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Review

Autophagy in regulatory T cells: A double-edged sword in disease settings

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ABSTRACT

Autophagy is an evolutionarily conserved catabolic process that directs cytoplasmic proteins, organelles and microbes to lysosomes for degradation. It not only represents an essential cell-intrinsic mechanism to protect against internal and external stresses but also shapes both innate and adaptive immunity. Regulatory T cells (Tregs) are a developmentally and functionally distinct T cell subpopulation engaged in sustaining immunological self-tolerance and homeostasis. There is compelling evidence that autophagy is actively regulated in Tregs and serves as a central signal-dependent controller for Tregs by restraining excessive apoptotic and metabolic activities. In this review, we discuss how autophagy modulates the stability and functionality of Tregs in different disease settings, and provide a perspective on how manipulation of autophagy enables better control of immune response by targeting the generation of Tregs and the maintenance of their stability.

1. Introduction

Autophagy is an evolutionarily conserved catabolic process that directs cytoplasmic proteins, organelles and microbes to lysosomes for degradation (Shaw et al., 2013). Although autophagy was initially recognized as a “self-eating” survival pathway that enables nutrient recycling during starvation, it has now been connected to multiple cellular responses, including several aspects of immune response (Kabat et al., 2016b). Initial links between autophagy and host immunity came from the observations that autophagy can target intracellular bacteria for degradation. However, subsequent studies revealed that autophagy plays a much broader role in immune responses, including antigen processing, thymic selection, lymphocyte homeostasis, and the regulation of immunoglobulin and cytokine secretion. Especially in T cell-mediated immune responses, the role of autophagy has been extensively studied (Table 1). Autophagy is induced after TCR and cytokine stimulation, but it seems that the effect of autophagy on diverse T cell subsets might actually differ. For example, activation of autophagy through dietary or pharmacological modulation directly promotes the survival and metabolic adaptation of Tregs in the intestine while limits

Abbreviations: Tregs, regulatory T cells; GVHD, graft versus host disease; SLE, systemic lupus erythematosus; LAMP-2a, lysosome-associated membrane protein 2a; HSC70, heat shock cognate 71 kDa protein; ER, endoplasmic reticulum; ATGs, autophagy-related proteins; PAS, phagophore assembly site; Foxp3, forkhead box P3; TEC, thymic epithelial cells; tTregs, thymus-derived Tregs; TCR, T cell receptor; IL-2, interleukin-2; IRF4, interferon regulatory factor 4; pTregs, peripherally derived Tregs; TGF-β, transforming growth factor-β; SCT, stem cell transplantation; HSC, hematopoietic stem cells; GVL, graft versus leukemia; aGVHD, acute GVHD; cGVHD, chronic GVHD; TIGIT, T cell immunoreceptor with Ig and ITIM domains; mTOR, mammalian target of rapamycin; STAT3, signal transducer and activator of transcription 3; DCs, dendritic cells; T1D, type 1 diabetes; GATA-3, GATA binding protein 3; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CD, Crohn’s disease; UC, ulcerative colitis; IBD, inflammatory bowel disease; SNP, single-nucleotide polymorphism; HIF-1, hypoxia-inducible factor-1; HREs, hypoxia-response elements; BNIP3, Bcl-2/E1B 19 kDa interacting protein; TSC, tuberous sclerosis complex; ROS, reactive oxygen species; iNKT cells, invariant natural killer T cells; ILCs, innate lymphoid cells; TNF-α, tumor necrosis factor-α; MHC, major histocompatibility complex; PRRs, pattern recognition receptors; TLR4, toll-like receptor 4; Nod1, nucleotide-binding oligomerization domain-containing protein 1; NLRX1, NOD-like receptor X1; HIV-1, human immunodeficiency virus-1; HBV, hepatitis B virus; TEM, transmission electron microscope; CHB, chronic hepatitis B; ULK1, Unc-51-like kinase 1; PI3KC3, class III PI3K; PI3P, phosphatidylinositol-3-phosphate; WIPIs, WD repeat domain phosphoinositide-interacting proteins; DFCP1, zinc-finger FYVE domain-containing protein 1; LC3, microtubule-associated protein light chain 3; PE, phosphatidylethanolamine; LIR, LC3-interacting region

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Table 1
Summary of studies related to the roles of autophagy in T cells using mouse models deficient in autophagic components.

