Review

Involvement of autophagy in T cell biology

Ozlem Oral[1], Ozlem Yedier[2]*, Seval Kilic[2]* and Devrim Gozuacik[2]
[1]Nanotechnology Research and Application Center, Sabanci University, and [2]Molecular Biology, Genetics, and Bioengineering Program, Faculty of Engineering and Natural Sciences, Sabanci University, Istanbul, Turkey
*Equally contributed second authors

Summary. Autophagy is an essential cellular pathway that sequesters various cytoplasmic components, including accumulated proteins, damaged organelles or invading microorganisms and delivers them to lysosomes for degradation. The function of autophagy has been reported in various tissues and systems, including its role in the regulation of cellular immunity. Autophagy plays a fundamental role at various stages of T cell maturation. It regulates the thymocyte selection and the generation of T cell repertoire by presenting intracellular antigens to MHC class molecules. Autophagy is crucial for metabolic regulation of T cells, and therefore supports cell survival and homeostasis, particularly in activated mature T cells. Furthermore, deletion of specific autophagy-related genes induces several immunological alterations including differentiation of activated T cells into regulatory, memory or natural killer T cells. In this review, we emphasize the impact of autophagy on T cell development, activation and differentiation, which is pivotal for the adaptive immune system.

Key words: Autophagy, T cells homeostasis, T cells function, Adaptive immune system

Introduction

Autophagy is an intracellular recycling mechanism responsible for the degradation of long-lived proteins, abnormal aggregates and damaged organelles which cannot be degraded by the ubiquitin proteasome system. There are three different types of autophagy, namely microautophagy, chaperone-mediated autophagy and macroautophagy. Microautophagy is mediated by direct lysosomal engulfment of the cytoplasmic cargo, which is trapped in the lysosome by a random process of membrane invagination. In addition to protein degradation, microautophagic vesicles are also involved in the maintenance of lysosome organelle size and membrane composition (Mijaljica and Devenish, 2011; Kaushik and Cuervo, 2012). Chaperone-mediated autophagy can selectively degrade soluble cytosolic proteins containing the recognition motif KFERQ via a cytosolic chaperone, heat shock cognate protein of 70 kDa (HSC70) which directs the substrate to the lysosome surface. This protein-chaperone complex binds to lysosome-associated membrane protein type 2A (LAMP 2A), which acts as a receptor for this pathway and allows translocation of substrate into the lumen of the lysosome (Massey et al., 2006). In macroautophagy (herein referred to as autophagy), double-membrane vesicles target large substrates such as toxic protein aggregates, degenerated organelles or invading microorganisms and deliver them to lysosomes for degradation (Kuma and Mizushima, 2010; Rabinowitz and White, 2010) (Fig. 1).

According to the type of degraded substrates, the autophagic pathway is also classified as selective or

nonselective. Starvation-induced autophagy is generally considered as a nonselective type of autophagy, while engulfment of specific organelles (mitophagy, pexophagy, ribophagy, ER-phagy), protein aggregates (aggrephagy), lipid droplets (lipophagy), or pathogenic microorganisms (xenophagy) are considered as a selective type of autophagy (Beau et al., 2008; van der Vaart et al., 2008; Kraft et al., 2009).

Autophagy is essential for almost all cell types for maintenance of cellular homeostasis. It recycles metabolic components and thereby supports cell survival under stress conditions, such as nutrient limitation, oxidative stress, and protein or organelle accumulation. Hence, autophagy is important for various cellular functions, and abnormalities of this fundamental process underlie the pathogenesis of a number of human diseases. For example, the involvement of autophagy abnormalities were reported in cancer, neurodegenerative disorders, cardiovascular diseases, and metabolic diseases (Schneider and Cuervo, 2014). Additionally, invading pathogens can be directly degraded by autophagy (Deretic et al., 2013).

Autophagy was found to be essential for the biology of T cells, a lymphocytic cell type that plays a central role in cell-mediated immunity and in the adaptive immune system. In this review, we will focus on the role of autophagy in T cell biology and its involvement in the adaptive immune system.

Molecular mechanisms of autophagy

Autophagy is an evolutionary conserved mechanism and, initially, more than 35 autophagy-related (ATG) genes were identified in yeast. Subsequent studies revealed mammalian orthologs of these genes and the basic mechanisms of autophagy were shown to be preserved in the mammalian system. The processes of autophagy consist of three major phases: nucleation, elongation and fusion/degradation. Each of these phases occurr in a sequential manner, and they are strictly regulated by coordinated action of ATG genes (Mizushima et al., 2011).

