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FORUM REVIEW ARTICLE

Autophagy Signaling Through Reactive Oxygen Species

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Abstract

Autophagy is a degradative pathway that involves delivery of cytoplasmic components, including proteins, organelles, and invaded microbes to the lysosome for digestion. Autophagy is implicated in the pathology of various human diseases. The association of autophagy to inflammatory bowel diseases is consistent with recent discoveries of its role in immunity. A complex of signaling pathways control the induction of autophagy in different cellular contexts. Reactive oxygen species (ROS) are highly reactive oxygen free radicals or non-radical molecules that are generated by multiple mechanisms in cells, with the nicotinamide adenine dinucleotide phosphate (NADPH) oxidases and mitochondria as major cellular sources. These ROS are important signaling molecules that regulate many signal-transduction pathways and play critical roles in cell survival, death, and immune defenses. ROS were recently shown to activate starvation-induced autophagy, antibacterial autophagy, and autophagic cell death. Current findings implicate ROS in the regulation of autophagy through distinct mechanisms, depending on cell types and stimulation conditions. Conversely, autophagy can also suppress ROS production. Understanding the mechanisms behind ROS-induced autophagy will provide significant therapeutic implications for related diseases. *Antioxid. Redox Signal.* 14, 2215–2231.

Introduction

The ancient cellular degradation pathway, autophagy, is now accepted as a multifunctional process involved in various cellular activities and affects human health in many aspects. The links of autophagy to conditions such as neurodegenerative diseases, cancers, cardiovascular diseases, infections, and inflammatory diseases make it a prime target for developing therapeutic treatments for these diseases (104). Numerous factors and signaling pathways have been shown to contribute to autophagy induction in different cellular contexts. Among them, ROS are indicated to be essential signals to activate autophagy by a variety of stimulating conditions (6, 19, 54, 140). ROS are a group of highly active oxygen free radicals and their derivatives. Although deleterious at high concentrations, under physiologic conditions, ROS play many beneficial roles in cell survival, death, and immunity as important signaling messengers (155). Studies of autophagy induction by ROS reveal the existence of multiple mechanisms. In this review, we provide an overview of the autophagy process, of signaling pathways that regulate autophagy in stress conditions and immunity, ROS functions, and autophagy regulation under oxidative stress. Furthermore, we review the current understanding of the mechanisms of autophagy induction by ROS, and the interplay between their signaling networks.

Autophagy and Its Signaling Pathways

Autophagy core machinery

Autophagy is a cellular degrading process that involves lysosomal breakdown of cytoplasmic components. Chaperone-mediated autophagy (CMA), microautophagy, and macroautophagy are three major forms of autophagy (104). Macroautophagy, hereafter referred to as autophagy, has been extensively studied and is the topic of this review. Characterized by the formation of intracellular double/multimembranous vesicles, called autophagosomes, autophagy sequesters cytoplasm, proteins, organelles, and invaded microbes for lysosomal delivery and degradation (104) (Fig. 1). Autophagy is typically maintained at a low basal level under nonstress condition. However, upon stress or stimulation, autophagic activity can be greatly induced. The autophagy pathway can be dissected into distinct steps, including induction, vesicle nucleation, selective cargo recognition, autophagosome formation, autophagosome-lysosome fusion, cargo degradation, and nutrient recycling (55). More than 30 key components of the autophagy machinery encoded by autophagy-related genes (ATGs) function at different steps of this process (75). For example, the Atg1 (homologue of mammalian ULK1) kinase complex (Atg1-Atg13-Atg17-Atg29-Atg31 in yeast, and ULK1/2-Atg13-FIP200-Atg101 in mammals) is critical for autophagy induction (102). The class III

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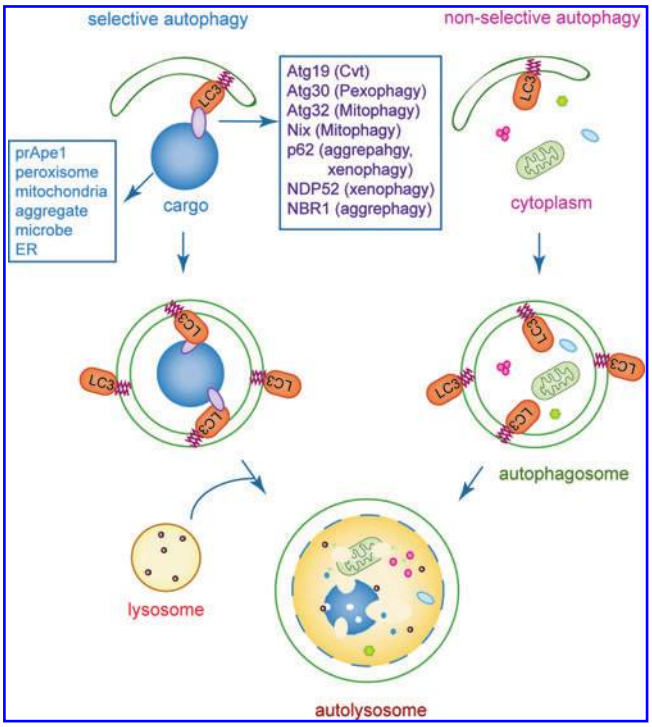

FIG. 1. A schematic diagram of the selective and nonselective autophagy pathways. Atg8/LC3 associates with an autophagosome that enwraps random cytoplasm in nonselective autophagy or specific cargoes in selective autophagy, and fuses with the lysosome to degrade sequestered material in an autolysosme. Cargoes and corresponding receptor proteins in selective autophagy are listed in boxes. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

phosphatidylinositol 3-kinase (PtdIns3K) complex, comprising Vps34 (the PtdIns3K), Vps15, Atg14, and Beclin 1/Atg6, functions at the stage of the phagophore [the autophagosome formation site or preautophagosomal structure (PAS) in yeast] initiation and assembly (39). Furthermore, two ubiquitin-like conjugation complexes are essential for phagophore elongation, expansion, and finally autophagosome formation. One is the Atg12-Atg5-Atg16 complex, in which Atg12 is catalyzed by the E1-like enzyme Atg7 and the E2-like enzyme Atg10, and then conjugated to Atg5 (105). The resultant Atg12-Atg5 conjugate further interacts with Atg16 and forms a complex that associates with autophagosomes (103). The other ubiquitin-like system is the Atg8 (homologue of mammalian LC3)-phosphatidylethanolamine (PE) conjugate (57). Under the activity of Atg7, Atg3 (another E2-like enzyme) and Atg12-Atg5 complex (acts as an E3), Atg8/LC3 is attached to the phospholipid PE and remains on the autophagosomal membrane throughout the autophagy process (47, 57, 65). Before its conjugation, Atg8/LC3 must be processed by Atg4, a cysteine protease, to expose a glycine residue that is a prerequisite for PE conjugation (73). Atg4 also regulates the deconjugation of Atg8/LC3-PE to recycle free Atg8/LC3 after the autophagosome is completely formed (73).

Selective autophagy

Autophagy can be classified into nonselective bulk autophagy and selective autophagy that targets specific cargoes (Fig. 1). Although starvation-induced bulk autophagy has long been thought to be nonspecific, recent proteomic studies have suggested a highly regulated and ordered manner of organelle degradation by autophagy under amino acid–starvation conditions (80). It was shown that at early time points after starvation, cytosolic proteins and proteasomes were degraded by autophagy, and later (about 12-h starvation), ribosomes were degraded in lysosomes. Organelle degradation started only at late time points (about 24 h) after starvation (80). Several selective forms of autophagy have been identified. For example, in yeast *Saccharomyces cerevisiae* (*S. cerevisiae*), the constitutively active cytoplasm-to-vacuole-targeting (Cvt) pathway specifically delivers precursor aminopeptidase 1 (Ape1) complexes to the vacuole (analogue of mammalian lysosome) for processing and activation (90). Selective targeting and degradation of peroxisome (35), mitochondria (71), and endoplasmic reticulum (ER) (10) by autophagy are called pexophagy, mitophagy, and reticulophagy, respectively. Furthermore, misfolded protein aggregates, such as Huntingtin protein aggregates, can be selectively degraded by autophagy (named aggrephagy) (133). Finally autophagy is able to target invaded viruses, bacteria, and parasites by a process called xenophagy (117).

