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Autophagy as an innate immunity paradigm: expanding the scope and repertoire of pattern recognition receptors

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Abstract

Autophagy is rapidly developing into a new immunological paradigm. The latest links now include overlaps between autophagy and innate immune signaling via TBK-1 and IKKα/β, and the role of autophagy in inflammation directed by the inflammasome. Autophagy's innate immunity connections include responses to pathogen and damage associated molecular patterns including alarming such as HMGB1 and IL-1β, Toll-like receptors, Nod-like receptors including NLRC4, NLRP3 and NLRP4, and RIG-I-like receptors. Autophagic adaptors referred to as SLRs (sequestosome 1/p62-like receptors) are themselves a category of pattern recognition receptors. SLRs empower autophagy to eliminate intracellular microbes by direct capture and by facilitating generation and delivery of antimicrobial peptides, and also serve as inflammatory signaling platforms. SLRs contribute to autophagic control of intracellular microbes, including *Mycobacterium tuberculosis*, *Salmonella*, *Listeria*, *Shigella*, HIV-1 and Sindbis viruses, but act as double edged sword and contribute to inflammation and cell death. Autophagy roles in innate immunity continue to expand vertically and laterally, and now include antimicrobial function downstream of vitamin D3 action in tuberculosis and AIDS. Recent data expand the connections between immunity related GTPases and autophagy to include not only IRGM but also several members of the Gbp (guanlyate-binding proteins) family. The efficacy with which autophagy handles microbes, microbial products and sterile endogenous irritants governs whether the outcome will be with suppression of or with excess inflammation, the latter reflected in human diseases that have strong inflammatory components including tuberculosis and Crohn's disease.

Introduction

The *sensu stricto* autophagy (often referred to as macroautophagy) is a ubiquitous eukaryotic process dependent on Atg factors and internal membrane formation in the cytoplasm that form unique organelles called autophagosomes [1]. Autophagosomes capture diverse cytoplasmic cargo with a variety of end purposes: (a) quality control of disused or defunct organelles such as irreversibly depolarized or leaky mitochondria; (b) removal of toxic macromolecular aggregates too large for handling by smaller capacity or single-molecule-handling proteolytic systems of the cell (e.g. proteasome); (c) digestion of bulk cytoplasm expressly to replenish amino acids and energy during starvation or growth factor

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withdrawal; (d) acting on or in concert with the molecular machineries and organelles at the interface between cell survival and cell death; and (e) controlling and acting as an effector or a regulator of innate and adaptive immunity and inflammation, which is the focus of this review.

One of the first reviews on this topic, written half a decade ago by the author [2] referred to several studies that have appeared at the time, suggesting rather gingerly a potential role for autophagy in immunity. Five to six years later, the immunological roles of autophagy have become one of the better established physiological functions of autophagy, as summarized in a number of recent comprehensive reviews [3-5]. The anti-inflammatory role of autophagy and its anti-microbial action [4] have not remained “unnoticed” by prospective pathogenic microbes in the process of their adaptation to a potentially susceptible host. Many highly successful pathogens have evolved mechanisms to counter or harness autophagy attesting to the significance of autophagy as a bona fide antimicrobial defense [5]. Autophagy roles in immunity have been established through in vitro, ex vivo, and in vivo models [4,5]. Importantly, immunological autophagy, via a variety of cellular and molecular mechanisms [6-8], shows unequivocal genetic links to inflammatory bowel Crohn's disease [9] and tuberculosis (reviewed in [5]). The genetically established role of autophagy in human immunity is arguably the most critical evidence for the health relevance of immunological autophagy [5,9].

The present review reflects the author's bias that the innate immunity role of autophagy is not just another physiological application of autophagy but is a key evolutionary driver and mainstream effector-regulator of autophagy that may have shaped several facets of this fundamental biological process. Another underlying theme of this opinion article is to consider autophagic adaptors [10], termed in the immunological context as **s**equestosome 1/p62-**l**ike **r**eceptors (SLRs) [5], as another category of pattern recognition receptors (PRRs) on par with Toll-like receptors (TLRs), Nod-like receptros (NLRs), and RIG-I-like receptors (RLRs).