Activation of autophagy is negatively regulated by the mammalian target of rapamycin (mTOR) protein, which is a key component of the mTOR complex I (mTORC1) and the major nutrient sensing pathway in cells (Ravikumar et al., 2004; Mizushima et al., 2011). Under nutrient-rich conditions, ULK1/2 (UNC51-like kinase 1, mammalian homolog of yeast ATG1) interacts with mTORC1 and mTORC1 phosphorylates and inactivates ULK1/2 and its partner ATG13. Phosphorylation of ULK1/2 and ATG13 blocks autophagy. Under stress conditions, including hypoxia, growth factor or nutrient deprivation, ULK1/2 dissociates from mTORC1, leading to its activation through dephosphorylation. The active ULK1/2 complex (ULK1/2-ATG13-FIP200-ATG101) conveys signals for the recruitment of other ATG proteins to autophagosome nucleation sites. These autophagosome nucleation sites are marked with phosphatidylinositol 3-phosphate (PI3P) molecules produced by a hVPS34 Class III phosphatidylinositol 3-kinase (PI3K) complex (Mizushima et al., 2011). BECN1 (Beclin1) protein is a subunit of the PI3K complex and has a central role in the regulation of hVPS34 activity. Recruitment of PI3P to autophagosome nucleation sites gives rise to the formation of autophagosome precursors called "isolation membranes" and triggers subsequent phases of autophagosome elongation.

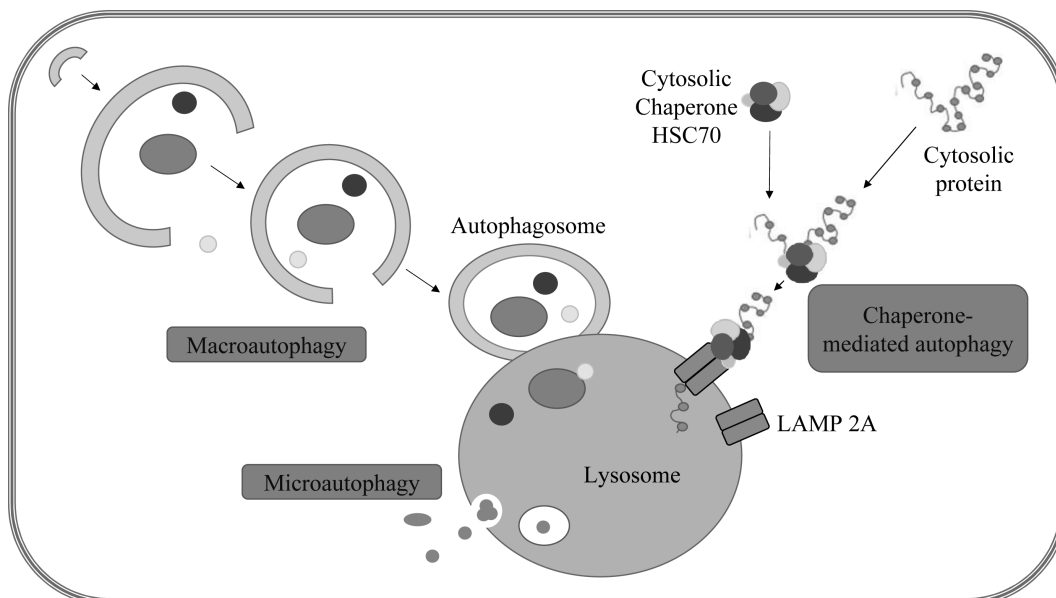Elongation, which is a critical step in auto-

Fig. 1. Different types of autophagy mechanism.

phagosome formation, is controlled by two ubiquitin-like conjugation systems, namely ATG12–ATG5 and microtubule-associated protein 1 light chain 3 (MAP1LC3 or shortly LC3) lipidation system (Kirisako et al., 2000; Mizushima et al., 2001). In the first system, ATG12 is activated by an E1-like enzyme, ATG7, and then transferred to an E2-like enzyme, ATG10. Covalent conjugation of ATG12 with ATG5 allows ATG16L1 binding to form a complex of higher molecular weight (Mizushima et al., 2001; Fujita et al., 2008). In the second system, a cytosolic free form of LC3-I, previously cleaved by ATG4B in its C-terminal, is activated by ATG7 (E1-like enzyme), transferred to ATG3 (E2-like enzyme), and then covalently conjugated to a lipid molecule, phosphatidylethanolamine (PE). The lipidated LC3, called LC3-II form, is localized both on isolation membranes and autophagosomes (Satoo et al., 2009). During these two ubiquitin-like conjugation systems, the ATG12-ATG5-ATG16L1 complex is suggested to play an E3-like activity for LC3 lipidation (Otomo et al., 2013). LC3 lipidation is an essential process for autophagosome elongation, and LC3-I to LC3-II conversion is commonly used as a marker of autophagic activity. LC3-II proteins located in the inner membrane of autophagosomes mediate binding of autophagy receptors such as p62/SQSTM1 and allow recruitment of cargo proteins into the autophagosomes in a selective manner (Fig. 2).

