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T cell intrinsic roles of autophagy in promoting adaptive immunity

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Abstract

Autophagy, an ancient cellular response where autophagic vacuoles are formed within the cytosol, is induced in response to a variety of cellular insults, including growth factor or nutrient withdrawal, organelle damage and misfolded proteins. Autophagy is rapidly induced in T lymphocytes following antigenic stimulation and blockade of autophagic signaling greatly reduces T cell clonal expansion, suggesting that autophagy is primarily involved in promoting T cell survival. In contrast, a recently identified negative feedback loop involving FADD and caspase-8, limits the level of autophagy in T cells. Failure to activate caspase-8 during T cell mitogenesis leads to hyperactive autophagy and cellular death through a programmed necrotic mechanism. These findings suggest that crosstalk between these cellular processes is essential for T cell activation and homeostasis.

Keywords

autophagy; caspases; apoptosis; programmed necrosis; necroptosis; RIP kinases; T cells; T cell homeostasis

Introduction

The fundamental tenet of the clonal selection theory, first proposed by Niels Jerne, and validated by Burnet and Medawar, requires massive and irrevocable cellular changes to incite the proliferation of a small number of antigen reactive cells at the start of an immune response. Given that such immune responses are often directed toward microbial pathogens, themselves capable of much more rapid proliferation than lymphocytes, it is clear that such profound changes in the cellular physiology of antigenically stimulated lymphocytes must take place if the immune system is to provide any benefit to the host. For T cells, these physiological changes allow for proliferation at a theoretical maximum rate in mammalian cells, with some estimates of 4-6 hour doubling times ¹. To accommodate the energetic demands required for such rapid proliferation, naïve T cells shift from a primarily respiratory energetic pathway to a less conservative but more strident glycolytic metabolism ². Recent evidence suggests that these physiological changes are associated with the generation of a specialized set of subcellular vesicles known as “autophagosomes,” supporting the notion that autophagy is essential for lymphocyte activation and differentiation during adaptive immune responses ³.

Autophagy and its role in cell biology

Cellular autophagy, which translates from Greek as “to eat” (phagy) “oneself” (auto), is an intracellular membrane trafficking system that delivers cytoplasmic material to lysosomes for

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degradation. Following autophagic induction, cytoplasmic material is sequestered by the phagophore, a lipid membrane which expands around the sequestered material to form a double membrane organelle termed an autophagosome⁴. The resulting autophagosome then fuses with lysosomes, exposing the cytoplasmic contents within the autophagosome to lysosomal hydrolases. This process results in the liberation of macromolecules that can be reused for other cellular processes⁵. In this way, autophagy can help maintain homeostasis during times of cellular stress, such as energy deprivation, infection or hypoxia. While this sequestration of cytoplasmic constituents can occur nonspecifically, it has become evident that cargo such as organelles, bacteria and protein aggregates may be specifically targeted to autophagosomes^{4,6,7}.

Autophagy is a multi-step process conserved among all eukaryotes and is impacted by lipid and protein kinase signaling cascades involving numerous autophagy (Atg) proteins. First, the mammalian target of rapamycin (mTor)-Atg1 pathway plays a pivotal role as a sensor of growth factors, energy stores and stress signals⁸⁻¹⁰. All these divergent signals repress autophagy through activation of mTOR, which phosphorylates Atg13, preventing its association and the activation of yeast Atg1 (mammalian Ulk1/2) kinase via phosphorylation of FIP200¹¹. Secondly, the class III phosphatidylinositol-3-phosphate (PI3P) kinase (PI3K) Vps34, in complex with Beclin 1, a member of the Bcl-2 family with a BH3 domain, is important for the nucleation of autophagosomes¹². Finally, vesicle elongation utilizes Atg7, Atg10 and Atg3, which are required for two downstream ubiquitin-like conjugation systems that promote the covalent conjugation of Atg5 to Atg12 and the conversion LC3-I (Atg8) into the phosphatidylethanolamine-conjugated LC3-II form¹³⁻¹⁷. LC3-II is the most widely used marker for the study of autophagy, as it remains bound to autophagosomal membranes throughout the pathway¹⁸.

Autophagic control over cell survival and death

The pro-survival role of autophagy has been clearly demonstrated at both the cellular and organismal level during development, microbial infection, and disease associated with protein aggregate accumulation^{19,20}. Conversely, cell death associated with autophagy, termed type II cell death²¹, has been demonstrated during drosophila salivary gland cell degradation *in vivo* and in the *in vitro* culture of mammalian cells²²⁻²⁴. In Bax/Bak double deficient MEFs, various apoptotic inducers depend on Atg proteins for the induction of cell death^{23,24}. However, it is now generally accepted that autophagy is first a survival mechanism and only under special circumstances results in type II cell death^{25,26}. To date, no evidence has been presented in which autophagy alone plays a direct role in mammalian cell death *in vivo*.

