Published in final edited form as:

Curr Opin Gastroenterol. 2009 November ; 25(6): 512–520. doi:10.1097/MOG.0b013e32833104f1.

Autophagy, Immunity and Human Disease

Robert J. Heath^{a,b} and Ramnik J. Xavier^{a,b,c}

^aCenter for Computational and Integrative Biology, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114, USA

^bGastrointestinal Unit, Center for Inflammatory Bowel Disease, Massachusetts General Hospital, Harvard Medical School, 55 Fruit Street, Boston, MA 02114, USA

^cThe Broad Institute of MIT and Harvard, 7 Cambridge Center, Cambridge, MA 02142, USA

Abstract

Purpose of review—To give an overview of autophagy and its effects on innate, and adaptive immunity and touch on some of the roles of autophagy in disease.

Recent findings—Precise regulation of autophagy is necessary to maintain metabolic equilibrium, immune homeostasis, delineate cell fate and influence host cell responses to cytosolic pathogens. A growing number of studies have implicated that inactivation of autophagy selective responses contributes to inflammatory disorders, neurodegeneration and cancer but, the precise steps at which disease associated ATG genes affects autophagy pathways is unknown at present.

Summary—In Eukaryotic cells autophagy is constitutively active at low levels, while significant up-regulation occurs in response to a multitude of stresses. Autophagy has achieved notoriety as a perturbed biological process in many disease states and an exponential increase of studies attribute roles for autophagy in innate and adaptive immunity. Understanding how individual disease associated ATG genes function will lead to a better understanding of, and potentially novel therapies for treating the diseases in which they are involved.

Keywords

Autophagy; Crohn's disease; innate immunity; adaptive immunity

Introduction

Autophagy is a cytoplasmic homeostasis pathway; permitting cells to digest their own cytosol, remove protein aggregates, intracellular microbes and defective organelles. Autophagy is involved in special types of cell functions such as waste disposal during states of mitosis and senescence, exocytosis of Paneth cell granules [1], and Atg proteins have recently been implicated in microbe vacuole responses [2]. GWAS studies have implicated autophagy in numerous disease states and recent studies have revealed that ATG5 is associated with systemic lupus erythematosus (SLE) and ATG16L1 and IRGM are associated with Crohn's disease (CD).

Correspondence should be addressed to: Robert J. Heath or Ramnik J. Xavier, Center for Computational and Integrative Biology, Massachusetts General Hospital, Richard B. Simches Research Center, 185 Cambridge Street, 7th Floor, Boston, MA 02114, USA; rheath@ccib.mgh.harvard.edu, Xavier@molbiol.mgh.harvard.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Building the Autophagosome

To date 31 autophagy-related (ATG) genes have been identified in yeast, with orthologues well conserved throughout eukaryotes. The main regulatory pathway for starvation-induced autophagy is the mTOR (Mammalian Target Of Rapamycin) pathway, which controls cell growth by integrating growth factor signalling, the cellular energy state and a number of other stress signals. Inhibition of mTOR signalling rapidly induces autophagy, which in yeast is mediated by Atg1 and in mammals is believed to involve Ulk1 (Unc-51-like kinase) and Ulk2 [3]. mTOR can form two complexes, mTORC1 and 2, with mTORC1 acting as the nutrient sensing complex responsible for the regulation of ribosome and protein biogenesis and cell growth [4]. Until recently little was known about how this complex regulates autophagy. Two groups [5,6] have shed light on this process. They found that the mammalian Atg13 homologue in complex with ULK1/2 and FIP200 (focal adhesion kinase family-interacting protein of 200kDa) associates with mTORC1 in a nutrient dependent manner. ULK1/2 and Atg13 are phosphorylated by mTOR, which inhibits the activity of ULK1/2. Thus the mTORC1 suppresses autophagy by sequestering the ULK1/2-Atg13-FIP200 complex.

Initiation

Phosphatidylinositol-3OH kinase (PI3K) (VPS34) and Beclin1 (Atg6) containing complexes are important in the nucleation of nascent autophagophores (Fig. 1). The human orthologue of Atg14 (ATG14L) has recently been identified [7]. The Atg14 containing PI3K complex (Atg14, Atg6/Vps30, Vps15 and Vps34) localizes with early membrane puncta preceding other autophagosome markers, such as ATG16L1 [7] and also associates with the ER membrane [8]. This Beclin1/Bcl-2 interaction has recently been shown to negatively regulate autophagy, providing evidence for the links between autophagy and apoptosis [9]. In addition, two positive regulators of autophagy; Ambra1 and UVRAG, can associate with this PI3K complex [10-12]. The UVRAG-Beclin 1-VPS34 complex acts as a promoter of autophagosome maturation, but the same complex with the addition of Rubicon (Rubicon-UVRAG-Beclin1-VPS34), can suppress autophagosome maturation [8,13].

Elongation

Elongation is driven by two protein conjugation systems: ATG16L1 complexed with an Atg5-12 conjugate, which marks the site of initiation and LC3 II, a derivative of LC3 I with a lipidated phosphatidylethanolamine at its C-terminus (Figure 1). The Atg5/12 conjugate associates with Atg16 and localizes to the site of autophagosome formation. In conjunction with the Atg3/7 complex, the Atg5/12/16 complex acts as an E3-like enzyme, driving LC3 I lipidation, which results in LC3 II conversion and elongation of the autophagosome. The lipidated LC3-PE (LC3-II) remains embedded within the lumen of the autophagosome [14].