Autophagy as an intracellular membrane trafficking pathway with potential links to inflammatory platforms

Autophagosomes are believed to emerge at least in part from the ER membranes [11] via an ER cradle model, with the autophagic phagophore (isolation membrane) being formed from or in the vicinity of a PI3P-possitive (albeit PI3P is atypical for the ER) and DFCP-positive transient omegasome structures (reviewed in [1]) (Fig. 1). This likely occurs with participation of additional compartments and organelles supplying either assembly/signaling platforms, phospholipids, or membrane intermediates including those originating from the biosynthetic secretory pathway [12-14], plasma membrane [15], and ER-mitochondria contact sites [16]. The processes of autophagosomal formation and autophagic flux are under the control of three systems [1]: (i) The first system represents the well known protein conjugation systems (Atg5-Atg12-Atg16L1 and Atg7-Atg3-Atg8/LC3/GABARAP) culminating in the C-terminal lipidation of Atg8 paralogs with phosphatidylethanolamine resulting in, for example, LC3-II, the emblematic marker of mammalian autophagosomes [1]. (ii) The second of these systems is centered upon the Ser/Thr kinase Atg1 (mammalian Ulk1 and Ulk2) controlled by the upstream nutritional regulator mTOR and, perhaps equally if not more importantly, directly by the cellular energy charge sensor and regulator AMPK [17]. (iii) The third system is centered around Atg6 (Beclin 1), which interacts with many proteins including the evolutionarily ancient Class III PI3K Vps34 [18].

Ulk1/2 and one of the Beclin 1 interactors, Atg14L, mark the sites for the initiation of autophagosome formation on the ER [1]. Significantly, these sites on the ER in mammalian

cells receive very early on, at the Ulk1 recruitment step, the autophagic adaptor proteins sequestosome 1/p62 and NBR1 [19]. NBR1 and p62 will be featured in other parts of this review as we explore links with innate immunity and inflammation. These adaptors have been primarily and exceptionally well characterized in the autophagic capture of diverse intracellular cargo [10], but will be considered here in the context of their immunological function (along with several other members) as SLRs (Fig. 2). These two archetypal SLRs are located at the very nidus of autophagosome formation in mammalian cells even before their engagement in cargo recognition and capture [19]. Of note, SLRs have been studied independently of autophagy as complex inflammatory signaling platforms independently of their role as autophagic adaptors [20,21]. This physically connects inflammatory signaling to the very initiation steps of the autophagic pathway. We propose that potential for inflammation may be intimately linked to the earliest stages of autophagy inception in mammalian cells.

Convergence of pro-inflammatory, immune, and physiological signaling pathways in control of autophagy

Much of the innate immunity signaling occurs through or involves the members of the IκB family of kinases (IKK), which fall into two categories – canonical (IKKα, IKKβ) and IKK-related kinases (IKKε and TBK-1). TBK-1, a key regulator of type I interferon response to viral infections, has been shown to play a role in autophagy [22]. TBK-1 phosphorylates optenurin, a TBK-1-interactor analogous to NEMO (IKK-γ; which serves as a platform for the canonical IKK complex). The phosphorylated optineurin, acting as an SLR, assists through its LC3-interacting region (LIR) in the autophagic uptake and elimination of *Salmonella* [22].

The canonical IKKs, IKKα and IKKβ turned out to participate in transducing the classical signal for autophagy induction – starvation [23,24]. This signaling is not based on NF-κB [23,24]. It appears that IKK induces autophagy in response to starvation by engaging 4 systems [23]: IKK-dependent depletion of cytosolic p53 (cytosolic p53 suppresses autophagy); IKK pathway-associated AMPK activation, likely engaging TAK1 signaling upstream of IKK since TAK1 is a known kinase activating AMPK [25], with AMPK directly phosphorylating and activating Ulk1 [17] and inhibiting mTOR complex 1 via Raptor and TSC2; and IKK-sponsored activation of JNK-1, the details of which are not known, with JNK-1 then phosphorylating Bcl-2 to release Beclin 1 (reviewed in [18]). These relationships between autophagy and IKK signaling underscore the links between autophagy, innate immunity, and inflammatory signaling.

Autophagy and conventional PRRs: TLRs

Continuing along with the theme of connections between autophagy and innate immunity responses, autophagy pathway and machinery shows physical, signaling, and regulatory interactions with pattern recognition receptors (PRR), such as Toll-like receptors (TLR), RIG-I like receptors (RLR), Nod-like receptors (NLR), and inflammasomes [5] (Fig. 3).

TLRs were historically the first class of PRRs to be connected with autophagy [5]. Autophagy is induced by signaling from TLRs as recently summarized or discussed [3-5]. Importantly, the details of how TLRs connect to autophagy have been elegantly delineated [26]. TLR4 triggers immunological autophagy via TRAF6 E3 ligase that ubiquitinates Beclin 1, followed by Bcl-2 dissociation from the BH3 domain of Beclin 1 [26]. Incidentally, processes following induction by NF-κB downstream of TRAF6 may lead to increase in A20 deubiquitinating enzyme [26], potentially explaining the reported

sporadically negative results with TLR stimulation and autophagy induction as well as a the initially observed negative role of NF-κB on autophagy (reviewed in [3]).