The fusion/degradation step occurs at multiple stages within the autophagic process. Autophagosome/lysosome fusion involves the concerted function of the V-ATPase complex, lysosomal-associated membrane proteins (LAMPs) and Ras-associated protein (RAB) GTPases such as RAB7, RAB22, and RAB24 (Bucci et al., 2000; Kauppi et al., 2002; Munafo and Colombo,

2002). Moreover, autophagosome/lysosome fusion is induced by dynein motor proteins which mediate autophagosome movement towards lysosomes along microtubules (Jahreiss et al., 2008). Fusion between the outer membrane of autophagosomes with lysosomal membranes requires SNARE (soluble N-ethyl-maleimidesensitive factor attachment protein receptor) proteins (Fader et al., 2009; Itakura et al., 2012). STX17 (syntaxin17) is recruited to completed autophagosomes and interacts with cytosolic SNAP29 (synaptosomal-associated protein 29) and the SNARE proteins VAMP7 (vesicle-associated membrane protein 7) or VAMP8, which are located on lysosomal membranes. After fusion, the lytic autophagosomes are called "autolysosomes" and the inner membrane and cargo are degraded by hydrolytic enzymes in lysosomal lumen. Degraded cargo constituents are eventually transported back to the cytosol for reuse.

Role of autophagy in T cell development

Hematopoietic stem cells (HSCs) are the stem cells that give rise to blood cells through the process of hematopoiesis. HSCs differentiate into common myeloid progenitors (CMPs) and common lymphoid progenitors (CLPs). Monocytes, macrophages, neutrophils, basophils, eosinophils, erythrocytes and megakaryocytes or platelets are originated from CMPs, while T cells, B cells, and natural killer cells are originated from CLPs (Dzierzak and Speck, 2008). Differentiation of HSCs into CLPs is associated to their quiescence and self-renewal capacity. In order to keep these capacities, HSCs reside in an oxygen-low niche in the bone marrow. They use anaerobic metabolism, defined by low mitochondrial activity and high glycolysis, thereby producing low
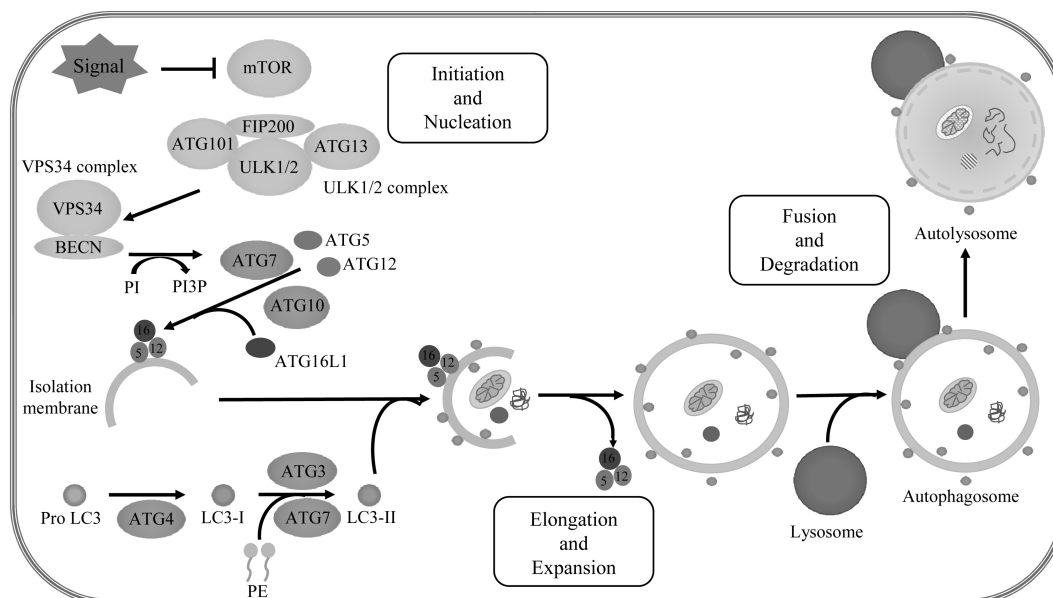

Fig. 2. Molecular mechanism of autophagy.

reactive oxygen species (ROS) levels. These features make HSCs extremely stress resistant. However, HSCs can quickly switch their anaerobic metabolism to oxidative phosphorylation, producing high levels of ROS, which is necessary for the differentiation of HSCs into CLPs. In contrast to HSCs, CLPs cannot renew themselves, thus they have to be perpetually replenished by HSCs (Suda et al., 2011).

