second relates to the mechanisms of recognition and delivery of IL-1 β to autophagic organelles and subsequent steps in secretion across the plasma membrane. These two questions are addressed below.

2. Chaperone-dependent import of IL-1 β into the autophagosomal intermembrane space provides a mechanism for the release of soluble IL-1 β in the extracellular medium

One mechanism that would ensure the release of soluble mIL-1 β is its translocation into the lumen of a precursor membrane compartment. Reconstitution of mIL-1 β secretion in nonmacrophage cells co-expressing pro-IL-1 β and pro-caspase-1 have shown that mIL-1 β is indeed unfolded and translocated across a membrane organelle in a manner that depends on the chaperone protein Heat Shock Protein HSP90 (Fig. 1A) (20). This membrane compartment appears to be a phagophore that progress into a closed autophagosome. HSP90 appears important for the unfolding and translocation of mIL-1 β into a vesicle intermediate that will progress into an autophagosome and deliver mIL-1 β to plasma membrane for secretion (20). This process is dependent on two linear amino acid motifs (but different from chaperone-mediated sequence KFERQ) within the IL-1 β protein sequence, which allow mIL-1 β to bind HSP90 (20). This way, mIL-1 β that translocates into autophagosomes is confined in the intermembrane space between the inner and the outer membrane of the double membrane autophagosome (20). This allows, when the autophagosome outer membrane fuse with the plasma membrane, for free mIL-1 β to be released from intermembrane space to outside of the cell.

3. TRIM16: a prototype receptor for secretory autophagy that interacts with the SNARE SEC22b

Are there mechanisms of recognition and delivery of IL-1 β to autophagic organelles and what are the subsequent steps in secretion across the plasma membrane? The discovery of TRIM16, a member of the tripartite motif (TRIM) family that functions as the first specialized receptor for secretory autophagy starts to shed light on this question (21).

Using yeast two-hybrid screen TRIM16 (alternative name: estrogen-responsive B Box protein) has been shown to directly interact with proIL-1 β . TRIM16 was found to be concomitantly released in the extracellular space with IL-1 β , but its function has remained obscure until recently (31).

The notion that TRIM16 acts as a specialized receptor involved in unconventional secretion of IL-1 β came from Kimura et al. (21). Indeed, TRIM16 binds to mAtg8s (GABARAP) (32) and mobilizes the core autophagy regulators ATG16L1, ULK1 and Beclin1 in response to damaged endomembranes (see below) (33). Strikingly, TRIM16 co-recruits its secretory autophagy cargo (e.g. mIL-1 β) and ATG proteins involved in the phagophore formation. Thus, TRIM16 links the autophagy apparatus with the recognition of cytosolic mIL-1 β .

In addition to binding mAtg8s (32), TRIM16 regulates IL-1 β secretion by interacting first with galectin-8 upon lysosomal damage. Lysosomal damage has been used as a trigger to study IL-1 β secretion. Lysosomal damage is caused by silica, monosodium urate crystals, and polymer precursors, such as Leu-Leu-OMe (LLOMe).

LLOMe accumulates in lysosomes and bursts its membranes, a process that activates both autophagy and inflammasome (34, 35). This leads to the cleavage of proIL-1 β into mIL-1 β . Galectin-8 recognizes damaged lysosomes and coalesces on them, which in turn brings its binding partner TRIM16 to the damaged lysosomes (21). As TRIM16 also associates with mIL-1 β , the complex TRIM16/Galectin8/IL1B is next transferred to a pool of R-SNARE SEC22b-containing membranes destined to deliver IL-1 β outside of the cell. This occurs through TRIM16, which contains a SNARE/longin-like domain interacting with the R-SNARE SEC22b, known primarily to be on ERGIC membranes and to play a role in ER-Golgi anterograde and retrograde transport (36, 37). The ERGIC membranes have also been recently recognized to support the formation of small vesicles active in LC3 lipidation upon autophagy induction (38).

The TRIM16-SEC22b interaction allows the transfer of mIL-1 β to LC3-positive membranes that will later form autophagosomal profiles, which do not fuse with lysosomes. This is reflected in the finding that mIL-1 β secretion is not affected by knockdown of STX17 (21), a SNARE required for the maturation of autophagosomes into autolysosomes (7). Instead, SEC22b interacts with plasma membrane syntaxins, syntaxin-3 (STX3) and syntaxin-4 (STX4) (39, 40) leading to mIL-1 β secretion. The secretory autophagosomal membrane fuses with plasma membrane via a SNARE fusion complex, including a 4-helix bundle composed of the R-SNARE SEC22b, a Qa-SNARE STX3 or STX4 and Qbc SNAREs SNAP-23 or SNAP-29 (Fig. 1B). This process could either allow the capture of mIL-1 β

inside the autophagosome lumen using TRIM16 as a receptor/adaptor but is also compatible with the process described by Zhang et al. (20), as TRIM16 colocalize with HSP90 upon lysosomal damage (21), with HSP90 playing a role in translocation of mIL-1 β across autophagosome membranes.