Mouse models	Phenotype	References
Atg5$^{-/-}$ chimeras by transferring day 14 fetal liver cells from *Atg5*$^{-/-}$ and wild-type mice into lethally irradiated *CD45.1*$^{+}$ congenic hosts	Reduced numbers of total thymocytes and peripheral T and B lymphocytes; increased death of CD8 + T cell; insufficient proliferation of T cell after TCR stimulation	Pua et al. (2007)
Atg3$^{f/f}$-*Lck*-Cre mice; *Atg3*$^{f/f}$-*ER*-Cre mice in which Atg3 can be inducibly deleted	Defective survival of naïve CD4^{+} and CD8^{+} T cells; expanded mitochondria and endoplasmic reticulum	Jia and He (2011)
Atg5$^{f/f}$-*Lck*-Cre mice	Decreased thymocytes and peripheral T cell numbers; decreased cell survival; increased mitochondrial mass	Stephenson et al. (2009)
Atg7$^{f/f}$-*Lck*-Cre mice		
Atg7$^{f/f}$-*Lck*-Cre mice	Defective IL-2 and IFN-γ production by effector Th cells; reduced proliferation after stimulation	Hubbard et al. (2010)
Atg7$^{f/f}$-*ER*-Cre mice		
Atg7$^{f/f}$-*Lck*-Cre mice	Expanded endoplasmic reticulum and mitochondrial content; increased ROS production; defective calcium homeostasis; decreased survival of mature primary T lymphocytes	Pua et al. (2009), Jia et al. (2011)
Atg7$^{f/f}$-*Cd4*-Cre mice	Increased apoptotic death stimulated with anti-CD3 and anti-CD28	Ch'en et al. (2011)
Becn1$^{f/f}$-*Cd4*-Cre mice	Increased death of T cells upon stimulation; increased cell death-related proteins	Kovacs et al. (2012)
Pik3c3 $^{f/f}$-*Cd4*-Cre mice	Increased mitochondrial mass and accumulation of ROS; reduced survival of naïve T cells; impaired intrathymic development of invariant NKT cells; inflammatory wasting syndrome in aged animals	Willinger and Flavell (2012), Parekh et al. (2013)
Pik3c3 $^{f/f}$-*Cd8*-Cre mice		
Atg16l1 $^{f/f}$-*Cd4*-Cre mice	Enhanced Th2 responses and decreased Treg numbers; spontaneous intestinal inflammation	Kabat et al. (2016a)
Atg16l1 $^{f/f}$-*Foxp3*-Cre mice		

Abbreviations: TCR, T cell receptor; Lck, lymphocyte protein tyrosine kinase; ER, estrogen receptor; IL-2, interleukin-2; IFN-γ, interferon-γ; ROS, reactive oxygen species; Becn1, Beclin 1; and NKT cells, natural killer T cells.

mucosal Th2 cell expansion (Kabat et al., 2016a).

As a specialized T cell lineage to mediate immunosuppression in response to immune and inflammatory signals, Tregs have emerged as an attractive target to steer immune responses in a desired direction. The manipulation of Tregs arms the immune system capable of destroying infected cells and cancer cells or limiting tissue destruction by autoreactive immune cells (Li and Rudensky, 2016; von Boehmer and Daniel, 2013). Unfortunately, how the functional integrity of Tregs is maintained under normal or disease condition is poorly understood. There is compelling evidence that autophagy is active in Tregs, by which it supports the lineage stability, functional identity and survival fitness of Tregs (Parekh et al., 2013; Kabat et al., 2016a; Wei et al., 2016; An et al., 2017; Marcel and Sarin, 2016). This review intends to summarize the contribution of autophagy to Treg stability and functionality and discuss its implication in disease settings.

2. The autophagy pathways

Generally, autophagy means "self-eating" at the subcellular level, which includes at least three distinct pathways: microautophagy, chaperone-mediated autophagy, and macroautophagy (Mizushima and Klionsky, 2007). Microautophagy involves budding of small cytosol-containing vesicles, and this budding occurs directly into the lysosomal lumen (Schmid and Munz, 2007). During chaperone-mediated autophagy, unfolded proteins translocate across the lysosomal membrane directly, via the LAMP-2a transporter assisted by the cytosolic and lysosomal HSC70 chaperones (Schmid and Munz, 2007). By contrast, macroautophagy is used for the sequestration and degradation of cytoplasm in a process that uses specialized cytosolic vesicles that ultimately fuse with the lysosome (Xie and Klionsky, 2007). On the other hand, chaperone-mediated autophagy is induced after macroautophagy as a secondary response to starvation in mammals, and macroautophagy (hereafter referred to as autophagy) is the primary response to nutrient limitation (Mizushima and Klionsky, 2007).

Signals that induce autophagy usually originate from various stresses in different conditions such as endoplasmic reticulum (ER) stress, oxidative stress, protein aggregation, starvation, hypoxia and others. In general, induction of autophagy is accompanied by the recruitment of autophagy-related proteins (Atgs) into a specific subcellular location termed as phagophore assembly site (PAS) and nucleation of an isolation membrane to form a cup-shaped structure termed as phagophore (Dikic and Elazar, 2018). The curved isolation membrane elongates gradually, leading to the expansion of phagophore into a sphere around a portion of the cytosol. The isolation membrane next seals into autophagosome, a double-membraned vesicle, which then traps the engulfed cytosolic material as autophagic cargo. The microtubules are next delivered into the lysosome once most Atgs are cleared, which leads to the fusion of the outer autophagosomal membrane and the lysosome membrane to form an autolysosome (Dikic and Elazar, 2018; Abada and Elazar, 2014; Lamb et al., 2013; Mizushima and Komatsu, 2011). The autolysosome then releases a single-membrane autophagic body into the lysosome lumen for degradation together with its carried cargo (Fig. 1).