Autophagy is crucial for the CLPs replenishment, and maintenance of quiescence and self-renewal ability of HSCs through degradation of mitochondria and control of ROS levels. Compared to differentiated cells, basal LC3-II and Atg5 levels were sustained high in HSCs in order to control ROS levels (Salemi et al., 2012). Supporting this work, both *in vitro* (Salemi et al., 2012) and *in vivo* (Liu et al., 2010; Mortensen et al., 2011a,b) chemical or genetic inhibition of autophagy resulted in the loss of self-renewability and quiescence, and the reduction in number of HSCs. In addition, the number of differentiated progenitor cells (CMPs and CLPs) was reduced and resulted in decreased T cell numbers in mice (Mortensen et al., 2011a).

Cycling CLPs in the bloodstream migrate into the thymus to develop into mature T lymphocytes. In the thymus, CLPs expand by cell division and generate immature T cells (Savino et al., 2016). Immature T cells, called thymocytes, are processed in a number of distinct maturational stages, namely beta-selection, positive-selection and negative-selection within both cortical and medullary portions of the thymus. The ability of mature T cells to recognize foreign antigens is mediated by the T-cell receptor (TCR), which is a surface protein that is able to recognize peptides presented on major histocompatibility complex class (MHC) (Schwarz and Bhandoola, 2006). In fact, the purpose of thymocyte development is to produce mature T cells with a diverse array of functional T cell receptors, through the process of TCR gene rearrangement (Sleckman, 2005). Cortical thymic epithelial cells (cTECs) are involved in beta-selection and positive-selection processes, which lead to the rearrangement of TCR genes via peptide presentation. In the cortex, the earliest thymocytes express neither CD4 nor CD8, and are therefore classified as double-negative cells (DN CD4−CD8−). Following beta-selection, thymocytes generate CD4+CD8+ double-positive cells (DP CD4+CD8+), which then undergo positive-selection in order to keep only those thymocytes that have a TCR capable of binding MHC (Miyazaki et al., 2014). Upon positive-selection, CD4+CD8+ double-positive T cells become single-positive: either CD4+ or CD8+. Initial negative-selection occurs in the cortex; however double positive cells can not be able to undergo negative selection because of the limited peptide repertoire expressed by cTECs. Therefore, negative-selection mainly occurrs in the medulla at the single positive stage. Medullar thymic epithelial cells (mTECs) and dendritic cells drive the negative-selection of thymocytes. In order to examine their auto-reactivity, single positive thymocytes are exposed to a more complex set of self-antigens and auto-reactive T cells are efficiently eliminated. CD4+ or CD8+ single positive T cells, which successfully pass the negative-selection step, are immunologically tolerant towards self-antigens, and they are released from the thymus to peripheral tissues in order to respond to foreign pathogens (Hinterberger et al., 2010). In the
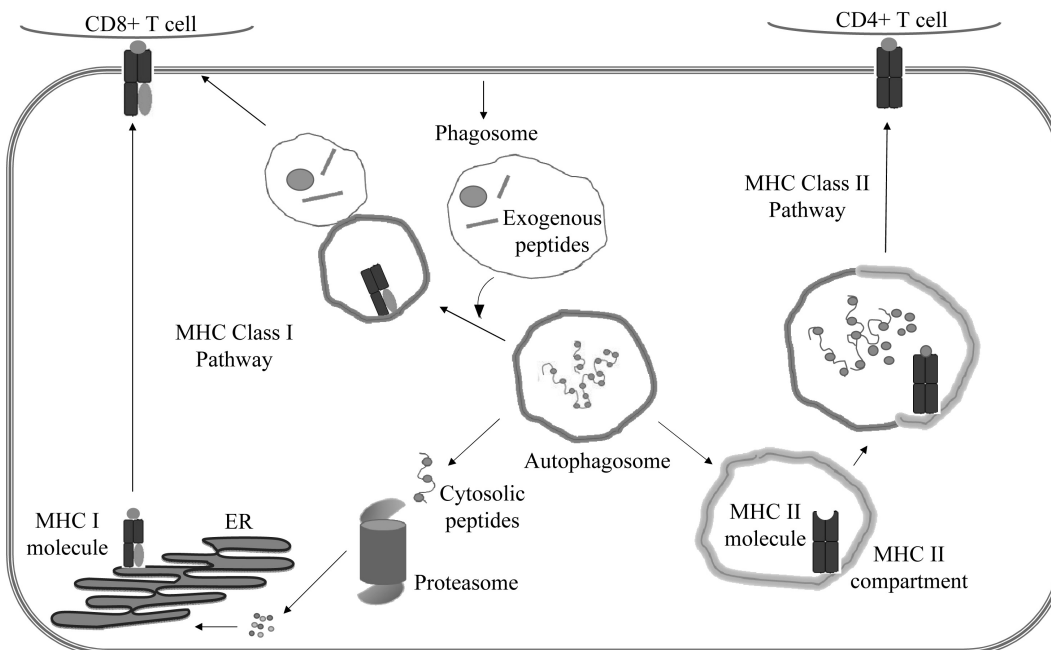

