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Autophagy and the immune function in aging

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Abstract

Just when you thought that you had heard it all about autophagy - the conserved cellular process that mediates turnover of cellular constituents in the lysosomes - studies keep coming out highlighting new types of autophagy, new functions for autophagy or even new autophagy-independent roles for the proteins associated with this process. The field of immunology has been riding the autophagic wave since the beginning of its revival; first due to its role in the host defense against pathogens, and more recently through the better understanding of the unique characteristics and functions of different autophagic pathways in immune cells. Here, we describe some of these new functions that are tightening the connection between autophagy and acquired or innate immunity and their malfunctioning with age.

Introduction

The identification of the molecular players that participate in autophagy, the process that mediates the degradation cellular components in lysosomes, and the numerous associations established between autophagy malfunctioning and human disease – including those of immune origin– were a major push for the revival of autophagy in the late 90's [1]. However, autophagy is far from fading in the scientific stardom, and it keeps instead emerging as hot topic because of its multifunctional nature and reciprocal functional interactions with important cellular processes [2]. Immunology has not been an exception to this extended autophagy honeymoon. Immunologist became initially interested in the role of autophagy in the cellular defense against pathogens [3] (xenophagy, see Box 1). Soon it became evident that the interplay between autophagy and innate immunity was more complex, which made it necessary to analyze specialized functions of autophagy in different

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cell populations of the immune system [4]. Current research in innate and adaptive immunity has also embraced some of the newly elucidated roles for autophagy, such as its contribution to the cellular energetic balance, remodeling of the proteome or unconventional secretion [5-7]. In some instances, research in Immunology has been the driving force in new discoveries in autophagy. For example, Crohn's disease was one of the first human diseases linked to mutations in an autophagy gene [8], and studies on phagocytosis discovered new autophagy-independent functions of autophagy related proteins (Atg) [9]. Here, we review recent findings that have kept autophagy in the headline of Immunology news and comment on the implications of the newly identified roles of autophagy in the regulation of the adaptive immune response and its decline with age.

Box 1

Autophagic pathways: the basics

Three basic autophagic pathways co-exist in mammals:

Macroautophagy: the gene products (Atg proteins) that participate in macroautophagy act sequentially in the assembly of lipid and proteins from different sources [10] to form a double membrane around cargo identified by receptor proteins that sequesters it from the cytosol. These double membrane vesicles (autophagosomes) use the microtubule tracks to encounter and fuse with lysosomes, where luminal hydrolases degrade the cargo [1].

Macroautophagy can occur “in bulk” – when cargo is sequestered in a random manner – or selectively – when the cargo is identified through interactions between cargo-receptors and structural components of the autophagosome. Examples of selective macroautophagy include mitophagy (mitochondria), lipophagy (lipid droplets), ribophagy (ribosomes), aggregophagy (aggresomes) or xenophagy (extracellular pathogens). In the case of xenophagy [3], the endocytic or phagocytic vesicles used for pathogen internalization rather than fusing with lysosomes are identified in the cytosol by the autophagy machinery that delivers them to lysosomes. Other pathogens find their way out of the internalizing phagocytic vesicles into the cytosol where they become autophagy substrates.

Microautophagy: Cargo sequestration in vesicles is also the first step in microautophagy, but in this case, vesicles form from the invagination of the limiting membrane of lysosomes (in yeast microautophagy [12]) or late endosomes (in endosomal microautophagy [15]). These cargo-containing vesicles then pinch off into the lumen for degradation (Fig. 1).

Chaperone-mediated autophagy (CMA): In CMA, client proteins are selectively identified by a cytosolic chaperone that delivers them to the lysosomal membrane [11]. Once bound to the integral lysosomal membrane protein (LAMP-2A), the substrate protein unfolds and reaches the lumen through a LAMP-2A-enriched translocation complex.

Autophagy: what is new?

Analysis of the way in which autophagic cargo is delivered to the lysosomes has revealed the co-existence in most mammalian cells of three different autophagic pathways (Fig. 1): macroautophagy, microautophagy and chaperone-mediated autophagy (CMA). The steps, effectors and regulators of these pathways have been extensively reviewed elsewhere and are also summarized in Box 1 [1,2,10-12].