3. Characterization of Tregs

The immune system has evolved to mount an effective defense against pathogens and to minimize deleterious immune-mediated inflammation (Josefowicz et al., 2012). Over the past two decades, our understanding of how T cell subsets differentiate and acquire a degree of stability that allows them to be considered a distinct T-cell lineage has greatly expanded (Huehn et al., 2009; Wilson et al., 2009). Particularly, Tregs express the transcriptional factor forkhead box P3 (Foxp3) to program their development and functionality (Fontenot et al., 2003; Khattri et al., 2003; Hori et al., 2003). Together with other immuno-suppressive cells, those Foxp3-expressing Tregs play a crucial role in maintaining the homeostasis of immune system against autoimmunity (Zeng et al., 2015).

It is believed that Foxp3^{+} Tregs develop extrathymically and intrathymically (Fig. 2). In the thymus, Foxp3 induction can start at the CD4/CD8 double positive stage but occurs preferentially at the CD4 single positive stage or during the transition to this stage (Fontenot et al., 2005). Studies suggested that antigen presented by either cortical or medullary thymic epithelial cells (TEC) is sufficient to induce Foxp3 expression, and thereby committing developing thymocytes to the Treg cell lineage (Liston et al., 2008; Aschenbrenner et al., 2007). These thymus-derived Tregs (tTregs) recirculate through secondary lymphoid tissues as quiescent CD44^{lo}CD62L^{hi} tTregs (Smigiel et al., 2014). In the periphery, activation signals involving T cell receptor (TCR) ligation, CD28 co-stimulation and/or interleukin-2 (IL-2) induction upregulate the expression of interferon regulatory factor 4 (IRF4), which then orchestrates their transition into the CD44^{hi}CD62L^{lo} effector tTreg (Liston

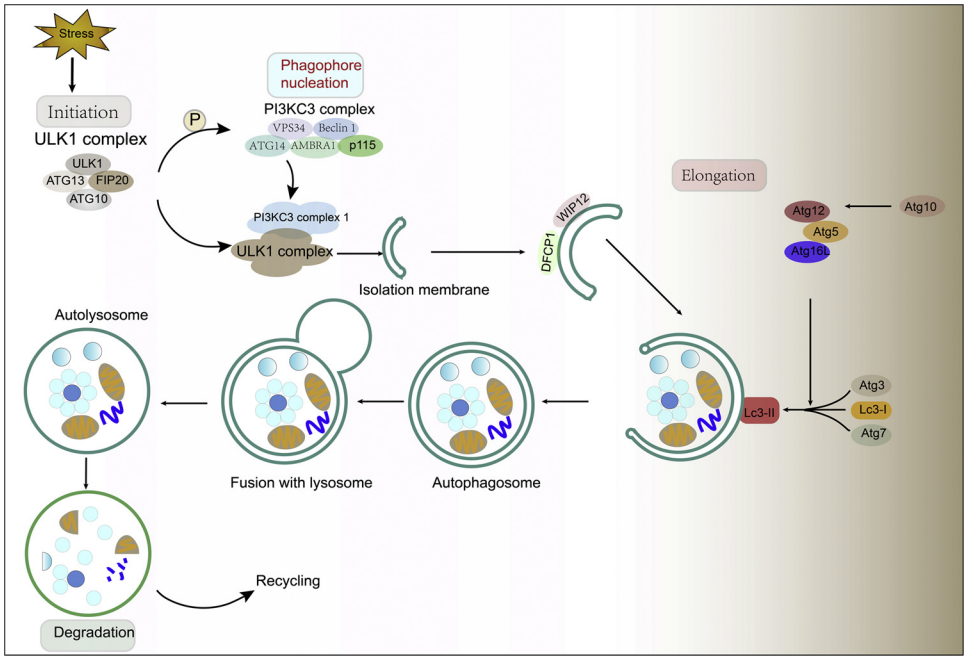

Fig. 1. Overview of the autophagy efflux and molecular machinery. Autophagy can be induced by certain environmental or intracellular cues. The common target of signals that activate the autophagic process is the Unc-51-like kinase 1 (ULK1) complex, which then triggers nucleation of the phagophore by phosphorylating components of the class III PI3K (PI3KC3) complex I, which in turn activates local phosphatidylinositol-3-phosphate (PI3P) production at the isolation membrane. PI3P then recruits the PI3P effector proteins WD repeat domain phosphoinositide-interacting proteins (WIPIs; here WIPI2) and zinc-finger FYVE domain-containing protein 1 (DFCP1) via interaction with their PI3P-binding domains. WIPI2 was recently shown to bind ATG16L1 directly, thus recruiting the ATG12~ATG5–ATG16L1 complex that enhances the ATG3-mediated conjugation of ATG8 family proteins (ATG8s), including microtubule-associated protein light chain 3 (LC3) proteins to membrane-resident phosphatidylethanolamine (PE), thus forming the membrane-bound, lipidated forms; for example, in this conjugation reaction, LC3-I is converted into LC3-II, the characteristic signature of autophagic membranes. ATG8s not only further attract components of the autophagic machinery that contain an LC3-interacting region (LIR) but also are required for elongation and closure of the phagophore membrane. Autophagosomes then fuse with lysosomes to form an autolysosome. The autolysosome then releases a single-membrane autophagic body into the lysosome lumen for degradation together with its carried cargo.

and Gray, 2014). In this case, the expression of Foxp3 and suppressive function in those tTregs can be maintained in inflamed periphery tissues, which constitutes a typical feature of a stable T-cell lineage (Siewert et al., 2008).