Fig. 3. Role of autophagy in antigen-presenting pathways. Antigens, which escape from autophagosomes into the cytosol, can be processed via conventional MHC class I pathway. In an alternative pathway, autophagosomes can cross-present some selected exogenous antigens to CD8+ T cells through the intersection with phagosomes. In MHC class II pathway, autophagosomes fuse with MHC II compartments and then deliver the antigens to MHC II molecules.

periphery, CD4+T cells and CD8+T cells are called naive forms of helper T (Th) cells and cytotoxic T (Tc) cells, respectively. Although, naive T cells are considered as mature T cells, they have not encountered their cognate antigen in the periphery.

The contribution of autophagy in early T cell development stage has been shown in several mouse models. Reconstitution of autophagy-deficient stem cells with chimeric Rag-1 deficient mice, which cannot generate T cells themselves, resulted in the formation of the lymphocyte compartment only from transferred cells. On the other hand, complementation of Rag-deficient blastocysts with BECN1 deficient embryonic stem cells showed a remarkable decrease in CLP and thymocyte numbers, while peripheral T cell numbers were almost similar to chimeric mice reconstituted with wild-type embryonic stem cells (Arsov et al., 2011). In contrast, reduced numbers of CLPs, thymocytes and peripheral T cells were observed after the transfer of fetal liver cells from an Atg5-deficient mouse (Pua et al., 2007). Importantly, the frequencies of different thymocyte subpopulations were altered in *BECN1* deficiency but not in *Atg5* deficiency, indicating that different stages of the autophagic process may be involved in developing tyhmocytes and mature T cells.

At later stages of T cell development, a conditional deletion of *Atg* genes using a CD4 Cre mouse model demonstrated that the number of thymocytes was not affected in these mice (Willinger et al., 2012; Parekh et al., 2013). Nevertheless, when deletion occurred at early stages in the thymus, reduced numbers of total thymocytes were detected, even though CD4/CD8 subsets were normally distributed (Arsov et al., 2011; Jia et al., 2011; Pua et al., 2009; Stephenson et al., 2009). This evidence indicates the crucial role of autophagy for CD4−CD8− double-negative thymocyte survival or proliferation. Moreover, autophagy may also be important for the transition of thymocytes from the double-negative to the double-positive stage.

Also, autophagy was reported to regulate positive-selection of thymocytes via MHC II peptide presentation in cTECs (Nedjic et al., 2008). In the mouse thymus, it was shown that TECs had a high constitutive level of autophagy compared to other cells, and genetic inhibition of autophagy by *Atg5* gene deletion led to the altered selection of certain MHC II-restricted T-cell specificities, resulting in severe multi-organ inflammations. These results indicate the importance of autophagy in the generation of MHC II-peptide repertoire of TECs consisting of a wide array of antigens (Mizushima et al., 2004; Nedjic et al., 2008). Thus, autophagy contributes to the formation of T-cell repertoire by promoting MHC II presentation of specific peptides during the positive-selection process.

While the function of autophagy is well established in the positive-selection of T cells, its role during the negative-selection process is controversial. Studies have shown colocalization of the autophagic marker LC3 with MHC II loading vesicles in both cTECs and mTECs (Nedjic et al., 2008; Kasai et al., 2009). During negative-selection, autophagy is necessary to remove redundant peptides in mTECs and antigen presenting cells (APCs). However, when the antigen levels are low, autophagy-dependent peptide presentation in mTECs becomes indispensable, indicating the crucial function of autophagy during negative-selection, characterized by the elimination of auto-reactive CD4+T cells (Aichinger et al., 2013). Transplantation of *Atg5*-deficient thymi into wild-type mice caused an infiltration of auto-reactive CD4+T cells into multiple organs and induced development of an auto-immune disease, as a result of defective cytoplasmic antigen presentation by autophagy-deficient TECs (Nedjic et al., 2008). In contrast, in another mouse study, conditional deletion of the *Atg7* gene in TECs did not result in any sign of autoimmune disease, although normal T cell frequencies were consistent in both studies (Nedjic et al., 2008; Sukseree et al., 2012). Thus, both cTECs and mTECs can present intracellular peptides on MHC II via autophagy and thereby shape the T-cell repertoire during positive- and negative-selection of T cells.

Consequently, accumulating data indicate that autophagy plays an essential function in T cell development and its role may be different in the early versus late stages of development, as evidenced by altered thymocyte numbers and T-cell repertoires.

