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When apoptosis meets autophagy: deciding cell fate after trauma and sepsis

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Abstract

Apoptotic cell death is considered to be an underlying mechanism in immunosuppression and multiple organ dysfunction after trauma–hemorrhage and sepsis. Although studied intensively over the last decade, the role of other cell death mechanisms under similar pathophysiological conditions has remained elusive. Recently, autophagy has emerged as an important mediator of programmed cell death pathways. Here, we review recent advances in our understanding of apoptosis and autophagy and the crosstalk between these processes. We explore the coexistence of these two processes and the effects of autophagy on apoptosis after trauma–hemorrhage and sepsis. The inter-relationship between autophagy and apoptosis might unveil novel therapeutic approaches for the detection and treatment of trauma–hemorrhage and sepsis.

Introduction

Trauma–hemorrhage and sepsis

Numerous advances have improved the short-term survival of patients in intensive care units. Despite these advances, sepsis leading to multiple organ failure still remains the major cause of death in severely injured patients who survive initial trauma, hemorrhage or burns [1–3]. The underlying pathophysiological effects, including tissue damage and organ dysfunction, might be caused not only by inadequate perfusion but also by inflammatory and immune responses. Moreover, the release of cytokine and non-cytokine factors in trauma–hemorrhage or sepsis might inflict tissue injury and contribute to multiple organ dysfunction and cell apoptosis. An important advance in trauma–hemorrhage and sepsis research has been defining the key role of apoptosis in vital organs and the immune system [4].

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Disclosure statement

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Apoptosis

Apoptosis acts as an important mechanism of immunosuppression during sepsis by causing depletion of immune cells and inducing an anti-inflammatory cytokine response [5]. Lymphocytes are crucial cells in the response to trauma–hemorrhage and sepsis, and interactions between the innate and adaptive immune system are increasingly being found to have important roles in this response. Studies by Hotchkiss *et al.* [6,7] have established that septic patients and animals exhibit significant lymphocyte apoptosis. Other cells within the body also fail to function normally, and it has been demonstrated that increased apoptosis of dendritic cells, macrophages and monocytes and mucosal epithelial cells are present in septic patients [8]. In addition to immune cell apoptosis, myocardial depression is modulated by the effects of apoptosis on the production of proinflammatory cytokines after trauma, hemorrhage, sepsis and ischemia–reperfusion [9–11]. In this regard, tumor necrosis factor- α (TNF- α)-induced caspase activation can mediate endotoxin-related cardiac dysfunction [9]. Although the focus of apoptosis research has been on caspases and their possible role as executors of programmed cell death (PCD), caspase activation was considered to be an indispensable hallmark of apoptosis [5]. Recently, increasing evidence has pointed to the existence of alternative pathways of cell death that are independent of caspases.

Autophagy

Although apoptotic cell death in trauma, hemorrhage and sepsis has been intensively studied over the last decade, the role of autophagy in cell death has been somewhat overlooked. Autophagy has recently emerged as an important mediator of the PCD pathway. Autophagy is a catabolic process that involves lysosomal degradation and recycling of cytoplasmic constituents. This process has been implicated in several pathologies, including cancer and neuro-degeneration. Under certain circumstances, inhibition of autophagy can trigger apoptosis, and upregulation of autophagy protects against the onset of apoptosis [12]. By contrast, autophagy can function as a second mode of PCD (Type II PCD) that is distinct from apoptosis [13]. Molecular links between apoptosis and autophagy have also been reported to be important in determining cell survival or death. Thus, the question emerges, is autophagy involved in apoptotic cell death after trauma-hemorrhage and sepsis? Is autophagy a survival pathway, a suicide pathway, or both? This review discusses recent findings in apoptotic and autophagic pathways, the role of autophagy, and the nature of crosstalk between autophagic and apoptotic pathways. Finally, we address the question, when apoptosis meets autophagy, can it decide the fate of a dysfunctional cell after trauma–hemorrhage and sepsis?

Autophagy

Autophagy occurs at low basal levels in all cells and functions in homeostasis. It is rapidly upregulated when cells need to rid themselves of damaged cytoplasmic components or to eliminate damaged organelles, for example mitochondria, during oxidative stress, infection, ischemia–reperfusion or mitochondrial dysfunction (Figure 1). Nutritional status, hormonal factors (including sex hormones), temperature, oxygen tension and cell density have important roles in controlling autophagy [12]. Stimulation of autophagy can be either a protective mechanism or a process that contributes to cell death during apoptosis. By contrast, in the absence of apoptosis, autophagy also can trigger Type II PCD, which is distinct from apoptotic cell death (Type I PCD) (Box 1 and Table 1). Autophagy is morphologically characterized by vacuolization, degradation of cytoplasmic contents and little chromatin condensation [13]. The autophagic process involves the vesicular sequestration of cytoplasmic proteins, organelles or pathogens, resulting in a double-membrane vesicle formation called an autophagosome. This is followed by fusion of the autophagosome with a lysosome to form an autolysosome, where the captured material, together with the inner membrane, is degraded [12].