Autophagy and NLRs

Connections between NLR signaling and autophagy (Fig. 3) exist in species from *Drosophila* to mammals (reviewed in [5]). Murine Nod1 and Nod2 have been reported to interact with Atg16L1 and may modulate autophagy in the context of Crohn's disease by several mechanisms [4] including influencing the localization of Atg16L1 at the point of microbial entry at the plasma membrane [27]. Intriguingly, this independently supports the model of plasma membrane as a source of Atg16L1 vesicular precursors leading to LC3-positive autophagosomal profiles [15]. Polymorphisms in the *ATG16L1* gene have been linked with predisposition to Crohn's disease mammals with the caveat of a low penetrance of the risk allele ATG16L1*300A. This has been explained in a recent study indicating that phenotypic expression of ATG16L1*300A depends on an “immunological trifecta”: genetic predisposition, endogenous flora in the gut, and viral infection [6]. The reader is directed to a recent review in which these aspects have been extensively analyzed [4].

In contrast to Nod2, NLRC4 (a.k.a. Ipaf) and NLRP4 have inhibitory effects on autophagy [28]. They both inhibit autophagic initiation, whereas NLRP4 additionally inhibits autophagic maturation [28]. Both NLRC4 and NLRP4 interact with Beclin 1 complexes apparently via their NACHT nucleotide-binding domains interacting with ECD (evolutionarily conserved domain) of Beclin 1. In addition, NLRP4 shows some affinity for VPS class C proteins (recall that VPS class C factors play a role in endosomal and autophagosomal maturation). NLRP1, NLRP10, and importantly NLRP3 (a key component of the principal form of inflammasome reacting to diverse agonist), also interact with Beclin 1 but the functional consequences of these interactions are yet to be reported [28]. Interestingly, NALP4 is found together with Beclin 1 in large cytoplasmic macromolecular complexes (500-700 kDa) [28], which is of further interest given that NLRC4-, NLRP1 and NLRP3 form inflammasome complexes in that size range. The emerging interrelationships between the inflammasome and autophagy will be treated in a separate section below.

Autophagy and RLRs

RLRs connections with autophagy (Fig. 3) are notable for the usual emphasis in published reports on the negative regulation of RLR signaling by autophagy including autophagy factors Atg5-Atg12 and Atg9 (reviewed in [3]). Atg9 has been reported to negatively regulate trafficking, assembly and activation of TBK-1 in type I interferon response elicited by intracellular double stranded DNA (dsDNA) [3]. However, in the study showing that Atg9 negatively regulates assembly of STING (stimulator of IFN genes), TBK-1, and interferon responses, the core autophagy conjugation system did not follow this pattern, since Atg7 and Atg16L1 knockout MEFs were not derepressed for type I interferon response to dsDNA and the STING puncta assembled normally in the absence of Atg7 and Atg16L1 [29]. As a forerunner of yet to be fully explored positive interactions between RLRs and autophagy, it has been demonstrated that RLRs can activate autophagy with biologically significant effects [30]. In these experiments, dsRNA (polyinosine-polycytidylic acid) in conjunction with MDA-5, an RLR, induced autophagy. It is worth noting here that there is much room for expansion of the standalone role of autophagy upon RLR agonist stimulation.

Autophagic adaptors SLRs, as a new class of PRRs, link autophagy and innate immunity signaling

Autophagic targets in the cytoplasm, ranging in nature, size, and complexity from protein aggregates to whole organelles are recognized and collected by proteins that act as autophagic adaptors [10]. The two principal adaptors, p62 and NBR1 (Fig. 2), have been well characterized and show domain and sequence similarity [10]. These autophagic adaptors have been termed SLRs [5] to emphasize that they represent a new family of innate immunity receptors, as another category of PRRs engaged in recognition and capture of intracellular microbes. Broadly defined, SLRs feature a cargo recognition and capture domain, contain an LC3 interacting region (LIR) [31-34], and invariably contain additional protein interaction modules that cause or participate in inflammatory processes [21,22,32,35]. This latter feature of SLRs has the potential to trigger inflammatory signaling, perhaps concomitantly with microbial target capture or when the process of autophagic elimination does not proceed smoothly [21,22,32,34,35].

