Review Article

Macrophage Autophagy in Atherosclerosis

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Macrophages play crucial roles in atherosclerotic immune responses. Recent investigation into macrophage autophagy (AP) in atherosclerosis has demonstrated a novel pathway through which these cells contribute to vascular inflammation. AP is a cellular catabolic process involving the delivery of cytoplasmic contents to the lysosomal machinery for ultimate degradation and recycling. Basal levels of macrophage AP play an essential role in atheroprotection during early atherosclerosis. However, AP becomes dysfunctional in the more advanced stages of the pathology and its deficiency promotes vascular inflammation, oxidative stress, and plaque necrosis. In this paper, we will discuss the role of macrophages and AP in atherosclerosis and the emerging evidence demonstrating the contribution of macrophage AP to vascular pathology. Finally, we will discuss how AP could be targeted for therapeutic utility.

1. Introduction

Atherosclerosis-related cardiovascular diseases are the leading cause of mortality worldwide. In addition to lipid dysfunction and arterial lipid accumulation, immune-inflammatory responses are major factors in directing the initiation and development of atherosclerosis [1, 2]. Macrophages play a central role in each stage of disease pathogenesis [3]. Interestingly, recent investigation into macrophage autophagy (AP) has demonstrated a novel pathway through which these cells contribute to vascular disease [4–7]. In this paper, we will discuss the role of macrophages and AP in atherosclerosis and the contribution of macrophage AP to vascular pathology. Finally, we will discuss how AP could be targeted for therapeutic utility in atherosclerosis.

2. The Origin of Vascular Macrophages

Macrophages are defined as diverse, scavenging, and bactericidal tissue-resident cells with critical immune functions. They are present in every endothelial and epithelial surface of the body, exhibit stellate morphology, and express markers including F4/80, CD11b, CD115, macrosialin (CD68), and CD83. They also express an array of Fc receptors, receptors for complement components, scavenging receptors, and pathogen recognition receptors such as Toll-like receptors (TLRs) and Nod-like receptors (NLRs). When activated, tissue macrophages phagocytose and kill microorganisms and secrete proinflammatory cytokines. In addition, the proinflammatory cytokines and chemokines they release upon activation contribute to the recruitment and activation of lymphocytes. However, it is these very functions that drive their well-established role in inflammatory conditions such as atherosclerosis.

The origin of tissue macrophages has been receiving much attention recently, with many long-held concepts proving incorrect. Indeed, many tissue macrophage populations do not arise from blood monocytes but maintain themselves locally in tissues after they are seeded by yolk sac macrophages [8, 9]. However, to our knowledge, the origin of vascular macrophages in the steady state is unclear and during inflammation, it is clear that input from circulating

monocytes is critical [10]. Monocytes originate from common CSF-1R$^+$CX3CR1$^+$Flt3$^+$ macrophage/dendritic cell precursors (MDPs) [11] and expand in response to macrophage colony-stimulating factor (M-CSF) [12]. Monocytes in the mouse can be divided into 2 subsets, classical (Ly6Chi CCR2+) and nonclassical monocytes (Ly6Clo CCR2lo) [13], with analogous subsets present in humans [14]. Classical monocytes exit the bone marrow in a CCR2-dependent manner to seed sites of inflammation [15], whereas it is as yet unclear how and if nonclassical monocytes arise from the bone marrow [16].

A central feature of atherosclerosis is the accumulation in the lesion of monocyte-derived, lipid-laden macrophages termed foam cells and, indeed, monocyte recruitment into plaques is critical for, and increases with, disease progression [10, 17, 18]. Consistent with this, mice deficient in M-CSF-derived macrophages (op/op) have reduced development of atherosclerosis [19]. However, perhaps the most compelling evidence of the role of monocyte-derived cells in atherosclerosis is borne out of successful therapeutic studies in mice targeting chemokine/chemokine receptors critical for monocyte chemoattraction to the plaque [20, 21]. Activation of blood vessel endothelium results in the arrest and extravasation of circulating monocytes into the plaque [22], and the extent of recruitment is regulated at least in part by blood monocyte levels [23]. Hypercholesterolemia correlates with an increase in the frequency of classical monocytes, and it is primarily this subset of monocytes that seeds the plaque [24]. Nevertheless, the capacity of nonclassical monocytes to patrol blood vessel walls [11] could be pertinent to the inflammatory process during atherosclerosis, and indeed this subset has been demonstrated to enter plaques [25, 26].

3. The Role of Macrophages in Atherosclerosis

Upon entry into the vascular wall, monocytes undergo maturation into macrophages that are critical for the inflammatory response. Although there is undoubtedly heterogeneity in plaque macrophages, the majority of macrophages in the plaque are classically rather than alternatively activated, and this is discussed in recent reviews [27]. Concomitant with this maturation process, macrophages engulf vast amounts of lipid in the form of apoB-containing lipoproteins into membrane-bound droplets to form foam cells [3]. Macrophages utilize scavenger receptors like CD36 and scavenger receptor type A *inter alia* to recognize modified low-density lipoproteins (LDL) [28, 29], and uptake of oxLDL alone can drive inflammatory gene expression in macrophages through a novel recognition pathway involving a CD36-dependent TLR4-TLR6 heterodimer [30]. In addition, signals through scavenging and c-type lectin-like receptors, TLRs, and numerous intracellular sensors can drive macrophage activation. Intriguingly, several endogenous ligands for TLRs such as heat shock proteins (HSPs) can be found in high concentration in atherosclerotic plaques [31] and have been proposed as a potential pathway to activate macrophages and perpetuate atherosclerosis [32]. Interestingly, recent work focusing on the effects of cholesterol crystals on macrophages has uncovered a novel pathway of

macrophage activation in atherosclerosis [33, 34]. The intracellular apparatus consisting of NLRP3, ASC, and caspase-1 all cooperate to drive the generation and subsequent release of active IL-1β and IL-18 by macrophages in response to cholesterol crystals and play an important role in the development of atherosclerosis [33]. Thus, there are a plethora of described pathways, and additional putative mechanisms, that drive macrophage activation during atherosclerosis.

Once activated, macrophages produce an array of proinflammatory cytokines such as TNFα, IL-12, IL-6, IL-1β [35], and leukotrienes [36] that drive inflammation during atherosclerosis. This, together with their production of inflammatory chemokines such as MCP-1, IL-8, and MIP-3α, results in further recruitment of monocytes, neutrophils and other inflammatory cells. Macrophage-derived TNFα and IL-1β also activate the vascular endothelium to upregulate adhesion molecules and chemokines [22, 37] and thus promote monocyte migration as part of a positive feedback loop. Activation of macrophages is also enhanced by IFNγ released by NK cells and T cells, and macrophage-derived cytokines, such as IL-12 and IL-15, can in turn drive proatherogenic T cells [38]. In addition to the production of inflammatory mediators, macrophage activation results in the induction of several bactericidal systems such as the NADPH oxidase enzyme. This converts oxygen into the superoxide anion and other free radicals, and these reactive oxygen intermediates (ROIs) are toxic to microbes but can damage host tissue due to their capacity to cause DNA degradation and inactivation of metabolic enzymes, and indeed perpetuate atherosclerosis [39]. Activated macrophages also release nitric oxide (NO) which combined with superoxide, generate peroxynitrite which causes cell injury [39, 40]. Further, myeloperoxidase- (MPO-) generated reactive nitrogen species from monocytes contributes to the conversion of LDL to an atherogenic form [41]. In addition, macrophages express nonspecific esterase, lysosomal hydrolases, and ectoenzymes [42], and secrete an array of cathepsins [43] and matrix metalloproteinases (MMPs) [44] that degrade collagens and the extracellular matrix, likely contributing to thinning of the fibrous cap and plaque instability.

Combined with overzealous macrophage activation in atherosclerotic plaques is the impairment of macrophage functions critical for the control and resolution of inflammation. Indeed, an important function of macrophages under both resting and inflammatory conditions is the rapid uptake of apoptotic cells from tissues, termed efferocytosis. Efferocytosis is mediated by a range of receptors such as CD36 [45] and MerTK [46], and chronic apoptosis of lipid-filled foam cells is combined with defective efferocytosis during atherosclerosis [47], likely contributing to the formation of the necrotic core. Interestingly, the receptors involved in the recognition of apoptotic cells, such as CD36 and $\alpha v \beta 3$, may also be involved in the recognition of necrotic cells [48]. This may be pertinent when one considers that the vast majority of cell death in advanced plaques is the result of necrosis, a process that drives inflammation and formation of the necrotic core [49].

Finally, an increasing body of evidence indicates that macrophages have developed several strategies to survive

and proliferate in the atherosclerotic plaque, such as the unfolded protein response and AP [50]. In particular, recent investigation into macrophage AP in atherosclerosis has demonstrated another pathway through which these cells contribute to vascular pathology [4–7].

4. Autophagy Machinery and Regulation

AP (derived from Greek words, "auto" meaning "self" and "phagy" meaning "to eat") is an evolutionarily conserved controlled cellular catabolic process involving the delivery of cytoplasmic contents to the lysosomal machinery for ultimate degradation and recycling. In mammalian cells, several types of AP have been identified; they are differentiated on the basis of their physiological functions and the mode of cargo delivery to the lysosomal compartment, such as chaperone-mediated AP, microAP, macroAP, and others [51]. MacroAP has been studied most extensively as compared with other types of AP and this paper will focus on macroAP (herein referred to as "AP"). The AP mechanism involves the formation of characteristic double-membrane vesicles, called autophagosomes or autophagic vacuoles, in which cytoplasmic material is sequestered. The origins of this structure remain incompletely understood; it may be generated from multiple sources including the endoplasmic reticulum (ER) [52, 53], the outer mitochondrial membrane [52, 54], and the plasma membrane [55, 56]. The autophagosomes are targeted to lysosomes to form single-membraned autolysosomes with degradative capacity. During the degradative phase, a series of lysosomal enzymes (e.g., cathepsins and other acid hydrolases) digest the contents of autolysosomes, that are then released to the cytosol for recycling or reuse for anabolic pathways and to get rid of toxic harmful cellular substances [57, 58].

In mammalian systems, basal AP is a continuous process serving as a quality control system to clear and recycle damaged and/or unwanted components of the cell including organelles and protein aggregates. This pathway is stimulated by numerous cellular or subcellular stresses, together with nutrient or growth factor deprivation, reactive oxygen species (ROS), hypoxia, DNA damage, protein aggregates, dysfunctional organelles, or intracellular pathogens to counter the stress for cell survival [58]. AP is mostly considered as a cell survival and cytoprotective process but under chronic stress situations, it is also associated with cell death (hence called "autophagic cell death" rather than "cell death with autophagic features"), though the meaning of AP in these situations remains controversial [59]. It is now well acknowledged that AP can exert a critical and decisive influence on a great variety of human physiological and pathophysiological processes, such as cancer, neurodegenerative disorders and cardiovascular diseases [60]. Moreover, the AP machinery also orchestrates various responses to exogenous stimuli such as microorganisms [61]. For instance, AP plays a key role in the defense against bacterial infection [62, 63]. AP is also required for antigen presentation via major histocompatibility complex (MHC) class II, which plays a key role in immune driven diseases [64], including atherosclerosis [65].