Recent structural studies of Atg4B and the Atg4B-LC3 complex have revealed the mechanism of LC3 processing and delipidation [15,16]. Atg4B cleaves a C-terminal peptide in LC3 to generate the processed LC3-I. Formation of the ATG4B-LC3 complex causes a large conformational change unmasking the active site of ATG4B. Humans have four homologues of Atg4, but only Atg4B seems capable of efficiently cleaving LC3 precursors and LC3-PE [17,18].

FNBP1L (Formin-binding protein 1 like) has recently been identified as an ATG3 interactor [19], which serves as a BAR domain conferring scaffold function for antibacterial autophagy, but is dispensable for classical autophagy.

Closure and maturation

Closure of the autophagosome through to fusion with the lysosome, are less well understood than the early stages [20]. In addition to its role in elongation of the autophagosomal membrane, LC3 may play an important role in the closure of the forming autophagosome (Fig. 1) [21]. Once the autophagosome has formed it must be moved to the perinuclear region of the cell for fusion with the lysosome, which requires microtubules and dynein [22]. Upon closure of the autophagosome the Atg16 complex rapidly dissociates, LC3 however remains attached. One speculative possibility is that dynein is recruited to the phagosome through an interaction with LC3 once the Atg16 complex has dissociated [20].

Maturation of the autophagosome occurs through fusion of the outer autophagic membrane with the lysosomal membrane resulting in the formation of the autolysosome. The canonical Rab-SNARE system of vacuole-vacuole homotypic fusion is believed to be involved in autophagosome-lysosome fusion, although the exact SNARE remains elusive.

Autophagy and immunity

In vitro, numerous studies have demonstrated functions for autophagy in defence against pathogens including, *Salmonella typhimurium*, *Shigella flexneri*, *Mycobacterium tuberculosis*, group A *Streptococcus* and *Toxoplasma gondii* [23]. The role of autophagy towards mucosal associated commensal microbes is unknown. In most pathogen models studied thus far, autophagy appears to be a protective anti-microbial response. There are, however, exceptions where autophagy is subverted to increase the replication rate of the pathogen or the autophagosome is arrested to form a novel replication niche, as is the case for poliovirus.

The peptidoglycan-recognition protein PRGP-LE is an innate microbial sensor that recognizes bacterial diaminopimelic acid (DAP)-type peptidoglycan. PRGP-LE recognizes cytosolic *L. monocytogenes* and is essential for inducing autophagy-mediated inhibition of bacterial growth, necessary for host survival [24]. PRGP-LE resistance requires Atg5 and acts independently of Toll and IMD pathways. In the case of PRGP-LE cytoplasmic bacteria that have escaped from the endosome are targeted by autophagy. In situations where the pathogen is enclosed within a vesicular structure (i.e. *Salmonella* inside a vacuole) the membrane dynamics are not fully understood. It has been postulated the failure to maintain the vacuolar niche leads to the autophagic targeting and subsequent destruction of *Salmonella Typhimurium*.

Autophagy and Innate Immunity

In addition to the role autophagy plays in restricting the cytoplasmic niche for pathogens, a number of other innate immune processes are enhanced and controlled by autophagy. Capture of a pathogen within an autophagosome and subsequent fusion of the lysosome gives rise to a concentrated pool of pathogen-derived molecules (PAMPs), which are likely to be ligands for immune receptors (Fig. 2) [25]. Many TLR-ligands can cause the induction of autophagy in stimulated cell lines [26], seemingly rendering the cell permissive to infection and, in immune effector cells, provides sustenance to fuel the *de novo* synthesis of pro-inflammatory molecules and anti-microbial pathways (e.g. ROS production) [27]. In addition, TLR signalling may also recruit autophagy proteins to the phagosomal membrane and deliver the phagosome contents to the lysosome [2]. In most cell types in the body, cytosolic sensors of viral replication recognize RNA viruses via the RLR family genes RIG-I and MDA-5 [28]. This is in contrast to plasmacytoid dendritic cells (pDCs), where autophagy plays a significant role in the recognition of ssRNA by delivering viral replication intermediates from the cytosol to endosomes where it engages and activates TLR7 [29]. Tal

et al. [30], have recently shown that mitochondrial-associated ROS plays a role in the positive regulation of RLR signalling. In the absence of Atg5, dysfunctional mitochondria accumulate in cells and an associated increase in ROS localized to mitochondria is seen with an associated increase in RLR signaling. The homeostatic clearance of damaged mitochondria by autophagy is therefore important for inhibiting ROS accumulation and maintaining RLR signalling.

Inflammasome and autophagy

The inflammasome activates caspase-1, leading to the processing and secretion of the pro-inflammatory cytokines interleukin 1 β (IL-1 β) and IL-18. To date three inflammasome complexes have been identified, the composition of which varies depending on the stimulus (Fig. 2). The NLRC4 inflammasome is activated by intracellular flagellin in a TLR dependent manner. A number of aflagellate pathogens (i.e. *Salmonella flexneri* and *M. tuberculosis*) have also been reported to activate the NLRC4 inflammasome [31,32] suggesting a flagellin-independent pathway to activation also exists. The NLRP1-inflammasome is activated by the bacterial cell wall protein and NOD2 ligand, MDP. NLRP3 is activated by a plethora of bacterial ligands including MDP, LPS, bacterial RNA, double stranded RNAs, and the anti-viral compounds R837 and R848 [33,34]. Furthermore, significant associations have been made between SNPs in the regulatory regions of NLRP3, NLRP3 expression and IL-1 β production [35]. Activation of the inflammasome appears to be down-regulated by autophagy. Macrophages from ATG16L1 or Atg5 deficient mice have enhanced IL-1 β responses to TLR4 activation by LPS [36]. Chimeric mice generated from ATG16L1-deficient fetal liver cells injected into irradiated hosts are highly susceptible to DSS-induced colitis. These mice however, do not develop spontaneous colitis and the DSS induced phenotype can be partially rescued by anti-IL-1 β or anti-IL-18 antibodies. Autophagy may therefore sequester and degrade the inflammasome, consequently reducing the output of IL-1 β . In addition there are cell specific feedback loops between autophagy and inflammasome function.