There is ample evidence that Tregs can also be induced *de novo* from naive peripheral CD4$^+$ T cells (Siewert et al., 2008; Kretschmer et al., 2005). For example, TCR recognition of antigens originated from the commensal microbiota or diet components can support the differentiation of such peripherally derived Tregs (pTregs) (Kretschmer et al., 2005; Curotto de Lafaille et al., 2008; Kretschmer et al., 2006; Sun et al., 2007; Coombes et al., 2007). Those pTregs possess a distinct TCR repertoire indicating that they are nonredundant and equipped with important role in acquired tolerance to, for example, food antigens or commensal gut flora (Huehn et al., 2009; Kretschmer et al., 2005; Sun et al., 2007). Indeed, those pTregs contribute to the peripheral Treg pool, but they do not have a stable phenotype comparable to that of tTregs. For example, antigenic stimulation of conventional CD4$^+$ T cells in the presence of transforming growth factor-β (TGF-β) leads to the induction of Foxp3 expression and the acquisition of suppressor function (Chen et al., 2003), and this conversion could be enhanced by the vitamin A metabolite retinoic acid, rapamycin and sphingosine 1-phosphate receptor agonist FTY720 (Battaglia et al., 2006; Zhou et al., 2009a). However, these cells have an unstable phenotype with transient expression of Foxp3 as most of them lose Foxp3 expression following

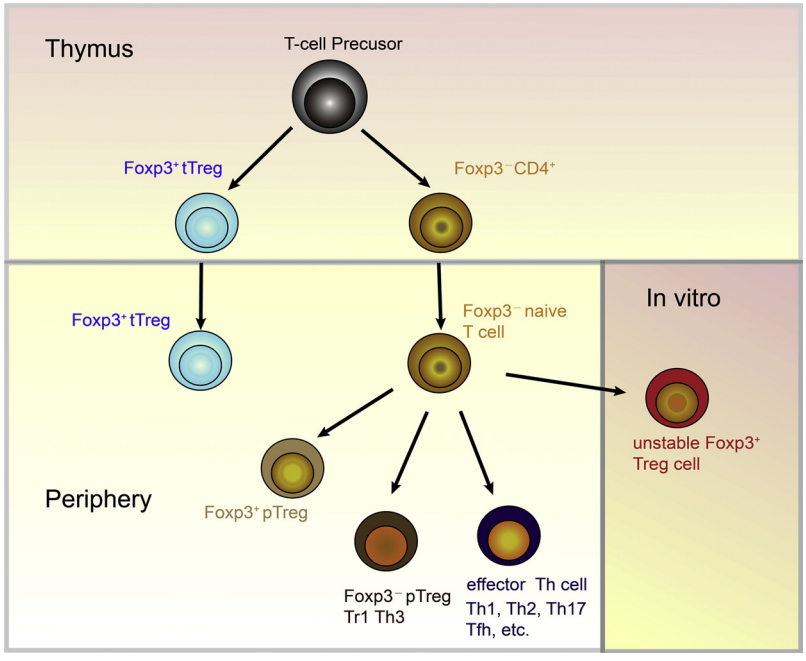

Fig. 2. Thymic and periphery Treg generation. In the thymus, tTregs differentiate from T-cell precursor in a process which is known to involve interaction with antigen presented by either cortical or medullary TEC. These thymically derived Tregs recirculate through secondary lymphoid tissues. pTregs differentiate in secondary lymphoid organs and tissues. The peripheral population of Foxp3$^+$ Treg cells comprises both tTregs and pTregs. In vitro, Tregs can also be induced de novo from naive peripheral Foxp3$^-$ T cells driven by treatment with TGF-β and may be enhanced by the vitamin A metabolite retinoic acid. However, these cells have an unstable phenotype and transient expression of Foxp3.

restimulation with antigen in the absence of exogenous TGF-β (Huehn et al., 2009).

Upon inflammatory challenge, cytokine receptor and TCR driven signals elicit Treg suppressor activity through the induction of various effectors (Arvey et al., 2014). Therefore, environmental signals dynamically program the activation states and functional capacities of Tregs. In general, Foxp3 is the transcription factor responsible for determining the development and function of Tregs (Huehn et al., 2009). However, as aforementioned, Tregs can lose Foxp3 expression and convert into pathogenic effector T cells under certain inflammatory conditions, suggesting that activating environmental factors may destabilize Foxp3 expression (Zhou et al., 2009b; Bailey-Bucktrout et al., 2013; Komatsu et al., 2014). Apart from lineage stability, anti-apoptosis is also critical for the survival and functional integrity of Tregs in maintaining immune homeostasis (Pierson et al., 2013).