Recently, progress has been made in characterizing the AP protein machinery and signaling cascades. It has been demonstrated that, in mammalian cells, the proteins encoded by AP-related genes (Atg) generally form multiprotein complexes that are crucial for autophagosome formation. The core machinery of mammalian AP incorporates five functional subgroups: (i) the unc-51-like kinase (ULK) complex, including ULK1/2, Atg13, and FIP200, involved in AP induction; (ii) the class III phosphatidylinositol 3-kinase (PI3K) complex, consisting of Vps34, Beclin 1, p150, and Barkor (Atg14-like protein). The lipid kinase activity of Vps34 is indispensable for generating phosphatidylinositol (3)-phosphate (PI3P) at the PAS (phagophore assembly site) for the recruitment of other Atg proteins and the formation of the autophagosome; (iii) two ubiquitin-like protein (Atg12 and LC3) conjugation systems involved in the expansion of autophagosome membranes; (iv) Atg9 and its recycling system that contributes to the delivery of membranes forming the autophagosome; (v) the proteins needed for the fusion between autophagosomes and lysosomes for ultimate degradation. The detailed literature concerning these topics can be found elsewhere [51, 58]. However, several non-Atg proteins and different macromolecular signaling complexes are shown to contribute in the regulation of this process [51, 66, 67].

Mammalian target of rapamycin (mTOR), and particularly its complex 1 (mTORC1), acts as a major checkpoint. In normal conditions (presence of growth factors and nutrients), mTORC1 induces phosphorylation of ULK1/2 and Atg13, which inhibits ULK complex activity and, in turn, AP. Many diverse signals, such as growth factors, amino acids, glucose, energy status, and different forms of stress, regulate the mTOR pathway [68]. In conditions that trigger AP, such as nutrient starvation or stimulation with the antibiotic rapamycin, the mTORC1 serine/threonine kinase activity is inhibited; mTORC1 dissociates from the ULK complex, which becomes active. mTORC1 also incorporates upstream activating signals that inhibit AP via the class I PI3K (and protein kinase B, PKB, also known as Akt) pathway [69]. AMPK (adenosine $5'$-monophosphate-activated protein kinase) and Sirtuin1 (Sirt1) also control starvation-induced AP through a coordinated fashion depending upon the energy status (ATP/AMP levels) of the system [70], but at the same time AMPK negatively regulates mTORC1 [71, 72]. A second regulatory step required for the autophagosome formation involves the "Beclin 1 core complex." There are several Beclin 1 complexes: the UVRAG (ultraviolet irradiation resistant-associated gene) [73] or the Rubicon (RUN domain and cysteine-rich domain containing, Beclin 1-interacting) [74, 75] complex, with UVRAG or Rubicon, in place of Barkor, respectively. These 3 complexes act differently: the Barkor complex has a role in the formation of autophagosomes, the UVRAG complex acts in autophagosome maturation, whereas the Rubicon complex inhibits autophagosome maturation [76, 77]. Moreover, other Beclin 1 binding partners have been shown to modulate AP, including ambra-1 (activating molecule in Beclin 1-regulated AP) [78] or Bif-1 (Bax-interacting factor 1) [79]. Under resting conditions, antiapoptotic Bcl-2 protein family members, such as Bcl-2 and Bcl-X$_L$,

constitutively bind Beclin 1 and act as negative regulators of AP, showing intricate interlinked complex control between AP and apoptosis processes [80–82]. The tumor suppressor gene, *p53*, has been reported to play a dual role in AP [83]. p53 can induce AP through activation of AMPK [84] and upregulation of DRAM (damage-regulated modulator of AP) [85], where cytoplasmic p53 can inhibit AP (transcription independent activity) [86]. The NF-κB transcription factor, and its certain upstream regulators, connects and integrates diverse stress response signals including immune signals with the AP pathway [87–89]. Others regulators that induce AP include tumor suppressors, such as PTEN, TSC1 and TSC2 complexes, and the death-associated kinase (DAPK); stress-activated signaling molecules, such as c-Jun N-terminal kinase 1 (JNK1), and those that respond to endoplasmic reticulum (ER) stress (PERK, eIF2α-kinase, and IRE1), and molecules involved in innate immune signaling, such as TLRs and immunity-related GTPases [90].

5. AP and Macrophages

In the last decade, it has been established that AP exerts important functions in many aspects of immune and inflammatory responses [91, 92]. AP is under the control of immune receptors and cytokine signaling [91, 92] and is stimulated upon microbial recognition by pattern recognition receptors (PRRs) [93–96] or activation with T helper 1 cytokines [97].

AP plays a critical role in host defense by promoting the elimination of pathogens via autolysosomes (referred to as xenophagy) as well as the delivery of microbial nucleic acids and antigens to endo/lysosomal compartments for activation of innate and adaptive immune responses [98]. *In vitro*, it has been demonstrated that AP has a crucial role in macrophage phagocytosis of different pathogens, such as *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Herpes simplex virus type I*, *Toxoplasma gondii* and many others [99, 100]. Immunity-related GTPase family M, an IFN-inducible GTPase, promotes AP that is involved in the elimination of mycobacteria in macrophages [101, 102]. Consistent with this, macrophages lacking Atg7 fail to eliminate live yeasts in phagolysosomes [103]. In mice, knockout of Atg5 in macrophages and neutrophils increases susceptibility to infection with *L. monocytogenes* and the protozoan *T. gondii* [104].

It has been shown that AP machinery and pathways interact with several PRRs. Firstly, TLRs were connected with AP [61, 103, 105]. Activated TLRs recruit adaptor proteins such as myeloid differentiation marker 88 (MyD88) and tumor necrosis factor receptor- (TNFR-) associated factor 6 (TRAF6), an E3 ubiquitin ligase and scaffold protein. In macrophages, both MyD88 and another adaptor protein, TRIF (TIR domain-containing adaptor inducing interferon-beta), interact with Beclin 1. Exposure of murine macrophages to a TLR4 ligand (lipopolysaccharide, LPS) also reduces the association between Beclin 1 and Bcl-2. TLR4 triggers AP via TRAF6 that ubiquitinates Beclin 1 and determines the Beclin 1/Bcl-2 dissociation [106]. Furthermore, it has been reported that TLR7-induced AP in murine

macrophage cell lines depends on MyD88 and Beclin 1 [96]. The stimulation of TLR2 with zymosan (a cell component of fungi) triggers the recruitment of LC3 to the phagosomes although the signaling pathways seem MyD88-independent [94]. The soluble TLR2 ligand, Pam3CSK4, only when fused with latex beads, is able to induce the maturation of phagosomes in primary macrophages, suggesting that TLR2 signaling is necessary but not sufficient for the induction of AP [94].

Others receptors involved in the detection of invasive pathogens, the cytosolic Nod- (nucleotide-binding oligomerization-domain-) like (NLRs) and RIG-I-like (RLRs) receptors, have been demonstrated to be players in the autophagic response to bacteria [107]. Among the members of the NLR family, Nod1 and Nod2 detect intracellular bacteria through their ability to sense bacterial peptidoglycan [108]. Activation of Nod1 and Nod2 initiates a proinflammatory response dependent mainly on the activation of the transcription factor NF-κB and on the recruitment of the adaptor protein RIP2 [109, 110]. Similar to TLRs, mutations in genes encoding Nod proteins have been associated with chronic inflammatory disorders [111].

Travassos and colleagues demonstrated that, in mouse macrophages and other cell lines, Nod1 and Nod2 recruited the AP protein ATG16L1 to the plasma membrane at the bacterial entry site by a mechanism independent of the adaptor RIP2 and transcription factor NF-κB [107]. In contrast to Nod2, NLRC4 (Ipaf) and NLRP4 exert inhibitory effects on AP. NLRC4 acts negatively at the initiation stage, whereas NLRP4 acts at both the initiation and maturation stages. NLRs, including those essential for inflammasome assembly and activation such as NRLP3, are found in complexes with Beclin 1 in several human cell lines including acute monocytic leukemia cells [112].

Recently, the autophagic adaptors, sequestosome 1/p62-like receptors (SLRSs), have been proposed as a new category of PRRs in order to link AP and innate immunity signaling [113]. The autophagic adaptors, NBR1 and p62, present at the earliest stages of autophagosome formation, have been studied independently of AP as complex inflammatory signaling platforms [114, 115]. A recent study performed with both *Drosophila* blood cells and mouse macrophages show that constitutive p62-mediated selective AP is required for cell spreading and Rho1-induced cell protrusions. It is suggested that p62 may mediate selective autophagic degradation of a regulator of the Rho pathway. Moreover, it is becoming apparent that proteins, organelles, and pathogens can be targeted for autophagic clearance by selective mechanisms, although the extent and roles of such degradation are unclear. These results illuminate a specific and conserved role for AP as a regulatory mechanism for cortical remodeling, with implications for immune cell function [116]. Moreover, AP can drive the rapid cellular changes necessary for proper differentiation. In fact, it has been shown that this process plays a crucial role in monocyte differentiation into macrophages when this differentiation is induced by colony stimulating factor-1 (CSF-1) as well as by M-CSF [117, 118]. SLRs can participate in the promotion of autophagic killing of intracellular microbes. The p62 adaptor protein can deliver specific ribosomal and bulk ubiquitinated cytosolic proteins to

autolysosomes where they are proteolytically converted into products capable of killing *M. tuberculosis*. Thus, p62 brings cytosolic proteins to autolysosomes where they are processed from innocuous precursors into neoantimicrobial peptides, explaining in part the unique bactericidal properties of autophagic organelles [119]. However, there are also potential links between AP and conventional antimicrobial peptides, such as cathelicidin, a peptide obtained by conventional proteolysis from larger precursors. Cathelicidin expression and its antimycobacterial action are induced by vitamin D3, an activator of AP, in human monocytes/macrophages [120]. Moreover, vitamin D3 triggers AP in human macrophages that inhibit HIV-1 infection [121].

AP and the inflammasome undergo complex functional interactions. Indeed, damage-associated molecular patterns (DAMPs), toxins, and several particulates and nanomaterials can induce AP directly or via the activation of the inflammasome pathway [113, 122, 123]. Cytokines can induce AP in some cases or, conversely, suppress it in other cases (reviewed in [103, 124, 125]). Some of the immune signals that induce AP include IFN-γ, TNF, and CD40-CD40L interactions. In contrast, AP is negatively regulated by the T helper type 2 cytokines, IL-4 and IL-13 [61]. Several observations have revealed a link between autophagic protein deficiency and proinflammatory cytokine secretion in macrophages. Macrophages lacking Atg16L1 and Atg7 produce high amounts of IL-1β and IL-18, but not TNF or IFN-β, in response to LPS. In these macrophages, the enhanced IL-1β production is induced by Toll/IL-1 receptor domain-containing adaptor inducing IFN-β (TRIF)-dependent generation of ROS. Further, deletion of Atg5 in macrophages enhances retinoic acid-inducible gene-I-like receptor- (RLR-) mediated type I IFN production in response to single-stranded RNA viruses. These data indicate the importance of Atg in the inflammatory response [103, 126, 127]. Increased activation of IL-1β and IL-18 has been observed in macrophages and monocytes genetically deficient in Beclin 1 and LC3B; this occurs through the increased activation of the NLRP3 (NALP3) inflammasome pathway [128, 129]. IFN-γ has also been reported to induce AP in macrophage cell lines, but less in primary mouse and human macrophages [97]. Taken together, this evidence suggests that AP in macrophages can be triggered directly via at least some PRRs and indirectly via certain cytokines induced upon PRR activation [130].