Autophagy in naive T cell (CD4+ and CD8+) homeostasis

Peripheral naive T cells require maintenance of endoplasmic reticulum (ER) and mitochondrial content for their maturation (Pua et al., 2009). In cells, autophagy is the major mechanism regulating ER and mitochondria levels (Pua et al., 2009, Jia and He, 2011). In naive T cells, deletion of *Atg* genes in thymocytes resulted in expanded levels of ER and mitochondria (Jia and He, 2011; Jia et al., 2011; Parekh et al., 2013). In addition, ER was not localized to one pole, but conversely distributed throughout the cell, and ER stress was induced in these cells. In line with mitochondrial expansion, ROS production was increased (Pua et al., 2009; Willernger and Flavell, 2012) and ER-like structures were accumulated, leading to the induction of cell death and accounting for a reduced number of lymphocytes in the mouse models (Pua et al., 2009; Stephenson et al., 2009; Jia and He, 2011; Jia et al., 2011; McLeod et al., 2011; Kovacs et al., 2012; Willenger et al., 2012; Parekh et al., 2013). In another study, suppression of autophagy by cellular FLICE-like inhibitory protein (cFLIP) was demonstrated in naive T cells (Lee et al., 2009; He and He, 2013). The role of autophagy in long-term survival of peripheral naive T cells has also been shown. In a resting stage, promotion of naive T cells survival depends on TCR interactions with stromal cells and interleukin-7 (IL-7)-induced anti-apoptotic signals (Sprent and Surh, 2011). Short-term culture of *Atg3*-deficient naive T cells with IL-7 did not

affect the survival of these cells. However, compared to control cells, *Atg3*-deficient cells that were cultured for 24 days showed an increased susceptibility to death and this correlated with an increased level of ER and mitochondrial volume, suggesting that cell loss was related to defects in organelle homeostasis (Jia et al., 2011). In contrast, T cell specific deletion of *BECN1* gene resulted in normal mitochondrial homeostasis, even though apoptosis was induced in the peripheral CD4+ and CD8+ naive T cells. Moreover, pro-apoptotic proteins CASP-8, CASP-3 and BIM as well as pro-survival protein BCL-2 were up-regulated in these cells, suggesting that BECN1 affected not only autophagy, but also pro-apoptotic or anti-apoptotic pathways in T cells (Kovacs et al., 2012). Another autophagy-related protein, VPS34, was described to regulate intracellular trafficking of the IL-7 receptor, leading to increased T cell death and reduced IL-7 receptor expression (McLeod et al., 2011).

Autophagy in mature T cells

T cell activation

Activation of T cells requires TCR stimulation by binding of an antigen and concomitant stimulation of CD28 and Interleukin-2 (IL-2) signaling. Following activation, naive T cells begin to proliferate and differentiate into subtypes and secrete cytokines. Afterwards, they either die or become memory T cells. Activity of mTOR, which is directly controlled by the energy metabolism of cells, is indispensible for T cell activation. Upon T cell activation, AKT/mTOR signaling is induced, rendering acvitation of the NF-κB signaling, which then regulates transcription of effector genes in T cells (Macian et al., 2002). In fact, stable and high intracellular calcium levels are also important for the stimulation of these nuclear factors (Negulescu et al., 1994; Sena et al., 2013). Following TCR signaling, calcium is rapidly released from the ER, leading to the opening of calcium release-activated calcium channels on the plasma membrane, and resulting in the influx of extracellular calcium. Elevated calcium levels in T cells activate an inhibitor of the mTOR, AMP-activated protein kinase (AMPK) (Tamas et al., 2006). Instant activation of AMPK supports the production of mitochondrial ROS and ATP, thereby providing the energy for a full activation of T cells.

In autophagy-deficient peripheral naive T cells, an expansion of the ER and mitochondria was reported to be associated with enhanced levels of ROS and cell death (Ireland and Unanue, 2011; Aichinger et al., 2013). Upon TCR activation, cytoplasmic calcium levels were decreased due to increased net efflux of calcium from the expanded ER, which resulted in defective proliferation and cytokine secretion from these cells (Pua et al., 2007; Stephenson et al., 2009; Hubbard et al., 2010; Ireland and Unanue, 2011; Jia and He, 2011; Kovacs et al., 2012; Aichinger et al., 2013; Parekh et al.,

2013). These data demonstrated that control of the ER and mitochondrial mass by autophagy is important for the activation and subsequent function of T cells. Both mTOR and AMPK signaling are important regulators of autophagy activation via phosphorylation of the ULK complex components. Genetic and chemical inhibition of autophagy caused a reduction in IL-2 and AMPK phosphorylation levels as well as in ATP production. Stimulation of ATP production by exogenous energy sources partially restored ATP and IL-2 production and reversed the function of autophagy-deficient T cells (Hubbard et al., 2010). Therefore, energy that is provided by the autophagic activity is required for a full activation of T cells.