Autophagy: to die or not to die?

An apparent conundrum is that autophagy acts both in cytoprotection and in cell death. The pro-survival function of autophagy has been demonstrated at the cellular and organ level in different contexts, including during nutrient and growth-factor deprivation, microbial infection and ischemia–reperfusion [14,15]. For example, increased autophagy after ischemia–reperfusion injures cardiomyocytes and allows cell survival by inhibiting apoptosis [16]. However, it remains unclear how autophagy stops cells from undergoing apoptosis. One study has suggested that the mechanism might involve sequestration of damaged mitochondria, thus preventing cytochrome *c* release and blocking formation of a functional apoptosome in the cytoplasm [17]. Another study showed that autophagy can protect cells from caspase-independent death that occurs after mitochondrial outer membrane permeabilization in the presence of caspase inhibitors [18].

There is also evidence that over-stimulation of autophagy can lead to cell death, possibly through activating apoptosis [19]. It is likely that excessive autophagy destroys a large amount of proteins and organelles beyond a certain threshold, causing a bioenergetic catastrophe culminating in cell death [20]. A study has shown that autophagy is involved in mouse macrophage death triggered by co-stimulation of lipopolysaccharide (LPS) and caspase inhibitor [21]. Both H₂O₂ and 2-methoxyestradiol (2-ME) induce apoptosis in cells and have recently been shown to induce autophagy leading to cell death in HEK293 and U87 cells [22]. This group also found that inhibition of autophagy significantly reduces H₂O₂- or 2-ME-mediated cell death. These studies suggest that inhibition of the apoptotic pathway can switch the cellular response from apoptosis to autophagy. Furthermore, autophagy is activated by many kinds of stress, including ischemia and inflammation [23,24]. Although the role of apoptosis in trauma-hemorrhage and sepsis has been well defined, the role of autophagy has not yet been ascertained. Thus, whether autophagy plays a protective or detrimental role in response to apoptotic insult in attenuating trauma–hemorrhage injury and sepsis might shed light on a new approach for combating these pathological conditions.

The regulation of autophagy

The regulation of autophagy includes four steps, depicted in Figure 2a. Briefly, the initial step of autophagy is regulated by class I and class III phosphoinositide 3 kinases (PI3Ks). Activation of class I PI3K inhibits autophagy through activation of Akt and mTOR (mammalian target of rapamycin). By contrast, activation of class III PI3K in a complex with autophagy-associated protein Beclin1 (also known as autophagy-related gene [ATG] [6]) promotes autophagy [25]. Xu *et al.* [21] found that LPS treatment induces autophagy in macrophages, which is inhibited by small interfering RNA (siRNA) knockdown of Beclin1 or pre-treatment with 3-methyladenine (3-MA; a pharmacological inhibitor of autophagy) or Wortmannin (a PI3K inhibitor). Although other studies have shown the importance of class I PI3K and mTOR pathways in response to LPS stimulation in immune cells, these have not been examined in the manifestation of autophagy. Ohtani *et al.* [26] found that class I PI3K and mTOR regulated the T helper 1 (Th1)–Th2 balance through regulation of interleukin (IL)-12 expression in LPS-treated dendritic cells. dos Santos *et al.* [27] reported that PI3K–mTOR pathway activation has an important role in response to LPS stimulation in macrophages. Furthermore, Bommhardt *et al.* [28] showed that constitutive activation of the Akt–protein kinase B (PKB) signaling pathway reduced sepsis-induced lymphocyte apoptosis and improved the survival of septic animals. These studies collectively provide evidence that the PI3K–Akt signaling pathway has an important role in protecting against immune cell death during sepsis. It is therefore tempting to speculate that autophagy might be involved in PI3K–Akt-mediated immune cell protection against sepsis-induced apoptotic cell death.

Apoptosis in trauma–hemorrhage and sepsis

Both extrinsic (death-receptor) and intrinsic (mitochondrial) pathways are known to be involved in the induction of apoptosis [5] (Figure 2b). The extrinsic pathway is mediated by caspase-8, whereas the intrinsic pathway is mediated by caspase-9. Caspase-8 can be activated by ligation of cell surface death receptors and their ligands, such as TNF, TNF-related apoptosis-inducing ligand (TRAIL) and FAS (TNF receptor superfamily, member 6) ligand (CD95L). The intrinsic pathway is controlled by members of the mitochondrial membrane-bound B cell leukemia/lymphoma 2 (Bcl-2) family. Studies have reported that in patients with trauma, hemorrhage or sepsis, an essential role of anti-apoptotic Bcl-2 protein for cell survival has been identified [29,30]. Moreover, mitochondria have a key role in mediating apoptosis induced by many different stimuli, such as release of cytochrome *c* into the cytosol, which is regulated by Bcl-2 family proteins [5].