In addition, it has been reported that cells undergoing autophagic cell death can induce a proinflammatory response in human macrophages. Indeed, upon engulfing MCF-7 cells undergoing autophagic cell death, human macrophages generate a proinflammatory response involving the secretion of IL-6, TNFα, IL-8, and the anti-inflammatory cytokine IL-10 [131]. Interestingly, AP also regulates phagocytosis of dead cells [132, 133]. Importantly, AP can influence adaptive immunity by regulating antigen presentation and the maintenance of lymphocyte function and homeostasis [134].

In conclusion, AP can influence inflammatory responses through several pathways in a cell-intrinsic manner, affecting both pro- and anti-inflammatory signaling and subsequent immune-driven diseases such as atherosclerosis.

6. Role of AP in Atherosclerosis

AP in atherosclerosis has been extensively investigated with particular focus on vascular smooth muscle cells (SMCs) and endothelial cells (ECs). Transmission electron microscopy (TEM) of SMCs in the fibrous cap of experimental or human plaques reveals features of AP such as formation of myelin figures [135]. This data is supported by western blot analysis of advanced human plaques showing elevated levels of LC3-II [136].

Several AP triggers are present in the atherosclerotic plaque, such as inflammatory mediators [137], ROS production [138] and accumulation of oxidized LDL [139, 140]. TNFα stimulation increases the number of vacuolated cells and the expression of LC3-II and Beclin 1 in SMCs isolated from atherosclerotic plaques [137]. Osteopontin, a protein involved in vascular inflammation, and advanced glycation end products (AGEs) have been shown to induce AP in human and rat SMCs, respectively [141, 142]. Similarly, 7-ketocholesterol, one of the major oxysterols present in atherosclerotic plaques, triggers extensive vacuolization and intense protein ubiquitination and increases the LC3-II expression in cultured SMCs [143]. SMCs in the fibrous cap are surrounded by a thick layer of basal lamina and therefore are subjected to hypoxia, caused by inadequate vascularization [144], and experience nutrient and growth factor deprivation, well-known conditions leading to the induction of AP. Dying SMCs in the fibrous cap of advanced human plaques show ubiquitinated inclusions in their cytoplasm and may undergo autophagic death [143, 145].

AP in vascular endothelial cells can be induced by several compounds in the circulation or in the subendothelial layer of the plaque. For example, oxLDL intensifies AP in human umbilical vein EA.hy926 cells [139], and mitochondrial-derived ROS activate AMPK that in turn increases AP leading to EC survival [146]. AP plays an important role in preserving vascular endothelial function by reducing oxidative stress, increasing nitric oxide bioavailability and reducing vascular inflammation [147]. Importantly, AP is reduced with ageing in vascular tissues [147].

The general consensus is that basal AP can protect plaque cells against oxidative stress by degrading damaged intracellular material [148] and promoting cell survival. The protective role of AP in stabilizing the plaque was confirmed *in vitro* showing that SMC death induced by low concentrations of statins was attenuated by the AP inducer 7-ketocholesterol [149]. Similarly the exposure of ECs in culture to oxLDL or AGEs [139, 150] induced AP which protected against EC injury [151]. Moreover, Salabei et al. [152] showed that the antiproliferative effect of verapamil in SMCs, beneficial in controlling vascular-injury-induced neointimal formation, was associated with the onset of AP. Although verapamil strongly upregulated AP, it did not promote SMC cell death yet appeared to suspend cell division resulting in an anti-proliferative state.

In contrast to basal AP, excessive stimulation of AP in SMCs or ECs may cause autophagic cell death [151], leading to reduced synthesis of collagen, thinning of the fibrous cap, plaque destabilization, lesional thrombosis, and acute clinical events [136].

7. Macrophage AP in Atherosclerosis

The investigation of macrophage AP in atherosclerosis has been complicated by the strong phagocytic activity of these cells. It is difficult to determine via conventional electron microscopy whether the vacuoles in their cytoplasm result from autophagocytosis or heterophagocytosis [136, 151]. In addition, the autophagosomal marker protein LC3 is poorly expressed in macrophages and overexpression of other lysosomal marker proteins may give rise to false-positive signals in immunoelectron microscopy [136, 151].

Importantly, pharmacological modulation of macrophage AP has been shown to affect vascular inflammation. Stent-based delivery of everolimus (mTOR inhibitor; a well-known AP inducer; see Section 8) in atherosclerotic plaques of cholesterol-fed rabbits leads to a marked reduction of macrophages via autophagic cell death without altering the SMC plaque content [4]. The observed macrophage cell death was characterized by bulk degradation of long-lived proteins, processing of LC3, and cytoplasmic vacuolization, which are all markers of AP [4]. Further, local administration of imiquimod (TLR7 ligand) to rabbit atherosclerotic carotid arteries induced macrophage AP, without affecting SMCs and ECs. However, in this case the induction of macrophage AP triggered cytokine production and the upregulation of VCAM-1 and enhanced leukocyte infiltration in the artery [5]. The authors speculate that the moderate AP induced in the macrophages of imiquimod-treated lesions does not lead to cell death but to plaque inflammation. Of note, TLR7 stimulation could lead to the activation of other immune cells homing to atherosclerotic vessels such as plasmacytoid dendritic cells, recently shown to play a key role in promoting experimental atherosclerosis in mice [153–155].

Two recent elegant papers add new dimensions to the understanding of the role of macrophage AP in regulating atherosclerotic plaque development [6, 7]. Razani et al. [6] demonstrated that during lesion formation, autophagic markers (p62 and LC3/Atg8) were expressed in the atherosclerotic plaques of apolipoprotein-E (apoE)$^{-/-}$ mice, colocalizing mainly with monocyte-macrophages (MOMA-2, CD11b +ve) and plaque leukocytes (CD45 +ve). The AP protein, p62/SQSTM1, is known to accumulate when autophagy flux through lysosomes is defective [156]. AP induction by prolonged fasting resulted in decreased levels of p62 protein in the aorta of apoE$^{-/-}$ mice, suggesting that changes in aortic p62 protein reflect *in vivo* AP status. p62 levels are raised with increasing age/plaque burden in atherosclerotic aortas, suggesting that initially AP is functional and becomes severely compromised with disease progression.

Beclin 1/Atg6 heterozygous-deficient (Beclin-Het) mice on the apoE$^{-/-}$ background showed similar extent of atherosclerosis compared to apoE$^{-/-}$ mice that were wild-type at the Beclin locus, demonstrating that autophagy haploinsufficiency had no effect on pathology. On the contrary, complete deficiency of macrophage AP increased vascular inflammation and plaque formation. To pursue this notion, the authors used macrophage-specific Atg5-null (Atg5-mϕKO) mice with complete absence of an AP gene in macrophages. Plaque formation, serum IL-1β levels, and aortic IL-1β expression were all increased in Atg5-mϕKO/apoE$^{-/-}$ mice fed a high-fat diet (HFD) as compared to apoE$^{-/-}$ controls. In addition, AP deficiency was associated with elevated plaque macrophage content.

Given the increased levels of IL-1β observed in Atg5-null mice, the authors suggest a link between AP deficiency and inflammmosome hyperactivation. Indeed, deficient AP, through mechanisms that might include lysosomal leakage, generation of ROS, and impaired mitophagy, could result in the activation of the inflammasome. However, the effects of AP on IL-1β production are complex and context dependent and the assumption that AP suppresses the inflammmosome in vascular inflammation merits further investigation.

The protective role of macrophage AP in atherosclerosis was confirmed by Liao et al. [7]. The authors provide evidence that AP prevents macrophage apoptosis and defective efferocytosis, both promoting plaque necrosis in advanced atherosclerosis. Firstly, authors demonstrated, in primary macrophages from mice transgenic for a GFP-tagged version of the AP effector LC3-II, that several proatherosclerotic stimuli induced AP and promoted autophagic flux through lysosomes. Inhibition of AP by silencing Atg5 or in Atg5-deficient macrophages enhanced apoptosis and NADPH oxidase-mediated oxidative stress, rendering the apoptotic cells less recognizable to efferocytosis. Importantly, the same findings were confirmed *in vivo*. Aortic root lesions of HFD-fed GFP-LC3/low-density lipoprotein receptor (LDLr)$^{-/-}$ mice contained macrophages expressing Atg5 and displaying the punctate pattern of GFP-LC3 fluorescence typical of AP. The number of macrophages expressing p62 increased as lesions progressed. These data confirm the presence of AP in atherosclerotic vessels and suggest that autophagic flux through lysosomes decreases as disease progresses. Lesion size and necrotic area were higher in Atg5$^{fl/fl}$Lysmcre$^{+/-}$/LDLr$^{-/-}$ versus control mice. The number of lesional macrophages was not increased, however, macrophage-rich regions of the Atg5$^{fl/fl}$Lysmcre$^{+/-}$/LDLr$^{-/-}$ plaques had more apoptotic cells positive for TUNEL, activated caspase-3, DHE, and p47. In summary, macrophage AP deficiency increased apoptosis and oxidative stress in plaque macrophages, promoted plaque necrosis, and impaired lesional efferocytosis in LDLr$^{-/-}$ mice. These data complement results obtained by Razani et al. [6], confirming a protective role played by macrophage AP in the two most widely used mouse models of atherosclerosis.

Another important aspect to consider is the contribution of lipophagy to vascular pathology. As recently reviewed [157–159], lipophagy, a special kind of AP, contributes in cholesterol egress from lipid-laden cells to high-density lipoprotein (HDL) via lysosomal lipases. AP can play a role in

the hydrolysis of stored cholesterol droplets in macrophages, thus facilitating cholesterol efflux [160]. Interestingly, Wip1 phosphatase, a known negative regulator of Atm-dependent signaling, has been recently shown to play a major role in controlling AP and cholesterol efflux in apoE$^{-/-}$ mice. Deletion of Wip1 resulted in suppression of macrophage conversion into foam cells, thus preventing the formation of atherosclerotic plaques [161].

In conclusion, macrophage AP becomes dysfunctional in atherosclerosis and its deficiency promotes vascular inflammation, oxidative stress, and plaque necrosis, suggesting a mechanism-based strategy to therapeutically suppress atherosclerosis progression.