The selective cargo recognition depends on cargo-receptor proteins that can be recognized by the autophagy machinery (summarized and illustrated in Fig. 1). Yeast *S. cerevisiae* ScAtg19 is a receptor protein on precursor Ape1 complex and binds to an adaptor protein, ScAtg11, to be recruited to PAS, where it interacts with ScAtg8 (143). A newly identified ATG protein, ScAtg32, is a mitochondrial outer-membrane protein, which acts as a receptor to bind ScAtg11 during mitophagy (67, 114). Similarly, mammalian mitochondria-localized protein Nix [also known as Bcl2/E1B 19 kDa-interacting protein 3 (BNIP3)-like protein (BNIP3L)] was recently shown to be a receptor on mitochondria, mediating mammalian mitophagy through interaction with LC3 via the N-terminal LC3 interacting region (111). Yeast *Pichia pastoris* (*P. pastoris*), a model organism for pexophagy studies, expresses a receptor protein PpAtg30 on peroxisomal membrane and interacts with PpAtg11 for autophagy recognition (37). Moreover, several recent studies have shown that the ubiquitin (both mono- and polyubiquitin)-binding protein p62/SQSTM1 is a cargo receptor that links autophagy machinery to different cargo targets, including polyubiquitinated protein aggregates (121), monoubiquitin-tagged peroxisomes (72), and bacterial pathogens, such as *Salmonella enterica* serovar typhimurium (*S. typhimurium*) (175), *Shigella flexneri* (*S. flexneri*) (36), and *Listeria monocytogenes* (*L. monocytogenes*) (169). P62/SQSTM1 interacts with both LC3 and ubiquitinated cargo through an LC3-interacting region and a C-terminal ubiquitin-associated (UBA) domain, respectively (58, 121). Interestingly, Kirkin and colleagues (74) recently reported that another ubiquitin-binding protein NBR1 (neighbor of BRCA1 gene 1), which shares similar domain organizations to p62/SQSTM1 and directly interacts with p62/SQSTM1, also acts as a receptor for selective autophagy targeting of ubiquitinated protein aggregates (74). However, NBR1 does not play a major role in autophagy of *S. typhimurium* (175), suggesting that NBR1 and p62 may have different cargo preferences. In addition, a recent study showed that autophagy targeting of *S. typhimurium* required another adaptor protein NDP52 (nuclear dot protein, 52 kDa), which recognizes the ubiquitin coat on the bacteria and brings them to autophagosomes via binding to LC3 (151).

Further identification of LC3-interacting proteins may reveal other receptors that are responsible for selective autophagy cargo recognition.

Autophagy in Health and Disease

Initially considered simply a degradation mechanism, autophagy is now believed to have numerous cellular functions and can play complex roles in human health and disease. Autophagy is a "double-edged sword" in the pathogenesis of these diseases. Extensive reviews of autophagy in health can be found elsewhere (55, 104, 144).

Autophagy in neurodegenerative diseases

In certain neurodegenerative diseases and myopathies, autophagy plays a beneficial role by preventing neuronal or muscle cell death because of its cytoprotective property. The accumulation of large aggregates/inclusion bodies has been shown to hamper normal neuron functions and is toxic to cells (97). A growing body of studies indicate that autophagy contributes to the clearance of abnormal protein aggregates that are linked to neurodegenerative diseases, such as polyglutamine expansions (for example, Huntingtin protein associated with Huntington disease) (9, 161), α-synuclein (associated with Parkinson disease) (9, 161), tau (associated with frontotemporal dementia) (9, 161), and mutant copper-zinc superoxide dismutase 1 (CuZnSOD1) (associated with amyotrophic lateral sclerosis) (40). *In vivo* studies of fly, worm, and mouse models of neurodegeneration showed that genetic inhibition of autophagy enhances degeneration symptoms, whereas pharmacologic induction of autophagy alleviates degeneration (60, 129). Furthermore, flies and mice deficient in Atg7 expression develop abnormal neuronal function, neuron death, and are subject to early death (48, 64, 76, 77). Currently, autophagy is appreciated to be cytoprotective, not only by the elimination of large protein aggregates, but also by degradation of those soluble, toxicity-prone, monomeric proteins, thus maintaining neural cell homeostasis and preventing cell death (48). It is worthwhile to note that the accumulation of autophagic vacuoles are often seen in brain-tissue samples of patients with neurodegenerative diseases (97), raising the possibility that inefficient autophagy or the hindrance of autophagic flux caused by impaired fusion with lysosomes may be involved in neuropathologies. Consistent with this, a recent study by Nixon and colleagues (85) demonstrated that autophagosome maturation and lysosomal degradation were impaired in fibroblasts from patients with early-onset familial Alzheimer disease, which is caused by mutations in presenilin-1. Loss of presenilin-1 led to failed lysosomal targeting of the v-ATPase V0a1 subunit and resulted in defective autolysosome acidification and proteolysis capacity.

Autophagy and cancer

Autophagy plays dual roles in tumorigenesis: the promotion of cell death as a tumor suppressor and the prevention of cell death as an oncogenic mechanism. This issue has been extensively reviewed elsewhere (12, 61, 86), and here we briefly discuss autophagy in cancer with respect to ROS. One of the major causes of tumorigenesis involves DNA damage and mutations initiated by radiation, oxidative stress, aging, or other factors. Mitochondrial DNA is more susceptible to damage due to the lack of repair systems and histone protein protection (61). Mitochondria generate large amounts of ROS as a byproduct of the electron-transport chain (107), which, when unregulated, can result in mitochondrial DNA damage and oxidization of both the inner and outer membranes, allowing permeabilization (155). Dysfunctional mitochondria may accumulate more ROS, which in turn, elevate cellular oxidative stress and further damage other mitochondria (61, 107). It is thought that autophagy removes damaged mitochondria, thus alleviating oxidative stress to prevent carcinogenesis (61). Moreover, recent studies reported ROS in signaling of autophagic cell death in a number of antitumor drug treatments (see the last section of autophagy and ROS), suggesting a tumor-suppressor function for autophagy.

However, autophagy is also a cytoprotective mechanism that prevents cell death under starvation or stress conditions. Studies have shown that ROS can induce autophagy, which, instead of causing cell death, protects cells from apoptosis or necrosis (see the last section of autophagy and ROS). Therefore, autophagy plays both promotion and suppression roles in tumorigenesis (6). Clearly, the role that autophagy plays in cancer is complex, and further discussion on the crosstalk between ROS signaling and autophagy is explored in the last section.

Autophagy and immunity

Autophagy has been extensively studied for its role in immunity. It is now believed that autophagy is an important innate immune defense mechanism and plays multiple roles in adaptive immunity [extensively reviewed in (31, 117, 158)]. Autophagy limits the intracellular growth of invaded pathogens by degrading them through the autophagosome–lysosome pathway. A wide range of bacteria, viruses, and parasites have been shown to be targets of autophagy, although some of them have evolved strategies to either evade autophagy recognition or use autophagy for their survival and replication (117). Autophagy also allows the detection of certain single-stranded RNA (ssRNA) viruses through the transport of viral-replication intermediates into acidic endosomes for recognition by host pathogen pattern-recognition receptor (PRR), Toll-like receptor 7 (TLR7) (84). Moreover, autophagy is involved in pro-inflammatory cytokine production via either positive or negative effects in different cell types. In the earlier study of TLR7 activation, autophagy was required for viral infection-initiated type I interferon (IFN) production in plasmacytoid dendritic cells (84). However, other studies using *ATG5* knockout mouse embryonic fibroblasts (MEFs) for ssRNA virus infection found enhanced production of type I IFN in these autophagy-deficient cells, suggesting a suppression of cytokine release by autophagy (63, 148). In addition, studies of mice expressing an inefficient level or a truncated form of Atg16L1 showed upregulated transcription of adipocytokine genes, or in the latter study, elevated production of interleukin (IL)-1β and IL-18 when mice were stimulated with bacterial endotoxin, lipopolysaccharide (LPS) (15, 136). Therefore, autophagy has paradoxic roles in both pro- and antiinflammatory responses.