4. The role of autophagy in Tregs to prevent GVHD

Allogeneic hematopoietic stem cell transplantation (SCT) is thought to be the most effective therapy for the majority of hematopoietic malignancies. After transplantation, donor hematopoietic stem cells (HSC) are assumed to reconstitute the recipient's hematopoietic and immune systems. The curative property of SCT lies within the graft versus leukemia (GVL) effect, in which donor T and NK cells mediate the clearance of residual tumor cells. This process is absolutely dependent on donor T cells contained within the graft; however, these T cells are also the primary mediators of graft versus host disease (GVHD), a serious complication with a high mortality rate (Le Texier et al., 2017). Therefore, establishing a viable transplant protocol aimed at maximizing GVL but minimizing GVHD is a formidable challenge in clinical settings.

GVHD is divided into acute and chronic forms, which involve distinct pathological processes: acute GVHD (aGVHD) has strong inflammatory components, whereas chronic CVHD (cGVHD) displays more autoimmune and fibrotic features (Blazar et al., 2012). However, both aGVHD and cGVHD can be characterized as resulting from an imbalance between the effector and regulatory arms of the immune system (Chen et al., 2009; Beres and Drobyski, 2013). Indeed, reduced frequency of Tregs was observed in patients with GVHD (Zorn et al., 2005), and Treg adoptive transfer ameliorates both aGVHD and cGVHD following SCT in preclinical models (Hoffmann et al., 2002; Giorgini and Noble, 2007; McDonald-Hyman et al., 2016; Leveque-El Mouttie et al., 2016). Surprisingly, GVT responses were preserved, which was possibly due to the retention of cytolytic T cell function or differences in Treg versus effector T cell homing patterns (Edinger et al., 2003). Moreover, a phase I clinical trial using Tregs for the prevention of aGVHD has been reported (Brunstein et al., 2011). Unfortunately, although Treg adoptive transfer after SCT manifests a therapeutic potential to promote tolerance, the low frequency of Tregs, time required to expand them in vitro to relevant numbers, and their instability after transfer limit the translation of this Treg-based immunotherapy into the clinic.

Generally, granulocyte-colony stimulating factor (G-CSF) is employed to mobilize peripheral blood stem cells in the majority of allogeneic stem cell transplants. G-CSF mobilization significantly modulates the transcription profile of $CD4^+CD25^+$ Tregs, and promotes their expansion in the donor and recipient (Leveque-El Mouttie et al., 2016; MacDon et al., 2014). Genome wide microarray study revealed that the expression of Atgs (e.g., Atg2a, Atg16l1 and Atg4c) is enhanced in Tregs from G-CSF treated donors, implicating a role of autophagy in this process (MacDon et al., 2014). More recently, this notion was confirmed and further extended by Texier and colleagues (Le Texier et al., 2016). They confirmed an intrinsic requirement of autophagy for the maintenance of Tregs in the periphery, and further identified a $TIGIT^+$ Treg subset that is uniquely dependent on autophagy at steady state. TIGIT is a co-inhibitory molecule, whose expression identifies a subset of activated Tregs implicated in the suppression of autoimmunity. They also identified the enrichment of this autophagy-dependent $TIGIT^+$ Treg subset in the bone marrow, the ability of G-CSF to mobilize this subset from the bone marrow, and their importance in the control of GVHD following SCT (Le Texier et al., 2016). Consistently, another study demonstrated that metformin, an autophagy-promoting drug, provides protection for mice against GVHD-induced mortality (Park et al., 2016). This protective effect was associated with the inhibition of mammalian target of rapamycin (mTOR) and signal transducer and activator of transcription 3 (STAT3) pathways, as well as the conversion of Th17 into Tregs via enhanced autophagy (Park et al., 2016). Collectively, these data indicate a critical role of autophagy in Tregs to prevent GVHD following SCT.

5. The impact of autophagy on Tregs in the setting of autoimmune and inflammatory diseases

Although autoimmune diseases have broad variations in terms of presentation, disease course, prognosis, as well as outcomes, the pathogenesis underlying autoimmunity is considered to involve dysfunction of the entire immune system, including B and T cells of the adaptive arm and dendritic cells (DCs), macrophages, and neutrophils of the innate arm (Perl, 2012; Huang and Perl, 2018). Furthermore, diminished Treg activities have been observed in various autoimmune conditions. For example, Tregs are functionally compromised along with an enhanced production of inflammatory cytokines in rheumatoid arthritis (Ehrenstein et al., 2004). In addition, whereas Treg frequency in the peripheral blood of type 1 diabetes (T1D) patients is unaltered, their suppressive abilities are diminished compared with Tregs from healthy controls (Visperas and Vignali, 2016). In a lupus mouse model, the number of Tregs decreases along with aging and disease progression (Humrich et al., 2010; Mizui and Tsokos, 2018), and their suppressive function is decreased in patients with active SLE (Bonelli et al., 2008; Valencia et al., 2007). Similar as earlier studies in mice (Wei et al., 2016), autophagy was augmented in Tregs over effector T cells in the healthy controls. Nevertheless, this difference is abolished in SLE patients, as autophagy is enhanced in naive and proinflammatory effector $CD4^+$ T cells but profoundly diminished in Tregs (Kato and Perl, 2018). Increased activity of the IL-21-mTORC1 axis in SLE leads to the blockade of autophagy in Tregs. In contrast, blockade of mTORC1 by 3-day rapamycin treatment enhances TGF-β production, and dual blockade of mTORC1 and mTORC2 by 4-week rapamycin treatment induces autophagy, which restores the expression of GATA-3 and CTLA-4, and recovers the function of Tregs (Kato and Perl, 2018). However, an autophagy-independent effect of rapamycin cannot be completely excluded in this study.