Regulation of signaling pathways is another function of autophagy during T cell activation. The NF-κB pathway is known to be essential for TCR activation in naive T cells (Kingeter et al., 2010). However, accumulating data indicate that autophagy down-regulates the NF-κB pathway during T cell activation (Paul et al., 2012). TCR activates the inducible transcription factor NF-κB via stimulating transcription of numerous effector molecules, including IL-2 (Skaug et al., 2009; Vallabhapurapu and Karin, 2009). The adaptor protein, BCL10, plays a key role in transmitting signals from the TCR to NF-κB. In the absence of BCL10, T cells are unable to efficiently proliferate and differentiate in response to TCR engagement (Ruland et al., 2001; Schulze-Luehrmann and Ghosh, 2006; Thome et al., 2010). Autophagy has been shown to selectively degrade Bcl10 only in effector T cells (activated T cells produced by clonal selection) and not in naive T cells (Paul et al., 2012). Thus, autophagy seems to regulate NF-κB signaling by both activation and inhibition of its activity during T cell activation.

Recently, the function of autophagy on the apoptotic signaling pathway was also investigated in activated T cells. In autophagy-deficient T cells, an increased level of pro- and anti-apoptotic proteins was demonstrated. These results suggest that autophagy functions as a degradation pathway for pro-apoptotic proteins and thereby supports the survival of activated T cells (Pua et al., 2009; Jia et al., 2011; Kovacs et al., 2012; Oral et.al., 2012; Parekh et al., 2013). In contrast, knockdown of *Beclin1* or *Atg7* protected cells from apoptosis, showing that autophagy can also induce apoptosis in activated T cells (Li et al., 2006). Currently, conflicting data exist on the role of autophagy in apoptosis or apoptosis-independent cell death signaling and the outcome is mostly defined in a context-dependent way (Oral et al., 2016).

Antigen presentation

In the presence of infection, APCs activate naive T cells by presenting antigens via MHC I and MHC II molecules. Cytosolic and nuclear peptides can be presented on MHC I and mature naive CD8+ T cells are able to recognize these antigens, while extracellular

antigens are presented on MHC II and mature naive CD4+ T cells can recognize MHC II-presented peptides (Dengjel et al., 2005; Dorfel et al., 2005; Aichinger et al., 2013). For presentation of peptides, APCs use the major secretory pathways and the lysosomal pathway via autophagy. Therefore, autophagy plays an important role in the presentation of antigens to T cells via MHC molecules.

During MHC I presentation, antigens are processed into peptides by the proteasome and then transported into the ER, where they are loaded onto MHC I molecules. Most classical studies indicated that MHC I antigen presentation did not require autophagy. However in the periphery, APCs seem to use autophagy to cross-present only selected exogenous peptides to CD8+ T cells (Paludan et al., 2005; Dorfel et al., 2005; Schmid et al., 2007). For example, influenza A viral peptide (Uhl et al., 2009), human cytomegalovirus protein (Tey and Khanna, 2012), ovalbumin and melanocyte differentiation antigen (Li et al., 2008) were shown to be routed into the MHC I pathway via intersection of autophagosomes with phagosomes (Fig. 3).

In the MHC II pathway, antigens that are processed in the endolysosomal compartments are degraded by lysosomal proteases. These antigenic fragments are loaded onto MHC II molecules and then transported to the cell surface for presentation to CD4+ T cells (Vyas et al., 2008). Due to the important role of the lysosomal system in MHC II antigen presentation, autophagy was found to be directly linked to this presentation pathway. Cytosolic antigens were shown to be delivered to CD4+ T cells via the fusion of autophagosomes with the MHC II compartment (MIIC). Multiple studies reported that autophagy selectively presented viral (Paludan et al., 2005; Schmid et al., 2007) and bacterial antigens to MIIC (Nimmerjahn et al., 2003; Jagannath et al., 2009; Russmann et al., 2010). Furthermore, secreted bacterial antigens were found to be engulfed by autophagosomes, suggesting that autophagy may sequester antigens when they cannot be captured by phagosomes (Jagannath et al., 2009). In addition to viral and bacterial antigens, tumor peptide presentation on MHC II has also been shown to be promoted by autophagy (Dorfel et al., 2005; Li et al., 2008). Although the underlying mechanism of antigen delivery to MHC molecules is controversial, autophagy is now a well-accepted pathway of antigen presentation and therefore functions as a key regulator of T cell priming and response to pathogens and infections (Fig. 3).