Autophagy also participates in adaptive immunity in a number of ways, ranging from antigen presentation to immune

cell maturation, activation, and development. Autophagy was recently shown to deliver endogenous antigens to class II major histocompatibility complex (MHC) loading compartments for $CD4^+$ T-cell presentation (30, 118). Furthermore, mice with Atg5 deficiency display impaired growth and maturation of T and B lymphocytes, implicating autophagy in lymphocytic proliferation and development (100, 126). Last, a recent report suggests that autophagy activity is constitutive and high in thymic epithelial cells, and contributes to T-cell selection and tolerance (110). Together, these findings shed light on the importance of autophagy in immunity.

The direct link between autophagy and immune diseases is demonstrated in the immune disorder, inflammatory bowel disease (IBD), in which the autophagy gene, *ATG16L1*, has been found to be a susceptibility gene for this disease (46, 130). IBD, of which Crohn disease (CD) and ulcerative colitis are two major forms, is a chronic disorder caused by abnormal inflammation in the intestinal tract (125). The pathogenesis of CD is complex, involving both environmental and genetic factors. The CD-associated *ATG16L1* (T300A) variant has normal function in canonic autophagy but is less efficient in autophagy targeting of bacteria in epithelial cells (81). However, the opposite result was obtained in fibroblasts, as the T300A mutation had no effect on antibacterial autophagy (38), raising the question whether the CD-associated *ATG16L1* allele affects autophagy only in specific cell types or affects other autophagy-independent functions. *In vivo* studies of the role of autophagy in IBD have shown that mice hypomorphic for *ATG16L1* expression have abnormal Paneth cells, and similar phenotypes were observed in tissue sections from human CD patients carrying the *ATG16L1* risk allele as well as Atg5-deficient mice (15). Interestingly, mice hypomorphic for Atg16L1 do not have the Paneth cell–dysfunction phenotypes when raised in an enhanced barrier facility instead of a conventional raising environment. However, if these mice are persistently infected with a specific virus, murine norovirus, they will develop the phenotype of Paneth cell abnormalities (16). This study suggests that the combination of an environmental factor, in this case, a viral infection, and the susceptibility gene *ATG16L1* determines the CD-associated ATG16L1 phenotypes in mice (16). A separate study showed that mice expressing a C-terminal truncated *Atg16L1* produced elevated levels of cytokine IL-1β and IL-18 on LPS stimulation, exhibiting more severe inflammation in their colons than did wild-type mice when challenged with dextran sulfate sodium (136).

Genes of two other autophagy-regulating factors were subsequently identified to be CD-susceptibility genes. One is *IRGM*, the human homologue of mouse Irgm1, a member of the murine immunity-related GTPase (IRG) family (130, 131, 150). *IRGM* is involved in autophagic clearance of intracellular *Mycobacterium tuberculosis* and *S. typhimurium* (95, 146). The other gene is *NCF4*, the gene that encodes the human $p40^{phox}$ subunit of the Nox2 NADPH oxidase complex, which recently was shown to regulate TLR, phorbol 12-myristate 13-acetate, and phagocytosis-induced autophagy targeting of phagosomes and bacteria (54, 101, 130, 131). Individuals lacking functional components of the Nox2 NADPH oxidase develop chronic granulomatous disease (CGD) and are more susceptible to bacterial and fungal infections compared with healthy controls (142). It is worth noting that CGD patients often develop clinical symptoms and pathologic features similar to those of CD patients (92). Therefore, the antibacterial function of Nox2 in autophagy may also play a role in CD pathogenesis.

CD has been associated with mutations of other genes, such as *NOD2* (nucleotide-binding and oligomerization domain-containing 2), an intracellular pathogen-recognition protein), the first identified CD-susceptibility gene (56). Nod2 is involved in activating transcription factor nuclear factor-kappa-B (NF-κB), which regulates the expression of a large number of factors that control immune responses (56). A recent study links Nod2 and Atg16L1 by showing that activation of Nod1 and Nod2 induces autophagy of bacteria by recruiting Atg16L1 to the bacterial entry sites on the plasma membrane (153). In cells homozygous for the CD-associated *NOD2* frameshift mutation (L1007insC), mutant Nod2 is retained in the cytosol and therefore fails to bring Atg16L1 to the plasma membrane, impairing autophagy targeting of bacteria (153). Meanwhile, work done by Simmons and colleagues (25) showed that stimulation of Nod2 by its bacterial ligand, muramyldipeptide, induced autophagy in primary human dendritic cells (DCs) (25). The Nod2-mediated autophagy was necessary for bacterial clearance and MHC class II antigen presentation in DCs. Additionally, muramyldipeptide failed to induce autophagy in DCs from CD patients expressing the susceptibility variants of *NOD2* (including L1007insC, R702W, or G908R). Deficient bacterial targeting and antigen-specific $CD4^+$ T-cell responses were observed in these cells.

Together, these studies suggest that autophagy can influence the development of IBD on multiple levels, including intestinal homeostasis, bacterial clearance, proinflammatory cytokine production, antigen presentation, as well as Paneth cell functions. Understanding the mechanisms behind autophagy and the pathogenesis of IBD will aid in the development of potential pharmacologic treatments for this disease.

Autophagy Signaling

Autophagy is a highly controlled process that can be induced by a wide range of stimuli and conditions. Although the signaling required for autophagy induction is not fully understood, it is certain that a complex of factors, signaling pathways, and mechanisms are involved in this process in different cellular contexts. Reviews of autophagy signaling can be found elsewhere (12, 51, 99, 117), and here we summarize the upstream signaling pathways only in the context of stress and immunity.

Signaling by growth factors, amino acids, and energy

Conditions of stress, such as metabolic stress (nutrient/growth factor/energy deprivation), low oxygen (hypoxia), excessive oxygen (oxidative stress), and ER stress, can induce autophagy in a cytoprotective manner to provide nutrients/energy or for the removal of harmful, damaged organelles. A key regulator of autophagy is TOR (the target of rapamycin), a conserved serine/threonine protein kinase that is inhibited by deprivation of nutrients or growth factors from the extracellular milieu (66). Mammalian TOR (mTOR) directly phosphorylates ULK1 and mAtg13 and inhibits ULK1 kinase activity, which is essential for autophagy induction (102). Thus, mTOR is a central negative regulator of autophagy. Growth factors, such as insulin, bind to membrane re-

ceptors to activate class I PtdIns3K. This process generates PI(3,4,5)P_3, which recruits protein kinase B (PKB/Akt) and its activator PDK1 (phosphoinositide-dependent kinase 1) to the plasma membrane, resulting in activation of PKB/Akt (34). Active PKB/Akt indirectly activates mTOR through inhibition of negative regulators [tuberous sclerosis complex (TSC1/2)] of mTOR and activating the mTOR activator Ras homologue enriched in brain (Rheb) (49) (Fig. 2). Amino acids are able to stimulate mTOR activity either directly or indirectly through regulating yet another factor that phosphorylates mTOR to activate it (27) (Fig. 2). A tyrosine phosphatase PTEN (phosphatase and tensin homologue) dephosphorylates PI(3,4,5)P_3 and downregulates PKB/Akt activity, thereby upregulating autophagy (4).

mTOR-independent autophagy regulation by amino acids also exists (Fig. 2). Studies in human colon cancer cell line HT-29 have shown that amino acids inhibit Raf-1, a serine/threonine kinase that directly activates the MEK-ERK1/2 pathway (123). Small G-protein Ras recruits Raf-1 to the membrane and activates it (123). Activated Raf-1-MEK-ERK1/2 signaling stimulates autophagy through activating GAIP (Gα-interacting protein), which accelerates the hydrolysis of GTP-bound trimeric G-protein $G_{\alpha i3}$ to GDP-bound $G_{\alpha i3}$ (123, 124). The $G_{\alpha i3}$-GDP upregulates autophagy in these cells (124). However, Ras also activates PKB/Akt to downregulate Raf-1 activity (123). Thus Ras appears to have both positive and negative effects on autophagy induction through different pathways.

Recent studies reported an essential role for ROS in starvation-induced autophagy in a number of mammalian cell lines, including U87, HEK293, HeLa, CHO, and MEF (19, 140). Nutrient starvation leads to the increased generation and accumulation of ROS, likely from mitochondrial sources, and ROS are critical for the subsequent triggering of autophagy (19, 140) (Fig. 2) (discussed in the last section).