Crohn's disease (CD) and ulcerative colitis (UC) are the two main forms of inflammatory bowel disease (IBD), and are manifested by the typical onset during young adulthood and a lifelong course characterized by periods of remission and relapse (Huttenhower et al., 2014). Although the precise etiology of IBD is yet to be fully addressed, several factors that make a major contribution to disease pathogenesis have been identified. They fall into three distinct categories: genetic factors, the host immune system, and the environmental factors such as the gut microbiota (Maloy and Powrie, 2011). The hunt for genetic predisposition to IBD onset has culminated in the Immunochip project, which has identified > 160 loci containing IBD susceptible genes (Van Limbergen et al., 2014). Among these genes, a single-nucleotide polymorphism (SNP) in the essential autophagy gene ATG16L1 was associated with an increased risk for Crohn's disease (Hampe et al., 2007; Rioux et al., 2007). Recently, a T300A mutation was identified in the coding region of ATG16L1, which increases ATG16L1 sensitization to caspase-3-mediated processing and impairs autophagy, indicating the contribution of a decreased autophagy function to IBD risk (Murthy et al., 2014). Furthermore, polymorphisms in several other autophagy-related genes, such as IRGM, LRRK2 and SMURF1, are also involved in

IBD susceptibility (Van Limbergen et al., 2014). To identify the mechanisms through which autophagy may regulate intestinal homeostasis, Atg16l1$^{\Delta CD4}$ mouse model was generated. Selective deletion of Atg16l1 in CD4 T cells resulted in chronic intestinal inflammation along with increased humoral responses toward commensal and dietary antigens, and loss of Tregs in mice. Mechanistically, Atg16l1 deficiency impacts intestinal CD4$^+$ T cell subsets as manifested by the markedly enhancing Th2 expansion while decreasing Treg numbers by limiting their survival and metabolic adaptation in the intestine (Kabat et al., 2016a). Taken together, activation of autophagy might be beneficial in disorders with a signature of decreased Treg and elevated Th2 responses, including intestinal inflammation and allergic diseases with hypersensitivities.

6. Autophagy modulates Tregs in favor of tumor development

It is believed that cancer cells exploit autophagy as a highly plastic and dynamic mechanism to either suppress initial stage in carcinogenesis or promote the survival and growth of established tumors (Maes et al., 2013). Given that the direct effect of autophagy on tumor development and progression has been extensively reviewed (Maes et al., 2013), our focus here is to address how autophagy impact Tregs in favor of tumor development.

During cancer progression, autophagy is activated in response to various forms of cellular stresses such as hypoxia, nutrient deprivation, extracellular matrix detachment, and ER stress (Viry et al., 2014). Specifically, autophagy is activated in response to hypoxia in a hypoxia-inducible factor-1 (HIF-1)-dependent or -independent manner. In the absence of oxygen, HIF-1 binds to hypoxia-response elements (HREs) in target genes (Harris, 2002). For example, HIF-1 directly binds to HRE within the *Bcl-2/E1B 19 kDa interacting protein* (*BNIP3*) promoter to transcribe BNIP3 expression, which then disrupts the BECN1-Bcl-2 complex, and the freed BECN1 next initiates autophagy (Kothari et al., 2003). In terms of HIF-1-independent induction of autophagy, severe hypoxia leads to ER stress and induces ATF4-dependent autophagy through LC3 as a survival mechanism (Rzymski et al., 2010). Similarly, AMPK serves as a primary energy sensor in cells and is activated in response to the increase of AMP/ATP ratio to phosphorylate the tuberous sclerosis complex (TSC), an upstream inhibitor of mTOR1 (Inoki and Guan, 2009), while mTOR1 functions as an autophagy repressor by inhibiting protein kinase ATG1 (Kamada et al., 2000). Furthermore, starvation-induced autophagy is regulated by reactive oxygen species (ROS) or miRNA as well (Li et al., 2013; Tekirdag et al., 2013).