The intracellular pathogen recognition and capture occurs in the case of *Salmonella* via multiple SLRs (p62, NDP52, optineurin) [22,33,35] recognizing either classical or branched ubiquitin chains in association with or in the vicinity of cytosolic salmonellae. Although ubiquitin binding domains of different SLRs involved may recognize different ubiquitin chains (Fig. 2), it is possible that they act along the same pathway, involving cooperation of p62, NDP52 and optineurin, albeit p62 domains on cytosolic bacteria appears to be physically segregated from the NDP52/optineurin domains [22,33,35]. Other microorganism utilizing these SLRs and parts of this pathway are *Shigella* via p62 [32] and NDP52 [36], Streptococci via NDP52 [35], *Listeria* via p62 [31] and NDP52 [36] and possibly Sindbis virus via p62 [34]. Through one or more LIRs, SLRs loaded with their cargo connect to nascent autophagosome [22,31-35]. SLRs' ability to recognize microbial targets is controlled by kinases, as recently revealed in the case of TBK-1-dependent phosphorylation of a Ser residue juxtaposed to the LIR domain of optineurin (Fig. 2); this modification enhances both optineurin binding to LC3 and autophagic capture of *Salmonella* via a pathway that involves optineurin along with p62 and NDP52 [22]. Although it seems that microbial targets are predominantly earmarked for autophagy by ubiquitin tags recognized by SLRs, additional lipid signals or tags (e.g. diacylglycerol) have been invoked in these processes with *Salmonella* [37].

SLRs act as dual-function innate immunity devices. As described above, SLRs endow autophagic machinery with the capacity to find its microbial targets in the cytoplasm, whereas they can via proinflammatory signaling induce other innate immunity mechanisms of the cell (e.g. p62 via TRAF6; Fig. 2). Since autophagic adaptors are consumed during autophagy, their proinflammatory signaling should be reduced when autophagy and microbial elimination proceed efficiently. However, if autophagic clearance of microbial targets does not proceed efficiently, the innate immunity signaling from SLRs and their interacting components (e.g. in the case of p62, TRAF6 and atypical PKC, as reviewed in [21]) feature may serve to expand the scope of pathogen containment by summoning other cellular and tissue innate immunity mechanisms.

The above and additional features of SLRs, e.g. that p62 can promote NF-kB induction and caspase-8 aggregation (Fig. 2), activation and cell death (reviewed in [21]), need to be further explored in the context of infection. We envision that SLRs have the potential to act as multi-stage regulators capable of orchestrating gradually escalating levels of innate defenses with the ultimate purpose of limiting microbial spread albeit at increasing inflammatory damage cost. Important examples of this duality are the role of p62 in elimination and/or inflammatory signaling with *Shigella* [32] and Sindbis virus [34]. The

duality of SLR functions in pathogen clearance and pro-inflammatory signaling may further be reflected in pathologies and NBR1's potential relationship to asthma [20]. NBR1 along with p62 plays a role in bone formation and remodeling [38,39], whereas mutations in the *SQSTM* gene encoding p62 are associated with activation of NF-κB in Paget's disease of the bone. NBR1 is also important in NFATc1 activation required for Th2 differentiation [20]. The association between TRIM5α and p62 in the context of HIV-1 infection [40] may engender yet to be explored inflammatory effects and autophagy connections. The elements outlined above primarily based on p62 and partially on NBR1, may nevertheless apply to other SLRs since for example NDP52 and optineurin link up with TBK-1 [22,35], and should depend in principle on the presence or absence of domains enabling interactions with proinflammatory signaling partners.

SLRs, antimicrobial peptides, and vitamin D3

SLRs can also act in a completely different manner to promote autophagic killing of intracellular microbes. In the case of p62, this SLR can collect cytoplasmic precursors to be converted in autolysosomes into neo-antimicrobial products [41]. The antimicrobial peptides generated in autolysosomes or autophagolysosomes can be derived by limited proteolysis from cytosolic proteins such as ubiquitin [42] and ribosomal proteins [41]. Thus, autophagolysosomes can acquire additional microbicidal properties by converting innocuous cytoplasmic proteins into products that have been shown to kill *M. tuberculosis* [41,42] and likely will apply to other microbes.

There are also indications of a relationship between autophagy and conventional antimicrobial peptides, i.e. cathelicidin (LL-37) [43] an antimycobacterial and antiviral peptide that is derived by conventional proteolysis from larger precursors. Although the details of the interplay between cathelicidin and autophagy are not clear at present (published work indicates that cathelicidin affects autophagy and not the other way around [43]), this is of interest both in the context of bacterial (tuberculosis) [43] and viral (HIV-1) [44] infections. Cathelicidin expression and its antimycobacterial action are induced by vitamin D3 [43], a known activator of autophagy. Significantly, vitamin D receptor gene variations are well know to predispose to tuberculosis in human populations, whereas low vitamin D3 levels are found in individuals with AIDS.