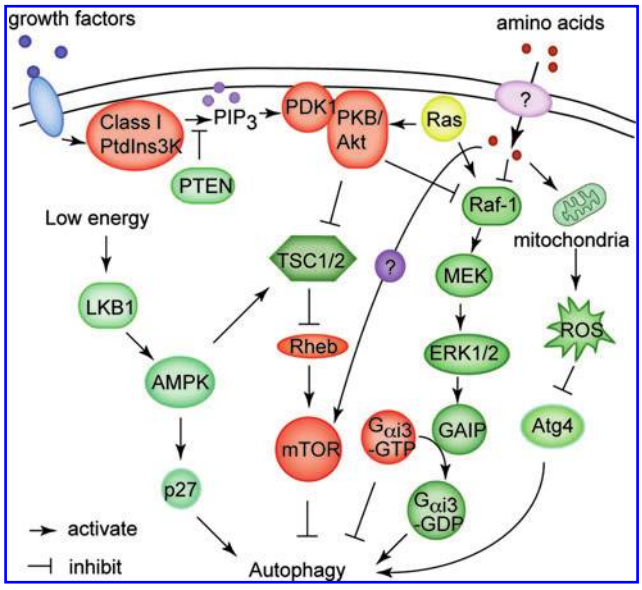

FIG. 2. Signaling pathways that regulate autophagy by growth factors, amino acids, and energy. Autophagy activators are illustrated in green, whereas autophagy inhibitors are in red. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

Low-energy (ATP) conditions can also induce autophagy through the activation of the LKB1 kinase-AMPK (5′-AMP–activated protein kinase) pathway, which phosphorylates and activates the TSC1/2 complex to inhibit mTOR (59). AMPK has also been shown to phosphorylate and stabilize the cyclin-dependent kinase inhibitor p27, upregulating cellular autophagy (87) (Fig. 2).

Signaling by hypoxia, oxidative stress, and ER stress

Solid tumors often encounter hypoxic microenvironments. Recent studies have shown autophagy to be upregulated by hypoxia in cancer cell lines, although controversial conclusions were obtained as to whether autophagy acts as a survival or a death mechanism. Discrepancies are possibly because of differences in cell type and stress level (7, 94, 122, 152, 172). The central transcription factor induced by hypoxia is the hypoxia-inducible factor-1 (HIF-1), which controls the transcription of a large number of genes critical for cellular adaptation to the low-oxygen environment (94). *BNIP3* and *BNIP3L* are two targets of HIF-1 that encode atypical BH3-only proteins for interaction with Bcl-2 or Bcl-X_L. HIF-1-BNIP3/BNIP3L–dependent hypoxia-induced autophagy was shown to protect cells from death by removing dysfunctional mitochondria, thereby reducing cellular ROS levels (7, 152, 172). It has been proposed that the autophagy protein Beclin 1, also a Bcl-2–interacting protein, may be in competition with BNIP3/BNIP3L to bind Bcl-2. The resultant increase in free Beclin 1 will enhance autophagosome formation (94) (Fig. 3). HIF-1–independent autophagy induction by hypoxia has been reported. For example, AMPK activation or unfolded protein response (UPR)-mediated signaling has been shown to regulate autophagy with hypoxia (122, 132) (Fig. 3).

Although prolonged hypoxia is toxic to cells, high levels of oxygen can also be damaging by enhancing production of mitochondrial ROS, resulting in increased oxidative stress. It is now appreciated that ROS are critical activators of autophagy in a variety of cellular contexts, and we discuss in detail ROS and autophagy regulation in the last section.

In addition to the previously mentioned stresses, ER stress also can induce autophagy (79, 113, 137, 168) (Fig. 3). Accumulation of unfolded or misfolded proteins is one of the major causes of ER stress. If not properly degraded, misfolded proteins form aggregates and cause ER dysfunction. The unfolded protein response (UPR) is an essential signaling pathway responding to ER stress that activates many cellular protective mechanisms to help with protein refolding, degradation, and maintenance of ER homeostasis (106). The UPR sensor, inositol-requiring transmembrane kinase and endonuclease1 (Ire1), is conserved in all eukaryotes and is the only UPR sensor in yeast (106). Mammals have at least two other UPR sensors, the protein kinase–like ER kinase (PERK) and activating transcription factor 6 (ATF6) (106). These three sensors control three signal-transduction pathways leading to transcription of a large profile of genes, among which are autophagic genes. Studies have shown that the treatment of neuroblastoma cells with ER stressors induces autophagy, dependent on Ire1 and its downstream effector, c-Jun N-terminal kinase (JNK) (113). In another study, polyglutamine expansion aggregates–induced ER stress triggers autophagy through PERK-mediated activation of eukaryotic translation initiation factor 2α (eIF2α) (79). Besides UPR, other

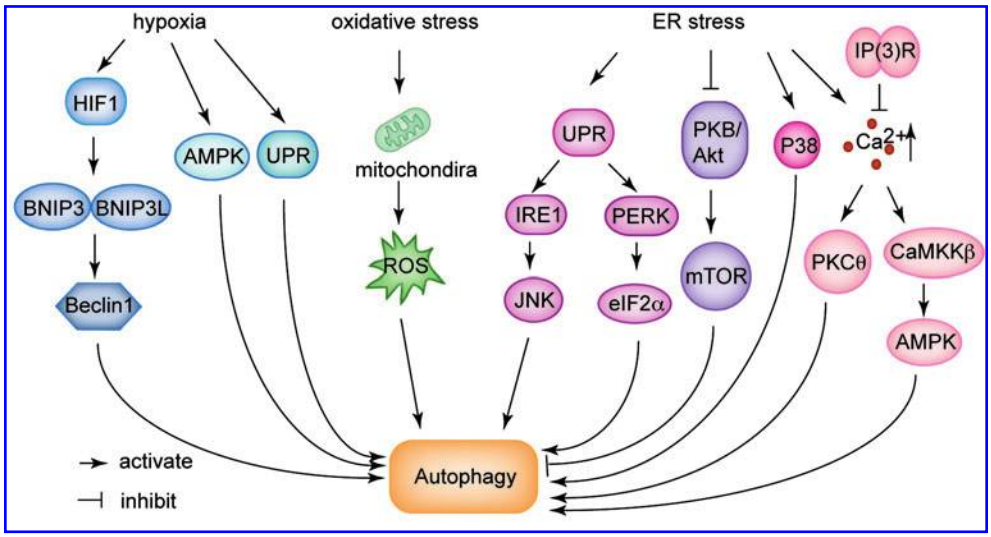

FIG. 3. Induction of autophagy by hypoxia, oxidative stress, and ER stress. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

mechanisms have been reported to mediate ER stress–induced autophagy, such as inhibition of the PKB/Akt-mTOR pathway (127), Ca^{2+}-dependent protein kinase C theta (PKCθ) activity (137), and the p38 mitogen-activated protein kinase (MAPK) pathway (70). In addition, ER stress leads to the release of Ca^{2+} from the ER, and increased cytosolic Ca^{2+} induces autophagy through calmodulin-dependent kinase kinase-*β* (CaMKK*β*)-dependent activation of AMPK (53). Furthermore, inhibition or knockdown of the ER-membrane Ca^{2+} channel, 1,4,5-inositol trisphosphate receptor [IP(3)R], also activates autophagy, although in a Ca^{2+}-independent manner (26).

Signaling in immunity

A growing number of pathogenic microbes have been shown to induce autophagy. However, the mechanisms of autophagy signaling in this field remain largely unknown. Autophagy regulation in immunity is summarized in Fig. 4. Pathogen-detection receptors, including TLRs and NOD-like receptors (NLRs), are crucial host immune factors that sense extra- or intracellular microbes and initiate downstream signaling pathways to activate antimicrobial immune responses (28). A number of TLRs have been reported to stimulate autophagy in murine and human phagocytes (29, 163). Furthermore, agonist-stimulated receptor (such as TLRs and FcγR) signaling during phagocytosis was shown to induce autophagy targeting of the phagosome and to promote phagosomal maturation (54, 138). Recent studies by our laboratory and others imply an essential role for Nox2 NADPH oxidase–generated ROS in autophagy targeting of phagosomes in response to TLR signaling (54, 101).