The hallmark of the early phase of trauma–hemorrhage and sepsis has been reported to be the pro-inflammatory state, which is mediated by immune cells, including lymphocytes (B and T cells), dendritic cells, neutrophils and macrophages. However, a profound decrease in numbers of immune cells, especially B and T cells, in sepsis results in substantially impaired immune responses. This is attributed to the extensive cell death by apoptosis [5,8]. Apoptotic immune cell death has also been suggested to dictate the outcome of trauma–hemorrhage and sepsis [8,31]. Interestingly, accumulation of apoptotic cells can activate macrophages to release high mobility group box 1 (HMGB1), a crucial cytokine mediator of organ damage in severe sepsis, and other proinflammatory cytokines [32]. Neutrophils are also crucial components of the innate immune response to infectious challenges. Patients with sepsis manifest a delay in neutrophil apoptosis [33], leading to neutrophil accumulation that appears to cause organ dysfunction in septic patients and in trauma–hemorrhage animals [33,34]. In addition to immune cells, cardiomyocytes are also an important target of the pathologic effects of apoptosis during trauma–hemorrhage and sepsis [10,35]. As discussed earlier, cells can respond to stress stimuli through both apoptosis and autophagy. It will be interesting to unravel the issue of whether autophagy is also involved in the regulation of apoptosis after trauma hemorrhage and sepsis.

Autophagy in inflammation and the immune response

In keeping with its primary function as a cytoplasmic clean-up process, autophagy is an important host mechanism for the removal of intracellular bacteria [36]. It has been suggested that autophagy promotes cellular survival during pathogen invasion because of enhanced degradation of intracellular pathogens and consequent decreases in microbial replication or enhanced degradation of specific cytotoxic microbial virulence products [14]. However, a growing number of studies indicate a role for autophagy in delivery of microbial antigenic material to the innate and adaptive immune system, as well as in maintaining lymphocyte homeostasis [25,37]. Decrease major histocompatibility complex (MHC) class II-expressing cells often contributes to immune suppression in septic patients and trauma–hemorrhage animals [38,39]. Studies have reported that autophagy promotes MHC class II presentation of cytosolic antigens [40,41], thereby connecting autophagy with adaptive immunity (Figure 3). For example, Brazil *et al.* [40] demonstrated that inhibition of autophagy with 3-MA abrogated MHC class II presentation of endogenously synthesized complement C5 protein. Recently, Schmid *et al.* [41] demonstrated constitutive autophagosome formation in MHC class II-positive cells, including B cells, dendritic cells and epithelial cells. They also showed that autophagosome-associated protein LC3 (microtubule-associated protein 1 light chain 3) led to enhanced MHC class II presentation to CD4⁺ T cell clones, suggesting that autophagy efficiently delivers cytosolic proteins for MHC class II presentation and can improve helper T cell stimulation. In addition, autophagy might also have a role in T-cell survival and proliferation. A study using lethally irradiated mice repopulated with hematopoietic cells from

fetal livers of ATG5^{-/-} mice showed that CD4⁺ and CD8⁺ T cells from ATG5^{-/-} mice fail to undergo efficient proliferation after T cell receptor stimulation [42].

The relationship between autophagy and immunity is bidirectional. The autophagic machinery does not just function in innate and adaptive immune responses: in parallel, autophagy is stimulated by several immune mediators (cytokines, receptors and ligands) that are involved in innate and adaptive immunity control [20,25]. Immune signaling molecules, including interferon (IFN)- γ and TNF family members (TNF, CD40 ligand and TRAIL), have been shown to positively regulate autophagy in macrophages and cancer cells [37,43,44]. By contrast, autophagy is negatively regulated by Th2 type cytokines, such as IL-3, in epithelial cell line HT-29 [45] (Figure 3). In fact, IFN- γ and TNF have been shown to be crucial for protection against infection by bacteria and other pathogens and are potent inducers of autophagy in murine macrophages and human tumor cell lines [37,43,44]. Additionally, Toll-like receptor 4 (TLR4), known as an important mediator of immune function in trauma–hemorrhage and a key therapeutic target in sepsis [2,46,47], has recently been reported to serve as a previously unrecognized environmental sensor for autophagy. It was shown that autophagy was regulated through a Toll/IL-1 receptor domain-containing adaptor-inducing IFN- β (TRIF)-dependent, myeloid differentiating factor 88 (MyD88)-independent TLR4 signaling pathway in LPS-treated macrophages [48]. A link between autophagy and the removal of apoptotic cell corpses has recently been suggested a possible role for autophagy in the prevention of tissue inflammation [25]. Qu *et al.* [49] reported that mice lacking ATG5 or Beclin1 displayed a defect in apoptotic corpse engulfment, suggesting that ATG5 or Beclin1 might provide apoptotic cells with signals to ensure their clearance by phagocytes during PCD. It is noteworthy that clearance of apoptotic cells by phagocytic cells is crucial in inflammation and sepsis. Thus, theoretically, in sepsis and trauma–hemorrhage, autophagy is likely to be crucial for mediating immune responses, as well as for releasing signals to clean up accumulated apoptotic cells.