8. Pharmacological Manipulation of Autophagic Pathways

Several pharmacological agents that are able to modulate AP have already been identified, such as mTOR inhibitors, AMPK modulators, IP$_3$ and calcium lowering agents, and lysosome inhibitors [162, 163].

mTOR inhibitors are the most studied AP inducers, and among them, rapamycin (also known as sirolimus) was the first drug to be identified. Rapamycin, a lipophilic macrolide antibiotic already in use to prevent the rejection of transplanted organs and to block restenosis after angioplasty, binds to the immunophilin FK506-binding protein of 12 kDa (FKBP12) inhibiting the kinase activity of mTOR, particularly TORC1 [164, 165]. AP induction with rapamycin enhances the clearance of toxic substrates, such as intracellular aggregate-prone proteins associated with neurodegenerative diseases, and protects against toxicity of these substrates in cell and animal models [166–168]. However, rapamycin also regulates numerous physiological processes that are independent of AP [68]. Indeed, rapamycin inhibits the translation of numerous proteins, causes immunosuppression and cell cycle arrest and alters cell size [169]. These side effects may be unwelcome consequences if rapamycin is used as an AP enhancer, and consequently there is a need for nonimmunosuppressive AP-inducing drugs. Of note, rapamycin and its analogs (temsirolimus, everolimus, and deforolimus) have had limited success as anticancer drugs, may be because they inhibit mTORC1, but not mTORC2 [170]. Consequently, a more complete blockade of the mTOR pathway has led to the development of ATP-competitive mTOR inhibitors of both mTORC1 and mTORC2 (e.g., PP242, AZD8055, WYE132, and Torin 1). Although these compounds clearly show preclinical evidence of antitumor activity, their effectiveness in the clinical setting has yet to be demonstrated [171–174].

Recently, further mTORC1 inhibitors, such as perhexiline, niclosamide, and rottlerin, have been identified as compounds that increase autophagosome number but further work is required to completely understand their activity [175].

Interestingly, several other drugs with well-known pharmacological actions can induce AP by mTOR-independent pathways. For example, mood-stabilizing drugs, such as carbamazepine, valproic acid, and lithium have been identified as AP inducers by reducing IP$_3$ levels [176, 177]. Furthermore, L-type calcium channel antagonists (e.g., verapamil), and antiarrhythmic drugs (e.g., amiodarone) induce AP by inhibiting levels of calcium [162]. The anticancer drug tamoxifen appears to function in part by upregulating the level of Beclin 1 and inducing AP [178]. Finally, the antidiabetic drug metformin has been shown to induce AP of several cancer cell types by activating AMPK [179–181].

A useful strategy for pharmacological manipulation of AP based on additive effects of drugs could be obtained using mTOR inhibitors in combination with mTOR-independent AP enhancers. It has been demonstrated that trehalose and small molecule enhancer rapamycin (SMERs) exerted an additive effect on the clearance of aggregate-prone proteins associated with Huntington's disease and Parkinson's disease when associated to rapamycin [168, 182].

AP inhibitors can be classified in: (i) early stage inhibitors including 3-methyadenine (3-MA), wortmannin and LY294002 that target the class III PI3K; (ii) late stage inhibitors, including chloroquine (CQ) or hydroxychloroquine (HCQ), bafilomycin A1, and monensin that prevent fusion of autophagosomes with the lysosomes [183]. Currently, several ongoing clinical trials registered with the National Cancer Institute (http://www.cancer.gov/clinicaltrials) are evaluating the efficacy of the combination of HCQ with cytotoxic drugs in a variety of cancers.

Importantly, many of the aforementioned pharmacological agents have been shown to be effective in the treatment of cardiovascular disorders, including cardiomyopathy, and heart failure in which AP is involved [184]. For example, reduction of infarct size has been demonstrated in mice treated with rapamycin [185]. Rapamycin, AICAR, and metformin improve cardiac function, reduce cardiac hypertrophy, and delay the onset of heart failure during overload pressure [186, 187]. On the other hand, a strong activation of AP, due to Beclin 1 upregulation, is observed in response to severe pressure overload, and this could be responsible for the transition from compensatory ventricular hypertrophy to pathological remodeling [188]. Finally, several indications support AP as a therapeutic target in experimental atherosclerosis [189]. Sirolimus and everolimus are antiatherogenic in mice [190–193]; on the other hand, AP induction by calorie-deprivation reduced atherosclerosis [194]. However, no clinical data are available regarding the efficacy of AP modulators in cardiovascular diseases.

In conclusion, several drugs that regulate AP have been identified, suggesting that the autophagic signaling may be manipulated to treat human disease. However, considering the dual role of AP in cytoprotection and cell death, there is a need for more specific molecules in order to target the pathways that control AP.

9. Conclusions

This paper summarizes recent evidence showing a protective role played by macrophage AP in atherosclerosis. AP becomes dysfunctional in atherosclerosis and its deficiency promotes vascular inflammation, oxidative stress, and plaque

necrosis. However, further work is needed to obtain a better understanding of this phenomenon in all stages of the pathology. We need to understand how AP is induced in atherosclerotic lesions, how lipophagy contributes to the pathology, the mechanisms governing the crosstalk between AP and apoptosis within the arterial wall, how they could influence plaque stability, and if they may prove to be effective therapeutic targets.

What degree of AP deficiency is proatherogenic? Is AP induction anti-atherogenic? The answer to these questions requires further investigation. Importantly, to date no studies have addressed the potential effect of AP on the multiple leukocyte subsets which have been shown to infiltrate the naïve and inflamed vessels playing a significant role in plaque formation and development [1, 195–197]. For example, AP regulates antigen presentation [198], T-cell development and homeostasis [113, 199], T-cell and dendritic cell activation [200, 201], and degranulation of mast cells [202]. However, none of these AP functions have been investigated in the context of vascular inflammation. As these studies progress we can expect to learn more about whether AP is indeed a good target for therapeutic intervention in atherosclerosis.

Conflict of Interests

The authors declare that there is no conflict of interests.

Acknowledgments

References

[1] E. Galkina and K. Ley, "Immune and inflammatory mechanisms of atherosclerosis," *Annual Review of Immunology*, vol. 27, pp. 165–197, 2009.

[2] P. Libby, P. M. Ridker, and G. K. Hansson, "Progress and challenges in translating the biology of atherosclerosis," *Nature*, vol. 473, no. 7347, pp. 317–325, 2011.

[3] K. J. Moore and I. Tabas, "Macrophages in the pathogenesis of atherosclerosis," *Cell*, vol. 145, no. 3, pp. 341–355, 2011.

[4] S. Verheye, W. Martinet, M. M. Kockx et al., "Selective clearance of macrophages in atherosclerotic plaques by autophagy," *Journal of the American College of Cardiology*, vol. 49, no. 6, pp. 706–715, 2007.

[5] I. De Meyer, W. Martinet, D. M. Schrijvers et al., "Toll-like receptor 7 stimulation by imiquimod induces macrophage autophagy and inflammation in atherosclerotic plaques," *Basic Research in Cardiology*, vol. 107, no. 3, p. 269, 2012.

[6] B. Razani, C. Feng, T. Coleman et al., "Autophagy links inflammasomes to atherosclerotic progression," *Cell Metabolism*, vol. 15, no. 4, pp. 534–544, 2012.

[7] X. Liao, J. C. Sluimer, Y. Wang et al., "Macrophage autophagy plays a protective role in advanced atherosclerosis," *Cell Metabolism*, vol. 4, no. 4, pp. 545–553, 2012.

[8] F. Ginhoux, M. Greter, M. Leboeuf et al., "Fate mapping analysis reveals that adult microglia derive from primitive macrophages," *Science*, vol. 330, no. 6005, pp. 841–845, 2010.

[9] C. Schulz, E. Gomez Perdiguero, L. Chorro et al., "A lineage of myeloid cells independent of Myb and hematopoietic stem cells," *Science*, vol. 336, no. 6077, pp. 86–90, 2012.

[10] E. L. Gautier, C. Jakubzick, and G. J. Randolph, "Regulation of the migration and survival of monocyte subsets by chemokine receptors and its relevance to atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 29, no. 10, pp. 1412–1418, 2009.

[11] C. Auffray, D. Fogg, M. Garfa et al., "Monitoring of blood vessels and tissues by a population of monocytes with patrolling behavior," *Science*, vol. 317, no. 5838, pp. 666–670, 2007.

[12] R. J. Tushinski, I. T. Oliver, and L. J. Guilbert, "Survival of mononuclear phagocytes depends on a lineage-specific growth factor that the differentiated cells selectively destroy," *Cell*, vol. 28, no. 1, pp. 71–81, 1982.

[13] F. Geissmann, S. Jung, and D. R. Littman, "Blood monocytes consist of two principal subsets with distinct migratory properties," *Immunity*, vol. 19, no. 1, pp. 71–82, 2003.

[14] M. A. Ingersoll, R. Spanbroek, C. Lottaz et al., "Comparison of gene expression profiles between human and mouse monocyte subsets (Blood (2010) 115, 3 (e10-e19))," *Blood*, vol. 116, no. 5, p. 857, 2010.

[15] N. V. Serbina and E. G. Pamer, "Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2," *Nature Immunology*, vol. 7, no. 3, pp. 311–317, 2006.

[16] M. A. Ingersoll, A. M. Platt, A. M. Potteaux, and G. J. Randolph, "Monocyte trafficking in acute and chronic inflammation," *Trends in Immunology*, vol. 32, no. 10, pp. 470–477, 2011.

[17] R. G. Gerrity, "The role of the monocyte in atherogenesis. I. Transition of blood-borne monocytes into foam cells in fatty lesions," *American Journal of Pathology*, vol. 103, no. 2, pp. 181–190, 1981.

[18] F. K. Swirski, M. J. Pittet, M. F. Kircher et al., "Monocyte accumulation in mouse atherogenesis is progressive and proportional to extent of disease," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 103, no. 27, pp. 10340–10345, 2006.

[19] J. D. Smith, E. Trogan, M. Ginsberg, C. Grigaux, J. Tian, and M. Miyata, "Decreased atherosclerosis in mice deficient in both macrophage colony- stimulating factor (op) and apolipoprotein E," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 18, pp. 8264–8268, 1995.

[20] R. R. Koenen and C. Weber, "Therapeutic targeting of chemokine interactions in atherosclerosis," *Nature Reviews Drug Discovery*, vol. 9, no. 2, pp. 141–153, 2010.

[21] E. J. A. Van Wanrooij, S. C. A. De Jager, T. Van Es et al., "CXCR3 antagonist NBI-74330 attenuates atherosclerotic plaque formation in LDL receptor-deficient mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 2, pp. 251–257, 2008.

[22] J. Mestas and K. Ley, "Monocyte-endothelial cell interactions in the development of atherosclerosis," *Trends in Cardiovascular Medicine*, vol. 18, no. 6, pp. 228–232, 2008.