Defining a clear distinction between type-I and type-II cell death is difficult as it becomes increasingly clear that autophagy can facilitate apoptosis in promoting cell death. During endoplasmic reticulum (ER) stress, autophagy was recently shown to act upstream of apoptosis and is required for human tumor cell death²⁶. In a CD4+ T lymphocyte model of HIV infection, both autophagy-associated cell death and apoptosis are observed²⁷. In this system, inhibition of autophagy by RNAi-mediated knockdown of Atg7 or Beclin 1 reduced caspase activation and cell death. Conversely, inhibition of apoptosis led to a further upregulation of autophagy, without completely attenuating cell death, suggesting that autophagy can lead to cell death and act cooperatively with apoptosis in the cell death process. Numerous proteins have also been shown to play a regulatory role in both apoptosis and autophagy, such as FADD, caspase-8, DAPk, p53, and the BH3 domain containing proteins Bcl-2, BNIP3 and NIX, among others^{7,28-31}. Due to the level of cross talk, the existence of “grey zones” between cell death modes requires further study¹⁸.

Autophagy, apoptosis and programmed necrosis

A cell may undergo cell death through different mechanisms, including necrosis, or accidental cell death, which can be caused by injury, cancer, and microbial infection, among others. However, in the absence of caspase activation, a form of programmed necrosis, termed necroptosis³², or type-III cell death, may occur. Little is known about the proteins involved in necroptosis, but unlike apoptotic protease cascades, it appears to be controlled primarily by cellular kinases. In most cells, ligation of the death receptors (DR) Fas or TNFR1 often leads to apoptosis. Blockade of apoptosis through genetic ablation of pro-apoptotic molecules such as FADD or caspase-8, or by treatment with the pan-caspase inhibitor zVAD, forces these cells to undergo necroptosis^{33,34}. Two RIP kinases, RIPK1 and RIPK3, are required for this alternative death pathway, as siRNA-mediated knockdown or treatment with the RIPK1 allosteric inhibitor Nec-1 blocks necroptosis downstream of DR ligation in the absence of FADD or casp8^{32,35-38}.

It has long been known that lymphocytes induce non-apoptotic casp8 activity upon stimulation of their antigen receptors^{39,40}. Blockade of this activity through expression of a dominant-negative version of FADD or the deletion of FADD or casp8 causes T cells to proliferate defectively and undergo a non-apoptotic form of cell death following mitogenic stimulation⁴¹⁻⁴⁸. This death cannot be rescued by overexpression of pro-survival members of the Bcl-2 family, raising an important question regarding the means by which T cells lacking the capacity to activate casp8 meet their early demise⁴⁹.

In addition to casp8 activation, autophagy also plays a vital role in maintaining the survival of activated lymphocytes, as T and B cells that lack Atg5 or Atg7 fail to proliferate^{3,50,51}. We have recently reported that upon T cell activation, casp8 is activated in a FADD-dependent manner, and this casp8 activity represses autophagic signaling, preventing the induction of cell death through Vps34, Atg7 and RIPK1³⁰. Ch'en and colleagues have reported similar findings regarding RIPK1, demonstrating that knockdown of this necroptosis inducing kinase restores the ability of casp8-deficient T cells to clonally expand following viral infection⁵². It should be noted that the role of RIPK1 in promoting autophagy versus necrotic cell death appears to be context dependent. In the case of TNF α induced necroptosis, RIPK1- and RIPK3-dependent necrosis induces autophagy, but inhibiting autophagy isn't sufficient to block this caspase-independent cell death³². Conversely, activation of T lymphocytes lacking the same signaling molecules (e.g. FADD and casp8) promotes a RIPK1-dependent necroptotic-like cell death^{30,53}. In the latter case, we found that blockade of autophagic signaling was sufficient to restore survival of T cells lacking the capacity to activate casp8, supporting the hypothesis that autophagy was upstream of and responsible for cell death. It is significant to emphasize that T cells require autophagic induction for efficient clonal expansion³, and also for cell death following growth factor withdrawal⁵⁴. Although the complete mechanistic basis for these roles of autophagy in T cells remains to be established, it has been demonstrated that blockade of autophagy leads to the detection of a significant fraction of damaged mitochondria^{51,55}. We have recently speculated that autophagy may serve as a crucial means to rapidly remodel the cytoplasmic physiology of activated T cells during the switch to glycolytic metabolism⁵³. The observation of numerous damaged mitochondria in cells lacking the capacity to induce mitophagy, the form of autophagy directed at eliminating such damaged organelles, supports this hypothesis. Given the crucial requirement for autophagy early in the process of clonal expansion, it is likely that T cells anticipate the need for this enhanced autophagy by transcriptionally upregulating numerous genes in the autophagy pathway.