Autophagy in the heart

Accumulation of autophagosomes has been noted in cardiac biopsies of patients with cardiac disorders, rodent models of these cardiac diseases and isolated stressed cardiomyocytes [50, 51]. Autophagy is an important process in the heart, and a defect in this process can be detrimental to the heart. However, in studies of the ischemic myocardium, autophagy has surprisingly received more attention as a cell protective mechanism. In fact, autophagy can be both a protective process and a precursor to cell death depending on the intensity and duration of the insult.

A protective role

Increased autophagy has been known to occur in hearts after ischemia–reperfusion, a model somewhat similar to trauma–hemorrhage. Sybers *et al.* [52] found an increase in autophagic vesicles after hypoxia with glucose deprivation combined with reoxygenation in fetal mouse hearts. It has also been shown that autophagosomes increase in response to sub-lethal ischemia in the perfused heart, suggesting that active autophagy might serve as a protective response to myocardial ischemia–reperfusion injury [53]. Studies have also reported that upregulation of autophagy during ischemia–reperfusion is cardioprotective and promotes survival of cells. Yan *et al.* [16] found that chronic ischemia induces autophagy and increases lysosomal activity in myocardium. They also identified that areas of the heart with increased autophagy displayed fewer apoptotic cells, suggesting that induction of autophagy might be related to apoptosis abrogation. An *in vitro* study has shown that upregulation of autophagy is protective [23] and that inhibition of autophagy in cardiomyocytes causes increased cell death in response to simulated ischemia–reperfusion, whereas enhancement of the autophagic response remains protective.

A lethal role

Conversely, there are reports showing that upregulation of autophagy can be detrimental to the heart. Valentin *et al.* [54] showed that inhibition of autophagy by both genetic and pharmacological inhibition of Beclin1 reduced cell death in cardiomyocytes subjected to simulated ischemia–reperfusion. In line with this, Beclin1 knockout mice exhibited a reduction in autophagy and apoptosis, as well as reduced infarct size in the heart after ischemia–reperfusion injury [55]. Matsui *et al.* [55] found that autophagy might be protective during ischemia, but it switched to playing a detrimental role during reperfusion. Taken together, these studies suggest that autophagy can have dual roles in the heart. Further studies are needed to elucidate under what conditions autophagy provides protection versus cell death.

Potential protective mechanisms

Decker and Wildenthal [53] observed that many autophagic vacuoles contain damaged mitochondria during reperfusion, suggesting that autophagy is upregulated to eliminate damaged mitochondria. Hamacher-Brady *et al.* [15] demonstrated that many autophagosomes contain mitochondria in the heart after ischemia–reperfusion. Accordingly, removal of damaged mitochondria by autophagy might protect cells by preventing activation of apoptosis. Although these findings were observed in ischemia–reperfusion injury, it is possible to speculate that a similar mechanism might operate in the heart after trauma–hemorrhage. Moreover, studies have suggested that apoptosis contributes to septic cardiomyopathy [9]. Previous studies have found that mitochondrial dysfunction and increased apoptosis are associated with cardiac dysfunction after trauma–hemorrhage [10]. Thus, the possible role of autophagy in cardiac dysfunction and in removing damaged mitochondria after trauma–hemorrhage and sepsis seem to be promising areas for further research.

Molecular connections between autophagy and apoptosis

Studies have suggested that there is crosstalk between the autophagic and apoptotic pathways. These two pathways are regulated by certain common factors and shared common components, each of which can possibly regulate and modify the activity of the other [14]. Many signals originally studied in the context of apoptosis activation induce autophagy, whereas signals that inhibit apoptosis also inhibit autophagy [20]. The interplay between autophagic and apoptotic pathways is emerging as a crucial decision-making process in determining the initiation of PCD.

Bcl-2 family proteins

Bcl-2 family proteins are known to be important regulators of apoptosis in patients and experimental models of trauma–hemorrhage and sepsis [5,30]. Similarly, there is increasing evidence that Bcl-2 family proteins can also regulate autophagy (Figure 3). Initially, Beclin1 (activation of autophagy) was identified as a Bcl-2-interacting protein. It was then discovered that the Bcl-2-binding domain of Beclin1 serves as a point of crosstalk between autophagic and apoptotic pathways at the level of autophagy induction. Bcl-2 downregulation has been shown to increase autophagy in human leukemia cells [56], whereas overexpression of Bcl-2 or BclX_L (hereafter referred to as Bcl-2/BclX_L) also increased autophagy in etoposide-treated embryonic fibroblasts [57]. Bcl-2 was also shown to suppress starvation-induced Beclin1-dependent autophagy [58]. Clearly, the relationship between Bcl-2 and autophagy is not yet fully understood. However, it is likely that the regulation of autophagy by Bcl-2 is a function of both cell types, and Bcl-2–Beclin1 interaction might be a turning point at the crossroad between cell survival and death.