New themes on autophagy mechanisms

Several recent findings in the autophagy field have gotten considerable attention. One of them is the existence of shared components among autophagic pathways which could underlie the basis of the compensatory activation of one autophagic pathway when another fails [13]. For example, hsc70 targets cytosolic proteins to CMA, endosomal microautophagy or macroautophagy depending on their folding state [14,15]. A second interesting finding is the fact that macroautophagy can proceed without some of the Atg proteins previously considered essential (i.e. Beclin, Atg5 or Atg7) [16,17]. The challenge now is to determine whether these unconventional variants are alternatives to canonical autophagy or if they coexist. Also worth mentioning is the now well-accepted idea that regulation of autophagy is not linear but that instead inputs from multiple pathways converge and often co-modulate autophagy [18]. Lastly, the recognition that autophagy-related proteins have functions independent of the autophagic process is also gaining momentum [7].

Autophagy functions

The roles of autophagy in cellular quality control and cellular defense have been actively researched but some of the “old” functions have also been revisited. For example, although the early connections between autophagy and the cell’s energetics were focused on the contribution of autophagy to the replenishment of the intracellular pool of free amino acids through protein breakdown, recent studies support that macroautophagy also contributes to sustain a positive energetic cellular balance through mobilization of intracellular lipid [6] and glycogen stores [19]. CMA also contributes to the regulation of cellular energetics by selectively degrading key enzymes involved in essential metabolic pathways [20]. The interplay between autophagy and cell death has also been revised in recent years, resulting in a stronger support for autophagy as a pro-survival mechanism, except for very specific scenarios mostly in invertebrate models. The early labeling of autophagy as a programmed type of cell death originated from the observation of autophagosomes in dying cells. However, later studies demonstrated that higher autophagosome content was often not a signature of increased autophagy but rather reflected the cell’s inability to degrade them, and therefore an expression of incomplete autophagy. These studies of the new and revisited functions of autophagy have also made an impact on our current understanding of the role of autophagy in the immune response.

Autophagy and the adaptive immune response

Studies in recent years have firmly established macroautophagy as a crucial component of the innate immune response [4]. Macroautophagy aids in the elimination of pathogens, but its role extends beyond that to include delivery of endogenous or exogenous molecules to intracellular compartments, modulation of the activity of the inflammasome, control of cytokine secretion and regulation of phagocytosis [21-25]. Though most of these functions are a consequence of the activation of conventional autophagy, non-autophagy functions of Atg proteins, such as LC3-associated-phagocytosis, also participate in the orchestration of the innate immune response [9,22]. The complex circuitries that regulate autophagy in macrophages and other phagocytic cells and the full spectrum of the functions regulated by autophagy in those cells have been the object of comprehensive recent reviews [4]. Here, we focus in the less well-studied functions that different forms of autophagy exert to modulate the adaptive immune response.

Autophagy and antigen presentation

Autophagy has emerged as an important mechanism of cross-delivery of intracellular pathogen's antigens to MHC-class II loading compartments (Fig. 2) [26-28]. Mice with macroautophagy-deficient macrophages become more susceptible to *Mycobacteria* infection and display increased bacterial burden, which has been attributed to defective pathogen killing and unregulated inflammatory response [29,30]. However, it is also likely that inefficient antigen presentation prevents adequate T cell activation and contributes to the deficient global response to *Mycobacteria*. Several recent findings underscore the contribution of macroautophagy to the development of an efficient adaptive immune response *in vivo*. For example, the protective efficiency of yellow fever vaccination depends on the ability of the virus to induce autophagy in dendritic cells [31], and likewise, chemical activation of autophagy potentiates immunization with BCG [32]. Furthermore, although the association of mutations in Atg16L or in NOD2 with inflammatory bowel disease has been explained on the basis of defective function of Paneth and goblet cells in the intestinal mucosa [8,33], it has also been reported that NOD2 activation regulates macroautophagy-mediated presentation of bacterial antigens and that, interestingly, dendritic cells from Crohn's disease patients with NOD2 mutations fail to upregulate MHC-II presentation in response to NOD2 ligands [34].

Beside pathogens, self-proteins can also be delivered through macroautophagy for MHC-II loading, implicating this pathway in the regulation of tolerance. In this respect, preferential involvement of autophagy in the presentation of citrullinated peptides, frequently associated with autoimmunity, has been proposed, as the presentation of this peptides was inhibited by silencing *Atg5* in B lymphoma cells [35]. The involvement of macroautophagy in the regulation of tolerance is not restricted to peripheral presentation of self-peptides but it extends to regulate thymocyte selection. Thymic epithelial cells use macroautophagy to present self-peptides and shape the TCR repertoire. Interestingly, nude mice receiving an *Atg5*-deficient thymic graft develop autoimmunity, supporting that macroautophagy participates also in negative selection [36]. Recent analyses suggest that autophagy becomes increasingly important to mediate thymocyte deletion when concentrations of antigen are

low, which is likely the case for many tissue-restricted genes expressed in thymic epithelial cells [37].