[23] C. Combadière, S. Potteaux, M. Rodero et al., "Combined inhibition of CCL2, CX3CR1, and CCR5 abrogates Ly6Chi and Ly6Clo monocytosis and almost abolishes atherosclerosis in hypercholesterolemic mice," *Circulation*, vol. 117, no. 13, pp. 1649–1657, 2008.

[24] F. K. Swirski, P. Libby, E. Aikawa et al., "Ly-6Chi monocytes dominate hypercholesterolemia-associated monocytosis and

give rise to macrophages in atheromata," *Journal of Clinical Investigation*, vol. 117, no. 1, pp. 195–205, 2007.

[25] S. Potteaux, E. L. Gautier, S. B. Hutchison et al., "Suppressed monocyte recruitment drives macrophage removal from atherosclerotic plaques of Apoe-/- mice during disease regression," *Journal of Clinical Investigation*, vol. 121, no. 5, pp. 2025–2036, 2011.

[26] F. Tacke, D. Alvarez, T. J. Kaplan et al., "Monocyte subsets differentially employ CCR2, CCR5, and CX3CR1 to accumulate within atherosclerotic plaques," *Journal of Clinical Investigation*, vol. 117, no. 1, pp. 185–194, 2007.

[27] J. L. Johnson and A. C. Newby, "Macrophage heterogeneity in atherosclerotic plaques," *Current Opinion in Lipidology*, vol. 20, no. 5, pp. 370–378, 2009.

[28] E. A. Podrez, M. Febbraio, N. Sheibani et al., "Macrophage scavenger receptor CD36 is the major receptor for LDL modified by monocyte-generated reactive nitrogen species," *Journal of Clinical Investigation*, vol. 105, no. 8, pp. 1095–1108, 2000.

[29] H. Suzuki, Y. Kurihara, M. Takeya et al., "A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection," *Nature*, vol. 386, no. 6622, pp. 292–296, 1997.

[30] C. R. Stewart, L. M. Stuart, K. Wilkinson et al., "CD36 ligands promote sterile inflammation through assembly of a Toll-like receptor 4 and 6 heterodimer," *Nature Immunology*, vol. 11, no. 2, pp. 155–161, 2010.

[31] P. A. Berberian, W. Myers, M. Tytell, V. Challa, and M. G. Bond, "Immunohistochemical localization of heat shock protein-70 in normal-appearing and atherosclerotic specimens of human arteries," *American Journal of Pathology*, vol. 136, no. 1, pp. 71–80, 1990.

[32] Z. Q. Yan and G. K. Hansson, "Innate immunity, macrophage activation, and atherosclerosis," *Immunological Reviews*, vol. 219, no. 1, pp. 187–203, 2007.

[33] P. Duewell, H. Kono, K. J. Rayner et al., "NLRP3 inflammasomes are required for atherogenesis and activated by cholesterol crystals," *Nature*, vol. 464, no. 7293, pp. 1357–1361, 2010.

[34] K. Rajamaki, J. Lappalainen, K. Oorni et al., "Cholesterol crystals activate the NLRP3 inflammasome in human macrophages: a novel link between cholesterol metabolism and inflammation," *PLoS ONE*, vol. 5, no. 7, Article ID e11765, 2010.

[35] R. Kleemann, S. Zadelaar, and T. Kooistra, "Cytokines and atherosclerosis: a comprehensive review of studies in mice," *Cardiovascular Research*, vol. 79, no. 3, pp. 360–376, 2008.

[36] R. Spanbroek, R. Grabner, K. Lotzer et al., "Expanding expression of the 5-lipoxygenase pathway within the arterial wall during human atherogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 3, pp. 1238–1243, 2003.

[37] J. S. Pober, M. P. Bevilacqua, and D. L. Mendrick, "Two distinct monokines, interleukin 1 and tumor necrosis factor, each independently induce biosynthesis and transient expression of the same antigen on the surface of cultured human vascular endothelial cells," *Journal of Immunology*, vol. 136, no. 5, pp. 1680–1687, 1986.

[38] D. M. Wuttge, P. Eriksson, A. Sirsjö, G. K. Hansson, and S. Stemme, "Expression of interleukin-15 in mouse and human atherosclerotic lesions," *American Journal of Pathology*, vol. 159, no. 2, pp. 417–423, 2001.

[39] C. N. Morrell, "Reactive oxygen species: finding the right balance," *Circulation Research*, vol. 103, no. 6, pp. 571–572, 2008.

[40] T. Nguyen, D. Brunson, C. L. Crespi, B. W. Penman, J. S. Wishnok, and S. R. Tannenbaum, "DNA damage and mutation in human cells exposed to nitric oxide in vitro," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 89, no. 7, pp. 3030–3034, 1992.

[41] E. A. Podrez, D. Schmitt, H. F. Hoff, and S. L. Hazen, "Myeloperoxidase-generated reactive nitrogen species convert LDL into an atherogenic form in vitro," *Journal of Clinical Investigation*, vol. 103, no. 11, pp. 1547–1560, 1999.

[42] D. A. Hume, "The mononuclear phagocyte system," *Current Opinion in Immunology*, vol. 18, no. 1, pp. 49–53, 2006.

[43] J. Liu, G. K. Sukhova, J. S. Sun et al., "Lysosomal cysteine proteases in atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 8, pp. 1359–1366, 2004.

[44] A. C. Newby, "Metalloproteinase expression in monocytes and macrophages and its relationship to atherosclerotic plaque instability," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 12, pp. 2108–2114, 2008.

[45] M. Febbraio, D. P. Hajjar, and R. L. Silverstein, "CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism," *Journal of Clinical Investigation*, vol. 108, no. 6, pp. 785–791, 2001.

[46] E. Thorp, D. Cui, D. M. Schrijvers, G. Kuriakose, and I. Tabas, "Mertk receptor mutation reduces efferocytosis efficiency and promotes apoptotic cell accumulation and plaque necrosis in atherosclerotic lesions of Apoe-/- mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 28, no. 8, pp. 1421–1428, 2008.

[47] D. M. Schrijvers, G. R. De Meyer, M. M. Kockx et al., "Phagocytosis of apoptotic cells by macrophages is impaired in atherosclerosis," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 25, no. 6, pp. 1256–1261, 2005.

[48] A. Böttcher, U. S. Gaipl, B. G. Fürnrohr et al., "Involvement of phosphatidylserine, $\alpha v\beta 3$, CD14, CD36, and complement C1q in the phagocytosis of primary necrotic lymphocytes by macrophages," *Arthritis and Rheumatism*, vol. 54, no. 3, pp. 927–938, 2006.

[49] W. Martinet, D. M. Schrijvers, and G. R. De Meyer, "Necrotic cell death in atherosclerosis," *Basic Research in Cardiology*, vol. 106, no. 5, pp. 749–760, 2011.

[50] W. Martinet, D. M. Schrijvers, and G. R. De Meyer, "Molecular and cellular mechanisms of macrophage survival in atherosclerosis," *Basic Research in Cardiology* , vol. 107, no. 6, p. 297, 2012.

[51] Z. Yang and D. J. Klionsky, "Mammalian autophagy: core molecular machinery and signaling regulation," *Current Opinion in Cell Biology*, vol. 22, no. 2, pp. 124–131, 2010.

[52] E. L. Axe, S. A. Walker, M. Manifava et al., "Autophagosome formation from membrane compartments enriched in phosphatidylinositol 3-phosphate and dynamically connected to the endoplasmic reticulum," *Journal of Cell Biology*, vol. 182, no. 4, pp. 685–701, 2008.

[53] M. Hayashi-Nishino, N. Fujita, T. Noda, A. Yamaguchi, T. Yoshimori, and A. Yamamoto, "A subdomain of the endoplasmic reticulum forms a cradle for autophagosome formation," *Nature Cell Biology*, vol. 11, no. 12, pp. 1433–1437, 2009.

[54] D. W. Hailey, A. S. Rambold, P. Satpute-Krishnan et al., "Mitochondria supply membranes for autophagosome biogenesis during starvation," *Cell*, vol. 141, no. 4, pp. 656–667, 2010.

[55] S. A. Tooze and T. Yoshimori, "The origin of the autophagosomal membrane," *Nature Cell Biology*, vol. 12, no. 9, pp. 831–835, 2010.

[56] B. Ravikumar, K. Moreau, L. Jahreiss, C. Puri, and D. C. Rubinsztein, "Plasma membrane contributes to the formation of pre-autophagosomal structures," *Nature Cell Biology*, vol. 12, no. 8, pp. 747–757, 2010.

[57] T. Yorimitsu and D. J. Klionsky, "Autophagy: molecular machinery for self-eating," *Cell Death and Differentiation*, vol. 12, supplement 2, pp. 1542–1552, 2005.

[58] G. Kroemer, G. Marino, and B. Levine, "Autophagy and the integrated stress response," *Molecular Cell*, vol. 40, no. 2, pp. 280–293, 2010.

[59] G. Kroemer and B. Levine, "Autophagic cell death: the story of a misnomer," *Nature Reviews Molecular Cell Biology*, vol. 9, no. 12, pp. 1004–1010, 2008.

[60] B. Levine and G. Kroemer, "Autophagy in the pathogenesis of disease," *Cell*, vol. 132, no. 1, pp. 27–42, 2008.

[61] B. Levine, N. Mizushima, and H. W. Virgin, "Autophagy in immunity and inflammation," *Nature*, vol. 469, no. 7330, pp. 323–335, 2011.

[62] I. Nakagawa, A. Amano, N. Mizushima et al., "Autophagy defends cells against invading group A Streptococcus," *Science*, vol. 306, no. 5698, pp. 1037–1040, 2004.

[63] M. Ogawa, T. Yoshimori, T. Suzuki, H. Sagara, N. Mizushima, and C. Sasakawa, "Escape of intracellular Shigella from autophagy," *Science*, vol. 307, no. 5710, pp. 727–731, 2005.

[64] C. Paludan, D. Schmid, M. Landthaler et al., "Endogenous MHC class II processing of a viral nuclear antigen after autophagy," *Science*, vol. 307, no. 5709, pp. 593–596, 2005.

[65] J. Sun, K. Hartvigsen, M. Y. Chou et al., "Deficiency of antigen-presenting cell invariant chain reduces atherosclerosis in mice," *Circulation*, vol. 122, no. 8, pp. 808–820, 2010.

[66] B. Levine and D. J. Klionsky, "Development by self-digestion: molecular mechanisms and biological functions of autophagy," *Developmental Cell*, vol. 6, no. 4, pp. 463–477, 2004.

[67] Z. Xie and D. J. Klionsky, "Autophagosome formation: core machinery and adaptations," *Nature Cell Biology*, vol. 9, no. 10, pp. 1102–1109, 2007.

[68] D. D. Sarbassov, S. M. Ali, and D. M. Sabatini, "Growing roles for the mTOR pathway," *Current Opinion in Cell Biology*, vol. 17, no. 6, pp. 596–603, 2005.

[69] K. Inoki, Y. Li, T. Zhu, J. Wu, and K. L. Guan, "TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling," *Nature Cell Biology*, vol. 4, no. 9, pp. 648–657, 2002.