Autophagy and adaptive immunity

Autophagy also plays a role in the adaptive immune response, both towards immune system development, immune education and activation. Autophagy appears to be crucial for development of the T cell repertoire in the thymus [37]. Thymic epithelial cells express a diverse array of otherwise cell specific proteins, exhibit high levels of constitutive autophagy and a robust starvation response [37]. These higher autophagy levels lead to enhanced antigen presentation and an increased diversity of self-antigen presentation to T cells, driving negative selection. Thymic transplantation experiments have shown that selection of a number of MHCII-dependent TCRs, but not MHCI-dependent TCRs requires Atg5. In addition athymic mice with transplanted Atg5^{-/-} thymi develop autoreactive CD4⁺ T cells causing significant autoimmunity, severe colitis, lymphadenopathy and uterine atrophy. It is plausible that abnormal negative selection may be an early trigger in the breakdown of tolerance and colitis susceptibility in patients with Crohn's disease (discussed below).

Antigen presentation

CD4⁺ T cells are MHCII restricted and monitor peptides generated by lysosomal degradation (Fig. 2). CD8⁺ T cells are MHCI restricted and presented antigen generated by proteasomal degradation. Analysis has shown that 20-30% of the natural MHCII-bound peptide array originates from cytosolic and nuclear proteins, some of which result from known autophagy constituents [38]. Almost 50% of autophagosomes merge with MHCII antigen loading compartments [39]. Chemical inhibition and RNA interference of autophagy

components results in the loss of such MHCII presentation, therefore cytosolic peptide uptake is dependent on autophagy. Viral and bacterially derived antigens are presented on MHCII via this method, underscoring its physiological importance [40].

Mitochondria and T-cells

Lymphocytes undergo substantial cytoplasmic and organelle rearrangement during the selection, expansion and contraction of antigen specific clones. Unsurprisingly, autophagy has been found to play an important role in lymphocyte biology. Loss of Atg5 or Atg7 impairs the survival and proliferation of mature T cells *in vivo* [41,42]. Atg5 is also required for the survival of pre-B cells and mature B1 B cells [43]. Transcriptional profiling of Atg5^{-/-} thymocytes shows a profound mitochondrial signature that is borne out as an increased mitochondrial mass in Atg5^{-/-} peripheral T cells [42]. In autophagy compromised T cells (Atg7^{-/-}), this mass change does not occur. Subsequently cells accumulate reactive oxygen species and display imbalances in the expression of pro-and anti-apoptotic proteins. Together these results suggest a role for autophagy in controlling mitochondrial homeostasis, a potentially significant method of protecting the cell from ROS.

Crohn's Disease (CD) and autophagy

Genome-wide association studies have identified a strong link between polymorphisms in two autophagy genes, IRGM and ATG16L1, and susceptibility to Crohn's disease (Table 1) [44,45]. The ATG16L1 risk corresponds to a single amino acid substitution of threonine to alanine (T300A) [44]. Copy number variation in the promoter of IRGM correlates with the identified risk allele [46].

ATG16L1

ATG16L1 and IRGM are essential for the autophagy of intracellular pathogens [44,47]. The risk-and protection-associated variants of ATG16L1 appear equally capable of mediating basal autophagy levels and rescuing starvation/rapamycin induced autophagy after knockdown of endogenous ATG16L1. However, in a *Salmonella Typhimurium* model of infection the risk-associated variant of ATG16L1 is much less capable of mediating anti-bacterial autophagy [48]. ATG16L1 has also been found to be involved in the regulation of cytokine expression in Paneth cells [1]. Microarray profiling of Paneth cells from mice with a hypomorphic ATG16L1 allele (expressing ~ one third the wild type level of ATG16L1), revealed enhanced gene expression of acute-phase reactants, PPAR signalling molecules and adipocytokines [1]. This appears to be a cell-type specific phenotype, as thymocytes from the same mice do not exhibit similar alterations. The mechanism behind these transcriptional changes in Paneth cells is unknown. A second mouse model, expressing a truncated autophagy deficient ATG16L1 protein has an enhanced IL-1 β response in macrophages treated with LPS [36] and chimeric mice engrafted with ATG16L1^{-/-} foetal liver haematopoietic progenitor cells, have a dramatic susceptibility to dextran sodium sulphate (DSS)-induced colitis and associated high serum concentrations of IL-1 β and IL-18. The mechanism for enhanced cytokine secretion remains unknown, but it is possible that autophagy may sequester and degrade the inflammasome, thereby reducing the output of inflammatory cytokines. An alternative idea is that autophagy increases the degradation of inflammasome-activating compounds, such as microbial ligands

IRGM

In mice the interferon-induced Irg family contains 20 proteins, many of which are necessary for IFN- γ mediated resistance to intracellular pathogens [49]. Two IRG orthologues are found in humans, the widely expressed IRGM gene and the testis restricted IRGC gene.