It is not only macroautophagy that regulates antigen presentation (Fig. 2). Silencing of LAMP-2A, the CMA receptor at the lysosomal membrane, decreases loading of a constitutively expressed cytosolic protein in B lymphoblastoid cells [38]. Moreover, delivery of cytosolic proteins to late endosomal compartments in a manner that resembles microautophagy occurs in dendritic cells [15]. Cytosolic proteins are recognized by hsc70 that then binds to specific lipids in the endosomal membrane. Proteins eventually access the endosome lumen in small vesicles that form by invagination of the endosome membrane in a process mediated by SCRT complex proteins [15].

Autophagy and lymphocyte function

T cells display basal autophagy that controls organelle homeostasis [39,40]. Consequently, T cells deficient in essential Atg proteins accumulate defective mitochondria and present higher levels of ROS and increased cell death [40]. Defective mitochondrial homeostasis likely underlies the reduced number of peripheral T cells observed in mice bearing Atg-deficient T cells or those with a T cell-specific deletion of Vps34, a PI3 kinase class III required for autophagy initiation [41-43]. Interestingly, old mice with Vps34-deficient T cells develop intestinal inflammation that correlates with decreased suppressive function of regulatory T cells [41]. How the absence of Vps34 affects regulatory T cell development or function and whether macroautophagy plays a role in these processes remains unknown.

Macroautophagy is also activated in response to TCR engagement (Fig. 2), and cells with defective macroautophagy respond poorly to activation and show reduced levels of proliferation and cytokine production [42,44]. The specific roles of activation-induced macroautophagy in T cells and the signaling pathways that regulate it are not yet fully characterized, but it is likely that basal and activation-induced autophagy might serve different purposes. For example, autophagosome cargo shifts from mainly organelles to mostly soluble cytosolic components as cells transition from a resting to an activated state [44]. Furthermore, two specific roles for macroautophagy in activated T cells have been described: providing substrates to adapt to an increased metabolic demand; and modulating NF κ B activation through the p62-mediated selective degradation of Bcl10 (Fig. 2) [44,45].

Less is known about the role of autophagy in B cells. Studies using bone marrow chimeras showed that Atg5-deficiency led to reduced numbers of peripheral B cells, with specific defects in B1 populations and partial blockage of pro-B to pre-B cell differentiation [46]. Autophagy has also been shown to be necessary to fulfill the metabolic demands of activated plasma cells and ensure survival of short- and long-lived plasma cells to maintain an efficient antibody response *in vivo* [47].

Autophagy and aging

Malfunctioning of macroautophagy and CMA occurs in many organs and tissues with age [48,49]. Activation of macroautophagy has proven necessary for the extension of life-span observed in worms and flies after specific mutations or caloric restriction [50,51].

Macroautophagy is also necessary to sustain normal life-span, as mice with compromised autophagy in individual systems all show shorter life-span. More excitingly mice overexpressing one of the essential Atg proteins display a noticeable increase in lifespan [52]; and genetic correction of the age-dependent decline on CMA in liver has proven sufficient to prevent the functional decline of this organ in old rodents [53]. Interestingly, most interventions that improve life- and health-span, such as caloric restriction, or treatment with rapamycin, resveratrol, spermidine or metformin, also activate autophagy [54].

The mechanisms behind the autophagic malfunctioning in aging are still poorly understood (Fig. 3). For CMA, reduced stability of the lysosomal receptor for this pathway is responsible for the CMA decline in many organs [48]. In the case of macroautophagy, changes in Atg proteins at the transcriptional or protein level have been reported, but whether they are the primary defect behind the age-dependent failure requires additional clarification [54]. Besides primary defects, changes in autophagy with age can also be reactive to disease conditions, where autophagy may be activated to assure cell survival. However, this initial compensatory activation may be followed by failure of this pathway as the disease condition becomes chronic.

Malfunctioning of autophagy in old organism could contribute to loss of cellular function and often to cell death by limiting the cell's ability to sustain a healthy proteome and organelles, making them more vulnerable to cellular stressors and pathogens, and rendering them incapable to adapt to energetically demanding conditions (Fig. 3) [54].

