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REVIEW

Review: the Role and Mechanisms of Macrophage Autophagy in Sepsis

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Abstract— Sepsis is a systemic inflammatory response syndrome caused by infection. The core mechanism underlying sepsis is immune dysfunction, with macrophages, as important cells of the innate immune system, playing an essential role. Autophagy has been shown to be closely related to inflammation and immunity, and autophagy enhancement in sepsis can play a protective role by negatively regulating abnormal macrophage activation, modulating macrophage polarization phenotype, reducing activation of the inflammasome and release of inflammatory factors, and affecting macrophage apoptosis. However, excessive autophagy may also lead to autophagic death of macrophages, which further aggravates the inflammatory response. The mechanisms underlying these functions are relatively complex and remain unclear, but may be related to a variety of signaling pathways such as NF-κB, mTOR, and PI3K/AKT. The administration of drugs to assist in the regulation of macrophage autophagy has become a novel treatment for sepsis. The present review focuses on the role and the potential mechanisms of macrophage autophagy in sepsis.

KEY WORDS: macrophage; autophagy; sepsis; inflammation; immunity; apoptosis; polarization.

INTRODUCTION

Sepsis is defined as life-threatening organ dysfunction caused by an inappropriately extreme host response to infection [1]. Immune dysfunction is the core mechanism underlying the development of sepsis, which includes early immune system over-activation and late immune suppression [2]. The host's innate immune system is the first line of defense against infection. Multiple immune cells including neutrophils, monocytes/macrophages, dendritic cells, natural killer cells, T cells, and *etc.* play an important role in sepsis. Neutrophils migrate to the site of infection to exert phagocytosis and bactericidal action [3]; dendritic cells exhibit antigen presentation [4]; T cells participate in adaptive immune response [5, 6]; and NK cells perform nonspecific direct killing of the pathogen [7]. Different from the above immune cells, macrophages are widely distributed in various tissues and play multiple roles throughout all the stages of sepsis such as phagocytosis, bactericidal, antigen presentation, and secretion of inflammatory factors and chemokines [6, 8, 9]. At the early stage of sepsis, macrophages secret a large number of pro-inflammatory factors and chemokines, which aggravate the inflammatory response [10, 11], and the excessive apoptosis of macrophages in late sepsis results in immune dysfunction and organ injury [12, 13]. Immunosuppression observed in the late phase of sepsis makes host susceptible to acquires secondary infections and increases the mortality, the reasons of which include (I) numerous immune cells involving T cells and macrophages endure apoptosis, and macrophages may be involved in accelerating T cell apoptosis

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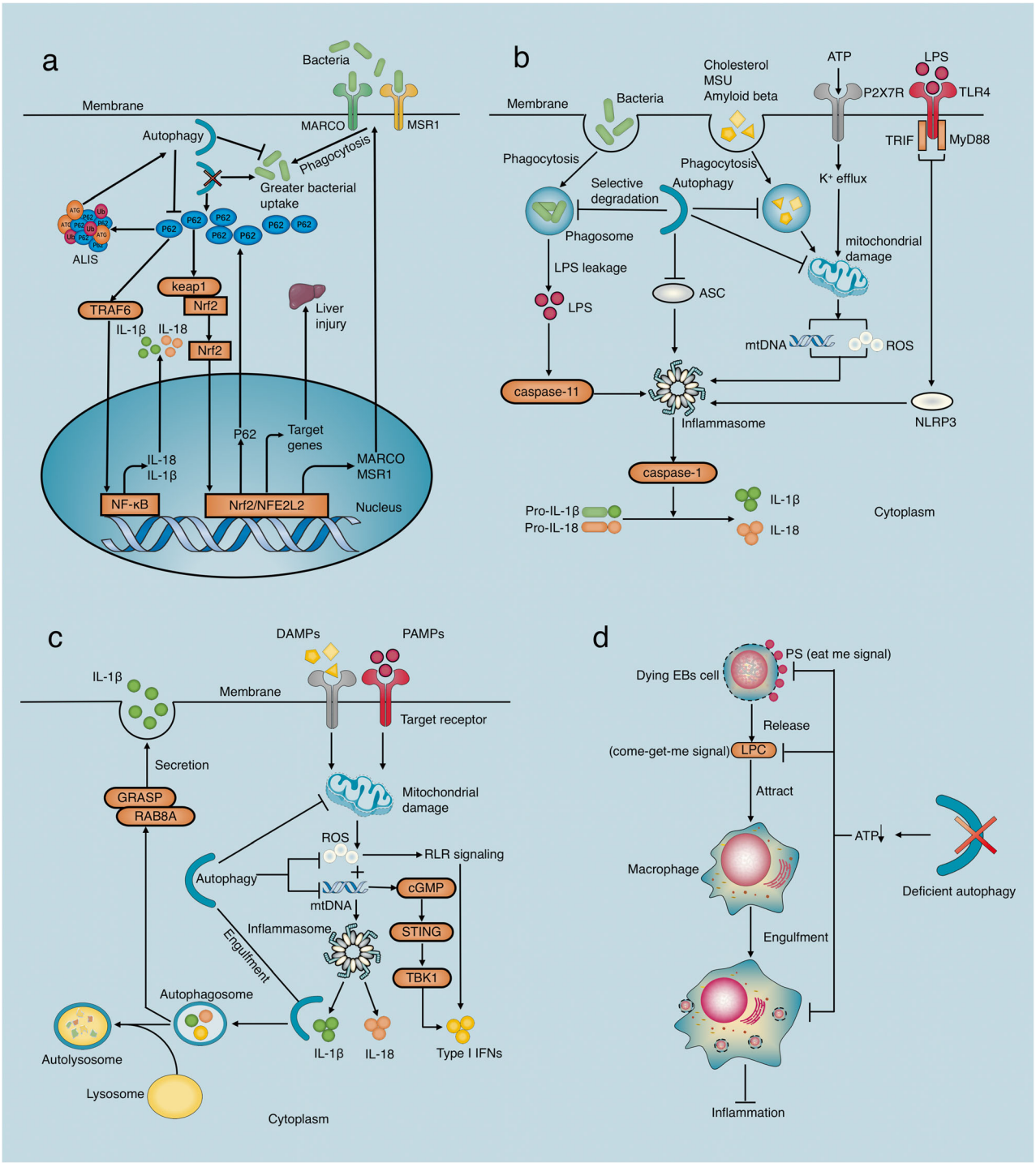

and impaired immune function caused by sepsis [6]. (II) Macrophage deactivation and endotoxin tolerance mediated by phenotypic changes of macrophages, decreased antigen presentation, and increased release of