Besides TLRs, NLR members, such as Naip5, Ipaf, PGRP-LE, and Nod2, can regulate autophagy as well. Naip5 is involved in promoting efficient autophagy of *Legionella pneumophila* (*L. pneumophila*) (2). Ipaf has been shown to negatively regulate autophagy of *S. flexneri* in macrophages (147). PGRP-LE is crucial for autophagy induction and limiting bacterial growth during *L. monocytogenes* infection of *Drosophila* (167). Nod2 mediates autophagy induction and targeting of *S. flexneri* (153). Autophagy can also be upregulated by IFN-γ and its downstream effector, Irgm1 (or human IRGM), to eliminate *M. tuberculosis* and the intracellular parasite, *Toxoplasma gondii* (117). Conversely, autophagy is negatively regulated by NF-κB during tumor necrosis factor (TNF)-α stimulation (32). Viral infection can induce autophagy through the cytosolic double-stranded viral RNA-sensing kinase PKR, activating the transcription factor eIF2α (149). In addition, viruses can inhibit autophagy by modulating autophagy proteins. An α-herpesvirus, HSV-1, produces the neurovirulence factor, ICP34.5, to target Beclin 1 directly and to block autophagy (116).

Reactive Oxygen Species and Their Functions

Intracellular sources of ROS

Free radicals are those chemical species harboring one or more unpaired electrons (106). They are highly unstable and reactive. In biologic systems, the major free radicals are oxygen free radicals, or reactive oxygen species (ROS), because of the involvement of oxygen in most free radical reactions. Oxygen free radicals are short-lived and tend to react with other biomolecules to form other ROS. Superoxide anion ($O_2^{-\bullet}$), hydroxyl radical ($OH^{\bullet}$), and hydrogen peroxide

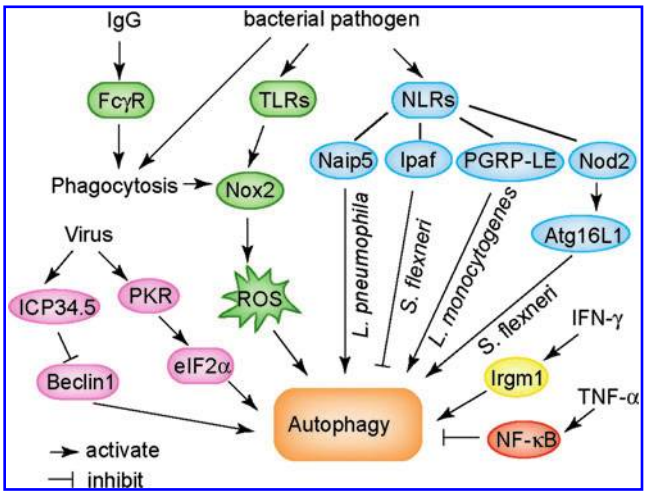

FIG. 4. Regulation of autophagy by immune signalings. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

(H_2O_2) are the most abundant ROS in living organisms (155). The formation of $O_2^{-\bullet}$ in biologic systems is mainly through NADPH oxidases (NOXs) (109), xanthine oxidase (XO) (50), and the mitochondrial electron-transport chain (ETC) (107) (Fig. 5).

NOX family NADPH oxidases are a family of multicomponent enzymes that convert molecular oxygen to superoxide anion in cellular compartments. The NOX family contains five members, Nox1 through Nox5. Closely related to Noxs are the dual oxidases (Duox), including Duox1 and 2 (109). They all share a similar membrane-bound catalytic component, but Duox1 and 2 have an extra peroxidase domain to catalyze superoxide conversion to other ROS. NOX members are expressed in a variety of cell types (109). The best-characterized NOX enzyme is Nox2 NADPH oxidase, which is the major NADPH oxidase expressed in phagocytes and plays a key role in host innate immunity (128). Nox2 NADPH oxidase is composed of a transmembrane catalytic complex, flavocytochrome b_{558} (comprising Nox2/gp91^{phox} and p22^{phox}), and cytosolic components p67^{phox}, p47^{phox}, p40^{phox}, and Rac2 (109). Upon activation by phagocytosis, p47^{phox}, which is in a trimer with p67^{phox} and p40^{phox} in the cytosol, is phosphorylated and undergoes a conformational change (109, 128). This allows it to bind p22^{phox}, translocate p67^{phox} and p40^{phox} to the membrane, and results in functional assembly of the complex (109). Nox2/gp91^{phox} catalyzes electron transfer from cytosolic NADPH to the oxygen molecules in the phagosomal lumen, generating $O_2^{-\bullet}$ (109, 128).

Another enzyme, xanthine oxidase (XO), also produces superoxide anions. XO and xanthine dehydrogenase (XDH) are the same enzyme but with different posttranslational modifications (50). Thus, the term xanthine oxidoreductase (XOR) generally refers to xanthine oxidase. XOR is involved in purine catabolism, converting hypoxanthine to xanthine and xanthine to urate (50). XO transfers electrons from hypoxanthine to the oxygen molecules and forms $O_2^{-\bullet}$. XOR is distributed in different human tissues, such as liver, intestine, skeletal muscle, and kidney, and in different cell types, such as epithelial cells, hepatocytes, enterocytes, goblet cells, capillary endothelial cells, and macrophages. XOR is found in the cytoplasm, perinuclear regions, and in intracellular vesicles (50).

The mitochondrial ETC is also a major source of superoxide formation in living cells (107) (Fig. 5). It is composed of four protein complexes (complexes I to IV), coenzyme Q (CoQ), and cytochrome *c*. Electrons are transferred from NADH at complex I to complex II and through CoQ to complex III, and then by cytochrome *c* to complex IV, where they are accepted by oxygen molecules to form water. However, about 0.2% of the oxygen consumed by mitochondria is only partially reduced and is converted to superoxide because of electron "leaking" from the ETC (83). Large amounts of $O_2^{-\bullet}$ are produced at the mitochondrial complex I when the NADH/NAD^+ ratio is high or reverse electron transport occurs (107). Complex III also produces superoxide but at a much lower level compared with complex I under physiologic conditions (154). Other mitochondrial enzymes can also be sources of $O_2^{-\bullet}$ generation under certain conditions, such as α-ketoglutarate dehydrogenase, α-glycerophosphate dehydrogenase, and dihydroorotate dehydrogenase (107).

$O_2^{-\bullet}$ is short-lived and subsequently converted into H_2O_2 by superoxide dismutase (SOD) (44). $O_2^{-\bullet}$ produced by NOXs or XOR is dismutated to H_2O_2 by the cytosolic CuZnSOD, whereas mitochondria have their own SOD, MnSOD, which functions specifically in H_2O_2 formation within the mitochondrial matrix (44). Peroxisomes are another major site of H_2O_2 generation (Fig. 5) (3). Peroxisomes contain enzymes that catalyze the oxidation of fatty acids or amino acids and produce H_2O_2 as a side product. In addition, the ER luminal thiol oxidase Ero1 catalyzes the electron transfer from dithiol to the oxygen molecule, and forms disulfide bonds in substrates as well as H_2O_2 as a byproduct (139) (Fig. 5). Under ER-stress conditions, ROS can be generated by Ero1, the