As mentioned earlier, class III PI3K (also known as Vps34) is contained in the Beclin1 interactome that activates autophagy. A study has demonstrated that distinct regions of Beclin1

are involved in binding to Vps34 and in binding to Bcl-2/BclX_L in human cancer cells [59]. Moreover, Bcl-2 binding to Beclin1 disrupts its interaction with Vps34 in some cell types; however, such effects are not universally required for the autophagy inhibitory effects of Bcl-2/BclX_L [60]. It seems that the Bcl-2/BclX_L–Beclin1–Vps34 multiprotein complex (compared to Beclin1–Vps34 complexes lacking Bcl-2/BclX_L) possesses decreased Vps34 PI3K activity [58,60]. In short, Beclin1 acts as an essential activator of Vps34. In conditions in which autophagy is inhibited, Beclin1 is inactivated by its interaction with multidomain proteins of the Bcl-2 family [60]. Thus, targeting of Beclin1 by Bcl-2 family members represents an essential mechanism of autophagy regulation.

Mitochondria

In the decision of cellular fate, mitochondria could have a detrimental role as the switch between autophagy and apoptosis. As mentioned above, autophagy can lead to removal of damaged mitochondria. These are not removed if autophagy is blocked, and mitochondria undergoing membrane potential loss can prime apoptosis [61]. It is also known that autophagy delays apoptosis, whereas inhibition of autophagy increases mitochondrial depolarization and apoptosis in camptothecin-treated MCF-7 (human breast adenocarcinoma cell line) cells, suggesting that the post-mitochondrial cascade is delayed by disposal of damaged mitochondria into phagosomes [62]. Interestingly, mitochondrial functions such as respiration can block autophagy in mouse embryonic fibroblasts, indicating that mitochondria need to be functional to allow induction of autophagy [63]. Mitochondrial dysfunction is often seen after trauma, hemorrhage, and sepsis [10,46,64] and has been suggested to cause apoptotic cell death in the heart and immune system [7,10]. These facts raise an additional possibility of the importance of mitochondria in relation to the autophagy–apoptosis axis in trauma–hemorrhage and sepsis.

Rethinking the approach to cell death

The role of TNF- α , a crucial mediator involved in the pathogenesis of trauma–hemorrhage and sepsis [3], might be laudatory because it induces autophagy as well as apoptosis, in which both reactive oxygen and nuclear factor- κ B (NF- κ B) have been shown to have crucial roles in the regulation of TNF- α [44]. Cauwels *et al.* [65] reported that mice treated with caspase inhibitor show exacerbated TNF toxicity caused by enhanced oxidative stress and mitochondrial damage, which results in hyperacute hemo-dynamic collapse, kidney failure and death. This study also suggests that a caspase-independent process might exist [65]. Thus, the involvement of TNF- α in autophagy might help to rekindle the anti-TNF- α therapy fire in septic patients. Another important target, which might be equally important in both of these cell death mechanisms, is p53. Under the predisposed conditions of DNA damage, autophagy is induced in a p53-dependent manner presumably through transactivation of inducers of autophagy [66]. Moreover, inhibition of p53 has been shown to decrease TNF- α -induced apoptosis and autophagy [67]. It is well known that p53 regulates apoptosis under similar stress conditions, although p53^{−/−} mice splenocytes are not protected from apoptosis and fail to improve survival in sepsis [68]. This failure of p53^{−/−} mice is not surprising if p53 also has a role in autophagy-mediated cell survival in sepsis. Although many aspects of these two pathways (TNF- α and p53) have not yet been investigated in cell death, some of these putative mechanisms are currently being explored in our laboratory in relation to trauma–hemorrhage and sepsis.

The anti-apoptotic protein Bcl-2 can inhibit Beclin1-dependent autophagy [60]. Moreover, the downstream signaling pathway of TLR4 has been demonstrated to induce autophagy [21,69]. Shi *et al.* [69] also showed that the TLR4 adaptor proteins MyD88 and TRIF either directly or indirectly recruit Beclin1 into the TLR4 signaling complex. This reduces interaction between Beclin1 and Bcl-2 and results in a biochemical modification of Beclin1 [69]. In an animal model of sepsis, transgenic mice overexpressing Bcl-2 in T cells had complete protection against T lymphocyte apoptosis and partial protection of B lymphocytes, as well as improved

survival [70]. Studies in an animal model of trauma–hemorrhage also showed that decreases in Bcl-2 expression were associated with hepatic and splenic apoptosis. In addition, it has been reported that TLR4 has a crucial role in immune cell function in trauma–hemorrhage and septic animals [46,71,72]. TLR4 upregulation in non-immune cells after initial TLR-mediated immune response might trigger secondary responses, such as activation of endothelial cells, that promote production of adhesion molecules, followed by neutrophil and macrophage infiltration and vascular permeability in septic animals, which can lead to tissue perfusion insufficiency and organ failure [73,74]. Therefore, determining whether autophagy is involved in Bcl-2 binding and the TLR4 signaling pathway will lead us to a new understanding of the mechanism in immune suppression after trauma–hemorrhage and sepsis.