◀ **Fig. 1.** The mechanisms of autophagy-regulated inflammation. **a** p62 is one of the most important substrates involved in autophagy, which can also activate autophagy *via* ALIS structures. Autophagy deficiency results in p62 accumulation, leading to the activation of multiple nuclear transcription factors (NF-κB, Nrf2/NFE2L2) and their target genes, which can increase the expression of inflammatory-related cytokines. Autophagy deficiency or overproduction of p62 can activate NF-κB *via* the promotion of oligomerization of the ubiquitin ligase, TRAF6, leading to increased production of IL-1β and IL-18. In addition, p62 can compete with the interaction between Nrf2 and Keap1, resulting in stabilization and nuclear translocation of Nrf2 and subsequent transcriptional activation of Nrf2 target genes, which can lead to liver injury or a further increase in p62. Moreover, accumulation of p62 in ATG7-deficient macrophages increases the activity of NFE2L2 and leads to greater expression of two class A scavenger receptors, MARCO and MSR1, resulting in greater bacterial uptake *via* phagocytosis when infected with *Mtb* and exacerbation of inflammatory lung injury. **b** Autophagy can inhibit the canonical activation of the inflammasome through selective degradation of damaged mitochondria caused by crystals (cholesterol, MSU, amyloid-β) and K+ efflux. Conversely, autophagy suppresses noncanonical inflammasome activation pathways by blocking LPS leakage *via* the elimination of phagosomes containing bacteria. **c** Autophagy can decrease the production of IL-1β, IL-18, and type I IFNs *via* the degradation of damaged mitochondria, ROS, and mtDNA and can also directly eliminate cytokines. However, autophagy may mediate the secretion of IL-1β under starved conditions through complex pathways involving GRASP and RAB8A. **d** Autophagy can promote the clearance of dead cells by expressing more "engulf-me signal," PS, and releasing more "come-get-me signal," LPC, in EBs *via* the possible mechanism of increasing ATP production. ALIS, aggresome-like induced structures; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2/NFE2L2, nuclear factor erythroid 2-related factor 2; Keap1, Kelch-like ECH-associated protein 1; TRAF6, TNF receptor-associated factor 6; MARCO, macrophage receptor with collagenous structure; MSR1, macrophage scavenger receptor 1; *Mtb*, *Mycobacterium tuberculosis*; LPS, lipopolysaccharide; ROS, reactive oxygen species; ASC, apoptosis-associated speck-like protein; GRASP, Golgi reassembly stacking protein; PS, phosphatidylserine; RAB8A, Ras-related protein Rab-8A; STING, stimulator of interferon genes; TBK1, TANK-binding kinase 1; LPC, lysophosphatidylcholine; EBs, embryoid bodies; NLRP3, nod-like receptor pyrin domain-containing 3.

anti-inflammatory factors [14, 15]. In summary, among various immune cells, macrophages play essential roles throughout all phases of sepsis with their ubiquitous presence and comprehensive effects in immune homeostasis and inflammatory process. Therefore, attention and regulation of macrophage immune function is of great significance for the treatment of sepsis.

Autophagy is a highly conserved process in biological evolution, maintaining intracellular homeostasis and cell self-renewal. Increasing evidence has shown that autophagy also plays an important regulatory role in the processes of inflammation and immunization [16–19]. The mechanism by which autophagy regulates inflammation is complex and is thought to be related to the following scenarios (Fig. 1): (I) modulation of inflammation-related transcription factors involving nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and nuclear factor erythroid 2-related factor 2 (Nrf2/NFE2L2) [20–23] (Fig. 1a); (II) regulation of inflammasome activation [24] (Fig. 1b); (III) regulation of the production and degradation of inflammatory cytokines [17, 18, 25] (Fig. 1c); (IV) promotion of the clearance of dead cells [26] (Fig. 1d); and (V) modulation of phagocytosis [20] (Fig. 1a).

As a new mechanism of immune regulation, autophagy participates in host immune defense against invading pathogens, regulates functions of innate immune cells such as neutrophils and macrophages, and affects their ability to defend and clear pathogens. The role of autophagy in neutrophil immune response during bacterial infection has been reviewed in detail [27]. Autophagy is not only involved in the antibacterial process of neutrophils such as extracellular traps formation, antimicrobial secretions, and phagocytosis but also affects cell fate when infection [27]. Similarly, autophagy is also important for the regulation of macrophage functions in a variety of diseases, especially in infectious diseases such as sepsis. The level of macrophage autophagy not only affects their activation, classification, and death but also affects the activation of the inflammasome and the release of inflammatory cytokines, which partly determine the development and prognosis of sepsis. Given the major roles of macrophages and autophagy in the progression of sepsis and their correlation in biological activities, it is quite valuable to clarify the contribution and underlying molecular mechanisms of autophagy-mediated regulation of macrophages and their implications for sepsis. In addition, the regulation of macrophage autophagy is also of great significance and may be an effective therapeutic target for sepsis.

MACROPHAGES AND SEPSIS

Macrophages recognize pathogens by surface-expressed receptors, subsequently engulfing and digesting them. As one of the major immune cells, macrophages are excessively activated during the early stage of sepsis. Macrophages with the pro-inflammatory phenotype (M1) increase and release massive amounts of inflammatory factors such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α), inducible nitric oxide synthase (iNOS), and a large number of chemokines such as chemokine (C-C motif) ligand 2–4 (CCL2–4) and chemokine (C-X-C motif) ligand 8–11 (CXCL8–11), which can induce the immune response mediated by type 1 helper T cells (Th_1 cells), uncontrolled inflammation, endotoxic

shock, and organ injury [15, 28]. During the late stage of sepsis, apoptosis of immune cells including macrophages increases sharply, resulting in immune suppression and aggravation of infection. Previous studies have shown that in human autopsies and animal models of sepsis, an excessive level of immune cell apoptosis was found, mainly in the spleen and thymus, including lymphocytes, macrophages, monocytes, natural killer cells (NK cells), and dendritic cells [5, 6, 29]. In addition, it has been shown that antagonizing apoptosis of immune cells rather than endotoxin or other inflammatory factors such as TNF-α or IL-1β significantly reduces organ damage and improves the prognosis of sepsis [5, 6, 29]. Therefore, it can be concluded that controlling the early excessive inflammatory response and improving the late immune suppression, by regulating the function of macrophages, would be an important and effective treatment for sepsis.