In addition to IL-1 β , TRIM16 and its partners are also needed for the unconventional secretion of ferritin (21), highlighting the broad implications of TRIM16 in secretory autophagy. This suggest that the first known secretory-autophagy receptor TRIM16 may not only be involved in the recruitment of mIL-1 β or ferritin to membranes but also in their transfer to Sec22b-containing LC3-positive membranes (38) that can fuse with or tether at the plasma membrane for secretion.

4. Gasdermin-dependent mechanism of IL-1 β export by hyperactivated macrophages

Pro-inflammatory stimuli that activate inflammasome are strong triggers of IL-1 β secretion by macrophages as a part of inflammatory responses. The release of IL-1 β has been shown to be mediated by Gasdermin D (GSDMD), which either act as a membrane pore facilitating export of IL-1 β across the plasma membrane (41) without causing full cell lysis (Fig. 1C) or causes hyper-permeabilisation of the plasma membrane (42) that requires pore forming GSDMD, suggested to represent a form of Type I UPS (22–24). The formation of Gasdermin D membrane pores was shown to depend on the phosphoinositide PI(4,5)P₂ (43), potentially linking this type of IL-1 β secretion to the classical examples of Type I UPS of FGF2 [(44) (Steringer and Nickel, this issue)] and HIV-TAT [(45); Beaumelle and colleagues, 2018, this issue)], both being shown to form self-made membrane pores in a PI(4,5)P₂ dependent manner.

Gasdermin D regulates pyroptosis, a form of programmed cell death caused by noncanonical activation via caspase-11 (in mice) or –4, –5 (in humans) induced directly via PAMPs (Pathogen-associated molecular patterns), such as LPS when they penetrate into the cytosol. Non-canonically activated caspase-11, –4 and –5 or canonically via inflammasome-activated caspase-1 cleave the cytosolic GSDMD to produce a N-terminal fragment, which oligomerizes and somehow translocates to the plasma membrane where it generates pores that cause cell death, lysis and mIL-1 β release [(22, 23) and reviewed in ref. (46)].

Studies by Kayagaki et al. (22) have shown that pyroptosis and IL-1 β processing can be partially separated, and that GSDMD KO mice show reduced IL-1 β release during early time points following canonical inflammasome activation, an effect that is lost or diminished at later time points. These studies have posited that GSDMD may play a role in plasma membrane damage and release of IL-1 β or in regulation of an IL-1 β secretion mechanism. Recent studies have confirmed a role for GSDMD in mIL-1 β secretion (41). This process appears to be at least partially independent of the pyroptotic cell death and is not a simple indiscriminate release of mIL-1 β during wholesale membrane disruption. GSDMD can serve as a conduit for secretion of IL-1 β family members, mIL-1 β and IL-18, from hyperactivated macrophages (Fig. 1C)(41).

This process seems to be active in still-alive cells displaying mitochondrial and phagocytic activity in the face of inflammasome assembly usually associated with cell death. Careful experiments in vitro and in vivo by Evavold et al (41) have shown that GSDMD pores preferentially release IL-1 β before cell death. This pathway may be a parallel or even more dominant pathway in activated macrophages.

Given how closely autophagy and inflammasome are co-regulated (47), it is possible that the gasdermin pore-dependent release of IL-1 β intersects with the secretory autophagy pathway. This is not known but poses interesting questions that should be explored. For example, autophagic membranes may affect N-terminal fragment GSDMD trafficking within the cell, e.g. affecting intracellular pools of heterogeneous membranes of unknown nature in which GSDMD N-terminal domain is found after GSDMD activation/cleavage en route to plasma membrane (43). However, it is also possible that GSDMD N-terminal fragment pore vs. secretory autophagy mechanisms of IL-1 β release are cell type dependent. It also remains to be seen whether eventual transition to cell death caused by GSDMD N-terminal fragment in activated macrophages (22, 23, 43, 48, 49) simply results in indiscriminate release of the cytoplasmic content.

Concluding remarks

Secretory autophagy of IL-1 β follows the route and membrane intermediates that likely do not fuse with lysosomes but instead are delivered directly to plasma membrane. Beyond IL-1 β secretion, a wide array of cargo varying in size and composition can be released from cells via different types of secretory autophagy (Table I). These processes include engagement of various trafficking routes and some secretory autophagosomes may fuse with organelles of the endosomal-lysosomal pathway such as Multivesicular bodies (MVBs), endosomes or lysosomes before secretion of its content via exocytosis [reviewed in ref. (50)]. The mechanisms involved in different secretory autophagy processes are an area of future investigations, and what at present may seem like a collection of phenomena may prove to be a well-tuned pathway used for cell-to-cell signaling, cytoplasmic homeostasis by expelling aggregates, organelles or microbes instead of following the route for degradative autophagy and potentially overwhelm the lysosomal capacity. Continuing studies in this area are needed to define and delineate secretory autophagy pathways and to establish the biological outputs that are linked to these processes.