In the context of HIV-1 infection, the interplay between autophagy, vitamin D3 and cathelicidin appears to be complex. Cathelicidin has been shown to boost anti-HIV-1 properties of blood mononuclear cells but it failed to induce autophagy [44]. Thus, at least in the context of HIV-1 the published work indicates that cathelicidin may not be upstream of autophagic processes. Vitamin D3-induced autophagy nevertheless suppressed HIV-1 [44] in keeping with prior observations that autophagy induction can inhibit HIV-1 in macrophages [45]. As a postscript to these considerations, it should be noted that autophagy is countered by HIV-1 proteins Nef [45], which interferes with autophagic maturation, and Env [46], which interferes with autophagy initiation. In this context, it is of potential interest that some autophagy-activating agonists, such as vitamin D3, can bypass the inhibitory effects of HIV-1 Nef and Env on autophagy [44].

DAMP signaling and autophagy

Alarmins or damage associated molecular patterns (DAMP) represent a number of diverse cellular components that undergo a change in their intracellular localization or are released from damaged cells serving as reporters of a need to contain cell or tissue injury under sterile or septic conditions. DAMPs induce autophagy [47,48]. HMGB1, an alarmin [49], undergoes a stepwise displacement from the nucleus (where it is a chromatin component) into the cytoplasm with eventual extracellular release, at each stage inducing autophagy

thorough intracytoplasmic events or extracellular receptor signaling [48]. As HMGB1 translocates from the nucleus to the cytoplasm it displaces Bcl-2 from Beclin 1 thus inducing autophagy [48]. As damage continues and HMGB1 ends up being released extracellularly, HMGB1 action widens to a tissue level where it can act both in an autocrine and paracrine fashion via the RAGE receptor and induce autophagy or cell death, an outcome modulated by a number of factors including HMGB1 oxidation state [47]. In addition to HMGB1, DAMPs such as ATP, IL-1β, and DNA complexes are known to induce autophagy (reviewed in [5]).

Basal autophagy prevents spurious inflammasome activation

Autophagy and inflammasome show complex functional interactions (Fig. 3). DAMPs such as ATP that signal to induce K^+ efflux, pore-forming toxins and ionophores (e.g. streptolysin O and nigericin), structurally diverse particulates such as silica, alum and asbestos, salt precipitates calcium pyrophosphate dehydrate and monosodium urate (linked to gout), and protein aggregates associated with inflammatory pathologies such as fibrillar amyloid β (Amβ), can activate inflammasome. These inflammasome agonists can induce potassium fluxes, ROS, and/or cathepsin B leakage from lysosomal compartments and activate inflammasomes leading to release of IL-1β, a key pro-inflammatory cytokine that in turn can induce autophagy [26,50].

The basal autophagy, at rates when cells are not exogenously stimulated, prevents spurious activation of inflammasome by ROS and DNA released from unkempt mitochondria or through additional mechanisms [51-53]. Leaky or depolarized mitochondria are continuously removed by mitophagy, a form of housekeeping autophagy acting in all cells at all times. If this basal autophagy is impaired, ROS and mitochondrial DNA cause unscheduled inflammasome activation [51,52]. Where might the negative/suppressive control of inflammasome by autophagy discussed above be of relevance for a known disease? The connections between autophagy and IL-1β could be of relevance for Crohn's disease. For example, the Atg16L1 mouse model of inflammatory colitis shows elevated IL-1β (reviewed in [3]). However, issues concerning the role of autophagy in Crohn's disease are complex, and may not involve only the inflammasome or even not primarily be expressed through inflammasome. For example, altered responses to multiple pro-inflammatory inputs are likely to be at play [4,6].

Is autophagy's role in inflammation limited to CD? This is unlikely. To take the central nervous system as one example, IL-1β levels are increased both in the brains and the serum of Alzheimer's patients and both neurons and glia have the capacity to activate inflammasome (with microglia relying on NLRP3 and neurons on NLRP1); both cell types are being subject to Amβ (an inflammasome agonist) action, with potential cell death outcomes known as pyroptosis (caspase 1-dependent “apoptosis-with-inflammation”) or pyronecrosis (caspase 1-independent cell death) in neurons and microglia, respectively. It should be explored whether autophagy prevents spurious inflammasome activation in the sterile environment of the nervous tissues, of potential relevance for Alzheimer's disease and other neurodegenerative disorders with inflammatory components. When the CNS is not sterile, e.g. in HIV-associated dementia, autophagy has been implicated in pathology-inducing relationship between the HIV-infected glia and HIV-uninfected neurons [54]. This was shown in an SIV encephalitis as a model and in brains of individuals with HIV-associated dementia, suggesting that retrovirally infected microglia produces mediators that suppress autophagy in neurons and negate is neuroprotective role [54]. This area deserves more study.