AUTOPHAGY AND SEPSIS

Autophagy is the process by which cytoplasmic material or pathogens are engulfed within a double-membrane-bound vesicle, termed an autophagosome, and fused with lysosomes for degradation and recycling of the sequestered substrates (Fig. 2). Autophagy, which is integrated into various functions and processes of the immune system, is an important defense mechanism to protect the body against external pathogens and danger signals, playing a crucial role in the induction and regulation of inflammatory reactions in innate immune cells, which are important factors affecting the progression of sepsis.

The classical idea reported by Saitoh *et al.* in *Nature* showed that loss of the autophagy protein, ATG16L1, enhances endotoxin-induced IL-1β production [25]. In addition, autophagy can alleviate inflammation by promoting the degradation of pro-inflammatory factors [25]. Autophagy-deficient mice are more susceptible to lipopolysaccharide (LPS), which is the main constituent of endotoxin [30]. Moreover, autophagy deficiency in T cells suppresses the immune response in sepsis and increases mortality [31]. Taken together, this evidence suggests that autophagy plays a protective role in sepsis, achieved through the following mechanisms: direct pathogen clearance [32], microbial toxin neutralization [33], modulation of cytokine release, mitochondrial integrity preservation [30], reduction in apoptosis [34, 35], and promotion of antigen presentation [18]. Consistent with these findings, studies have demonstrated that autophagy inhibition is closely related to organ dysfunction in sepsis [36–38]. However, it has also been shown that autophagy is involved in mitochondrial damage caused by sepsis and generates a noxious effect on the body [39].

A series of studies has illustrated that autophagy is activated in sepsis, negatively regulating the inflammatory response [25, 40, 41] and attenuating the inflammatory damage of various tissues and organs. It has been proven that

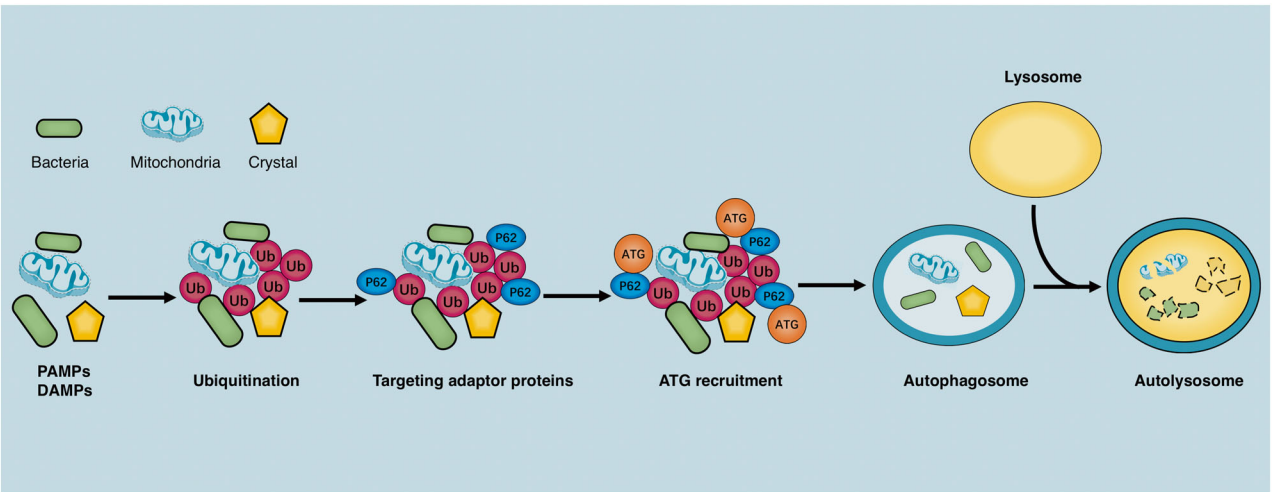

Fig. 2. The elimination process of damaged organelles and invading pathogens by autophagy. Autophagy can be activated by PAMPs (bacteria, viruses, fungi) and DAMPs (damaged organelles, crystals, ATP). The "cargos," such as damaged organelles and invading pathogens in the cytoplasm, are ubiquitinated and subsequently targeted by the adaptor proteins (P62/SQSTM1, NDP52, and optineurin), which recruit autophagy-related proteins to form autophagosomes and encapsulate these cargos. Following fusion with lysosomes, autolysosomes are formed to degrade and eliminate the cargos. PAMPs, pathogen-associated molecular patterns; DAMPs, damage-associated molecular patterns; NDP52, nuclear dot protein 52; SQSTM1, sequestosome 1.

heme oxygenase-1-mediated autophagy can protect against hepatocyte cell death and hepatic injury from sepsis in mice [42], and enhanced activity of hepatic autophagy can improve survival in septic mice by regulating autophagy and apoptosis-related gene expression [37, 43]. In addition, specific ablation of ATG7 from kidney proximal tubules worsens LPS-induced acute kidney injury [44]. Conversely, autophagy activation serves a renoprotective role in endotoxin-induced kidney injury [45, 46]. In a mouse model of sepsis created by cecal ligation and puncture (CLP), incompletion of the autophagic process was shown to likely contribute to sepsis-induced cardiac dysfunction [38], and autophagy activation by rapamycin restored cardiac performance and alleviated myocardial injury caused by CLP [38]. Moreover, enhanced autophagy in hippocampal neurons, as a result of the inhibition of the NF-κB signaling pathway, has been shown to play a protective role in septic brain injury [47]. In lung tissue, knockout of autophagy related gene 4B (ATG4B) increases pulmonary inflammation upon LPS challenge [36]. Conversely, enhancement of ATG12-dependent autophagy mediates a protective effect of GAPDH on lung injury in sepsis [48, 49]. Moreover, overexpression of the autophagy marker, light chain 3 (LC3), has been shown to improve survival and attenuate lung injury *via* an increase in the fusion of autolysosomes and the clearance of autophagosomes in septic mice [50].