Autophagy as an intracellular danger response

Perhaps a unifying feature through which to consolidate our understanding of autophagy in immunity harkens back to the “Danger Theory” that suggested potential surveillance by the “innate” immune system⁵⁶. It is clear that in many if not all cases, autophagic signaling is induced to generate autophagosomes destined to eliminate the sources of cellular “danger”, including damaged organelles and macromolecular structures, intracellular pathogens, or to mitigate conditions of nutrient deprivation. Autophagosomes are then generated to envelop these intracellular danger signals and shunt them to lysosomes for degradation, or in the case of nutrient deprivation, to shunt cytoplasmic contents to lysosomes to generate bioenergetic precursors. We hypothesize that, as with other forms of “danger” as detected by TLRs or NOD-like receptors, autophagosome formation itself leads to the activation of signaling pathways that allow the cell to appropriately respond to the intracellular stressors.

Our previous work has demonstrated the assembly of a complex that may form the basis for such signaling³⁰. We propose that RIP kinases (RIPK1 and RIPK3) are recruited to this complex, and control the outcome of autophagic responses. With limited autophagy (and thus, modest intracellular danger), lysosomal degradation of autophagosomal contents is sufficient to cope with the intracellular threat. However, in cases in which autophagy cannot bring the cell back from the brink, the only appropriate course of action is to induce cell death. For this, direct recruitment and activation of casp8 via FADD binding to Atg5⁵⁷ on pre-autophagosomal structures provides a direct mechanism for inducing caspase-dependent apoptosis. In this scenario, casp8 is antagonistic toward RIPK1 (and likely RIPK3) via direct proteolytic cleavage; RIPK1 may be antagonist toward casp8 and apoptosis by inducing an anti-apoptotic program dependent on NF- κ B. Failure to cleave RIPK1 and RIPK3 promotes necroptosis, thus ensuring that cells lacking the capacity to induce caspase-dependent apoptosis undergo this alternative death pathway. It is interesting to note that during DR-induced necroptosis in cells lacking the capacity to activate casp8, RIPK3 promotes the expression of a variety of genes that facilitate energy metabolism and the generation of reactive oxygen species (ROS)⁵⁸. Given that such ROS may damage the very mitochondria that produce them, it is tempting to speculate that casp8 activation is heightened during T cell activation to allow these cells to limit ROS generation. In this manner, T cells may place a particularly acute burden on this intracellular damage-sensing pathway during rapid clonal expansion; casp8 activation may prevent over-activation of respiratory metabolism induced by RIPK3.

Conclusions

As described here, autophagy is clearly a fundamental cellular mechanism required for adaptive immunity. As highlighted elsewhere, the process of autophagy, or its signaling intermediates, may also be required for innate immune responses mediated by Toll-like receptors^{59,60}. As well, autophagy is a response of all known eukaryotic cells to a variety of intracellular stresses, including nutrient starvation, organelle damage, and infection by intracellular parasites. Much work remains to be completed to clarify the basic mechanisms used by cells to promote the generation of autophagosomes. In addition, the relevance of autophagy to cell survival vs. cell death must be clarified in a variety of physiological contexts to demonstrate the relative contribution autophagy makes to cellular fate. Finally, the specific functions for autophagic signaling in the proliferation and differentiation of lymphocytes await further exploration. Despite these challenges, such exploration is likely to lead to important clues regarding the significant participation of this ancient intracellular process in controlling immunity.

Acknowledgments

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References and recommended reading

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

- of special interest

14: Demonstration that Atg5 is required for autophagy.

18: Guidelines for the evaluation of autophagic markers within a variety of cell types.

26: Demonstration of autophagic cell death induced by ER stress in mammalian cells.

27: Demonstration that HIV-1 induced death of CD4⁺ T cells depends on autophagy.

31: Loss of caspase-8 activity leads to heightened autophagy and non-apoptotic cell death.

32: Identification of necrostatin-1, a RIP kinase inhibitor that prevents programmed necrosis in response to TNF receptor ligation in FADD-deficient Jurkat cells.

36: Identification of the cellular genes involved in programmed necrosis using an shRNA library approach.

39 & 40: First demonstration of non-apoptotic caspase activation in mitogenically stimulated T cells.

52: Demonstration that RIPK1 induced programmed necrosis leads to the early demise of T cells lacking caspase-8.

54: This report first demonstrates the induction of autophagy in primary T cells and the potential role of this process in controlling T cell homeostasis.

55: First demonstration that autophagy is required for elimination of damaged mitochondria in proliferating T cells.

58: Demonstration that RIPK3, along with RIPK1, activates programmed necrosis following TNF receptor stimulation in cells lacking the capacity to activate caspase-8, and that RIPK3 modulates cellular metabolism.

- of outstanding interest

3: This is the first demonstration of an essential role for autophagy in efficient T cell clonal expansion.

22: First demonstration that autophagy is essential for controlling physiological cell death.

24: Demonstration of non-apoptotic death in mammalian cells lacking Bax and Bak is dependent on autophagic signaling.

30: Demonstration that loss of caspase-8 activity in primary T cells leads to hyperautophagy and necrotic cell death.

33: First demonstration of a programmed necrotic death pathway induced by TNF receptor ligation and dependent on RIPK1 kinase activity.

40: Demonstration that FADD is distinctly required for T cell clonal expansion.

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