IRGM has a role in the autophagy-mediated destruction of *Mycobacterium bovis BCG* [23,47] and *Salmonella typhimurium*, in an expression dependent manner [46]. In the *M. bovis BCG* model of tuberculosis, IRGM-dependent clearance of bacteria in macrophages is associated with IRGM localization to bacteria-containing compartments. This feature is common to both human IRGM and mouse IRGM1 [23,47].

Single nucleotide polymorphisms (SNPs) close to IRGM have been strongly correlated with Crohn's disease (CD) susceptibility in genome wide association studies (GWAS) [44,50]. A large 20Kb deletion of potential regulatory sequence is in perfect linkage with the functional polymorphisms associated with CD [46]. The regulation of IRGM in humans is complex. Different tissues and cell lines heterozygous for the two haplotypes (CD risk and CD protective) exhibit varying levels of expression of the two alleles. In a cell culture model of *S. typhimurium* infection decreased IRGM expression significantly inhibited the efficiency of anti-bacterial autophagy [46]. It is possible that IRGM is regulated in a cell-type specific manner and that altered expression induced by the CD risk deletion may result in cell specific phenotypes. In addition, IRGM is predicted to be subject to alternative splicing with five isoforms predicted in humans. Expression of these isoforms has not been documented at the protein level. It is possible that they may also contribute to IRGM function in a cell specific manner.

The evolution of the IRG gene family also appears to be rather complex. A number of non-human primates, through significant duplication and diversification events, have evolved up to 21 interferon-regulated genes. Yet humans possess only two, neither of which seems to be regulated by interferon or possesses canonical interferon-responsive elements [51]. Through study of Old World and New World monkeys, it appears that ~40 million years ago, IRGM became non-functional in a common ancestor of prosimian lemurs and was resurrected ~20 million years later in the common ancestor of humans and African great apes [51]. Alterations of the gene expression appear to be retroposon induced. It seems that the locus is continuing to evolve in humans, possibly reflecting our ever-changing relationship with intracellular parasites. The emerging evidence would suggest that dysregulation of autophagy genes may contribute to the pathogenesis of inflammatory bowel disease via several routes. Atg5 mediated removal of autoreactive CD4+ T cells, ATG16L1 mediated endotoxin-induced inflammatory signalling in macrophages and Paneth cell dysfunction through direct and indirect effects on autophagy related lymphocyte homeostasis. Much remains to be done to elucidate the precise mechanisms of ATG16L1 and IRGM polymorphisms in the pathogenesis of Crohn's disease, but autophagy appears to be paramount to mucosal immunity.

Cancer: Apoptosis and senescence

An appropriate response to stress is critical for maintaining healthy tissue and preventing diseases such as cancer. Cells respond to stress by adaptation, repair and recovery, or are diverted into senescence (irreversible cell cycle exit) or apoptosis. Autophagy is a major component of the cellular stress response. When stimulated by stress conditions autophagy is traditionally viewed as a cell survival mechanism, degrading cellular proteins and organelles to suppress damage and maintain metabolism and cellular fitness. Several of the Atg genes also function in tumourigenesis. Recent studies suggest that the stress of oncogene activation induces autophagy required for the establishment of senescence; a form of cell cycle arrest that serves to limit the proliferation of damaged cells. In a model of oncogene-induced. Young et al. found that autophagy is important in regulating the transition into into senescence at a time when a down-regulation of mTOR is also observed. Inhibition of autophagy also delayed senescence and the accumulation of senescence-associated secreted proteins. Thus autophagy is an important component of the senescence

program. The ubiquitin binding protein p62 (SQSTM1) is the hub of interactions between autophagy and the proteasome. p62 co-localizes with protein aggregates and LC3 (Fig. 3) and loss of autophagy results in poly-p62 aggregates that are associated with liver injury and neurodegeneration. Mathew et al. [52] demonstrate that autophagy defective tumour cells accumulate p62, ER chaperones, and protein disulphide isomerases (PDIs) in response to metabolic stress; suggesting that tumourigenesis may be a consequence of defective protein quality control. Moreover autophagy is likely the main mechanism of p62 turnover in tumour cells. Accumulation of p62 in autophagy deficient tumour cells also caused the accumulation of damaged mitochondria, elevated oxidative stress and DNA damage response activation. The inability of autophagy deficient cells to remove p62 was sufficient to promote tumourigenesis via altered NF- κ B regulation. Therefore it seems that autophagy suppresses tumourigenesis by inhibiting p62 accumulation (Fig. 3).

p53 is well characterized as a tumour suppressor protein that accumulates in cells in response to DNA damage, oncogene activation and other stresses. p53 can be considered the hub of numerous signalling pathways, facilitating the transient adaptation of cells to a range of stresses.