However, autophagy plays differing roles in the regulation of lung alveolar epithelial and vascular endothelial cells in the acute lung injury caused by sepsis. The role of autophagy in alveolar epithelial cells is pathogen- and level-dependent, with moderate autophagy induced by oxidative stress reducing the death of epithelial cells [51, 52] and excessive autophagy leading to increased programmed cell death [53]. A recent study demonstrated that autophagy induced by macrophage migration inhibitory factor (MIF) in endothelial cells, *via* the extracellular signal-regulated kinase (ERK) pathway, leads to pulmonary vascular leakage as a result of autophagic degradation of the junction proteins, zonula occludens-1 (ZO-1) and vascular endothelial cadherin (VE-cadherin), indicating that autophagy enhancement in pulmonary endothelial cells increases septic lung injury [54]. Neutrophil infiltration and macrophage activation appear in pulmonary tissues in sepsis. LPS can induce a dose- and time-dependent activation of mammalian target of rapamycin complex 1 (MTORC1) in pulmonary neutrophils, thus amplifying the inflammatory response *via* the inhibition of autophagy [55]. With respect to alveolar macrophage autophagy, it has been shown to play a protective role in sepsis through the inhibition of the pro-inflammatory response induced by CLP in mice [30]. The role of macrophage autophagy in sepsis will be discussed in detail in the following sections.

THE ROLE OF MACROPHAGE AUTOPHAGY IN SEPSIS

At present, autophagy, in particular macrophage autophagy, is considered an important part of the host immune defense, eliminating intracellular pathogens through heterophagy. Pathogens can induce autophagy through PAMPs, toxins, and proteins secreted by the bacterial secretory system T3/T4 (T3SS/T4SS). Following activation, autophagy can recognize and subsequently remove pathogens *via* multiple pathways including direct death, regulation of inflammatory processes, LC3-associated patterns (LAPs), antigen presentation and other immune functions, and release of bactericidal factors (ROS, NO) [56]. Thus, macrophage autophagy may regulate and change its role in inflammation and immunity with respect to different levels and causes, which could partly determine the prognosis of sepsis (Fig. 3).

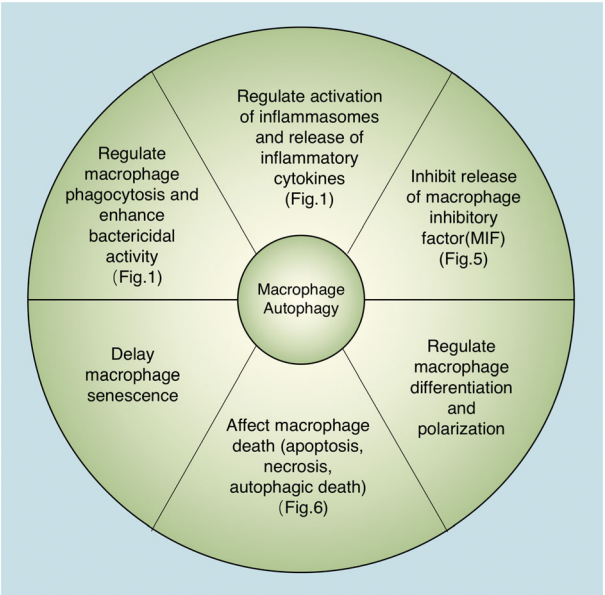

Fig. 3. The role of macrophage autophagy in sepsis. Macrophage autophagy can be induced by LPS or other stress factors in sepsis. Following activation, macrophage autophagy may affect the process and prognosis of sepsis through multiple pathways including the regulation of inflammation, MIF, polarization, and cell aging and death (apoptosis, necrosis, autophagic death). However, whether macrophage autophagy plays a protective or a harmful role in sepsis remains controversial. MIF, macrophage migration inhibitory factor.

The Mechanisms of Macrophage Autophagy Activation in Sepsis

As the most important pathogenic factor in sepsis, LPS has been shown to induce macrophage autophagy through toll-like receptor 4 (TLR4)-dependent pathways [41, 57–59] (Fig. 4). TLR4 signaling pathways can be divided into two categories: myeloid differentiation factor 88 (MyD88)-dependent and MyD88-independent. Xu *et al.* demonstrated that LPS can induce macrophage autophagy through a MyD88-independent TLR4 pathway involving toll-interleukin-1 receptor domain-containing adaptor-inducing interferon-β (TRIF), receptor interacting protein 1 (RIP1), and p38 mitogen-activated protein kinases (p38MAPK) [57]. In addition, Fujita *et al.* found that macrophage autophagy can also be activated by LPS *via* a MyD88-dependent TLR4 pathway, which is a p62-dependent type of selective autophagy of aggresome-like induced structures (ALIS) [59]. As a result of LPS engagement with TLR4, a series of signaling pathways including TRIF, MyD88, interleukin-1 receptor-associated kinase 4 (IRAK4), TRAF6, p38, and Nrf2 were shown to be activated, followed by increased transcriptional levels of p62, which resulted in the formation of ALIS *via* oligomerization [59]. Subsequently, ALIS are engulfed in autophagosomal membranes, which fuse with lysosomes to form autolysosomes for degradation [59]. Moreover, research conducted by Waltz *et al.* illustrated that LPS can induce macrophage autophagy through heme oxygenase-1 (HO-1)-dependent pathways [41].

