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Autophagy, nutrition and immunology

Ana Maria Cuervo^{a,c} and Fernando Macian^{b,c}

^aDepartment of Developmental and Molecular Biology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

^bDepartment of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA

^cInstitute for Aging Studies, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Abstract

Turnover of cellular components in lysosomes or autophagy is an essential mechanism for cellular quality control. Added to this cleaning role, autophagy has recently been shown to participate in the dynamic interaction of cells with the surrounding environment by acting as a point of integration of extracellular cues. In this review, we focus on the relationship between autophagy and two types of environmental factors: nutrients and pathogens. We describe their direct effect on autophagy and discuss how the autophagic reaction to these stimuli allows cells to accommodate the requirements of the cellular response to stress, including those specific to the immune responses.

Keywords

aging; antigen presentation; chaperones; lipids; lysosomes; starvation; T-cells

1. Introduction

Autophagy is the cellular process by which lysosomes contribute to the degradation of intracellular components, including proteins, organelles and even pathogens that reach the cytosol after cell invasion (Mizushima et al. 2008). The field of autophagy has undergone rapid expansion in recent years, due for the most part, to a better molecular characterization of the different steps of this process, which include cargo-recognition, sequestration from the cytosol, delivery to lysosomes, degradation and recycling of the essential components of the macromolecules degraded (Yang and Klionsky 2010a). Taking into account all types of autophagic pathways, about 40-45 genes have been identified to participate in each of the steps of this process (Kaushik et al. 2011a; Sahu et al. 2011; Yang and Klionsky 2010b). The possibility of manipulating these genes to upregulate or downregulate autophagy *in vivo* has broadened the spectrum of physiological functions attributed to this pathway and has

Correspondence: Ana Maria Cuervo, MD PhD, Dept. of Developmental and Molecular Biology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, Phone: (718) 430 2689, Fax: (718) 430 8567, ana-maria.cuervo@einstein.yu.edu; Fernando Macian-Juan, MD PhD, Dept. of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, Phone: (718) 430 2630, Fax: (718) 430 8567, fernando.macian@einstein.yu.edu.

helped identify previously unknown connections between autophagy malfunction and a growing number of human diseases (Mizushima et al. 2008).

Many studies have underlined the contribution of autophagy to cellular quality control through the removal and degradation of damaged intracellular components (Hara et al. 2006; Komatsu 2005; Rubinsztein et al. 2005). In fact, failure to perform this cellular surveillance as a result of impaired autophagic function seems to underlie the basis of common protein conformational disorders, including severe neurodegenerative disorders and myopathies (Wong and Cuervo 2010). Additional functions recently added to the physiology of autophagy include, among others, cellular remodeling and tissue differentiation (Mizushima and Levine 2010), development (Di Bartolomeo et al. 2010), cellular survival and response to stress (Wang and Levine 2010), cell death (Scarlatti et al. 2009), senescence, and even an anti-aging function based on the need for autophagy to attain longevity (Hansen et al. 2008; Melendez et al. 2003). Readers are directed to recent reviews on these and other functions of autophagy (Mizushima and Levine 2010; Mizushima et al. 2008; Wong and Cuervo 2010). In this review, instead, we focus on two additional functions of autophagy: the contribution of autophagy to the regulation of the cellular energetic balance - an old function recently revitalized and expanded - and a relatively new role of autophagy in innate and adaptive immunity. We summarize recent advances pertinent to these two autophagic functions and comment on the possible interrelation between them and their implications for human health and disease.

2. Autophagy, much more than cellular cleaning

Cytosolic cargo is recognized and targeted for autophagy through different mechanisms that set the basis for the distinction of several types of autophagic pathways. The three best characterized forms of autophagy include macroautophagy, microautophagy and chaperone-mediated autophagy (Fig. 1). However, variations of each of these processes have now been described that differ in whether the cargo is recognized in a selective manner or in bulk, and in the subset of molecules that assist in the autophagic process as depicted in Fig. 1. When macroautophagy is activated, a membrane forms *de novo* in the cytosol, and as it grows and seals, encloses cytosolic cargo inside a double membrane vesicle known as the autophagosome (Mizushima et al. 2001). Lysosomes then fuse with these vesicles and infuse the hydrolases (proteases, lipases, glycosidases and DNases) required for the complete degradation of cargo. Lipids and proteins from other cellular membranes are shuttled to the site of autophagosome formation and contribute to membrane growth through their coordinated assembly (Geng and Klionsky 2010; Mizushima et al. 1998; Yen et al. 2010).