Autophagy in T cell differentiation

Following activation of T cells, two types of cells are produced by clonal selection: effector T cells and memory T cells. Effector T cells include cytotoxic (Tc) and helper T (Th) cells, also known as CD8+T and CD4+T cells.

Previous studies showed that basal autophagy levels were much higher in CD8+T cells compared to CD4+T cells (Stephenson et al., 2009). Upon inhibition of autophagy the CD8+T cell frequency was reduced more than the CD4+T cell frequency (Stephenson et al., 2009; Jia and He, 2011; Kovacs et al., 2012; Willenger et al., 2012; Parekh et al., 2013), suggesting that autophagy may have an important role during the differentiation of T cells in the thymus, and in this regard, CD8+T cells seem to be more dependent on autophagy.

Environmental factors such as glucose or cytokine level can induce initial differentiation of effector T cells into specific Th cells or regulatory T (Treg) cells. In fact, these two metabolically different subtypes of CD4+T cells and Th cells further develop into T helper cell type 1 (Th1), cell type 2 (Th2) or cell type 17 (Th17) cells.

Although several factors influence differentiation, Th cells were differentiated when the mTOR pathway was induced in activated T cells. In contrast, if mTOR activation was low and AMPK levels were high, activated T cells differentiated into Treg cells (Michalek et al., 2011). In an *in vitro* study, *Beclin1*-deficient naive T cells were differentiated into Th1, Th2 or Th17 cells; however Th17 cells were more sensitive to cell death upon stimulation (Kovacs et al., 2012). This is correlated with another study, showing that Th2 cells exhibited more autophagy than Th1 cells, and this property made Th2 cells more resistant to cell death (Li et al., 2006). Therefore, Th17 cells do not seem to depend on autophagy for survival as much as other effector T cells. Although the impact of autophagy in different types of Th cells needs to be addressed in a more detailed manner in the future, it is likely that effector T cells regulate autophagy according to their metabolic activities and their effector functions.

Treg cells, also known as suppressor T cells, can either develop naturally in the thymus or they can be induced peripherally. But both cases require the expression of the transcription factor FOXP3. Treg cells play an important role in the inhibition of auto-reactive T cells that may escape during negative-selection in the thymus. Moreover, Treg cells suppress any potentially deleterious Th cell activities that could lead to an autoimmune disorder. According to their FOXP3 expression, two major classes of Treg cells have been described: FOXP3+ Treg cells and FOXP3– Treg cells (Shevach, 2000; Haribhai et al., 2011). In a mouse study, T cell-specific deletion of the autophagy-related PI3 kinase *Vps34* gene resulted in severe defects in the autophagic flux and led to the accumulation of cellular organelles. Furthermore, VPS34 was found to be required for the peripheral homeostasis and function of CD4+ Foxp3+ Treg cells (Parekh et. al., 2013). Very recently, Treg cell-specific deletion of two essential autophagic genes, *Atg7* or *Atg5*, has been shown to lead to the loss of Treg cells and development of inflammatory disorders. In this study, *Atg7*-deficient Treg cells showed increased apoptosis and they were defective in FOXP3 expression. In addition, autophagy deficiency increased mTORC1 activity, c-MYC expression and up-regulation of glycolytic metabolism

contributing to defective Treg function. These results indicate a crucial role of autophagy in the regulation of immune signals and metabolic homeostasis protecting functional integrity of Treg cells, and thereby Treg-cell mediated immune tolerance (Wei et al., 2016).

Memory T cells are long-lived cells that are produced after the first immune response to an antigen, and they are stimulated only if the organism is re-exposed to the same antigen. They are a subset of antigen-specific T cells that are responsible for long-term immunity against diseases. Memory T cells may either be of CD4+ or CD8+ type. Two recent independent studies demonstrated that T-cell specific deletion of autophagy-related genes, *Atg5* or *Atg7*, lead to defective survival of effector T cells and affected formation of CD8+ memory T cells after viral infection (Puleston et al., 2014; Xu et al., 2014). Therefore, autophagy is essential for CD8+ memory T cell differentiation. Additionally, induction of autophagy in CD4+T cells was shown to promote survival and proliferation of memory T cells upon several viral infections (Ren et al., 2012, 2015).

In mice with T-cell specific deletion of *Vps34* gene, a blockage of natural killer T (NKT) cell development was reported, indicating a role for autophagy in NKT cells development (Parekh et. al., 2013). Nevertheless, further studies are needed to clarify the role of autophagy in this type of immune cells.

Conclusion

Autophagy is an important survival mechanism under stress and energy-deficient conditions. Yet, physiological functions of autophagy are diverse and it has been implicated in multiple biological processes and cellular events. Accumulating data indicate a prominent role of autophagy in T cells, key cell types playing a central role in adaptive immunity. However, there are still many questions that need to be answered about the influence of autophagy in T cells and immune responses in general.

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