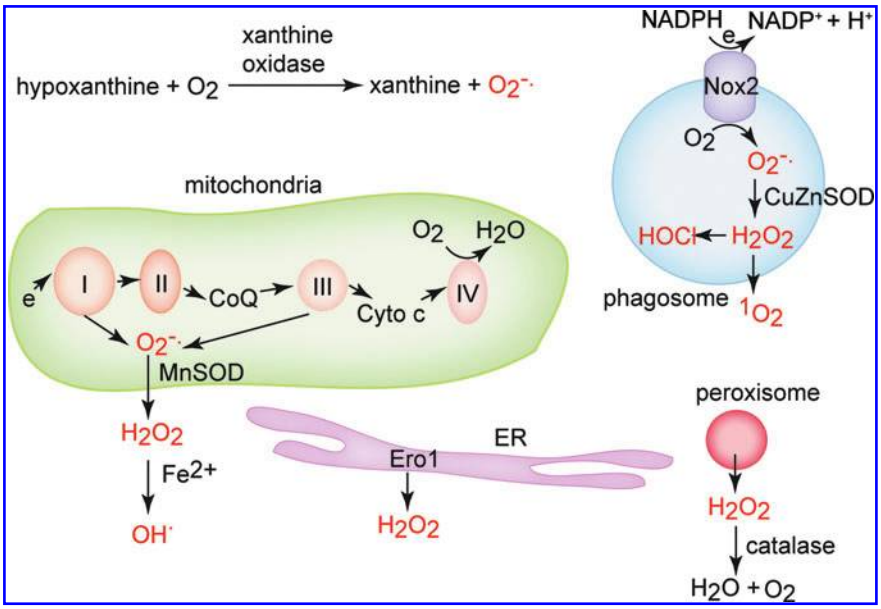

FIG. 5. Intracellular sources of ROS. Superoxide anions ($O_2^{-\bullet}$) are generated by xanthine oxidase in catalyzing the oxidation of hypoxanthine to xanthine; by NADPH oxidase (*e.g.*, Nox2 on phagosomal membrane in phagocytic cells) in converting NADPH to $NADP^+$; and by mitochondrial electron-transport chain reaction, in which electrons (e) are transferred from the mitochondria complex I (I) to CoQ via the complex II (II), then to cytochrome *c* via the complex III (III), and finally to O_2 via the complex IV (IV), and H_2O is produced. Electrons can leak from the electron-transport chain, and $O_2^{-\bullet}$ is generated from complexes I and III. $O_2^{-\bullet}$ is converted to H_2O_2 by CuZnSOD in the cytosol, or by MnSOD in the mitochondria. H_2O_2 can react with Fe^{2+} and produce $OH^\bullet$, or produce HOCl and $^1O_2$ when combined with Cl^- or $O_2^{-\bullet}$. Other sources of H_2O_2 include the ER and peroxisome. H_2O_2 can be further decomposed into H_2O and O_2 by catalase. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

mitochondria, and the Nox4 NADPH oxidase through the unfolded protein response (139).

When cells are exposed to high concentrations of O_2, and intracellular $O_2^{-\bullet}$ or H_2O_2 is not efficiently eliminated, cells are under oxidative stress. At this point, the iron-containing enzyme clusters will free Fe^{2+}, which reacts with H_2O_2 to generate $OH^{\bullet}$ (44). The $OH^{\bullet}$ radical is very short-lived and highly reactive. It immediately reacts with the molecules at the site where it is produced. By combining with Cl^- or $O_2^{-\bullet}$, H_2O_2 further forms hypochloric acid (HOCl) and singlet oxygen ($^1O_2$) (44) (Fig. 5).

Cells have evolved protective mechanisms to scavenge ROS and maintain cellular redox homeostasis. ROS scavengers include the enzymes SOD, catalase (decomposes H_2O_2 into H_2O and O_2), glutathione peroxidase (decomposes lipid and hydroperoxides), and antioxidants such as glutathione (GSH), ascorbic acid, and vitamins C and E (44, 155). Cells also have redox systems to maintain redox homeostasis. GSH and thioredoxin (TRX) are two major redox systems that have antioxidant function (62). Conversion of GSH to its oxidized form, glutathione disulfide (GSSG), is coupled with many ROS-decomposition reactions and disulfide bond reduction. TRX catalyzes the reduction of protein disulfide bonds. These antioxidant mechanisms keep the intracellular ROS at a tolerable level.

Dual Roles of ROS in Health

Cytotoxic ROS

Excessive ROS are harmful to cells because of their capacity for damaging cellular components, including DNA, proteins, and lipids. The deleterious effects of ROS are summarized in Fig. 6. ROS interaction with nucleic acids affects DNA structure, resulting in gene mutagenesis, an initial step of carcinogenesis (155). Cysteine residues in proteins can be oxidized by ROS to form Cys-SOH, Cys-SO_2H, and Cys-SO_3H or disulfide bridges (62). These modifications may affect protein structure, function, and stability. ROS interact with a variety of membrane ion-transport proteins, which leads to a disturbance of intracellular ion homeostasis and normal ion-regulated signaling pathways (78). Lipids are also sensitive to ROS (44). Oxidation of the unsaturated fatty acid chains of phospholipids can lead to the formation of lipofuscin, an undegradable pigment granule that is often seen accumulated in long-lived postmitotic cells and is related to aging (13). Lipid oxidation is also a cause of membrane damage. Mitochondria and lysosomes are the two major sites of lipofuscin formation. Accumulation of ROS in the mitochondria can also impair ATP production, cause membrane permeabilization, and lead to apoptosis (119). ROS-induced lysosomal membrane damage releases iron and acidic hydrolases into the cytosol and can lead to cell death. The toxicity of excessive ROS is linked to aging and many human diseases, such as neurodegenerative diseases, cancer, cardiovascular disease, ischemia, and diabetes. Mechanisms and details are extensively reviewed elsewhere (44, 119, 155).

Beneficial ROS

ROS are important secondary messengers in a number of signal-transduction pathways critical for cell growth and proliferation (98, 155) (Fig. 6). The role of ROS as signaling molecules relies on the evidence that many cytokines and growth factors can stimulate ROS production when they bind membrane receptors and initiate signal transduction (112, 155). These signaling pathways generate NADPH oxidases–derived ROS within confined compartments, such as endosomes, and ROS act as secondary messengers to regulate downstream pathways (112). For example, ROS can oxidize the thiol group in cysteine residues, often located in the catalytic domain of cellular enzymes. Studies have shown that ROS regulate the activities of tyrosine kinases (*e.g.*, Src) (5), serine/threonine kinases [*e.g.*, MAPKs (ERK, JNK, and p38), and PKB/Akt) (11, 98)] and protein phosphatases (*e.g.*, PTEN) (22). Moreover, ROS regulate the activity of certain transcription factors, such as NF-κB for immune responses (42), HIF-1 for hypoxic response (145), and p53 for cell growth and proliferation (88). Besides promoting cell growth, ROS also activate cell-death pathways, such as apoptosis, necrosis, and autophagic cell death, which is beneficial in cancer treatment (119).

In addition, ROS play important roles in immunity. Immune cells, such as macrophages and neutrophils, generate a large amount of ROS during phagocytosis of microbes through the activation of the Nox2 NADPH oxidase (128). This process is known as the oxidative burst, playing a key role in innate immunity against invading pathogens. Studies

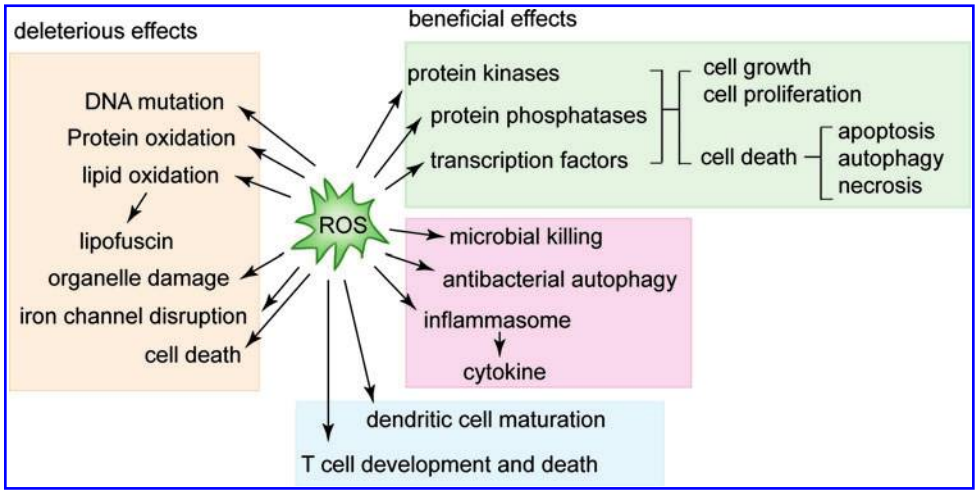

FIG. 6. A summary of the deleterious and beneficial effects of ROS. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

have shown that chronic granulomatous disease (CGD) patients who bear mutations in Nox2 component genes experience recurrent and severe infections with certain bacteria and fungi (142). Although the toxicity of ROS is believed to account for the direct killing of microbes within phagosomes (128), indirect microbicidal effects of ROS also exist. For example, ROS inhibit pathogen infection by modifying and detoxifying microbial virulence factors (23, 24). We recently demonstrated that Nox2 NADPH oxidase–generated ROS triggered antibacterial autophagy in mouse macrophages and neutrophils (54). Furthermore, ROS are shown to activate immune signaling pathways. As mentioned earlier, various stimulatory ligands can induce ROS-mediated activation of NF-κB (*e.g.*, TNF-α, IL-1β, and LPS), although in certain conditions, ROS have an inhibitory effect on TNF-induced NF-κB activation (42).