Apoptosis inhibitors: potential therapeutic effects related to autophagy

One of the novel strategies for treatment of trauma–hemorrhage and sepsis is caspase inhibitors. Caspase inhibitors have been reported to decrease lymphocyte apoptosis and improve survival in animal models of sepsis [75]. However, caspase inhibitors might not completely prevent cell death, and their use carries the risk of transforming an apoptotic process into caspase-independent cell death, which might potentially damage cells. For example, caspase inhibitor does not attenuate hepatocellular depression and liver injury in animals with hemorrhagic shock [76]. Moreover, caspase inhibitor does not completely prevent lymph-induced human endothelial cell apoptosis after trauma–hemorrhage, suggesting that caspase-independent pathways are involved [77]. Inhibition of caspases might induce hyper-acute TNF-induced shock in mice [65]. Patients with defects in caspase-8 are immunodeficient and suffer from recurring infections [78]. The concern regarding caution in the development of caspase inhibitors as a potential therapy for treating human sepsis has also been raised by Hotchkiss and Nicholson [5]. Thus, caspase inhibitors that not only result in inhibition of apoptosis but also affect other cell deaths might provide important clues in the development of therapeutic applications of these inhibitors.

Recent studies have shown that caspase inhibitors might be of therapeutic value when they do not inhibit autophagic cell death or promote necrotic cell death [79]. Yu *et al.* [80] have demonstrated that zVAD (Z-Val-Ala-dl-Asp), a general caspase inhibitor, induces cell death through induction of autophagy in L929 murine fibroblastic cells; however, Wu *et al.* [81] recently observed that zVAD not only inhibits caspases but also the lysosomal protease cathepsin B. This consequence of zVAD inhibition of both cell death by apoptosis and cell survival by autophagy is induction of necrotic cell death. In support of these observations, the study further demonstrated that the mTOR inhibitor rapamycin, or pharmacological inhibition of autophagy with chloroquine or 3-MA, protects cells from zVAD-induced death [81]. Other studies have also reported that T cells lacking FADD (FAS-associated via death domain) or caspase-8 activity are subject to hyperactive autophagic signaling and subvert a cellular survival mechanism into a potent death process [82]. Moreover, autophagy can delay cell death by apoptosis, and in apoptotic-defective cells, inactivation of the autophagy survival pathway promotes necrotic cell death [83,84]. It has also been suggested that defects in apoptosis are overcome by induction of metabolic catastrophe, a death process where cells that cannot die by apoptosis and cannot access the autophagy survival pathway when metabolically stressed are forced to die by necrosis [79]. Another study has shown that the combination of caspase inhibitor and mTOR inhibitor enhances radiosensitization more than individual treatment alone in lung cancer cells, indicating that combined inhibition of apoptosis and induction of autophagy represents a potential therapeutic strategy in cancer patients [85]. In this regard, autophagy emerges as an important mechanism controlling survival after treatment with caspase inhibitors.

Concluding remarks

Apoptosis is a form of cell death in trauma–hemorrhage and sepsis and leads to a striking loss of immune cells. The question that we are interested in here is ‘What about autophagy?’ (Box 2). As described earlier, in response to LPS administration, infection or ischemia–reperfusion injury, autophagy is generally a cell-survival response that can limit cellular damage and cell death. However, autophagy has also been observed in dying cells under those conditions. Based on currently available experimental data, autophagy can have dual roles. Although it is not known what factor determines whether autophagy will be harmful or protective to the cell, it is likely that the level and duration of autophagy are important. Also, interpretation of the ‘markers’ of autophagy should be considered to prevent misleading results. More importantly, autophagy might exert cytoprotective functions in infected cells and also mediate trafficking events required for innate and adaptive immunity. These immune-enhancing functions will make autophagy an attractive target for therapeutic manipulation after trauma–hemorrhage and sepsis. Thus, a complex interrelationship seems to exist between autophagy and the apoptotic cell death pathway, where regulators of apoptosis also function as regulators of autophagic activation. While apoptosis is certainly a mechanism of cell death in trauma–hemorrhage and sepsis, the definition and involvement of autophagy as a mediator of apoptosis in response to such conditions needs further clarification. Accordingly, the interaction between autophagy and apoptosis, as well as the effects on immune cell responses after trauma–hemorrhage and sepsis, might unveil novel therapeutic approaches for treating patients after trauma and hemorrhage and for patients in the early phase of sepsis.