It has become known that cytosolic p53 functions to induce apoptosis and inhibit autophagy (Fig. 3). Due to its role in genome stability, inhibition of autophagy is oncogenic. Consequently, loss of one allele of either of the haploinsufficient genes *Becn1* (beclin 1) or UVRAG is enough to induce carcinogenesis [10]. Likewise, numerous oncogenes, including Bcl2, Akt1 and PI3K, inhibit autophagy. The inactivation of tumour suppressor proteins, including Pten and Lkb1, also results in the inhibition of autophagy [53,54]. Cytosolic p53 can transactivate genes that induce autophagy [55], but normal levels of p53 mediate tonic inhibition of autophagy and its deletion or inhibition induces autophagy in humans, mice and *Caenorhadtis elegans*. Thus, physiological induction of autophagy must deplete the cytosolic pool of p53 to alleviate its inhibitive effects. It is not unreasonable to hypothesize that the nuclear transactivation-dependent metabolic effects of p53 and the cytosolic, transcription-independent inhibition of autophagy act in unison to coordinate the action of p53 in stress induced adaptation.

Inducers of autophagy

In addition to cellular homeostasis, autophagy can be a type of programmed cell death (type II programmed cell death) or play a cytoprotective role (i.e. during starvation) and autophagy has been proposed to play a role in tumour progression and promotion of cancer cell death. A report by Salazar et al., [56], links the antitumoural action of D⁹-Tetrahydrocannabinol (THC) to autophagy induction via ER stress in human glioma cells. THC upregulates Tribbles 3 (TRB3) that decreases the phosphorylation of Akt leading to the inhibition of mTORC and subsequent induction of autophagy and tumour reduction. Reactive oxygen species (ROS) can regulate apoptosis (programmed cell death type I), and ROS has also been shown to regulate autophagy (programmed cell death type II). Both hydrogen peroxide (H₂O₂) and superoxide (O₂⁻) have been implicated in autophagy, but the ROS responsible for regulating autophagy remains unclear. Chen et al. [57], demonstrate that under different starvation condition different ROS levels are observed even though the level of autophagy remains constant. In addition they show that exogenous addition of H₂O₂, which is commonly believed to induce autophagy by raising intracellular H₂O₂ levels, in fact raises intracellular O₂⁻.

Atg genes function in autophagy independent pathways

Atg5 has been proposed to curb *T. gondii* infection in an autophagy independent manner [58]. Similar to other autophagy-based resistance mechanisms, IFN- γ -mediated resistance to

T. gondii involves the formation of protein complexes around the pathogen-containing vacuole. Vacuolar membrane lysis ensues by Atg5-dependent recruitment of the interferon-induced GTPase Irga6. It has been suggested [58], that the role of Atg5 in *T. gondii* infection is similar to that observed when TLR ligand-coated beads are phagocytosed [2]. Furthermore, proteomic analysis of latex bead-loaded phagosomes isolated from macrophages shows enhanced LC3 recruitment to phagosomal membranes when autophagy is induced [59].

Conclusion

A growing number of studies are widening the scope of autophagy beyond its role in homeostasis to a full range of immunological processes and disorders of immunity. Relative to disease, autophagy can function as a survival mechanism, maintaining viability during stress by removing damaged organelles and toxic protein aggregates or intracellular pathogens. Autophagy can also act as a tumour suppressor mechanism restraining necrosis and inflammation and inhibiting accumulation of p62.

A number of unexpected roles have been revealed for autophagy genes in the regulation of immunity and inflammation. In adaptive immunity, autophagy regulates lymphocyte populations influencing the naïve T cell repertoire in the thymus and antigen specific T cells in the periphery. B cells are also affected and autophagy may also function to prevent immunological hyper-responsiveness in certain cell types including macrophages and specialized functions in Paneth cells. This, perhaps partly, underlies the associations between polymorphisms in ATG16L1 and IRGM with Crohn's disease. Autophagy is clearly pivotal to the proper function of the immune system and sentinel to metabolic stress induced disease.

Acknowledgments

RJX and RJH are supported by the following grants from the NIH: A1062773, DK83756, DK043351.

References and recommended reading

Papers of particular interest, published within the annual period of review have been highlighted as:

- of special interest
- of outstanding interest
- 1•• Cadwell K, Liu JY, Brown SL, et al. A key role for autophagy and the autophagy gene Atg16l1 in mouse and human intestinal Paneth cells. *Nature* 2008;456:259–263. [PubMed: 18849966] Demonstrates the role of autophagy in Paneth cells, relating to Crohn's disease.
- 2•. Sanjuan MA, Dillon CP, Tait SW, et al. Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis. *Nature* 2007;450:1253–1257. [PubMed: 18097414] This study demonstrates that LC3 labels the phagocytic membrane, expanding the role of autophagy proteins and suggesting a link between the two processes.
- 3. Chan EY, Kir S, Tooze SA. siRNA screening of the kinome identifies ULK1 as a multidomain modulator of autophagy. *J Biol Chem* 2007;282:25464–25474. [PubMed: 17595159]
- 4. Wullschlegel S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006;124:471–484. [PubMed: 16469695]
- 5•. Hosokawa N, Hara T, Kaizuka T, et al. Nutrient-dependent mTORC1 association with the ULK1-Atg13-FIP200 complex required for autophagy. *Mol Biol Cell* 2009;20:1981–1991. [PubMed: 19211835] This paper demonstrates that mTORC1 suppresses autophagy by interacting directly with the ULK1-Atg13-FIP200 complex.