2.1. Macroautophagy

Macroautophagy is activated in response to a number of different stressors with a pro-survival function (Mizushima et al. 2008). In addition, some level of basal macroautophagy also exists in almost all cell types and contributes to the maintenance of cellular homeostasis (Hara et al. 2006; Komatsu 2005; Raben et al. 2008). Both basal and inducible autophagy utilize the same set of genes and proteins for the formation of autophagosomes, but also have autophagy type-specific components and are subjected to different forms of regulation (Lee et al. 2010; Yamamoto et al. 2006). Macroautophagy was initially described as a form

of indiscriminate degradation by which cytosolic substrates are degraded “in bulk”. However, although this may still be true for soluble cytosolic proteins that are trapped along with other cargo as the autophagosomes form, selective recognition occurs in the case of organelles and particles (aggregates and pathogens) (Dikic et al. 2010; Kirkin et al. 2009; Tolkovsky 2009; Yokota and Dariush Fahimi 2009). The bases for this specificity are not completely defined yet. However, for many substrates, specific features or components on the surface of the organelle or particle serve as docking points for what are known as cargo recognition molecules, which bring along the autophagic machinery leading to the formation of the autophagosome membrane around the specific cargo. Molecules such as p62 or NBR1 have been recently described as cargo recognition proteins, which bind both to the cargo (often through ubiquitinated residues) and to Atg8/LC3, an essential component of the autophagosome membrane (Lamark et al. 2009). Selective macroautophagy has given rise to names such as mitophagy, ribophagy, ER-phagy and aggrephagy, to refer to the selective degradation of these components by macroautophagy (Dikic et al. 2010; Kirkin et al. 2009; Tolkovsky 2009; Yokota and Dariush Fahimi 2009) (Fig. 1).

2.2. Microautophagy

In bulk and selective degradation also takes place via microautophagy, but in this case sequestration of cargo occurs directly at the surface of the lysosomes. Invaginations of the lysosomal membrane containing the cargo pinch off as vesicles into the lumen where they are rapidly degraded (Ahlberg and Glaumann 1985). In yeast, microautophagy occurs at the vacuole (equivalent to the lysosome) and makes use of some macroautophagy genes and some microautophagy-specific genes (Yuan et al. 1997). Although this process is still poorly characterized in mammals, recent studies place microautophagy in late endosomes, where it makes use of the machinery previously described to be required for the formation of multivesicular bodies (Sahu et al. 2011) (Fig. 1). Substrate can be sequestered by the forming vesicles “in bulk” or in a selective manner following interaction with the membrane-bound chaperone hsc70, the constitutive member of the hsp70 family of chaperones (Sahu et al. 2011). Microautophagy activity can be detected under basal conditions in many cell types, but there is currently no information as to whether this pathway can be further upregulated under specific cellular conditions.

2.3. Chaperone-mediated autophagy

The intrinsic characteristics of the third type of autophagy, chaperone-mediated autophagy (CMA), make this pathway to mediate selective degradation of soluble proteins exclusively (Cuervo 2010; Kaushik et al. 2011a). Substrates for this pathway are cytosolic proteins that contain in their amino acid sequence a pentapeptide motif that is recognized by the cytosolic form of hsc70 (Dice 1990) (Fig. 1). Binding of the chaperone mediates delivery of the substrate protein to a receptor at the lysosomal membrane, the lysosome-associated membrane protein type 2A (LAMP-2A) (Cuervo and Dice 1996). Interaction of the substrate with LAMP-2A promotes the multimerization of this single-span membrane protein into a higher order complex required for translocation of substrates across the lysosomal membrane (Bandyopadhyay et al. 2008). A form of hsc70 resident in the lysosomal lumen assists in the translocation of the substrate that is then rapidly degraded in the lumen (Agarraberes et al. 1997). Organelles or particulate structures cannot be CMA substrates,

due to the fact that CMA requires substrates to directly cross the lysosomal membrane. Basal CMA activity occurs in all cells but it can be further upregulated in response to several stressors including prolonged starvation and conditions leading to protein damage, such as oxidative stress (Kiffin et al. 2004).. CMA activity directly depends on the levels of LAMP-2A at the lysosomal membrane, and in fact, much of the regulation of this pathway is directly linked to the dynamics of this protein at the lysosomal membrane (Cuervo and Dice 2000b). About 30% of cytosolic proteins bear the CMA-targeting motif in their sequence, which makes them putative CMA substrates. However, degradation through this pathway depends on the accessibility this motif to hsc70, suggesting that conformational changes in the substrate protein, posttranslational modifications or changes in interacting proteins that usually mask the motif, could be triggers for CMA degradation.

Although the different autophagic pathways are not redundant and each fulfills specific cellular needs, they often function in an interconnected manner. Thus, cells respond to blockage in one of these pathways by upregulating the others, and this compensation often ensures cell survival in those compromised conditions (Kaushik et al. 2008; Massey et al. 2006). The cross-talk among autophagic pathways could have important implications in pathologies associated with a primary defect in one of these pathways.