ROS also regulate cytokine production. For example, a pathogen-recognition molecule NLR family, pyrin domain-containing 3 (NLRP3) inflammasome senses pathogens and stress signals to activate maturation and secretion of IL-1β (141). Recent studies indicate a ROS-dependent stimulation of NLRP3 inflammasome on infections by influenza A virus, fungus *Candida albicans,* and *Aspergillus fumigatus* (1, 45, 135). NOX proteins have been implicated in ROS-dependent inflammasome activation, as one study shows abolished IL-1β production in $p22^{phox}$-knockdown THP1 cells stimulated by the airborne pollutant asbestos, which activates inflammasomes (33). In contrast, recent studies demonstrate the possibility of NOX-independent activation of inflammasomes. Primary monocytes isolated from CGD patients with mutated $p47^{phox}$ or absent $p22^{phox}$ expression showed even higher production of IL-1β on stimulation (156, 157), suggesting that NOX-derived ROS suppress inflammasome activation in these cells. As previously mentioned, the $p40^{phox}$-encoding gene *NCF4* has been identified to be a susceptibility gene to CD and rheumatoid arthritis (115, 130, 131), implying a role of ROS in the pathology of inflammatory diseases in general.

ROS also participate in adaptive immunity activation. Studies have shown that ROS treatment of DCs activates their maturation (134). ROS also modulate T-cell proliferation and apoptosis (17, 43, 52) and are therefore critical for maintaining T-cell homeostasis. Taken together, ROS are critical modulators in innate immune defense systems, inflammatory responses, and adaptive immunity.

ROS and Autophagy

Autophagy and oxidative stress

Autophagy as a cell-survival mechanism. Autophagy is a well-known stress-induced cellular survival mechanism. Under oxidative stress, large amounts of ROS oxidize proteins and damage their functions. These oxidized proteins may form aggregates and become toxic to cells. Autophagy is able to degrade these oxidized proteins (68, 120, 162, 174). Chaperone-mediated autophagy has been shown to be able to degrade oxidized protein substrates (68). Moreover, oxidative stress induces autophagy-mediated degradation of oxidized proteins in the plant *Arabidopsis* (162). A recent report demonstrated that oxidized low-density lipoprotein, a possible causative agent of atherosclerosis, induced autophagy in human umbilical vein endothelial cells, and its turnover was dependent on autophagy (174).

Autophagy suppresses cellular ROS level by eliminating ROS-producing compartments to protect cells from ROS-induced damage. For example, when mitochondrial ROS are significantly elevated, the mitochondrial membrane can be damaged, resulting in the leakage of ROS into the cytosol, damaging other organelles. Autophagy selectively targets and removes these damaged organelles (*e.g.*, mitochondria and ER) by mitophagy or reticulophagy (10, 71), serving as a quality-control mechanism. It has been shown that under metabolic stress, autophagy deficiency caused accumulation of abnormal mitochondria and elevated ROS levels in $atg5^{-/-}$ and $beclin1^{+/-}$ iBMK cells (93). Similarly, autophagy removes aberrant ER during ER stress and thus may limit ROS production from the ER stress (139). Recent findings demonstrating that autophagy targets phagosomes during phagocytosis (54, 138) suggest that autophagy may limit NADPH oxidase–generated ROS in phagosomes. One recent study indicated that autophagy also controls the antiviral signaling by eliminating dysfunctional mitochondria and ROS. SsRNA virus can be recognized by cytosolic RIG-I–like receptors (RLRs) and initiate antiviral signals. Autophagy deficiency causes the accumulation of dysfunctional mitochondria and ROS, which abnormally upregulate the RLR signaling pathway (148).

A growing body of studies show that autophagy is activated by ROS as a cytoprotective process. Bacterial endotoxin LPS induces ROS accumulation in cardiomyocytes and leads to death. Autophagy is stimulated to clear ROS to protect cells from LPS-induced cardiomyocyte damage (171). Furthermore, HYD1, a D-amino acid peptide that inhibits adhesion of prostate cancer cells to the extracellular matrix, was found to induce ROS-dependent autophagy as a cell-survival mechanism in multiple myeloma cells (108).

Autophagy as a cell-death mechanism. However, when oxidative stress reaches a level beyond the control of cellular protective mechanisms, cell death will occur through necrosis, apoptosis, or autophagic cell death. Massive induction of autophagy by ROS may cause excessive self-digestion of cell components and lead to death. Autophagic cell death makes autophagy an attractive target of study in cancer research. A rapidly growing body of studies show that exogenous ROS, inhibition of cellular antioxidant systems, inhibition of mitochondrial ETCs, metabolic stresses, cytokine (TNF-α), and anticancer drug treatments that induce cellular ROS production can all induce ROS-dependent autophagic cell death in various cancer cell lines under certain conditions (14, 18–21, 32, 41, 69, 89, 140, 159, 160, 164–166, 173).

In addition, autophagy was reported to contribute to cellular ROS accumulation and cell death (82, 170) (Fig. 7). In one study, caspase inhibition induced autophagy, which selectively degrades the H_2O_2 scavenger, catalase. This led to accumulation of cellular ROS and cell death (170). A recent report showed that glutamate-induced ROS accumulation and cell death in mouse brain cell lines were suppressed by autophagy and lysosomal inhibitors, indicating that they are sites of basal-level ROS generation (82)

Mechanisms of autophagy regulation by ROS

As mentioned earlier, besides exogenous ROS, stimuli (*e.g.*, TNF-α and LPS) that can trigger ROS-generation signaling

pathways may in turn induce autophagy (32, 163, 171). In most studies, mitochondria are the major source of ROS for autophagy induction. Recent studies by our laboratory and others demonstrate that NADPH oxidase–generated ROS contribute to autophagy induction (54, 101). TLR and FcγR-mediated phagocytosis in mouse macrophages and neutrophils stimulate Nox2 NADPH oxidase activity and generate ROS in phagosomes. The autophagy protein LC3 is recruited to the latex-bead or bacteria-containing phagosomes in a Nox2 NADPH oxidase and ROS-dependent manner (54). Autophagy targeting of phagosomes leads to fusion with lysosomes and microbial killing (54, 138). It was also observed in human neutrophils that TLR agonists, PMA, and phagocytosis triggered ROS-dependent autophagy induction (101). Besides Nox2 NADPH oxidase and phagocytic cells, other NOX-derived ROS also are involved in autophagy induction and targeting of intracellular *Salmonella* in nonphagocytic cells (54). These data imply a general role for NOX-generated ROS in autophagy activation as an innate immune-defense mechanism.

Although many studies revealed ROS to be a signal in autophagy induction, little is known about the mechanism of action. Figure 7 illustrates the current known modes of ROS-regulated autophagy. One model proposed by Elazar and colleagues (140) indicates that nutrient starvation increases intracellular H_2O_2, which inhibits the protease activity of Atg4 by oxidizing a critical cysteine residue near the catalytic site. It is not yet clear how inhibition of Atg4 activity activates autophagy. It is possible that the formation of ROS in mitochondria is transient on starvation and may act only on the proximate pool of Atg4, which prevents the cleavage of LC3 from the membrane and results in more LC3-PE in the cell. This may lead to more autophagosome formation, although other factors may be regulated by ROS as well and contribute to autophagy induction.

Some studies show that ROS activate autophagy by regulating mTOR activity. In malignant glioma, ROS disrupt mitochondrial membrane potential and induce autophagy through inhibiting Akt/mTOR signaling (173). In C6 glioma cells, ROS induce autophagy by inhibiting mTOR in a BNIP3-dependent manner (14).