Induced autophagy enhances inflammasome output

In contrast to the anti-inflammatory effects of basal autophagy, which suppresses unscheduled inflammasome activation [51-53], induced autophagy promotes IL-1β secretion [55]. This turned out to be related to the fundamental issue of how IL-1β gets secreted outside of the cell. IL-1β is a cytosolic protein without a leader peptide and thus cannot utilize the conventional secretory pathway (export via ER lumen, Golgi, post-Golgi carriers, and their fusion with plasma membrane). It has now been shown [55] that IL-1β, following inflammasome activation and processing of pro-IL-1β into mature IL-1β by caspase-1, utilizes the autophagy-based unconventional secretion pathway to exit the cytosol into the extracellular milieu where it acts in pro-inflammatory signaling. Significantly, the autophagy-based unconventional secretion pathway contributes to secretion of other alarmins, e.g. HMGB1, from the cells [55].

Other innate immunity systems and autophagy: IRGM and Gbp

In the context of inflammatory bowel disease, IRGM, a human autophagy factor and a member of the family of immunity related GTPases (IRG), has been genetically linked with Crohn's disease as a locus of polymorphisms associated with increased risk [9]. IRGM is also a risk locus for tuberculosis in human populations (reviewed in [5]). IRGM is important for physiologically (starvation) pharmacologically (rapamycin), and immunologically (IFN-γ) induced autophagy [8]. *IRGM* impacts autophagic maturation [7,56] and exerts its effects on autophagy via mitochondria where it binds to cardiolpin [8] (Fig. 5). Autophagy links with the immunity related GTPases such as IRGM have now been expanded to interferon-induced guanlyate-binding proteins Gbp1, Gbp2 and Gbp7, until recently orphaned for function [42,56]. The details of Gbp connections to autophagy include Gbp1 interaction with an SLR, p62/sequestosome 1, whereas Gbp7 acts in concert with Atg4 [42].

Concluding remarks

Autophagy is intimately intertwined with nearly all aspects of innate immunity, attesting to the contention that immunological functions are one of autophagy's mainstream roles. In this opinion article we have focused on the interrelationship between autophagy and conventional innate immunity systems including TLRs, RLRs, NLRs, and inflammasomes. Additionally, we have ascribed a PRR function to the autophagic adaptors (SLRs), such as p62/sequestosome 1, NBR1, NDP52, and optineurin, characterized by the presence of LC3-interacting region, ability to recognize and target for autophagy the invading intracellular microbes, and propensity to connect with proinflammatory signaling. A common theme emerges whereby different PRRs, now including a new category, the SLRs, coordinate cell-autonomous capture of intracellular microbes with other aspects of innate immunity responses. We propose that, among the PRRs, what sets apart the SLRs is that they have a unique capability of executing their antimicrobial function in full without having to trigger inflammation, but can switch to a pro-inflammatory mode when needed (Fig. 5). If the autophagic clearance of microbes fails, SLRs can trigger pro-inflammatory signaling at cellular and tissue levels to recruit additional forces and limit the spread of infection. In keeping with the dual anti-inflammatory and pro-inflammatory capabilities of autophagy as an innate immunity mechanism, basal autophagy keeps inflammasomes from being spuriously activated by intracellular triggers, but induced autophagy can augment inflammasome-dependent processing and secretion of potent pro-inflammatory DAMPs/alarmins IL-1β and HMGB1.

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Highlights

- Autophagy is a developing immunological paradigm with vertical and lateral connections in innate immunity
- Sequestosome 1/p62-like adaptors (SLRs) serve both as autophagic adaptors and innate immunity signaling platforms
- Balance between autophagic clearance via SLRs and their and other inflammatory signaling determines degree of collateral inflammatory damage
- Interactions between autophagy and inflammasome involve both suppression of inflammasome activation and enhancement through the autophagy-based unconventional secretion of inflammasome substrates