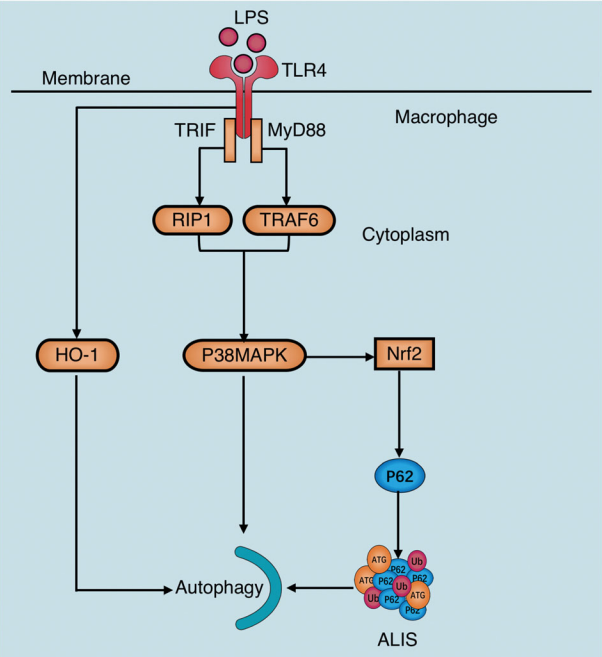

Fig. 4. The mechanism of LPS-induced macrophage autophagy. Following binding to TLR4, LPS induces macrophage autophagy *via* three possible pathways: (I) the MyD88-dependent pathway involving activation of TRAF6, p38MAPK, and Nrf2, which induce autophagy *via* an increase in p62 and ALIS; (II) the MyD88-independent pathway involving activation of RIP1 and p38MAPK; and (III) HO-1-dependent pathway; however, the details remain unclear. TLR4, toll-like receptor 4; MyD88, myeloid differentiation factor 88; TRIF, toll-interleukin-1 receptor domain-containing adaptor-inducing interferon-β; RIP1, receptor interacting protein 1; IRAK4, IL-1 receptor-associated kinase 4; p38MAPK, p38 mitogen-activated protein kinases; HO-1, heme oxygenase-1.

Macrophage Autophagy Regulates the Activation of the Inflammasome and Release of Inflammatory Factors

Macrophage autophagy induced in sepsis can reduce the secretion of IL-1β by targeting pro-IL-1β for lysosomal degradation and regulating the activation of the inflammasome, NLRP3 [60]. In contrast, LPS can stimulate autophagy-deficient macrophages to produce increased levels of inflammatory cytokines such as IL-1β and IL-18 [25]. In sepsis, autophagy deficiency in macrophages leads to the accumulation of damaged mitochondria, excessive production of ROS, increased activation of NLRP3, and induction of an excessive NLRP3-dependent inflammatory response (Fig. 2). Loss of autophagy also leads to the accumulation of damaged phagosomes containing bacterial components and LPS leakage, both of which increase noncanonical activation of NLRP3 (Fig. 2b), while stimulation of autophagy can reduce the inflammatory response mediated by this pathway [17].

Excessive activation of macrophages is the basis of many inflammatory and autoimmune diseases, including sepsis. It has been elucidated that autophagy induced by rapamycin can negatively regulate the abnormal activation of macrophages and reduce the inflammatory response *via* the following scenarios [61]: (I) inhibition of NLRP3 inflammasome activation through the p62/SQSTM1 pathway; (II) reduction in the production of mitochondrial reactive oxygen species (mtROS) and pro-IL-1β *via* the p62/SQSTM1-dependent Nrf2 activation pathway; and (III) suppression of the activation of the IL-1β-p38MAPK-NF-κB pathway and reduction in IL-1β, which decreases the activation of the p38MAPK-NF-κB pathway, resulting in the transcriptional downregulation of IL-6, IL-8, monocyte chemoattractant protein 1(MCP-1), and nuclear factor of kappa light polypeptide gene enhancer in B

cells inhibitor alpha (IκBα) in rapamycin-treated macrophages.

Macrophage Autophagy Regulates the Release of Macrophage Migration Inhibitory Factor

In sepsis, the release of a plethora of inflammatory factors (cascade effect) leads to dysfunction of endothelial cells and increased vascular permeability and leakage, which are responsible for septic shock. Studies have shown that migration inhibitory factor (MIF) is an important pathogenic factor in septic shock [54], which can induce macrophages to secrete TNF-α and synergize with IFN-γ to increase NO production. Endotoxic shock is significantly reduced in MIF knockout mice or in mice administered an MIF antibody. This protective effect may be mediated by the regulation of TLR4 expression, which is the MIF-dependent LPS receptor.

MIF can be secreted by multiple cells such as macrophages, hepatocytes, and endothelial cells. Following binding to receptors, MIF can activate the PI3K/AKT and MAPK/ERK signaling pathways to regulate the inflammatory response [54]. It has been shown that MIF can induce autophagy in endothelial cells *via* the inhibition of the mTOR signaling pathway and can cause vascular leakage through the activation of the ERK pathway [54]. In addition, another study found that autophagy deficiency in macrophages can lead to an increase in ROS-dependent MIF release [62]. Therefore, it can be concluded that the enhancement of macrophage autophagy may reduce the release of MIF and alleviate vascular endothelial injury; however, this needs to be validated by further research (Fig. 5). Moreover, another study demonstrated that MIF can induce autophagy *via* ROS generation in hepatoma cells [63]. Taken together, these findings suggest that there may be a feedback loop between autophagy and MIF, which could inhibit the excessive release of MIF *via* a negative feedback mechanism, alleviating inflammatory injury (Fig. 5). As described above, macrophages are the main source of MIF; therefore, the balance between macrophage autophagy and MIF release plays an important role in the regulation of sepsis progression.