[70] N. B. Ruderman, X. J. Xu, L. Nelson et al., "AMPK and SIRT1: a long-standing partnership?" *American Journal of Physiology-Endocrinology and Metabolism*, vol. 298, no. 4, pp. E751–E760, 2010.

[71] K. Inoki, H. Ouyang, T. Zhu et al., "TSC2 integrates wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth," *Cell*, vol. 126, no. 5, pp. 955–968, 2006.

[72] D. M. Gwinn, D. B. Shackelford, D. F. Egan et al., "AMPK phosphorylation of raptor mediates a metabolic checkpoint," *Molecular Cell*, vol. 30, no. 2, pp. 214–226, 2008.

[73] C. Liang, P. Feng, B. Ku et al., "Autophagic and tumour suppressor activity of a novel Beclin1-binding protein UVRAG," *Nature Cell Biology*, vol. 8, no. 7, pp. 688–698, 2006.

[74] K. Matsunaga, T. Saitoh, K. Tabata et al., "Two Beclin 1-binding proteins, Atg14L and Rubicon, reciprocally regulate autophagy at different stages," *Nature Cell Biology*, vol. 11, no. 4, pp. 385–396, 2009.

[75] Y. Zhong, Q. J. Wang, X. Li et al., "Distinct regulation of autophagic activity by Atg14 L and Rubicon associated with Beclin 1-phosphatidylinositol-3-kinase complex," *Nature Cell Biology*, vol. 11, no. 4, pp. 468–476, 2009.

[76] E. Itakura, C. Kishi, K. Inoue, and N. Mizushima, "Beclin 1 forms two distinct phosphatidylinositol 3-kinase complexes with mammalian Atg14 and UVRAG," *Molecular Biology of the Cell*, vol. 19, no. 12, pp. 5360–5372, 2008.

[77] Q. Sun, W. Fan, K. Chen, X. Ding, S. Chen, and Q. Zhong, "Identification of Barkor as a mammalian autophagy-specific factor for Beclin 1 and class III phosphatidylinositol 3-kinase," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 49, pp. 19211–19216, 2008.

[78] G. M. Fimia, A. Stoykova, A. Romagnoli et al., "Ambra1 regulates autophagy and development of the nervous system," *Nature*, vol. 447, no. 7148, pp. 1121–1125, 2007.

[79] Y. Takahashi, D. Coppola, N. Matsushita et al., "Bif-1 interacts with Beclin 1 through UVRAG and regulates autophagy and tumorigenesis," *Nature Cell Biology*, vol. 9, no. 10, pp. 1142–1151, 2007.

[80] M. C. Maiuri, G. Le Toumelin, A. Criollo et al., "Functional and physical interaction between Bcl-XL and a BH3-like domain in Beclin-1," *EMBO Journal*, vol. 26, no. 10, pp. 2527–2539, 2007.

[81] M. C. Maiuri, E. Zalckvar, A. Kimchi, and G. Kroemer, "Self-eating and self-killing: crosstalk between autophagy and apoptosis," *Nature Reviews Molecular Cell Biology*, vol. 8, no. 9, pp. 741–752, 2007.

[82] S. Sinha and B. Levine, "The autophagy effector Beclin 1: a novel BH3-only protein," *Oncogene*, vol. 27, supplement 1, pp. S137–S148, 2008.

[83] B. Levine and J. Abrams, "p53: the Janus of autophagy?" *Nature Cell Biology*, vol. 10, no. 6, pp. 637–639, 2008.

[84] Z. Feng, H. Zhang, A. J. Levine, and S. Jin, "The coordinate regulation of the p53 and mTOR pathways in cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 23, pp. 8204–8209, 2005.

[85] D. Crighton, S. Wilkinson, J. O'Prey et al., "DRAM, a p53-induced modulator of autophagy, is critical for apoptosis," *Cell*, vol. 126, no. 1, pp. 121–134, 2006.

[86] E. Tasdemir, M. C. Maiuri, L. Galluzzi et al., "Regulation of autophagy by cytoplasmic p53," *Nature Cell Biology*, vol. 10, no. 6, pp. 676–687.

[87] V. Baud and M. Karin, "Is NF-kappaB a good target for cancer therapy? Hopes and pitfalls," *Nature Reviews Drug Discovery*, vol. 8, no. 1, pp. 33–40, 2009.

[88] A. Criollo, L. Senovilla, H. Authier et al., "The IKK complex contributes to the induction of autophagy," *EMBO Journal*, vol. 29, no. 3, pp. 619–631, 2010.

[89] A. Criollo, M. Niso-Santano, S. A. Malik et al., "Inhibition of autophagy by TAB2 and TAB3," *EMBO Journal*, vol. 30, no. 24, pp. 4908–4920, 2011.

[90] A. Esclatine, M. Chaumorcel, and P. Codogno, "Macroautophagy signaling and regulation," *Current Topics in Microbiology and Immunology*, vol. 335, no. 1, pp. 33–70, 2009.

[91] B. Levine and V. Deretic, "Unveiling the roles of autophagy in innate and adaptive immunity," *Nature Reviews Immunology*, vol. 7, no. 10, pp. 767–777, 2007.

[92] D. Schmid and C. Münz, "Innate and adaptive immunity through autophagy," *Immunity*, vol. 27, no. 1, pp. 11–21, 2007.

[93] H. K. Lee, J. M. Lund, B. Ramanathan, N. Mizushima, and A. Iwasaki, "Autophagy-dependent viral recognition by plasmacytoid dendritic cells," *Science*, vol. 315, no. 5817, pp. 1398–1401, 2007.

[94] M. A. Sanjuan, C. P. Dillon, S. W. G. Tait et al., "Toll-like receptor signalling in macrophages links the autophagy pathway to phagocytosis," *Nature*, vol. 450, no. 7173, pp. 1253–1257, 2007.

[95] Y. Xu, C. Jagannath, X. D. Liu, A. Sharafkhaneh, K. E. Kolodziejska, and N. T. Eissa, "Toll-like receptor 4 is a sensor for autophagy associated with innate immunity," *Immunity*, vol. 27, no. 1, pp. 135–144, 2007.

[96] M. A. Delgado, R. A. Elmaoued, A. S. Davis, G. Kyei, and V. Deretic, "Toll-like receptors control autophagy," *EMBO Journal*, vol. 27, no. 7, pp. 1110–1121, 2008.

[97] J. Harris, S. A. De Haro, S. S. Master et al., "T Helper 2 cytokines inhibit autophagic control of intracellular mycobacterium tuberculosis," *Immunity*, vol. 27, no. 3, pp. 505–517, 2007.

[98] V. Deretic, "Autophagy in infection," *Current Opinion in Cell Biology*, vol. 22, no. 2, pp. 252–262, 2010.

[99] B. R. Dorn, W. A. Dunn Jr., and A. Progulske-Fox, "Bacterial interactions with the autophagic pathway," *Cellular Microbiology*, vol. 4, no. 1, pp. 1–10, 2002.

[100] G. B. Kyei, C. Dinkins, A. S. Davis et al., "Autophagy pathway intersects with HIV-1 biosynthesis and regulates viral yields in macrophages," *Journal of Cell Biology*, vol. 186, no. 2, pp. 255–268, 2009.

[101] M. G. Gutierrez, S. S. Master, S. B. Singh, G. A. Taylor, M. I. Colombo, and V. Deretic, "Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages," *Cell*, vol. 119, no. 6, pp. 753–766, 2004.

[102] S. B. Singh, A. S. Davis, G. A. Taylor, and V. Deretic, "Human IRGM induces autophagy to eliminate intracellular mycobacteria," *Science*, vol. 313, no. 5792, pp. 1438–1441, 2006.

[103] T. Saitoh and S. Akira, "Regulation of innate immune responses by autophagy-related proteins," *Journal of Cell Biology*, vol. 189, no. 6, pp. 925–935, 2010.

[104] Z. Zhao, B. Fux, M. Goodwin et al., "Autophagosome-independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens," *Cell Host and Microbe*, vol. 4, no. 5, pp. 458–469, 2008.

[105] V. Deretic, "Autophagy in immunity and cell-autonomous defense against intracellular microbes," *Immunological Reviews*, vol. 240, no. 1, pp. 92–104, 2011.

[106] C. S. Shi and J. H. Kehrl, "TRAF6 and A20 regulate lysine 63-linked ubiquitination of Beclin-1 to control TLR4-induced Autophagy," *Science Signaling*, vol. 3, no. 123, p. ra42, 2010.

[107] L. H. Travassos, L. A. M. Carneiro, M. Ramjeet et al., "Nod1 and Nod2 direct autophagy by recruiting ATG16L1 to the plasma membrane at the site of bacterial entry," *Nature Immunology*, vol. 11, no. 1, pp. 55–62, 2010.

[108] S. E. Girardin, L. H. Travassos, M. Hervé et al., "Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2," *Journal of Biological Chemistry*, vol. 278, no. 43, pp. 41702–41708, 2003.

[109] N. Inohara, T. Koseki, J. Lin et al., "An induced proximity model for NF-κB activation in the Nod1/RICK and RIP signaling pathways," *Journal of Biological Chemistry*, vol. 275, no. 36, pp. 27823–27831, 2000.

[110] D. W. Abbott, A. Wilkins, J. M. Asara, and L. C. Cantley, "The Crohn's disease protein, NOD2, requires RIP2 in order to induce ubiquitinylation of a novel site on NEMO," *Current Biology*, vol. 14, no. 24, pp. 2217–2227, 2004.

[111] J. P. Hugot, M. Chamaillard, H. Zouali et al., "Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease," *Nature*, vol. 411, no. 6837, pp. 599–603, 2001.

[112] N. Jounai, K. Kobiyama, M. Shiina, K. Ogata, K. J. Ishii, and F. Takeshita, "NLRP4 negatively regulates autophagic processes through an association with Beclin1," *Journal of Immunology*, vol. 186, no. 3, pp. 1646–1655, 2011.

[113] V. Deretic, "Autophagy as an innate immunity paradigm: expanding the scope and repertoire of pattern recognition receptors," *Current Opinion in Immunology*, vol. 24, no. 1, pp. 21–31, 2012.

[114] J. Moscat and M. T. Diaz-Meco, "p62 at the crossroads of autophagy, apoptosis, and cancer," *Cell*, vol. 137, no. 6, pp. 1001–1004, 2009.

[115] J. Q. Yang, H. Liu, M. T. Diaz-Meco, and J. Moscat, "NBR1 is a new PB1 signalling adapter in Th2 differentiation and allergic airway inflammation in vivo," *EMBO Journal*, vol. 29, no. 19, pp. 3421–3433, 2010.

[116] P. Kadandale, J. D. Stender, C. K. Glass, and A. A. Kiger, "Conserved role for autophagy in Rho1-mediated cortical remodeling and blood cell recruitment," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 23, pp. 10502–10507, 2010.

[117] A. Jacquel, S. Obba, L. Boyer et al., "Autophagy is required for CSF-1-induced macrophagic differentiation and acquisition of phagocytic functions," *Blood*, vol. 119, no. 19, pp. 4527–4531, 2012.