Box 1

Cell death

Considerations of ‘autophagy’ and ‘autophagic cell death’

When investigating autophagy, it is important to consider that it is a dynamic process regulated by autophagosome formation and trafficking to, and fusion with, lysosomes. For example, when fusion of autophagosomes with lysosomes is blocked, the former accumulates in spite of autophagy inhibition [12,87]. Thus, massive accumulation of autophagosomes is by no means a demonstration that the autophagic pathway is induced, and functional tests are required to investigate autophagy [88]. Therefore, it is important to discern the dynamic process and describe the assays for monitoring autophagy. Although ‘autophagic cell death’ defines cell death occurring with autophagy, it might misleadingly suggest a form of death occurring through autophagy. A consensus from recent studies is emerging that autophagy is largely a cell death impostor that, in reality, functions primarily to promote cellular and organismal health [89].

Nomenclature of cell death

The Nomenclature Committee on Cell Death (NCCD) provides a forum in which names describing distinct modalities of cell death are critically evaluated and recommendations on their definition and use are formulated [88]. Beginning in 2009, *Cell Death and Differentiation* will actively enforce a policy in which terms like ‘percent apoptosis’ and ‘percent autophagy’ must be replaced with language that precisely describes what has been measured as ‘percent cells’ with ‘condensed chromatin’, ‘percent cleaved caspase-3 positive’, ‘number of GFP-LC3 puncta/cells’, or ‘percent vacuolated cells’.

Programmed and non-programmed cell death

Programmed cell death (PCD): type I PCD refers to apoptosis (self-killing) and is the best-described form of PCD. Type II PCD refers to autophagy (self-eating), a lysosomal degradation pathway that is essential for homeostasis and reportedly contributes to cell

death as well. Non-programmed cell death: this type of death generally occurs as a consequence of cellular injury or in response to extreme changes in physiological conditions and typically takes the form of necrosis. Necrotic cell death is characterized by rapid cell and organelle swelling and loss of plasma membrane integrity. Clumping of chromatin is observed and DNA degradation occurs by cleavage at random sites.

Box 2

Outstanding questions

- Is autophagy involved in trauma–hemorrhage and sepsis? If so, is it a survival pathway, a cell-death pathway, or both?
- If autophagy has a protective role in trauma–hemorrhage and sepsis, can inhibition of the apoptotic pathway switch the cellular response from apoptosis to autophagy?
- As the combined inhibition of apoptosis and induction of autophagy is a potential therapeutic strategy in cancer patients, is the combination of apoptosis inhibitor and autophagy activator better than individual treatment alone in trauma–hemorrhage and sepsis?
- Does autophagy modulate MHC II antigen presentation in trauma–hemorrhage and sepsis, thereby regulating CD4⁺ T-cell immune responses? Can this modulation of MHC II antigen presentation also decide CD4⁺ T-cell apoptosis?
- Can treatment advances (such as insulin, activated protein C administration, low-dose steroids) that are believed to be potential therapies in sepsis or trauma affect autophagy?
- What experiments are needed to determine whether autophagy in an experimental setting is protective or detrimental?

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Glossary

Autolysosome	the double-membrane autophagosome fuses with the lysosome to form an autolysosome
Autophagic vacuole	an autophagosome or an autophagic structure that has fused with an endosome or lysosome. This term is used mainly in electron microscope studies
Autophagosome	an initial step of autophagic vacuoles. Cytosol, containing proteins or organelles, is sequestered by an expanding membrane, which results in formation of a double-membrane autophagosome. The double-membrane vesicles are responsible for delivering cytoplasmic material to lysosomes
Beclin1	a Bcl-2-interacting protein known to be a promoter of autophagy

Microtubule-associated protein 1 light chain 3 (LC3)	a commonly used marker of autophagy. LC3 protein is subjected to complex post-translational modifications. Immediately after synthesis, the carboxyl terminal region of LC3 is cleaved, generating a soluble form, designated LC3-I, which is located in the cytosol. During autophagy, LC3-I is modified by a series of ubiquitination-like reactions and by conjugating to phosphatidylethanolamine, thus becoming a membrane-bound form, termed LC3-II, which associates tightly with the autophagosomal membrane. Therefore, tracking the conversion of LC3-I to LC3-II is indicative of autophagic activity. As the half-life of LC3-II is short because autophagosomes are transient structures, LC3-II dots represent the autophagic activity at the moment in time
Mammalian target of rapamycin (mTOR)	an Akt downstream effector that has a crucial role in cell proliferation, growth and survival. Inhibition of mTOR induces autophagy
Programmed cell death (PCD)	death of a cell in any form, mediated by an intracellular program. Apoptosis is a type I PCD and is morphologically characterized by chromatin condensation, nuclear fragmentation, cell surface blebbing, cell shrinkage and formation of apoptotic bodies. Autophagy, a type II PCD, is characterized by accumulation of multi-lamellar vesicles that engulf cytoplasm and organelles, forming autophagosomes marked by LC3
Rapamycin	a potent immunosuppressant that possesses both antifungal and antineoplastic properties. It inhibits the activity of mTOR

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Figure 1.