Dysfunction of the autophagic systems may also be behind some cell- and tissue-specific functions that fail with age. Decreased autophagy in phagocytic cells may not only impair their ability to kill pathogens but also cause dysregulated activation of the inflammasome due to deficient mitophagy, with the subsequent increase in the production of inflammatory cytokines, likely contributing to the age-associated inflammaging phenotype. Indeed, activation of the NLPR3 inflammasome responds to mitochondrial ROS production and the release of oxidized mitochondrial DNA [55,56], and macroautophagy has been shown to play an essential role in regulating this process [57,58].

It was recently reported that CD8⁺ T cells isolated from old healthy humans presented significantly lower levels of basal macroautophagy [59]. How this dysfunction affects T cell responses and the mechanisms that may account for the reduced autophagy in T cells with age are still unknown. However, one possible mechanism might involve deficient mitophagy by analogy to reports of reduced mitophagy with age in other cell types [60]. It would be then expected that decreased mitophagy would result in ROS accumulation, leading to altered T cell responses and increased susceptibility to stress. In addition, the T cell energetic balance may also be compromised as a result of the autophagic failure with age. In fact, a recent study found that T cells isolated from patients with rheumatoid arthritis, which usually present characteristics of premature aging, failed to properly activate autophagy and were unable to utilize internal cell energy sources [61].

Although little is still known on how age affects autophagy in dendritic cells, a recent study reported that oxidized proteins accumulate in the endosomal compartment of dendritic cells from old mice, which correlates with decreased macroautophagy in those cells (Fig. 3). As a consequence, aged dendritic cells cannot efficiently present antigen and activate T cells [62]. Interestingly, *in vivo* administration of an antioxidant was able to restore the ability of aged mice to present antigen and elicit T cell responses, suggesting that therapeutic interventions aimed at restoring autophagy or palliating the effects of decreased autophagy with age might be a valid approach to improve immune function in the elderly [62].

Concluding remarks and a promising future

The challenge in the study of autophagy in immune cells originates in part by the fact that conventional regulatory mechanisms do not always apply, and that the autophagic process often fulfills very specific needs different from other organs and systems. The recent emphasis in “exceptions” in the autophagic process, the identification of novel autophagic functions and the better characterization of different autophagic pathways has provided a fertile ground for the future expansion of research on autophagy in immunity. Future efforts should be directed at elucidating how each autophagic pathway regulates specific processes, such as antigen presentation, cell activation or memory generation.

Autophagy is being flagged as an anti-aging mechanism and the growing number of successful attempts to improve health-span through modulation of autophagy has provided momentum to the search for more effective and specific chemical modulators of autophagy. A better characterization of the changes with age in autophagic pathways in immune cells, their time- course and whether the mechanisms behind this failure are universal or cell-type specific are needed before novel autophagic modulators can be considered for use in the prevention of immunosenescence or the restoration of the immune function in aging.

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Highlights

- Different forms of autophagy play key roles regulating innate and adaptive immunity
- New functions of autophagy are reshaping the understanding of how autophagy modulates immunity
- An age-associated dysregulation of autophagy in immune cells may underlie immunosenescence

Figure 1. Schematic of the main autophagic pathways in mammalian cells

Macroautophagy and chaperone-mediated autophagy deliver cytosolic cargo directly to lysosomes for degradation. In the case of endosomal microautophagy, cytosolic proteins are internalized in single membrane vesicles into late endosomes and undergo degradation in this compartment or in lysosomes upon endosome/lysosome fusion.

Figure 2. Autophagic functions in adaptive immunity

In dendritic cells, cytosolic proteins or those derived from intracellular pathogens can be delivered to be loaded into MHC-II through macroautophagy, CMA or endosomal microautophagy (e-microautophagy) to activate T cells. Whereas basal macroautophagy in T cells regulates organelle homeostasis (including mitochondria and endoplasmic reticulum), activation-induced macroautophagy in response to TCR engagement degrades cytosolic components to respond to increased metabolic demand and to modulate specific signaling pathways.

Figure 3. Age-related alteration in autophagy and its cellular consequences

The scheme depicted the most common mechanisms that contributed to malfunctioning of macroautophagy and chaperone-mediated autophagy with age. Boxes on the right summarized the main consequences that could follow autophagic malfunctioning with age separating those common to all cells (top) from those specific for immune cells (bottom).