[118] D. S. Polancec, V. Munic Kos, M. Banjanac et al., "Azithromycin drives in vitro GM-CSF/IL-4-induced differentiation of human blood monocytes toward dendritic-like cells with regulatory properties," *Journal of Leukocyte Biology*, vol. 91, no. 2, pp. 229–243, 2012.

[119] M. Ponpuak, A. S. Davis, E. A. Roberts et al., "Delivery of cytosolic components by autophagic adaptor protein p62 endows autophagosomes with unique antimicrobial properties," *Immunity*, vol. 32, no. 3, pp. 329–341, 2010.

[120] J. M. Yuk, D. M. Shin, H. M. Lee et al., "Vitamin D3 induces autophagy in human monocytes/macrophages via cathelicidin," *Cell Host and Microbe*, vol. 6, no. 3, pp. 231–243, 2009.

[121] G. R. Campbell and S. A. Spector, "Hormonally active vitamin D3 (1α,25-dihydroxycholecalciferol) triggers autophagy in human macrophages that inhibits HIV-1 infection," *Journal of Biological Chemistry*, vol. 286, no. 21, pp. 18890–18902, 2011.

[122] D. Tang, R. Kang, K. M. Livesey et al., "Endogenous HMGB1 regulates autophagy," *Journal of Cell Biology*, vol. 190, no. 5, pp. 881–892, 2010.

[123] H. Eidi, O. Joubert, C. Némos et al., "Drug delivery by polymeric nanoparticles induces autophagy in macrophages," *International Journal of Pharmaceutics*, vol. 422, no. 1-2, pp. 495–503, 2012.

[124] H. W. Virgin and B. Levine, "Autophagy genes in immunity," *Nature Immunology*, vol. 10, no. 5, pp. 461–470, 2009.

[125] V. Deretic and B. Levine, "Autophagy, immunity, and microbial adaptations," *Cell Host and Microbe*, vol. 5, no. 6, pp. 527–549, 2009.

[126] T. Saitoh, N. Fujita, M. H. Jang et al., "Loss of the autophagy protein Atg16L1 enhances endotoxin-induced IL-1β production," *Nature*, vol. 456, no. 7219, pp. 264–268, 2008.

[127] M. C. Tal, M. Sasai, H. K. Lee, B. Yordy, G. S. Shadel, and A. Iwasaki, "Absence of autophagy results in reactive oxygen species-dependent amplification of RLR signaling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 8, pp. 2774–2775, 2009.

[128] K. Nakahira, J. A. Haspel, V. A. K. Rathinam et al., "Autophagy proteins regulate innate immune responses by inhibiting the release of mitochondrial DNA mediated by the NALP3 inflammasome," *Nature Immunology*, vol. 12, no. 3, pp. 222–230, 2011.

[129] R. Zhou, A. S. Yazdi, P. Menu, and J. Tschopp, "A role for mitochondria in NLRP3 inflammasome activation," *Nature*, vol. 469, no. 7329, pp. 221–226, 2011.

[130] C. C. Mihalache and H. U. Simon, "Autophgy regulation in macrophages and neutrophils," *Experimental Cell Research*, vol. 318, no. 11, pp. 1187–1192, 2012.

[131] G. Petrovski, G. Zahuczky, K. Katona et al., "Clearance of dying autophagic cells of different origin by professional and non-professional phagocytes," *Cell Death and Differentiation*, vol. 14, no. 6, pp. 1117–1128, 2007.

[132] X. Qu, Z. Zou, Q. Sun et al., "Autophagy gene-dependent clearance of apoptotic cells during embryonic development," *Cell*, vol. 128, no. 5, pp. 931–946, 2007.

[133] J. Martinez, J. Almendinger, A. Oberst et al., "Microtubule-associated protein 1 light chain 3 alpha (LC3)-associated phagocytosis is required for the efficient clearance of dead cells," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 108, no. 42, pp. 17396–17401, 2011.

[134] L. Fesus, M. A. Demény, and G. Petrovski, "Autophagy shapes inflammation," *Antioxidants and Redox Signaling*, vol. 14, no. 11, pp. 2233–2243, 2011.

[135] M. M. Kockx, G. R. Y. De Meyer, N. Buyssens, M. W. M. Knaapen, H. Bult, and A. G. Herman, "Cell composition, replication, and apoptosis in atherosclerotic plaques after 6 months of cholesterol withdrawal," *Circulation Research*, vol. 83, no. 4, pp. 378–387, 1998.

[136] W. Martinet and G. R. Y. De Meyer, "Autophagy in atherosclerosis: a cell survival and death phenomenon with therapeutic potential," *Circulation Research*, vol. 104, no. 3, pp. 304–317, 2009.

[137] G. Jia, G. Cheng, D. M. Gangahar, and D. K. Agrawal, "Insulin-like growth factor-1 and TNF-α regulate autophagy through c-jun N-terminal kinase and Akt pathways in human atherosclerotic vascular smooth cells," *Immunology and Cell Biology*, vol. 84, no. 5, pp. 448–454, 2006.

[138] R. Scherz-Shouval and Z. Elazar, "Regulation of autophagy by ROS: physiology and pathology," *Trends in Biochemical Sciences*, vol. 36, no. 1, pp. 30–38, 2011.

[139] M. Nowicki, O. Zabirnyk, N. Duerrschmidt, J. Borlak, and K. Spanel-Borowski, "No upregulation of lectin-like oxidized low-density lipoprotein receptor-1 in serum-deprived EA.hy926 endothelial cells under oxLDL exposure, but increase in autophagy," *European Journal of Cell Biology*, vol. 86, no. 10, pp. 605–616, 2007.

[140] M. Muller, R. Salvayre, A. Nègre-Salvayre, and C. Vindis, "Oxidized LDLs trigger endoplasmic reticulum stress and autophagy: prevention by HDLs," *Autophagy*, vol. 7, no. 5, pp. 541–543, 2011.

[141] Y. H. Zheng, C. Tian, Y. Meng et al., "Osteopontin stimulates autophagy via integrin/CD44 and p38 MAPK signaling pathways in vascular smooth muscle cells," *Journal of Cellular Physiology*, vol. 227, no. 1, pp. 127–135, 2012.

[142] P. Hu, D. Lai, P. Lu, J. Gao, and H. He, "ERK and Akt signaling pathways are involved in advanced glycation end product-induced autophagy in rat vascular smooth muscle cells," *International Journal of Molecular Medicine*, vol. 29, no. 4, pp. 613–618, 2012.

[143] W. Martinet, M. De Bie, D. M. Schrijvers, G. R. Y. De Meyer, A. G. Herman, and M. M. Kockx, "7-Ketocholesterol induces protein ubiquitination, myelin figure formation, and light chain 3 processing in vascular smooth muscle cells," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 24, no. 12, pp. 2296–2301, 2004.

[144] J. C. Sluimer, J. M. Gasc, J. L. van Wanroij et al., "Hypoxia, hypoxia-inducible transcription factor, and macrophages in human atherosclerotic plaques are correlated with intraplaque angiogenesis," *Journal of the American College of Cardiology*, vol. 51, no. 13, pp. 1258–1265, 2008.

[145] W. Martinet and G. R. Y. De Meyer, "Selective depletion of macrophages in atherosclerotic plaques: myth, hype, or reality?" *Circulation Research*, vol. 100, no. 6, pp. 751–753, 2007.

[146] Q. Wang, M. Zhang, B. Liang et al., "Activation of AMP-activated protein kinase is required for berberine-induced reduction of atherosclerosis in mice: the role of uncoupling protein 2," *PLoS One*, vol. 6, no. 9, p. 25436, 2011.

[147] T. J. LaRocca, G. D. Henson, A. Thorburn et al., "Translational evidence that impaired autophagy contributes to arterial ageing," *Journal of Physiology*, vol. 590, no. 14, pp. 3305–3316, 2012.

[148] R. Kiffin, U. Bandyopadhyay, and A. M. Cuervo, "Oxidative stress and autophagy," *Antioxidants and Redox Signaling*, vol. 8, no. 1-2, pp. 152–162, 2006.

[149] W. Martinet, D. M. Schrijvers, J. P. Timmermans, and H. Bult, "Interactions between cell death induced by statins and 7-ketocholesterol in rabbit aorta smooth muscle cells," *British Journal of Pharmacology*, vol. 154, no. 6, pp. 1236–1246, 2008.

[150] L. Qin, Z. Wang, L. Tao, and Y. Wang, "ER stress negatively regulates AKT/TSC/mTOR pathway to enhance autophagy," *Autophagy*, vol. 6, no. 2, pp. 239–247, 2010.

[151] D. M. Schrijvers, G. R. De Meyer, and W. Martinet, "Autophagy in atherosclerosis: a potential drug target for plaque stabilization," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 31, no. 12, pp. 2787–2791, 2011.

[152] J. K. Salabei, A. Balakumaran, J. C. Frey et al., "Verapamil stereoisomers induce antiproliferative effects in vascular smooth muscle cells via autophagy," *Toxicology and Applied Pharmacology*, vol. 262, no. 3, pp. 265–272, 2012.

[153] Y. Döring, H. D. Manthey, M. Drechsler et al., "Auto-antigenic protein-DNA complexes stimulate plasmacytoid dendritic cells to promote atherosclerosis," *Circulation*, vol. 125, no. 13, pp. 1673–1683, 2012.

[154] N. MacRitchie, G. Grassia, S. R. Sabir et al., "Plasmacytoid dendritic cells play a key role in promoting atherosclerosis in apolipoprotein E-deficient mice," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 32, no. 11, pp. 2569–2579, 2012.

[155] G. Grassia, N. MacRitchie, A. M. Platt et al., "Plasmacytoid dendritic cells: biomarkers or potential therapeutic targets in atherosclerosis?" *Pharmacology & Therapeutics*, 2012.

[156] G. Bjorkoy, T. Lamark, S. Pankiv, A. Øvervatn, A. Brech, and T. Johansen, "Chapter 12 monitoring autophagic degradation of p62/SQSTM1," *Methods in Enzymology*, vol. 451, no. C, pp. 181–197, 2009.

[157] R. Singh, S. Kaushik, Y. Wang et al., "Autophagy regulates lipid metabolism," *Nature*, vol. 458, pp. 1131–1135, 2009.

[158] R. Singh and A. M. Cuervo, "Lipophagy: connecting autophagy and lipid metabolism," *International Journal of Cell Biology*, vol. 2012, Article ID 282041, 12 pages, 2012.

[159] K. Liu and M. J. Czaja, "Regulation of lipid stores and metabolism by lipophagy," *Cell Death and Differentiation*, vol. 20, pp. 3–11, 2013.

[160] M. Ouimet, V. Franklin, E. Mak, X. Liao, I. Tabas, and Y. L. Marcel, "Autophagy regulates cholesterol efflux from macrophage foam cells via lysosomal acid lipase," *Cell Metabolism*, vol. 13, no. 6, pp. 655–667, 2011.