Tumor cells deploy multiple mechanisms to escape from immunosurveillance. As suppressor T cells, Treg infiltration in tumors has been associated with adverse prognosis of cancer patients (Correale et al., 2010; Shen et al., 2010; Sato et al., 2005; Bonertz et al., 2009). This is not surprising, as high numbers of intratumoral Tregs indicate that an ongoing anti-tumor T cell response may be suppressed eventually. As autophagy enforces functional integrity of Tregs by coupling environment cues and metabolic homeostasis, the Foxp3CreAtg7$^{fl/fl}$ mouse model was generated and used to establish the intrinsic role of autophagy in Tregs (Wei et al., 2016; Le Texier et al., 2016) and subsequently employed to demonstrate the impact of Treg restricted autophagy deficiency on tumor control. Remarkably, tumor growth was severely inhibited in Foxp3CreAtg7$^{fl/fl}$ mice inoculated with MC38 colon adenocarcinoma cells. Consistently, high percentages of tumor-infiltrating CD8$^+$ cells, higher levels of IFN-γ expression in effector CD4$^+$ and CD8$^+$ T cells, and a profound loss of Tregs at the tumor site were noted in Foxp3CreAtg7$^{fl/fl}$ mice (Wei et al., 2016). Together, these results demonstrate a crucial role for autophagy in Treg-mediated suppression of anti-tumor immune responses.

7. The effect of autophagy on Tregs in infectious diseases

Currently, studies have shown the involvement of autophagy in inflammatory responses (Deretic et al., 2013). Firstly, autophagy influences the development, homeostasis and survival of inflammatory cells, including macrophages, neutrophils, invariant natural killer T (iNKT) cells, innate lymphoid cells (ILCs), T cell and B cell (O'Sullivan et al., 2016; Yang et al., 2018; Chen et al., 2014; Riffelmacher et al., 2017; Miller et al., 2008), which play critical roles in the development and pathogenesis of inflammation. Secondly, autophagy has a key role in the control of inflammatory cytokine production (Shibutani et al., 2015). For example, Loss of autophagy in macrophages greatly augments the production of IL-1β, as macrophages deficient in *Atg7* or treated with an inhibitor of the ATG14L complex catalytic subunit VPS34 produce large amounts of IL-1β in response to various inflammasome inducers (Saitoh et al., 2008; Crisan et al., 2011). Interestingly, IL-1β can itself induce autophagy in macrophages (Shi and Kehrl, 2008), suggesting that there may be a negative feedback loop to control IL-1-induced inflammation. Similarly, the secretion of IL-18, IL-6 and tumor necrosis factor-α (TNF-α) was also regulated by autophagy. Inhibition of autophagy enhanced the production of IL-18, but reduced the production of IL-6, IL-8 and TNF-α (Qian et al., 2017). Thirdly, Autophagy-related proteins contribute, at least by three pathways, to the loading of vesicular antigens onto major histocompatibility complex (MHC) molecules (Shibutani et al., 2015; Crotzer and Blum, 2010). During MHC II presentation, autophagosomes can fuse with endosomal MHC loading compartments and deliver cytoplasmic constituents for loading onto MHCII (Schmid and Munz, 2007). Loading of MHC I might also occur in these vesicular compartments, especially under conditions in which the classical processing of classical MHC I antigens is inhibited (Shibutani et al., 2015). For cross-presentation, autophagosomes may intersect with phagosomes bearing phagocytosed exogenous antigen that can then be routed into the MHC I pathway to prime CD8$^+$ T cells (Puleston and Simon, 2014). Thus, autophagy has multi-tiered immunological functions that influence inflammation.

Meanwhile, autophagy is also under the control of various immunological signals, including cytokines and ligands for pattern-recognition receptors (PRRs) (Shibutani et al., 2015). For instance, the Th1 cytokine, interferon-γ, is a potent inducer of autophagy; while the Th2 cytokines, IL-4 and IL-13, have been shown to have inhibitory effects, which suggests a role for autophagy as an effector arm of Th1-mediated immunity and may partly explain why Th1 cytokines afford protection against intracellular bacteria (Deretic, 2010). The stimulation of toll-like receptor 4 (TLR4) and TLR7 by their cognate ligands induces autophagy in macrophages (Xu et al., 2007; Delgado et al., 2008). As cytosolic receptors for bacterial peptidoglycan, nucleotide-binding oligomerization domain-containing protein 1 (Nod1) and Nod2 recruited the autophagy protein ATG16L1 to the plasma membrane at the bacterial entry site in non-phagocytic cells, macrophages and lymphocytes (Travassos et al., 2010). Nod2 stimulation also induces autophagy in DCs (Cooney et al., 2010). NOD-like receptor X1 (NLRX1) is required for the viral–induced autophagic machinery by interacting with the ATG16L1 complex via an intermediary partner, TUFM (Lei et al., 2012). However, other NLRs, including NLRC4, NLRP3, NLRP4 and NLRP10, negatively regulate autophagic processes through its ability to bind and inhibit the action of Beclin 1 (Jounai et al., 2011).