Induction of autophagy contributing to cell survival or death during apoptosis. Low levels of autophagy can promote survival by eliminating intracellular damaged proteins and organelles, apoptotic cell corpses and bacteria in keeping with its primary function as a cytoplasmic clean-up process. It can also regulate immune-cell function and inflammation. This process can increase cell survival by preventing cell apoptosis. However, excessive and long-term upregulation of autophagy eventually leads to destruction of essential proteins and organelles beyond a certain threshold, resulting in cell death. This prolonged upregulation can lead to apoptosis activation.

Figure 2.

Regulation of autophagy and apoptosis. **(a)** Autophagy. Induction of autophagy requires activity of Beclin1 and its interacting partner, class III phosphoinositide 3 kinase (PI3K), also known as Vps34. Bcl-2/BclX_L can bind to Beclin1 and inhibit autophagy. By contrast, autophagy is negatively regulated by class I PI3K through mammalian target of rapamycin (mTOR). The elongation and shape of autophagosomes are controlled by two protein (and lipid) conjugation systems, namely the autophagy-related gene (ATG)12 pathway and the microtubule-associated protein 1 light chain 3 (LC3 or ATG8)–phosphatidylethanolamine (PE) pathway. These pathways are similar to the ubiquitin conjugation systems and include E1-activating and E2-conjugating enzymes. ATG12 is a first modifier that is essential for the

formation of autophagosomal precursors and isolation membranes. ATG12 is activated by ATG7 (an E1 ubiquitin-like enzyme), transferred to ATG10 (an E2 ubiquitin-like enzyme) and conjugated to ATG5, which subsequently forms a complex with ATG16. The membrane-bound ATG12–ATG5–ATG16 complex is a prerequisite for recruitment of LC3-II, the product of the second ubiquitin-like conjugation. Initially, LC3 is cleaved by the protease ATG4 to generate LC3-I. LC3-I is then activated by ATG7, transferred to ATG3 (a second E2 ubiquitin-like enzyme) and conjugated to PE. The resulting LC3-II is recruited into the forming autophagosomal membrane. The Beclin-1–Vps34 autophagy complex can be inhibited by another Beclin-1-interacting partner, the anti-apoptotic protein Bcl-2. After completion of autophagosome formation, a second cleavage by ATG4 results in some removal of PE from LC3-II, reverting it into LC3-I, which is released from the membrane. The remaining membrane-attached LC3-II is degraded by lysosomal proteases. The LC3-II:LC3-I ratio has been demonstrated to correlate with the extent of autophagosome formation. **(b)** Signaling and processes of autophagy and apoptosis. Autophagy: cytosol, containing proteins or organelles (such as damaged mitochondria), is sequestered by an expanding membrane. This double-membrane structure fuses with the lysosome to form an autolysosome, generating the autophagolysosome. Apoptosis: the extrinsic pathway is mediated by caspase-8, whereas the intrinsic pathway is mediated by caspase-9. FADD (FAS-associated via death domain), an adaptor protein that couples death receptors (such as CD95) and Bcl-2/Bcl-X_L, inhibits the loss of mitochondrial membrane potential. Cytochrome *c* is released from the mitochondria and forms the apoptosome together with apoptotic-protease-activating factor 1 (APAF1) and pro-caspase-9. Active caspase-9 and caspase-8 can activate caspase -3, leading to cell death apoptosis.

Figure 3.

The regulation of autophagy in adaptive immunity. Autophagy might be involved in adaptive immunity by delivering antigens into the MHC class II loading compartment (MIIC). In MIICs, the antigen is processed and loaded onto MHC class II molecules for CD4⁺ T-cell stimulation. Activated CD4⁺ T cells can then enhance autophagy and autophagosome–lysosome fusion via IFN- γ and TNF family members (such as TNF, CD40L and TRAIL). In addition, autophagy is negatively regulated by the Th2-type cytokine IL-3.

Table 1

Distinct stimuli, and their effects on the processes of apoptosis and autophagy

Stimuli ^a	Processes	Cell-death mode	Morphological features
Death receptors, (Fas ligand, TNF- α , and TRAIL), ER stress, DNA damage [62,86]	Cytochrome <i>c</i> and pro-apoptotic factor release, caspase activation	Apoptosis	Nuclear fragmentation Chromatin condensation DNA fragmentation Cell shrinkage Plasma membrane blebbing Minor modification of cytoplasmic organelles
Nutrient starvation [58]	Cytoplasmic vacuolization, autophagosome formation	Autophagy	Little (or no) cell shrinkage and chromatin condensation Massive vacuolization of the cytoplasm Accumulation of double-membraned vacuoles

Abbreviations: ER, endoplasmic reticulum; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand.

^aThe stimuli have been tested in cell-based studies.