6. Jung CH, Jun CB, Ro SH, et al. ULK-Atg13-FIP200 complexes mediate mTOR signaling to the autophagy machinery. *Mol Biol Cell* 2009;20:1992–2003. [PubMed: 19225151]
7. Itakura E, Kishi C, Inoue K, et al. Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG. *Mol Biol Cell* 2008;19:5360–5372. [PubMed: 18843052] Identifies the mammalian orthologue of yeast ATG14 and defines the important role of the Atg14-Beclin 1-hVPS34 complex in the initiation of autophagy.
8. Matsunaga K, Saitoh T, Tabata K, et al. Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages. *Nat Cell Biol* 2009;11:385–396. [PubMed: 19270696]
9. Shi CS, Kehrl JH. MyD88 and Trif target Beclin 1 to trigger autophagy in macrophages. *J Biol Chem* 2008;283:33175–33182. [PubMed: 18772134]
10. Liang C, Feng P, Ku B, et al. Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG. *Nat Cell Biol* 2006;8:688–699. [PubMed: 16799551]
11. Fimia GM, Stoykova A, Romagnoli A, et al. Ambra1 regulates autophagy and development of the nervous system. *Nature* 2007;447:1121–1125. [PubMed: 17589504]
12. Liang C, Lee JS, Inn KS, et al. Beclin1-binding UVRAG targets the class C Vps complex to coordinate autophagosome maturation and endocytic trafficking. *Nat Cell Biol* 2008;10:776–787. [PubMed: 18552835]
13. Zhong Y, Wang QJ, Li X, et al. Distinct regulation of autophagic activity by Atg14L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex. *Nat Cell Biol* 2009;11:468–476. [PubMed: 19270693] Using mouse genetics and biochemistry, the group identifies the mammalian orthologue of yeast Atg14. The authors go on to show its involvement with Beclin 1 and a novel protein Rubicon and suggest novel roles for these complexes in regulating autophagy at multiple stages.
14. Kabeya Y, Mizushima N, Ueno T, et al. LC3, a mammalian homologue of yeast Apg8p, is localized in autophagosome membranes after processing. *EMBO J* 2000;19:5720–5728. [PubMed: 11060023]
15. Sugawara K, Suzuki NN, Fujioka Y, et al. Structural basis for the specificity and catalysis of human Atg4B responsible for mammalian autophagy. *J Biol Chem* 2005;280:40058–40065. [PubMed: 16183633]
16. Satoo K, Noda NN, Kumeta H, et al. The structure of Atg4B-LC3 complex reveals the mechanism of LC3 processing and delipidation during autophagy. *EMBO J* 2009;28:1341–1350. [PubMed: 19322194]
17. Hemelaar J, Lelyveld VS, Kessler BM, et al. A single protease, Apg4B, is specific for the autophagy-related ubiquitin-like proteins GATE-16, MAP1-LC3, GABARAP, and Apg8L. *J Biol Chem* 2003;278:51841–51850. [PubMed: 14530254]
18. Kabeya Y, Mizushima N, Yamamoto A, et al. LC3, GABARAP and GATE16 localize to autophagosomal membrane depending on form-II formation. *J Cell Sci* 2004;117:2805–2812. [PubMed: 15169837]
19. Huett A, Ng A, Cao Z, et al. A novel hybrid yeast-human network analysis reveals an essential role for FNB1L in antibacterial autophagy. *J Immunol* 2009;182:4917–4930. [PubMed: 19342671]
20. Noda T, Fujita N, Yoshimori T. The late stages of autophagy: how does the end begin? *Cell Death Differ* 2009;16:984–990. [PubMed: 19424283]
21. Fujita N, Hayashi-Nishino M, Fukumoto H, et al. An Atg4B mutant hampers the lipidation of LC3 paralogues and causes defects in autophagosome closure. *Mol Biol Cell* 2008;19:4651–4659. [PubMed: 18768752]
22. Kimura S, Noda T, Yoshimori T. Dynein-dependent movement of autophagosomes mediates efficient encounters with lysosomes. *Cell Struct Funct* 2008;33:109–122. [PubMed: 18388399]
23. Gutierrez MG, Master SS, Singh SB, et al. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. *Cell* 2004;119:753–766. [PubMed: 15607973]
24. Yano T, Mita S, Ohmori H, et al. Autophagic control of listeria through intracellular innate immune recognition in drosophila. *Nat Immunol* 2008;9:908–916. [PubMed: 18604211] First *in*

vivo demonstration of autophagy acting as an innate defence mechanism against bacterial infection.