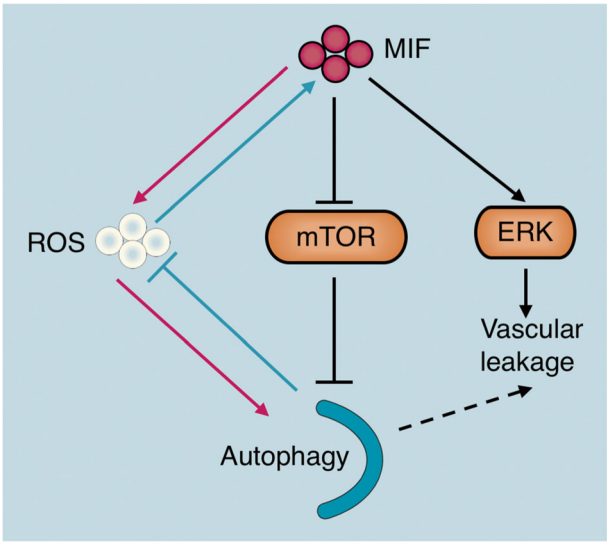

Fig. 5. The relationship between autophagy and MIF. MIF can induce autophagy by increasing ROS production and inhibiting mTOR. In contrast, autophagy may decrease MIF *via* the elimination of ROS. Thus, there may be a feedback loop between autophagy and MIF, which could inhibit the excessive release of MIF *via* a negative feedback mechanism, alleviating inflammatory injury. Furthermore, there is a two-way effect of autophagy on vascular leakage. Autophagy can attenuate vascular leakage by inhibiting the release of MIF and subsequent ERK activation; however, it has been demonstrated that vascular barrier destruction in sepsis may be related to autophagy enhancement. mTOR, mammalian target of rapamycin; ERK, extracellular signal-regulated kinase.

Autophagy Promotes Monocyte-Macrophage Differentiation

The precursor of macrophage is monocyte, whose life is short in blood. Monocytes are programmed to undergo apoptosis in the absence of stimulation [64]. On the contrary, once stimulated by an inflammatory response, they will activate the pro-survival pathways, migrate to the tissues, and differentiate into macrophages [65]. It is well-known that the factors of stimulating monocyte-macrophage differentiation mainly include granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF/CSF-1), *etc.* However, the molecular mechanisms of differentiation still remain largely unexplored, which may require activation of autophagy and caspase [66–68]. Autophagy, as a physiological process widely present in eukaryotic cells, has been shown to regulate the survival and differentiation of various cells [69–71] and plays an essential role in monocyte-macrophage differentiation in various disease environments involving inflammation [64, 66–68, 72], hypoxia [73], metabolic diseases [74], and even tumors [75]. Zhang *et al.* [64] demonstrate that autophagy is activated when monocytes are triggered to differentiate by GM-CSF or M-CSF both *in vitro* and *in vivo*, which may attribute to Beclin-1 activation mediated by JNK and block of Atg5 cleavage mediated by inhibition of calpain activity. The induction of autophagy can prevent monocyte from apoptosis and promote its differentiation into macrophage. In contrast, the inhibition of autophagy leads to

increased apoptosis and blockage of differentiation [64]. Consistent with the above results, Bonlakirba *et al.* found that the induction of autophagy is pivotal for the survival and differentiation of monocytes induced by CSF-1 and IL-34 [66]. Similarly, findings by Bruno *et al.* illustrate that autophagy contributes to the differentiation from bone marrow cells (BMCs) into macrophages stimulated by *Physalis angulata* (AEPa) [72]. In addition, autophagy is also crucial for the acquisition of phagocytic ability of differentiated macrophages. In ATG7-deficient mice, both the ability of monocyte-macrophage differentiation and macrophage acquisition of phagocytic functions are severely impaired [68]. However, little is known about the molecular mechanisms of autophagy affecting the monocyte-macrophage differentiation. Studies have suggested that autophagy may not only promote monocytes from programmed death to differentiation indirectly through crosstalk with the apoptotic pathway but also engage in the differentiation process directly [64]. In addition, the molecular mechanisms involved in the induction of autophagy by GM-CSF or CSF-1 are not fully understood. However, there are still some clues providing by several researches. Previous study reported that purinergic receptor P2RY6 is required for CSF1-induced monocyte differentiation [76]. Furthermore, it has been demonstrated that CSF1 increases the expression of the purinergic receptor P2RY6 and activates the CAMKK2-PRKAA1-ULK1 pathway that is responsible for autophagy induction in monocyte-macrophage differentiation. Instead, inhibition of this pathway abrogates CSF1-mediated induction of autophagy and monocytes differentiation [77]. Collectively, autophagy can promote the differentiation of macrophages, which is of great significance in the early stage of sepsis.

Macrophage Autophagy Regulates Macrophage Polarization

Research has revealed that there are two phenotypes of macrophage activation, broadly described as M1 and M2 polarization. Typically, the M1 phenotype is known as classical activated macrophages (CAM), while the M2 phenotype is called alternative activated macrophages (AAM), the latter of which can be further divided into three subtypes, termed M2a, M2b, and M2c. The two different phenotypes can be activated by diverse stimuli or different internal environments. It has been illustrated that there is a direct relationship between endotoxic shock and M1 polarization [15]. The overwhelming inflammation induced by the M1 phenotype has been reported to be closely related to sepsis [28], while the M2 phenotype plays a protective role by releasing multiple anti-inflammatory factors to restrict the inflammatory response and promote tissue repair [78, 79]. In addition, the transformation from the M1 to the M2 phenotype can protect the body from excessive inflammatory injury, and one of the mechanisms affecting macrophage polarization is autophagy [80].

Recent studies have demonstrated that inhibition of autophagy in a tumor microenvironment can attenuate M2 macrophage polarization [75]. Conversely, autophagic flux mediated by cathepsin S can polarize tumor-associated macrophages (TAMs) to the M2 phenotype, leading to tumor development [81]. Similarly, it has been found that the antiangiogenic drug, sorafenib, may exert a pro-tumorigenic effect in hepatocellular carcinoma by inducing macrophage autophagy and suppressing the expression of CD80, a marker of the M1 phenotype [82]. Overall, these findings indicate a potent correlation between macrophage autophagy and polarization, especially in tumor environments; however, the mechanism by which autophagy affects polarization remains unclear.