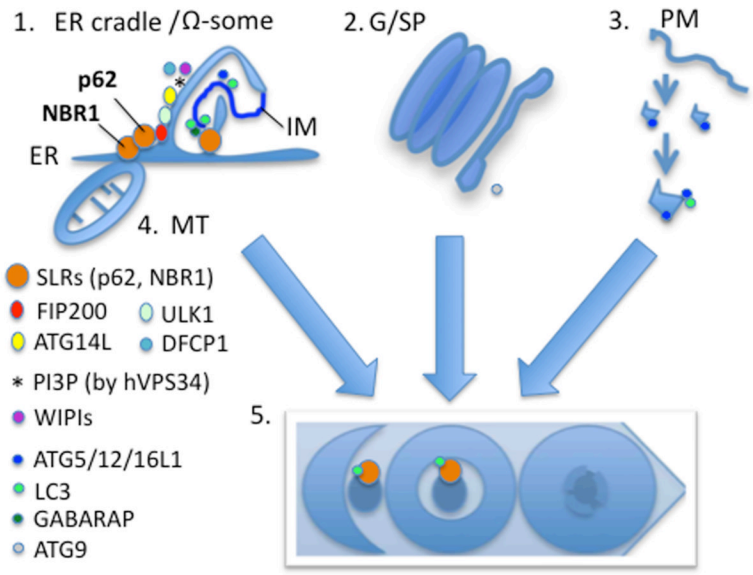

Fig. 1. Autophagy pathway

Four major contributors proposed as the source of autophagic membrane: 1) ER, endoplasmic reticulum; 2) G/SP, Golgi/secretory pathway; 3) PM, plasma membrane; 4) MT, mitochondria. 5) Autophagic pathway has been stylized into a logo flow chart (crescent, initiation/phagophore formation; double membrane, early/nondegradative autophagosome; single membrane with dense content, degradative autolysosome). SLRs, sequestosome 1/p6-lke receptors. PI3P, phosphatidylinositol 3-phosphate.

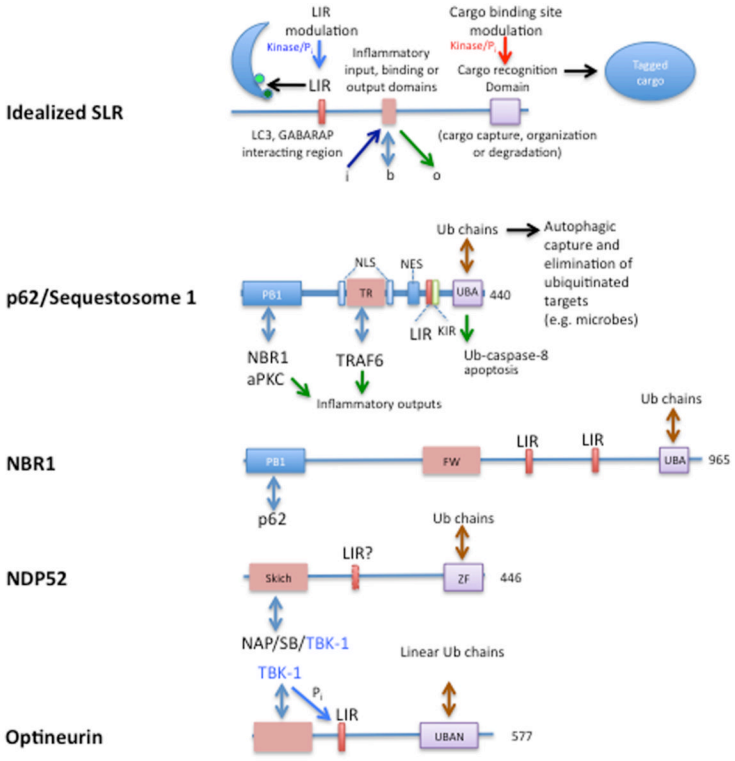

Fig. 2. SLRs, sequestome 1/p62-like receptors

Top, idealized SLR represents no particular member of the group, but is depicted in functional terms. LIR, LC3-interacting region allows an SLR to connect with autophagic organelles via Atg8 paralogs (LC3s and GABARAPs). Cargo recognition domain, so far based on ubiquitin binding: UBA (Ub-associated) and UBAN domains. The inflammatory input, binding, or output domain depicts the ability of SLRs to interact with proinflammatory factors (e.g. TRAF6, TBK-1), and receive signals, e.g. phosphorylation by protein kinases modifying SLR functions (e.g. TBK-1 phosphorylates a Ser residue juxtaposed to the LIR domain core motif (W/FXXL) improving its ability to associate with LC3, or a kinase modifying ability of the UBA domain of p62 to bind different ubiquitin chain subsets. FW, four tryptophans domain. KIR, Keap1-interacting region. NES, nuclear export signal. NLS, nuclear localization signal. PB1, protein-protein interaction (hetero- and homo-oligomerization) domain. ZF, zinc finger domain.