25. Howell SJ, Wilk D, Yadav SP, et al. Antimicrobial polypeptides of the human colonic epithelium. *Peptides* 2003;24:1763–1770. [PubMed: 15019208]
- 26•. Delgado MA, Elmaoued RA, Davis AS, et al. Toll-like receptors control autophagy. *EMBO J* 2008;27:1110–1121. [PubMed: 18337753] A TLR agonist screen is used to demonstrate that multiple TLR agonists can induce autophagy.
27. Delgado M, Singh S, De Haro S, et al. Autophagy and pattern recognition receptors in innate immunity. *Immunol Rev* 2009;227:189–202. [PubMed: 19120485]
28. Foy E, Li K, Sumpter RJ, et al. Control of antiviral defenses through hepatitis C virus disruption of retinoic acid-inducible gene-I signaling. *Proc Natl Acad Sci U S A* 2005;102:2986–2991. [PubMed: 15710892]
29. Lee S, Comer FI, Sasaki A, et al. TOR complex 2 integrates cell movement during chemotaxis and signal relay in *Dictyostelium*. *Mol Biol Cell* 2005;16:4572–4583. [PubMed: 16079174]
- 30•. Tal MC, Sasai M, Lee HK, et al. Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling. *Proc Natl Acad Sci U S A* 2009;106:2770–2775. [PubMed: 19196953] Demonstrates that autophagy is involved in the homeostatic regulation of innate antiviral defence via the clearance of dysfunctional mitochondria. Also demonstrates that mitochondrial associated ROS play a role in RLR signalling.
31. Suzuki T, Franchi L, Toma C, et al. Differential regulation of caspase-1 activation, pyroptosis, and autophagy via Ipaf and ASC in *Shigella*-infected macrophages. *PLoS Pathog* 2007;3:e111. [PubMed: 17696608]
32. Master SS, Rampini SK, Davis AS, et al. *Mycobacterium tuberculosis* prevents inflammasome activation. *Cell Host Microbe* 2008;3:224–232. [PubMed: 18407066]
33. Kanneganti TD, Body-Malapel M, Amer A, et al. Critical role for Cryopyrin/Nalp3 in activation of caspase-1 in response to viral infection and double-stranded RNA. *J Biol Chem* 2006;281:36560–36568. [PubMed: 17008311]
34. Muruve DA, Petrilli V, Zaiss AK, et al. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* 2008;452:103–107. [PubMed: 18288107]
35. Villani AC, Lemire M, Fortin G, et al. Common variants in the NLRP3 region contribute to Crohn's disease susceptibility. *Nat Genet* 2009;41:71–76. [PubMed: 19098911]
- 36••. Saitoh T, Fujita N, Jang MH, et al. Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1 β production. *Nature* 2008;456:264–268. [PubMed: 18849965] Using transgenic ATG16L1 knockout mice, demonstrates the role autophagy plays in IBD.
- 37••. Nedjic J, Aichinger M, Emmerich J, et al. Autophagy in thymic epithelium shapes the T-cell repertoire and is essential for tolerance. *Nature* 2008;455:396–400. [PubMed: 18701890] A critical study which demonstrates that autophagy and MHC restricted endogenous antigen presentation by thymic epithelial cells is important in tolerance.
38. Fengsrud M, Raiborg C, Berg TO, et al. Autophagosome-associated variant isoforms of cytosolic enzymes. *Biochem J* 2000;(Pt 3):352. 773–781.
- 39••. Schmid D, Pypaert M, Munz C. Antigen-loading compartments for major histocompatibility complex class II molecules continuously receive input from autophagosomes. *Immunity* 2007;26:79–92. [PubMed: 17182262] Demonstrates the role of autophagy in delivering cytosolic agents to endosomally located MHC II.
40. Jagannath C, Lindsey DR, Dhandayuthapani S, et al. Autophagy enhances the efficacy of BCG vaccine by increasing peptide presentation in mouse dendritic cells. *Nat Med* 2009;15:267–276. [PubMed: 19252503]
41. Pua HH, Dzhagalov I, Chuck M, et al. A critical role for the autophagy gene Atg5 in T cell survival and proliferation. *J Exp Med* 2007;204:25–31. [PubMed: 17190837]
42. Stephenson LM, Miller BC, Ng A, et al. Identification of Atg5-dependent transcriptional changes and increases in mitochondrial mass in Atg5-deficient T lymphocytes. *Autophagy* 2009;5
- 43•. Miller BC, Zhao Z, Stephenson LM, et al. The autophagy gene ATG5 plays an essential role in B lymphocyte development. *Autophagy* 2008;4:309–314. [PubMed: 18188005] Using a murine

Atg5 deletion specific to B-cells, demonstrates the role of autophagy in B-cell development and homeostasis.