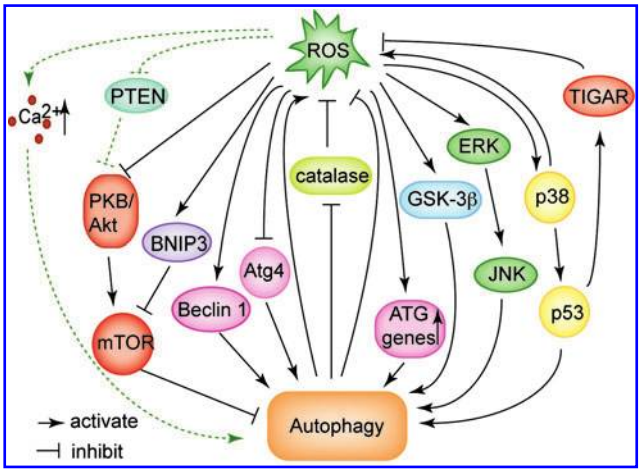

FIG. 7. Mechanisms of autophagy regulation by ROS. The green dashed lines indicate the potential signaling pathways through which ROS may regulate autophagy. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

In addition, MAPKs have been found to be downstream effectors of ROS in autophagy induction. A novel compound, 1,3-dibutyl-2-thiooxo-imidazolidine-4,5-dione, was recently found to induce autophagic cell death in tumor cells through stimulating ROS production and, in turn, activation of ERK and JNK (160). A mannose-binding lectin, *Polygonatum cyrtonema* lectin, induces autophagy in A375 cells through mitochondria-mediated ROS-p38-p53 pathway (89). ROS-induced ATG gene upregulation in skeletal cells requires p38 activation (96). However, p38 and p53 also regulate ROS production, possibly as positive-feedback responses (88, 91, 171). For example, LPS induces ROS-dependent autophagy in cardiomyocytes in a p38-dependent manner (171). One p53-target gene encodes TIGAR (TP53-induced glycolysis and apoptosis regulator), which indirectly modulates ROS level. Inhibition of TIGAR expression increases ROS production and enhances ROS-dependent autophagy (8). However, TIGAR regulates autophagy in an mTOR- and p53-independent manner. Another mechanism was reported that cadmium (toxic heavy metal) induces autophagy in mesangial cells through the ROS-GSK-3β pathway (159).

NOX-activated autophagy targeting of phagosomes may involve localized signals. ROS can cause phagosomal membrane lipid oxidation and microdamages to phagosomes. This may therefore serve as a signal to induce autophagy to repair the membrane damage, acting in a housekeeping role to degrade damaged organelles. Some ATG genes, including LC3, were found to be upregulated at transcriptional levels during TLR-, PMA-, and phagocytosis-stimulated autophagy induction, suggesting a possible role for ROS in regulation of ATG protein expression (101).

Although some ROS-regulated signaling pathways have been shown to modulate autophagy, it is not known whether they function in the same pathway. For example, ROS can inhibit PTEN (22), which has been shown to activate autophagy through the downregulation of the class I PI3K-PKB/Akt pathway. ROS can also affect the Ca^{2+} channel IP(3)R to increase the intracellular Ca^{2+} level (78), which has been reported to induce autophagy (26, 53). Although many studies use H_2O_2 as an ROS source and H_2O_2 is a relatively stable signaling molecule in many signal-transduction pathways, it is now thought that $O_2^{-\bullet}$, rather than H_2O_2, is the major ROS that regulates starvation- and mitochondria ETC inhibition-induced autophagy (19). Exogenous H_2O_2 treatment, ETC inhibition, as well as carbon starvation (medium lacking glucose, glutamate, and pyruvate) results in increased intracellular $O_2^{-\bullet}$ levels, but not H_2O_2 levels. Nitrogen starvation (lacking amino acid) increases both $O_2^{-\bullet}$ and H_2O_2 levels, but conversion of $O_2^{-\bullet}$ to H_2O_2 by SOD overexpression results in decreased $O_2^{-\bullet}$ and reduced autophagy, whereas H_2O_2 levels remain constant (19). Therefore, $O_2^{-\bullet}$ is the major ROS regulating autophagy. However, it remains difficult to conclude whether other forms of ROS have an effect on autophagy because of limitations of current techniques to distinguish and measure the different types of ROS and the complex redox-regulation systems that influence ROS levels.

Overall, still many unknowns exist regarding the downstream signals of ROS in autophagy regulation. It is likely that ROS act through different mechanisms to regulate autophagy under different cellular contexts and in different cell types. Examining the downstream signals of ROS that overlap with

autophagy-induction pathways may help to determine the mechanism of ROS-induced autophagy.

Conclusions

ROS control a wide range of cell signal-transduction pathways leading to cell survival, proliferation, and death. As essential antimicrobial molecules, ROS also regulate other innate immune defense mechanisms, such as activating NLRP3 inflammasome and autophagy. Autophagy is an important cell-survival and death mechanism and is now appreciated to be a crucial player in both innate and adaptive immunity. Regulation of autophagy induction involves complex signaling pathways that differ under various conditions. ROS generated from both mitochondria and NADPH oxidases have been shown to activate autophagy to protect cells from nutrient starvation, dysfunctional mitochondria, cell death, and invading pathogens. Mechanisms of autophagy regulation by ROS vary by different stimuli and cell types. The connection of NADHP oxidases to autophagy implies a critical signaling role for ROS in autophagy regulation in immune cells. Further studies aimed at the participation of ROS in other pathogen- or immune-related activation of autophagy and mechanisms in addition to these processes will aid in the understanding of the pathology of various infectious diseases.

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Abbreviations Used

AMPK = 5′-AMP-activated protein kinase
Ape1 = aminopeptidase 1
ATF6 = transcription factor 6
ATG = autophagy-related gene
BNIP3 = Bcl2/E1B 19-kDa–interacting protein 3
BNIP3L = BNIP3-like protein
CaMKKβ = calmodulin-dependent kinase kinase-β
CD = Crohn disease
CGD = chronic granulomatous disease
CMA = chaperon-mediated autophagy
CoQ = coenzyme Q
Cvt = cytoplasm-to-vacuole targeting
DC = dendritic cell
eIF2α = eukaryotic translation initiation factor 2α

Abbreviations Used (Cont.)

ER = endoplasmic reticulum
ETC = electron-transport chain
GAIP = Gα-interacting protein
GSH = glutathione
GSSG = glutathione disulfide
HIF-1 = hypoxia-inducible factor-1
H_2O_2 = hydrogen peroxide
HOCL = hypochloric acid
IBD = inflammatory bowel disease
IFN = interferon
IL = interleukin
IP(3)R = 1,4,5-inositol trisphosphate receptor
IRE1 = transmembrane kinase and endonuclease1
IRG = immunity-related GTPase
JNK = c-Jun N-terminal kinase
LPS = lipopolysaccharide
MAPK = mitogen-activated protein kinase
MEF = mouse embryonic fibroblast
MHC = major histocompatibility complex
NADPH = nicotinamide adenine dinucleotide phosphate
NBR1 = neighbor of *BRCA1* gene 1
NF-κB = nuclear factor-kappa-B
NLR = NOD-like receptor
NLRP3 = NLR family, pyrin domain-containing 3
NOD2 = nucleotide-binding and oligomerization domain containing 2
$O_2^{-\bullet}$ = superoxide anion
$^1O_2$ = singlet oxygen
$OH^\bullet$ = hydroxyl radical
PAS = preautophagosomal structure
PE = phosphatidylethanolamine
PERK = protein kinase-like ER kinase
PKB = protein kinase B
PKCθ = protein kinase C theta
PRRs = pathogen pattern-recognition receptors
PtdIns3K = phosphatidylinositol 3-kinase
PTEN = phosphatase and tensin homologue
Rheb = Ras homologue enriched in brain
RLR = RIG-I-like receptor
ROS = reactive oxygen species
SOD = superoxide dismutase
ssRNA = single-stranded RNA
TIGAR = TP53-induced glycolysis and apoptosis regulator
TLR = Toll-like receptor
TNF-α = tumor necrosis factor-α
TOR = target of rapamycin
TRX = thioredoxin
TSC1/2 = tuberous sclerosis complex 1 and 2
UBA = ubiquitin associated
UPR = unfolded protein response
XDH = xanthine dehydrogenase
XO = xanthine oxidase
XOR = xanthine oxidoreductase

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