Recent research has demonstrated that the transcription factor, NF-κB, is involved in the process by which macrophage autophagy regulates macrophage polarization. Following binding to toll-like receptor 2 (TLR2), hepatoma-derived factors can promote M2 polarization through selective autophagy of NF-κB mediated by p62 [83]. In contrast, inhibition of autophagy can rescue the activity of NF-κB and force M2-polarized macrophages to produce high levels of M1-like cytokines [84].

mTOR is not only the most critical point in autophagy regulation, it also exerts an important role in polarization of monocytes to TAMs. In bone marrow-derived macrophages, activation of the PI3K-mTOR pathway can increase M2 polarization, while inhibition of PI3K or mTOR exerts the opposite effect [85], suggesting that the PI3K-mTOR pathway is important in the regulation of macrophage polarization. Similarly, macrophages treated with LPS and the mTOR inhibitor, rapamycin, exhibit a greater M1 phenotype, whereas a greater M2 phenotype is seen following activation of mTOR *via* knockdown of the TSC2 (tuberous sclerosis 2) gene that suppresses mTOR [86]. In addition, PI3K-Akt is a well-known upstream regulator of the mTOR pathway, which has also been shown to affect macrophage polarization. Overall, it can be inferred that the PI3K-Akt-mTOR pathway may be important in the crosstalk between macrophage autophagy and polarization [87]; however, it has been shown that the interaction between autophagy and polarization is complex, which is exhibited in disease- and phase-dependent manners.

Nevertheless, we can conclude that macrophage polarization regulated by autophagy likely plays an important role in sepsis.

Macrophage Autophagy Affects Macrophage Death

The massive death of immune cells, in particular macrophages, is the main reason for immunosuppression in the late stage of sepsis, which may cause deterioration of infectious states. At present, it is believed that there are three modes of cell death including apoptosis (type I programmed cell death), necrosis, and autophagic death (type II programmed cell death). Autophagy has been found to be involved in all these processes, exerting vital functions; however, autophagy is a double-edged sword. There is evidence that moderate autophagy is of great significance in the maintenance of intracellular homeostasis, while excessive autophagy may lead to cell death [34, 35, 88, 89]. The activation level of autophagy depends on multiple factors including the type of disease and cell, microenvironment, and stimulus intensity [34, 35]. However, the specific causes and mechanisms underlying autophagic death remain unclear, which is generally believed to be the result of decompensation of cell functions during excessive stress.

According to the findings of several studies, the effect of autophagy on cell survival in sepsis is currently in dispute. On one hand, autophagy has been shown to lead to cell death. By way of illustration, Li *et al.* found that LPS aggravates lung injury by activating autophagy and autophagic death in alveolar epithelial cells through endoplasmic reticulum stress and an ERK-related pathway [90]. In addition, findings by Zhang *et al.* illustrate that the death of type II alveolar epithelial cells induced by LPS may be related to the enhancement of autophagy [91]. Furthermore, it has also been found that the death of macrophages induced by increased LPS may be associated with an imbalance between the levels of autophagy and apoptosis [92]. However, the relationship between autophagy and apoptosis is complex, and the effect of autophagy on apoptosis is currently in dispute (Fig. 6). There exists evidence that autophagy can promote apoptosis through multiple pathways [93, 94]. Interestingly, what is actually at play in these pathways is not the autophagy flux but specific autophagy proteins [95, 96]. Overall, we may conclude that macrophage autophagy can lead to macrophage death through the promotion of autophagic death or apoptosis in sepsis, which should be investigated in further experiments.

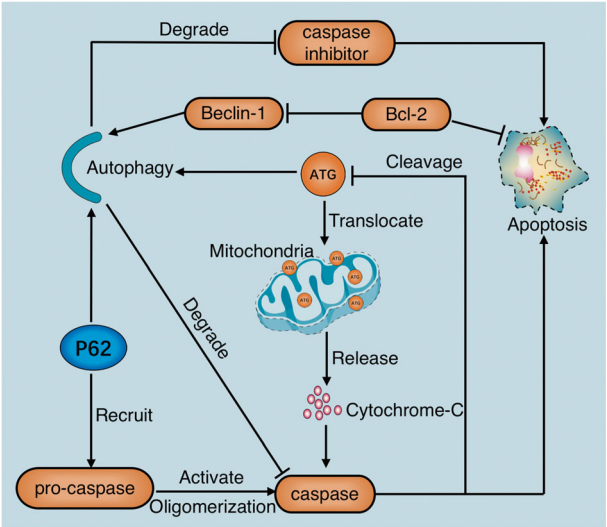

Fig. 6. The crosstalk between autophagy and apoptosis. Caspases are a family of proteases that play an essential role in both apoptosis and autophagy. The activation of caspases results in apoptosis. Autophagy can deplete caspase inhibitors such as dBruce and promote apoptosis. The autophagy substrate, p62, is involved in the recruitment of caspase-8, promoting its own oligomerization and further activation; however, autophagy can also degrade caspases and inhibit apoptosis. Moreover, apoptosis-related proteins may also act against autophagy. Caspases can suppress autophagy by cleaving multiple ATGs including beclin-1, ATG3, ATG4D, ATG5, ATG16L, and AMBRA1. The cleavage fragments of ATGs may translocate to the outer membrane of mitochondria and induce the release of cytochrome c, leading to caspase activation and subsequent apoptosis. Similarly, one of the anti-apoptotic protein family members, Bcl-2, may inhibit autophagy through the interaction with the autophagy-related protein, beclin-1, restricting its role in the formation of autolysosomes. Caspase, cysteinyl aspartate-specific proteinase; ATG, autophagy protein; Bcl-2, B cell lymphoma-2.

Conversely, autophagy can attenuate cell death, which may be attributed to autophagic regulation of other cell death patterns. Research conducted by Byrne *et al.* indicated that the components of the inflammasome can stimulate the turnover of macrophage autophagosomes and enhance autophagy to protect macrophages from pyroptosis [97]. In addition, autophagy has been shown to be required for the inhibition of necroptosis through the degradation of RIPK1, an enzyme that plays an essential role in necroptosis [98]. Furthermore, there is evidence that autophagy has a reverse effect on apoptosis, which can be summarized simply as follows: inhibition of autophagy leads to greater apoptosis; however, increased autophagy can have the opposite effect [34, 35]. The crosstalk between the autophagic and apoptotic pathways has been found to be mediated by multiple core molecules such as Bcl-2 family members, autophagy proteins, and apoptosis-

related proteins (caspases) (Fig. 6). Studies have demonstrated that inhibition of autophagy in renal tubular epithelial cells by 3-MA or the use of ATG5- or beclin-1-specific siRNA increases the activation of caspase-3, caspase-6, and caspase-7, increasing the level of apoptosis [99–101]. Conversely, overexpression of ATG5 or beclin-1 exerts the opposite effect [102]. In addition, apoptosis can react to autophagy (Fig. 6). Caspases have been demonstrated to be key proteins in the regulation of autophagy and apoptosis, which can suppress autophagy *via* cleavage of multiple autophagy proteins including beclin-1, ATG5, VPS34, ATG3, ATG4D, ATG16L, and AMBRA1. The cleavage fragments of autophagy proteins can be localized within mitochondria, increasing mitochondrial permeability and cytochrome c release, which eventually promote apoptosis. The affection of apoptosis on autophagy is a positive-feedback loop, leading to further deterioration of sepsis.