44. Rioux JD, Xavier RJ, Taylor KD, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007;39:596–604. [PubMed: 17435756]
45. Barrett JC, Hansoul S, Nicolae DL, et al. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008;40:955–962. [PubMed: 18587394]
- 46•. McCarroll SA, Huett A, Kuballa P, et al. Deletion polymorphism upstream of IRGM associated with altered IRGM expression and Crohn's disease. *Nat Genet* 2008;40:1107–1112. [PubMed: 19165925] Demonstrates that the IRGM non-coding polymorphism associated with Crohn's disease is associated with a large deletion haplotype.
47. Singh SB, Davis AS, Taylor GA, et al. Human IRGM induces autophagy to eliminate intracellular mycobacteria. *Science* 2006;313:1438–1441. [PubMed: 16888103]
- 48•. Kuballa P, Huett A, Rioux JD, et al. Impaired autophagy of an intracellular pathogen induced by a Crohn's disease associated ATG16L1 variant. *PLoS ONE* 2008;3:e3391. [PubMed: 18852889] Demonstrates that the ATG16L1 T300A CD risk-associated variant is destabilised upon infection with, and cannot support autophagic clearance of, *S. typhimurium*, using an *in vitro* cell culture model.
49. Henry SC, Daniell X, Indaram M, et al. Impaired macrophage function underscores susceptibility to Salmonella in mice lacking Irgm1 (LRG-47). *J Immunol* 2007;179:6963–6972. [PubMed: 17982087]
50. Parkes M, Barrett JC, Prescott NJ, et al. Sequence variants in the autophagy gene IRGM and multiple other replicating loci contribute to Crohn's disease susceptibility. *Nat Genet* 2007;39:830–832. [PubMed: 17554261]
51. Bekpen C, Marques-Bonet T, Alkan C, et al. Death and resurrection of the human IRGM gene. *PLoS Genet* 2009;5:e1000403. [PubMed: 19266026]
- 52•. Mathew R, Karp CM, Beaudoin B, et al. Autophagy suppresses tumorigenesis through elimination of p62. *Cell* 2009;137:1062–1075. [PubMed: 19524509] Demonstrates the important role of autophagy in the regulation of p62 and tumour suppression.
53. Liang J, Shao SH, Xu ZX, et al. The energy sensing LKB1-AMPK pathway regulates p27(kip1) phosphorylation mediating the decision to enter autophagy or apoptosis. *Nat Cell Biol* 2007;9:218–224. [PubMed: 17237771]
54. Ueno T, Sato W, Horie Y, et al. Loss of Pten, a tumor suppressor, causes the strong inhibition of autophagy without affecting LC3 lipidation. *Autophagy* 2008;4:692–700. [PubMed: 18424911]
55. Crichton D, Wilkinson S, O'Prey J, et al. DRAM, a p53-induced modulator of autophagy, is critical for apoptosis. *Cell* 2006;126:121–134. [PubMed: 16839881]
56. Salazar M, Carracedo A, Salanueva IJ, et al. Cannabinoid action induces autophagy-mediated cell death through stimulation of ER stress in human glioma cells. *J Clin Invest* 2009;119:1359–1372. [PubMed: 19425170]
- 57•. Chen Y, Azad MB, Gibson SB. Superoxide is the major reactive oxygen species regulating autophagy. *Cell Death Differ* 2009;16:1040–1052. [PubMed: 19407826] Demonstrates that superoxide is the main ROS regulator of autophagy and highlights the important point that not all reactive oxygen species contribute equally to autophagy.
58. Zhao YO, Khaminets A, Hunn JP, et al. Disruption of the *Toxoplasma gondii* parasitophorous vacuole by IFN γ -inducible immunity-related GTPases (IRG proteins) triggers necrotic cell death. *PLoS Pathog* 2009;5:e1000288. [PubMed: 19197351]
59. Shui W, Sheu L, Liu J, et al. Membrane proteomics of phagosomes suggests a connection to autophagy. *Proc Natl Acad Sci U S A* 2008;105:16952–16957. [PubMed: 18971338]

Figure 1.

Building the autophagosome.

The early stages of autophagosome formation are better understood than the late stages. 1. Initiation: autophagy is induced by an upstream signal. Nucleation of the isolation membrane occurs through the action of the Atg14-Beclin1-hVPS34 complex. This membrane may be derived from endoplasmic reticulum (ER). 2. Elongation: The phagophore enlarges to form a double membrane organelle. Atg16L1/Atg5-Atg5 acts as an E3 enzyme, converting LC3 I to lipidated LC3 II. Atg4 reverses LC3 II to LC3 I by removing phosphatidylethanolamine (PE). 3. Closure: Closure of the elongating phagophore results in the autophagosome. The exact mechanism of closure is unknown but LC3 is necessary for closure of the phagophore. 4. Maturation: once the autophagosome has formed it must be moved to the perinuclear region of the cells for fusion with lysosomes. Both dynein and microtubules are necessary for this process, but the regulation of this process is unknown. Once the autophagosome has fully closed the Atg16 complex rapidly dissociates, but LC3 remains attached. It is possible that LC3 mediates an interaction between dynein and microtubules to facilitate translocation of the autophagosome to the lysosomal region of the cell. Fusion with lysosomal organelles causes acidification of the autophagosome converting it into the degradative, single membrane autolysosome. The UVRAG-Beclin1-VPS34 complex can promote maturation of the autophagosome, while the Rubicon-UVRAG-Beclin1-VPS34 complex can suppress maturation of the autophagosome. Coding variants identified from GWAS studies are shown inset. The ATG16L1 allele T300A is associated with susceptibility to Crohn's disease and the ATG5 C/T coding variant, where T is the minor allele, is associated with susceptibility to systemic lupus erythematosus. CC-coiled-coil domain.

Figure 2.

Autophagy is involved in innate and adaptive immunity.

Autophagy is involved in the regulation of the inflammasome (innate immunity). The inflammasome is activated by numerous ligands leading to the activation of Caspase 1 and the secretion of the proinflammatory cytokines IL-1 β and IL18. Autophagy may degrade the inflammasome in order to dampen the inflammatory response. Furthermore, a coding variant in the NLRP3 region is a Crohn's disease risk factor. Autophagy is involved in MHC II antigen presentation (adaptive immunity). Antigen in the cytosol is captured by the autophagosome, which merges with the MHCII loading compartment (MIIC) where it is processed and presented on MHC II.

Figure 3.

Autophagy and cancer.

Autophagy is a major component of the stress response and is involved in tumour suppression. Under normal conditions cytosolic p53 can suppress oncogenic potential by triggering apoptosis or senescence. Cytosolic p53 can also transactivate genes that induce autophagy. Autophagy degrades the pool of p53 in order to alleviate its inhibitive effects. Nuclear, transactivation-dependent effects of p53 and cytosolic, transcription independent inhibition of autophagy pathways likely act in unison to coordinate p53 mediated stress adaptation. Oncogene induced activation of autophagy can induce senescence *in vitro*. The ubiquitin binding protein p62 links autophagy to the proteasome. In autophagy deficient tumour cells, p62 accumulates in response to metabolic stress. p62 is likely turned-over by autophagy and its accumulation alone is sufficient to promote tumourigenesis via altered NFkB regulation. Ub -ubiquitin., ROS-Reactive oxygen species.