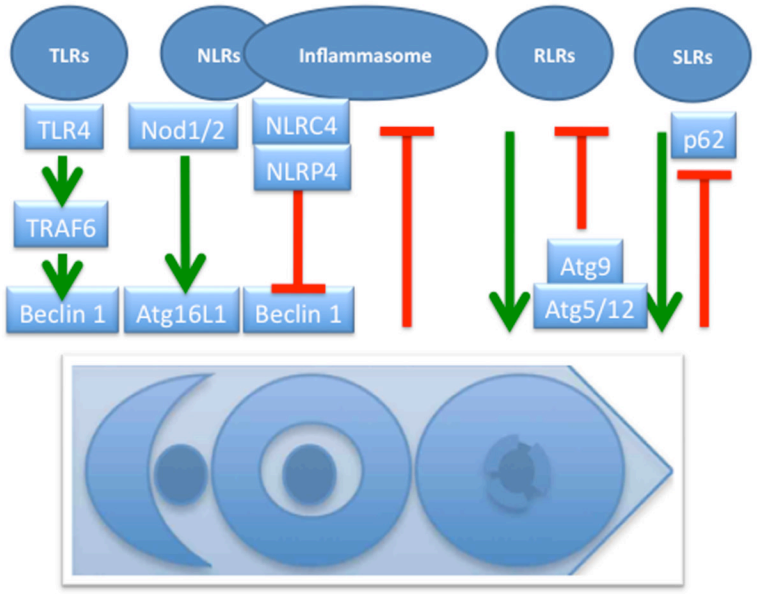

Fig. 3. Pattern recognition receptors, inflammasomes, and autophagy

NLRs, Nod-like receptors. RLRs, RIG-I-like receptors. SLRs, Sequestome 1/p62-like receptors. TLRs, Toll-like receptors. All are collectively referred to as pattern recognition receptors (PRRs). Positive regulation of autophagy by PRRs can occur via TRAF6 downstream of TLR4 (TRAF6 ubiquitinates Beclin 1 and releases it from inhibitory complexes with Bcl-2), or via direct interactions with Atg factors (e.g. Nod1/2-Atg16L1). Negative regulation of inflammasome by autophagy is presently ascribed to autophagic prevention of formation of the endogenous sources of inflammasome agonists (e.g. reactive oxygen species or DNA released from unkempt mitochondria). Atg5-Atg12 can negatively regulate RLR signaling by binding and interfering with RLR signaling complex formation (e.g. Atg5-Atg12 conjugate binding to VISA/IPS-1/Cardif/MAVS located on mitochondria). SLRs as autophagic adaptors play a positive role in autophagic elimination, whereas autophagy degrades (and thus downregulates) SLRs in the process of autophagy.

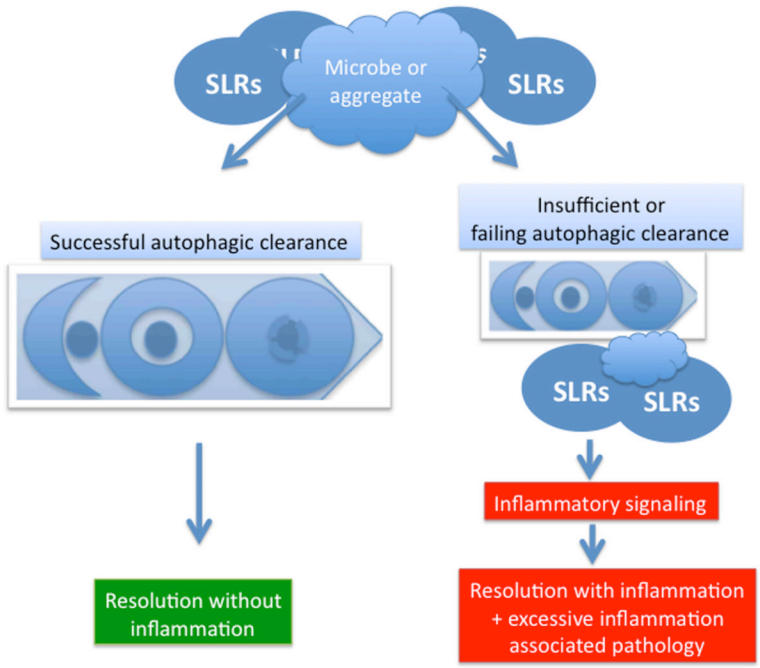

Fig. 4. A model of autophagic clearance with and without inflammation

Efficacy of autophagic elimination of insults (microbes, endogenous and exogenous particulate irritants such as inflammasome agonists) and SLRs dictates clearance of insult and resolution with and without inflammation. Left, efficacious clearance of insult along with removal of attached SLRs prevents inflammation. A stalled or inefficient autophagic removal of the insult (aggregates, microbes, etc) may lead to accumulation of SLRs and signaling through their pro-inflammatory partners. A realistic process in vivo falls somewhere within the spectrum between the two extremes, and may be skewed by direct or indirect pathological inputs and genetics.