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Abbreviations

Acb1	AcetylCoA binding protein 1
ATG	Autophagy-related
CUPS	Compartment for Unconventional Protein Secretion
EV	Extracellular Vesicle

GRASP	Golgi reassembly stacking protein
GSDMD	gasdermin D
HSP	Heat Shock Protein
IL-1β	Interleukin 1 β
LLOMe	Leu-Leu-OMe
LPS	lipopolysaccharide
mIL-1β	mature-interleukin 1 β
PAMP	Pathogen-associated molecular pattern
STX	syntaxin
TRIM	tripartite motif

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Figure 1. Proposed mechanisms of unconventional IL-1 β secretion

A. Chaperone (HSP90) mediated translocation of mature IL-1 β into LC3-positive profiles with subsequent exocytosis at plasma membrane. Note that IL-1 β in this model remains in the intermembrane space within the double membrane autophagosomes and that upon putative fusion at the plasma membrane IL-1 β is free to diffuse.

B. Lysosomal damage (a physiological inducer of inflammasome) activates canonical inflammasome and processing of pro-IL-1 β into mature IL-1 β (see details in panel C) after which mIL-1 β is captured by the secretory autophagy receptor TRIM16. TRIM16 has four relevant features: (i) it recognizes local lysosomal damage via its binding to Galectin-8

(which in turn recognizes exposure of luminal glycoconjugates to cytosol following membrane damage); (ii) it binds to mIL-1 β and thus collect its after inflammasome activation; (iii) it associates with mammalian Atg8s (mAtg8s) such as GABARAP; and (iv) it binds via its SNC1/longin hybrid SNARE-like domain to the R-SNARE Sec22b on ERGIC and LC3⁺ membranes. The complex also contains HSP90 providing a potential link to the chaperone mediated translocation mechanism depicted in panel A (denoted by number 1). The Sec22b- TRIM16 complex with mIL-1 β on LC3/mAtg8-positive vesicles can lead to luminal sequestration of mIL-1 β into double membrane autophagosomes once a phagophore forms and closes (net result denoted by number 2) with Sec22b forming 4-helix bundle SNARE complexes with Qa (STX3 or STX4) and Qbc (SNAP23 or SNAP29) SNAREs at the plasma membrane allowing exocytosis and secretion (denoted by number 3). Alternatively or in addition, Sec22b, previously described as being able to tether membranes to plasma membrane without fusion, could position/concentrate TRIM16-mIL-1 β complexes at the plasma membrane for pore secretion/translocation/release mechanisms such as those described in model C.

C. A gasdermin (GSDMD)-based model of IL-1 β release. Non-canonical inflammasome activation associated with pyroptotic cell death following recognition of microbial products such as LPS when present directly in the cytoplasm, is mediated via Caspases 4, 5 and 11. These capsases process GSDMD to release GSDMD N-terminal domain/fragment (GSDMD-NT), which assembles into complexes/pores on cytoplasmic of still unknown nature, and GSDMD-NT pores are delivered to the plasma membrane causing plasma membrane permeability and pyroptotic cell death. Recent data indicate that GSDMD-NT at the plasma membrane can act independently of cell death and indiscriminate plasma membrane permeability (depicted below dashed line) by selectively exporting IL-1 β from hyperactivated macrophages that are still alive into the extracellular milieu (depicted above the dashed line). This mechanism can also apply to canonical inflammasome activation, as caspase-1 (activated through conventional inflammasome mechanisms, e.g. as in panel B) can also process GSDMD. Note: Whereas the three mechanisms depicted in A-C may appear independent, the role of autophagy is implicated in all of them starting with the regulation of inflammasome activation and ending with known (e.g. HSP90 and Sec22b in A and B) or potential overlaps between B and C discussed in the text.

Table I

Cargo linked to secretory autophagy

Type of cargo	Reported cargo and references
Leaderless cytosolic proteins	Annexin 1 (51), Ferritin (21), Galectin-3 (51), HMGB1 (19, 52), mLL-1 β (19–21), IL-18 (19), Tubulin (51), AcbA/1 (25, 26)
Molecules with signal peptide	Cathepsin K (53), CCL2 (54), CFS1 (54), CSF2 (54), DKK3 (55), FAM3C (55), IDE (56), IL-2 (54), IL-6 (57–59), IL-8 (55, 57, 59), IL-27 (54), LIF (55), Lysozyme (60), Mucin (61–63), Von Willebrand Factor (64)
Amino acids	Alanine (65)
Aggregates	α -synuclein (66), amyloid β peptide (67)
Organelles, organellar components	Autophagosomes (68), Autophagolysosomes (56, 69), ER (70), Golgi (70), Lysosomes containing ATP (71), Mitochondria (72, 73), Internalized plasma membrane domains (70)
Vesicles	Exosomes (74)
Bacteria	<i>B. abortus</i> (75), <i>M. marinum</i> (76)
Viruses	Coxsackievirus (77), Epstein Barr Virus (EBV) (78, 79), Hepatitis C virus (HCV) (80–82), Influenza A virus (83), Morbillivirus (84), Poliovirus (85–87), Varicella Zoster Virus (VZV) (88, 89)