[161] X. Le Guezennec, A. Brichkina, Y. F. Huang et al., "Wip1-dependent regulation of autophagy, obesity, and atherosclerosis," *Cell Metabolism*, vol. 16, no. 1, pp. 68–80, 2012.

[162] A. Fleming, T. Noda, T. Yoshimori, and D. C. Rubinsztein, "Chemical modulators of autophagy as biological probes and potential therapeutics," *Nature Chemical Biology*, vol. 7, no. 1, pp. 9–17, 2011.

[163] P. Bischoff, E. Jossett, and F. J. Dumont, "Novel pharmacological modulators of autophagy and therapeutic prospects," *Expert Opinion on Therapeutic Patents*, vol. 22, no. 9, pp. 1053–1079, 2012.

[164] B. Ravikumar, C. Vacher, Z. Berger et al., "Inhibition of mTOR induces autophagy and reduces toxicity of polyglutamine expansions in fly and mouse models of Huntington disease," *Nature Genetics*, vol. 36, no. 6, pp. 585–595, 2004.

[165] Z. Berger, B. Ravikumar, F. M. Menzies et al., "Rapamycin alleviates toxicity of different aggregate-prone proteins," *Human Molecular Genetics*, vol. 15, no. 3, pp. 433–442, 2006.

[166] B. Ravikumar and D. C. Rubinsztein, "Role of autophagy in the clearance of mutant huntingtin: a step towards therapy?" *Molecular Aspects of Medicine*, vol. 27, no. 5-6, pp. 520–527, 2006.

[167] S. Sarkar, E. O. Perlstein, S. Imarisio et al., "Small molecules enhance autophagy and reduce toxicity in Huntington's disease models," *Nature Chemical Biology*, vol. 3, no. 6, pp. 331–338, 2007.

[168] S. Sarkar, J. E. Davies, Z. Huang, A. Tunnacliffe, and D. C. Rubinsztein, "Trehalose, a novel mTOR-independent autophagy enhancer, accelerates the clearance of mutant huntingtin and α-synuclein," *Journal of Biological Chemistry*, vol. 282, no. 8, pp. 5641–5652, 2007.

[169] N. Hay and N. Sonenberg, "Upstream and downstream of mTOR," *Genes and Development*, vol. 18, no. 16, pp. 1926–1945, 2004.

[170] K. E. O'Reilly, F. Rojo, Q. B. She et al., "mTOR inhibition induces upstream receptor tyrosine kinase signaling and activates Akt," *Cancer Research*, vol. 66, no. 3, pp. 1500–1508, 2006.

[171] K. Yu, C. Shi, L. Toral-Barza et al., "Beyond rapalog therapy: preclinical pharmacology and antitumor activity of WYE-125132, an ATP-competitive and specific inhibitor of mTORC1 and mTORC2," *Cancer Research*, vol. 70, no. 2, pp. 621–631, 2010.

[172] C. M. Chresta, B. R. Davies, I. Hickson et al., "AZD8055 is a potent, selective, and orally bioavailable ATP-competitive mammalian target of rapamycin kinase inhibitor with in vitro and in vivo antitumor activity," *Cancer Research*, vol. 70, no. 1, pp. 288–298, 2010.

[173] M. E. Feldman, B. Apsel, A. Uotila et al., "Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2," *PLoS biology*, vol. 7, no. 2, p. e38, 2009.

[174] C. C. Thoreen, S. A. Kang, J. W. Chang et al., "An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1," *Journal of Biological Chemistry*, vol. 284, no. 12, pp. 8023–8032, 2009.

[175] A. D. Balgi, B. D. Fonseca, E. Donohue et al., "Screen for chemical modulators of autophagy reveals novel therapeutic inhibitors of mTORC1 signaling," *PLoS ONE*, vol. 4, no. 9, Article ID e7124, 2009.

[176] R. S. B. Williams, L. Cheng, A. W. Mudge, and A. J. Harwood, "A common mechanism of action for three mood-stabilizing drugs," *Nature*, vol. 417, no. 6886, pp. 292–295, 2002.

[177] S. Sarkar, R. A. Floto, Z. Berger et al., "Lithium induces autophagy by inhibiting inositol monophosphatase," *Journal of Cell Biology*, vol. 170, no. 7, pp. 1101–1111, 2005.

[178] N. Furuya, X. H. Liang, and B. Levine, "Autophagy and cancer," in *Autophagy*, D. J. Klionsky, Ed., pp. 241–255, Landes Bioscience, Georgetown, Tex, USA, 2004.

[179] R. J. O. Dowling, M. Zakikhani, I. G. Fantus, M. Pollak, and N. Sonenberg, "Metformin inhibits mammalian target of rapamycin-dependent translation initiation in breast cancer cells," *Cancer Research*, vol. 67, no. 22, pp. 10804–10812, 2007.

[180] I. B. Sahra, K. Laurent, A. Loubat et al., "The antidiabetic drug metformin exerts an antitumoral effect in vitro and in vivo through a decrease of cyclin D1 level," *Oncogene*, vol. 27, no. 25, pp. 3576–3586, 2008.

[181] M. Buzzai, R. G. Jones, R. K. Amaravadi et al., "Systemic treatment with the antidiabetic drug metformin selectively impairs p53-deficient tumor cell growth," *Cancer Research*, vol. 67, no. 14, pp. 6745–6752, 2007.

[182] R. A. Floto, S. Sarkar, E. O. Perlstein et al., "Small molecule enhancers of rapamycin-induced TOR inhibition promote autophagy, reduce toxicity in Huntington's disease models and enhance killing of mycobacteria by macrophages," *Autophagy*, vol. 3, no. 6, pp. 620–622, 2007.

[183] Z. J. Yang, C. E. Chee, S. Huang, and F. A. Sinicrope, "Autophagy modulation for cancer therapy," *Cancer Biology and Therapy*, vol. 11, no. 2, pp. 169–176, 2011.

[184] S. W. Ryter, S. J. Lee, A. Smith, and A. M. Choi, "Autophagy in vascular disease," *Proceedings of the American Thoracic Society*, vol. 7, no. 1, pp. 40–47, 2010.

[185] S. A. Khan, F. Salloum, A. Das, L. Xi, G. W. Vetrovec, and R. C. Kukreja, "Rapamycin confers preconditioning-like protection against ischemia-reperfusion injury in isolated mouse heart and cardiomyocytes," *Journal of Molecular and Cellular Cardiology*, vol. 41, no. 2, pp. 256–264, 2006.

[186] B. Loos and A. M. Engelbrecht, "Cell death: a dynamic response concept," *Autophagy*, vol. 5, no. 5, pp. 590–603, 2009.

[187] B. Loos, S. Genade, B. Ellis, A. Lochner, and A. M. Engelbrecht, "At the core of survival: autophagy delays the onset of both apoptotic and necrotic cell death in a model of ischemic cell injury," *Experimental Cell Research*, vol. 317, no. 10, pp. 1437–1453, 2011.

[188] A. Nakai, O. Yamaguchi, T. Takeda et al., "The role of autophagy in cardiomyocytes in the basal state and in response to hemodynamic stress," *Nature Medicine*, vol. 13, no. 5, pp. 619–624, 2007.

[189] W. Martinet, I. De Meyer, S. Verheye et al., "Drug-induced macrophage autophagy in atherosclerosis: for better or worse?" *Basic Research in Cardiology*, vol. 108, no. 1, p. 321, 2013.

[190] C. Castro, J. M. Campistol, D. Sancho, F. Sánchez-Madrid, E. Casals, and V. Andrés, "Rapamycin attenuates atherosclerosis

induced by dietary cholesterol in apolipoprotein-deficient mice through a p27Kip1-independent pathway," *Atherosclerosis*, vol. 172, no. 1, pp. 31–38, 2004.

[191] M. A. Mueller, F. Beutner, D. Teupser, U. Ceglarek, and J. Thiery, "Prevention of atherosclerosis by the mTOR inhibitor everolimus in LDLR-/- mice despite severe hypercholesterolemia," *Atherosclerosis*, vol. 198, no. 1, pp. 39–48, 2008.

[192] R. Pakala, E. Stabile, G. J. Jang, L. Clavijo, and R. Waksman, "Rapamycin attenuates atherosclerotic plaque progression in apolipoprotein E knockout mice: inhibitory effect on monocyte chemotaxis," *Journal of Cardiovascular Pharmacology*, vol. 46, no. 4, pp. 481–486, 2005.

[193] L. Zhao, T. Ding, T. Cyrus et al., "Low-dose oral sirolimus reduces atherogenesis, vascular inflammation and modulates plaque composition in mice lacking the LDL receptor," *British Journal of Pharmacology*, vol. 156, no. 5, pp. 774–785, 2009.

[194] Z. Guo, F. Mitchell-Raymundo, H. Yang et al., "Dietary restriction reduces atherosclerosis and oxidative stress in the aorta of apolipoprotein E-deficient mice," *Mechanisms of Ageing and Development*, vol. 123, no. 8, pp. 1121–1131, 2002.

[195] P. Maffia, B. H. Zinselmeyer, A. Ialenti et al., "Multiphoton microscopy for 3-dimensional imaging of lymphocyte recruitment into apolipoprotein-E-deficient mouse carotid artery," *Circulation*, vol. 115, no. 11, pp. e326–e328, 2007.

[196] E. Galkina, A. Kadl, J. Sanders, D. Varughese, I. J. Sarembock, and K. Ley, "Lymphocyte recruitment into the aortic wall before and during development of atherosclerosis is partially L-selectin dependent," *Journal of Experimental Medicine*, vol. 203, no. 5, pp. 1273–1282, 2006.

[197] E. K. Koltsova, Z. Garcia, G. Chodaczek et al., "Dynamic T cell-APC interactions sustain chronic inflammation in atherosclerosis," *Journal of Clinical Investigation*, vol. 122, no. 9, pp. 3114–3126, 2012.

[198] J. M. Ireland and E. R. Unanue, "Autophagy in antigen-presenting cells results in presentation of citrullinated peptides to CD4 T cells," *Journal of Experimental Medicine*, vol. 208, no. 13, pp. 2625–2632, 2011.

[199] I. X. McLeod, W. Jia, and Y. W. He, "The contribution of autophagy to lymphocyte survival and homeostasis," *Immunological Reviews*, vol. 249, no. 1, pp. 195–204, 2012.

[200] S. Paul and B. C. Schaefer, "Selective autophagy regulates T-cell activation," *Autophagy*, vol. 8, no. 11, pp. 1690–1692, 2012.

[201] S. Morris, M. S. Swanson, A. Lieberman et al., "Autophagy-mediated dendritic cell activation is essential for innate cytokine production and APC function with respiratory syncytial virus responses," *Journal of Immunology*, vol. 187, no. 8, pp. 3953–3961, 2011.

[202] H. Nakano and H. Ushio, "An unexpected role for autophagy in degranulation of mast cells," *Autophagy*, vol. 7, no. 6, pp. 657–659, 2011.