Despite the existence of a two-way function, it is certain that autophagy is an important regulator of cell death. The manner by which macrophage autophagy actually affects macrophage death in sepsis is a topic that requires further exploration. Accordingly, targeting the regulation of macrophage autophagy may be an effective treatment for sepsis.

Macrophage Autophagy Affects Macrophage Aging and Phagocytosis

Autophagy is not only essential as an anti-inflammatory mechanism but also for the maintenance of the homeostasis and function of macrophages, which can regulate the level of inflammation and metabolism and prevent immune aging of cells. A reduction in autophagy decreases the inherent immune function of macrophages, which can be demonstrated by the fact that autophagy-deficient macrophages exhibit a higher basal level of inflammation and abnormal mitochondrial function and metabolism [103]. In addition, autophagy can prevent macrophages from developing aged characteristics and delay the process of aging, indirectly affecting the role of macrophages in sepsis.

It has been found that the enhancement of macrophage phagocytosis and bactericidal activity is closely related to macrophage autophagy activation [104], while a deficiency in macrophage autophagy may reinforce phagocytosis of pathogens by increasing the expression of scavenger receptors (Fig. 2a). The number of pathogens entering macrophages increases, but cannot be selectively degraded by autophagy, resulting in aggravation and deterioration of infection [20].

PHARMACOLOGICAL REGULATION OF MACROPHAGE AUTOPHAGY IN SEPSIS

Changes in macrophage autophagy play an important regulatory role in sepsis. Moderate macrophage autophagy can reduce inflammatory reactions and tissue damage in sepsis, improving survival; therefore, drugs that can regulate macrophage autophagy may be an effective treatment for sepsis.

Research conducted by Li *et al.* showed that epigallocatechin gallate (EGCG), one of the main components of green tea, can enhance macrophage autophagy by stimulating LC3-II production and autophagosome formation [105]. Moreover, EGCG can inhibit the production and release of the LPS-induced inflammatory mediator, HMGB1, and this effect can be blocked by the autophagy inhibitor, 3-MA, in addition to knockdown of the autophagy-associated gene, beclin-1 [105]. Furthermore, the protective effect of macrophages against EGCG-mediated sepsis can be partly impaired by the autophagy inhibitor, chloroquine [105]. Taken together, these data infer that EGCG reduces the inflammatory response of sepsis by promoting autophagy and the autophagic degradation of HMGB1 in macrophages.

Recent studies have demonstrated that docosahexaenoic acid (DHA), a member of the omega-3 unsaturated fatty acid family, not only regulates macrophage polarization [106] but also suppress the activation of the inflammasome and release of the pro-inflammatory cytokine, IL-1β, *via* inhibition of the NF-κB signaling pathway and the enhancement of autophagy [107]. This effect of DHA requires the mediation of free fatty acid receptor 4 (FFAR4) and is attenuated in ATG5-deficient mice. Maresin-1 has been discovered in recent years as a novel anti-inflammatory and pro-resolving mediator derived from DHA [108]. Research has shown that Maresin-1 can promote autophagy in bone marrow-derived macrophages *via* an mTOR-independent pathway including ALX-NF-κB [109].

As an important method in traditional Chinese medicine, moxibustion helps in the resistance to infection and the reduction in excessive inflammation and has been shown to increase autophagy and reduce inflammation in mouse peritoneal macrophages *via* downregulation of the mTOR/Akt pathway and upregulation of an mTOR-independent signaling pathway mediated by eIF2α [110].

Similarly, the drug-containing serum of Huang-Lian-Jie-Du Decoction has been demonstrated to promote macrophage autophagy *via* upregulation of the expression of the autophagy-related protein, beclin-1, and downregulation of mTOR expression [111].

CONCLUSION AND PERSPECTIVES

In summary, macrophages, as one of the most important cells of the innate immune system, play an important role in inflammatory and immune processes. In particular, the autophagic function of macrophages has an important impact on the development and prognosis of sepsis, at multiple levels and through a variety of mechanisms. Although autophagy is two-sided, studies have demonstrated that moderate levels of autophagy are beneficial for the maintenance of homeostasis and the reduction of organ injury in sepsis. Macrophage autophagy can reduce the activation of the inflammasome and the release of inflammatory cytokines to control the escalation of inflammation in sepsis. Macrophage autophagy can also regulate the release of macrophage migration inhibitory factor and attenuate damage to the pulmonary vascular barrier. In sepsis, autophagy may promote macrophage polarization to the M2 phenotype, which exhibits anti-inflammatory effects. Moreover, macrophage autophagy can affect cell death *via* complex pathways involving crosstalk with apoptosis, which may partly attenuate immunosuppression in the late stage of sepsis. Furthermore, macrophage autophagy may affect macrophage aging and phagocytic ability.

However, the molecular mechanisms underlying these effects remain unclear. It is noteworthy that current research data are mainly based on animal models and *in vitro* experiments. Whether the findings are suitable for humans is still unknown and requires further study. Moreover, autophagy is a continuous process that includes several stages involving the formation of autophagosomes and fusion of autolysosomes. The mechanism by which the autophagic level of macrophages changes during different stages of sepsis needs to be determined in further experiments. In spite of this, it has been demonstrated that drug regulation of macrophage autophagy can reduce the inflammatory response and tissue damage in sepsis. Therefore, it can be predicted that an in-depth study regarding the role and mechanism of macrophage autophagy would provide proof-of-concept of an effective treatment and a theoretical basis for sepsis.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest. The authors declare that they have no conflict of interest.

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