Upon viral infection, autophagy is usually evoked as part of viral life cycles. For example, human immunodeficiency virus-1 (HIV-1) activates the mTOR pathway in DCs, leading to autophagy exhaustion in favor of HIV-1 survival in DCs and transfer of HIV-1 to CD4$^+$ T cells (Blanchet et al., 2010). Similarly, hepatitis B virus (HBV) induces early autophagic pathway but inhibits autophagic degradation by impairing lysosome maturation in hepatocytes, by which HBV can be successfully replicated in hepatocytes (Sir et al., 2010a, b; Yang et al., 2015; Liu et al., 2014). The ongoing chronic HBV infection epidemic and its associated complications, exert formidable challenges and burden to human health. Chronic HBV infection is characterized by a weak immune response to HBV. It is believed that Tregs could be a key player in this impaired immune response since they are potent suppressors of

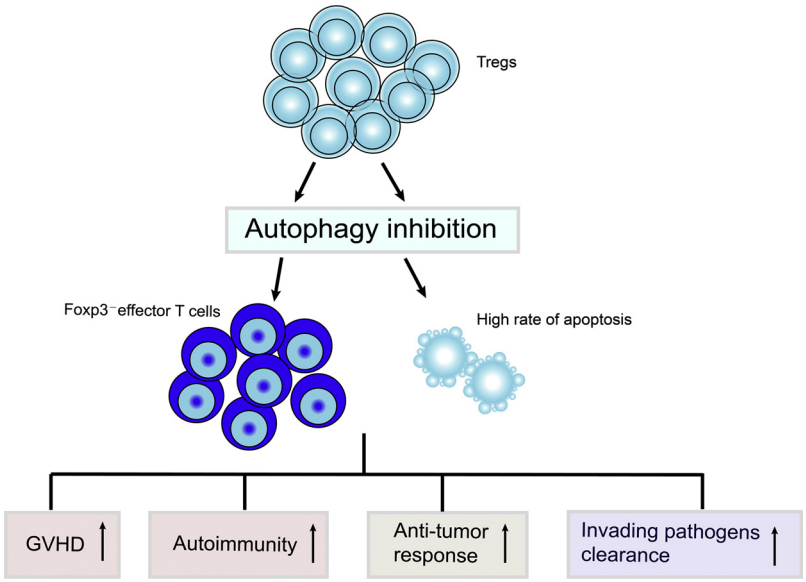

Fig. 3. The impact of autophagy on Tregs and their contribution to various diseases. Depletion of one of the essential autophagic genes, or inhibition of autophagic process would lead to impaired stability and functional integrity or loss of Tregs along with enhanced GVHD, higher anti-tumor immunity, as well as the development of inflammatory disorders and autoimmune diseases.

effector T cell function (Stoop et al., 2005). Unfortunately, it is currently unknown how Tregs maintain their anti-inflammatory features in response to multiple pro-inflammatory stimuli in the setting of chronic HBV infection. In a recent study, Cheng and colleagues measured spontaneous and induced autophagy of peripheral Tregs from 98 patients with chronic HBV infection, by which they assessed the levels of lipidated form of LC3-II (a marker for closed autophagosomes), and autophagic vacuoles with transmission electron microscope (TEM) (Cheng et al., 2017). Although no significant difference was found in spontaneous autophagy of either Tregs or $CD4^+$ naive cells as compared to healthy subjects, Tregs originated from chronic hepatitis B (CHB) patients displayed significantly higher autophagic activity following anti-CD3/CD28 stimulation. Since induced autophagy could maintain cell survival and functional stability of Tregs from CHB patients, this discovery might open new perspective in developing therapeutic strategies to activate specific anti-HBV immunity by diminishing autophagy in Tregs (Stoop et al., 2005).

8. Conclusion remarks

Autophagy is an evolutionarily conserved cellular process characterized by the formation of a double-membrane vesicle around invading intracellular bacteria or cellular cytoplasmic materials for efficient degradation (Choi et al., 2018). It typically occurs in response to stress or starvation in order to reutilize essential cellular proteins and amino acids (Geremia et al., 2014). Altered autophagy has been characterized in neurodegenerative diseases, aging, tumor, immune disorders and infectious diseases (Deretic et al., 2013). Upon the recognition of its role in immune system, autophagy was noted to be active in lymphocytes and almost involved in every aspect of their life cycle. Particularly, autophagy inhibition would render $Foxp3^+$ Tregs to be $Foxp3^-$ effector T cells along with enhanced apoptosis of $Foxp3^+$ Tregs. Therefore, autophagy inhibition generally is associated with enhanced GVHD, and allograft rejection, higher anti-tumor immunity, and increased risk for the development of autoimmune diseases (Kabat et al., 2016a; Wei et al., 2016) (Fig. 3). Given the fact that the functionality of other subset of T cells can also be affected by the net impact of global autophagy inhibition, targeted regulation of Treg autophagy would be essential to steer immune responses in a desired direction. For example, nanoparticles targeting characteristic surface molecules of Tregs such as CD25, CTLA-4, and GITR may provide the feasibility for delivery of drugs to Tregs in a relatively specific manner. Therefore, autophagy could be a promising target to manipulate Treg function in different disease settings. Nevertheless, the current data on T cell autophagy are mainly obtained from mouse models whose T cell subsets are deficient in one of the specific Atgs. Thus, more human related data are still wanting before translate above discoveries into clinical settings, particularly for those data in assessing the impact of autophagy on human T cell subsets in diseased conditions.

Declaration of interest

The authors declare that no competing interests exist.

Acknowledgments

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