3. Autophagy and cellular metabolism

3.1. Autophagy in the cellular response to nutritional deprivation

The discovery of the connections between autophagy and the cellular nutritional status goes back to the early days of autophagy in the 1970s. Starvation was the first stimulus described to activate macroautophagy (Dice et al. 1978; Mortimore and Poso 1987). Likewise, removal of serum from the media in cultured cells or prolonged starvation (>10h) in animals are also the best characterized stimuli for CMA and the ones that led to the discovery of this autophagic pathway (Berger and Dice 1985; Cuervo et al. 1995). In both cases, activation of autophagy would be beneficial for the energetic balance, because the recycling of amino acids resulting from protein breakdown in lysosomes serves to sustain protein synthesis even in the absence of amino acids coming from the extracellular environment through the diet (Singh and Cuervo 2011). In addition, these amino acids could also be utilized directly to obtain ATP through their entry at different steps of the Krebs cycle (Fig. 2).

Early work, done for the most part in rodents, demonstrated that the availability of nutrients and the hormonal changes associated to food intake regulate autophagic activity in liver (Mortimore 1970). Analysis of the rates of lysosome-mediated protein degradation *in vivo* revealed that glucagon exerts a stimulatory effect over macroautophagy, whereas insulin is a potent inhibitor of this pathway (Mortimore 1970). Levels of circulating amino acids also modulate macroautophagy, although in this case the effect is cell-type dependent, since amino acids that exert a potent inhibition on hepatocyte macroautophagy have less effect, for example in fibroblasts (Fuertes et al. 2003; Mortimore and Pösö 1984). The reasons for these differences remain unknown.

A vast body of studies supports that communication between the nutritional status and autophagy is mediated by intracellular nutrient sensors (Singh and Cuervo 2011). Among

these, the mammalian target of rapamycin (mTOR) has proven to be a major player in the regulation of macroautophagy. This kinase integrates nutritional cues (amino acids, insulin, ATP levels) and actively suppresses macroautophagy under normal nutritional conditions (Kanazawa et al. 2004). In the case of yeast, TOR directly phosphorylates one of the proteins essential for autophagy (Atg13) preventing it from interacting with other Atg proteins to initiate autophagosome formation (Huang and Klionsky 2002). In mammals, recent studies have shown a direct interaction between mTOR and components of the autophagic machinery, whereby mTOR directly sequesters the autophagy initiation complex away from the sites of autophagosome formation (Ganley et al. 2009; Hosokawa et al. 2009). Low levels of nutrients and insulin inactivate mTOR through the action of another major cellular nutrient sensor, the AMP-activated protein kinase (AMPK). Inactivation of mTOR then leads to the release of the autophagy initiation complex and the subsequent formation of autophagosomes (Singh and Cuervo 2011). Recent studies also support the existence of a type of mTOR-independent macroautophagy that can be activated even under normal nutritional conditions. This overwriting of the mTOR regulation occurs, for example, when the purpose of autophagic activation is the removal of abnormal or damaged cellular components rather than to provide building blocks for synthetic cellular activities (Yamamoto et al. 2006).

The signaling mechanisms that modulate CMA activation in response to nutritional stress remain unknown. It was proposed that ketone bodies, generated during the typical longer periods of starvation required for CMA activation, could act as signaling molecules for CMA. However, experimental testing revealed that the higher rates of CMA activity observed upon exposure to ketone bodies did not originate from their effect on CMA effectors but rather it was a result of the oxidative effect of these by-products on the CMA substrates (Finn and Dice 2005). Previous studies had indeed shown that oxidation of proteins bearing the CMA-targeting motif enhanced their degradation through this pathway (Kiffin et al. 2004).

In most cells, activation of macroautophagy and CMA during nutritional deprivation occurs in a sequential manner (Massey et al. 2006). Proteolysis resulting from macroautophagy activation reaches a peak after 4-6h of starvation and then gradually decreases as CMA activity becomes upregulated. Maximal CMA activation is attained at 24h into starvation and it remains at this level of activity for up to 3 days of starvation. The higher selectivity of CMA in the targeting of cytosolic proteins for degradation could be the reason behind the autophagic switch, as this would permit the utilization of non-essential proteins as a source of amino acids while preserving essential ones.

3.2. Autophagy as an alternative source for cellular energy

Although the amino acids resulting from autophagic protein breakdown could be utilized to generate ATP by feeding the Krebs cycle, the energetic balance of this conversion is relatively poor. Lipids, in contrast, are a more favorable source of energy and in fact, mobilization of intracellular lipid stores is utilized by cells under conditions of nutritional deprivation (Singh and Cuervo 2011).. Recent studies have demonstrated that macroautophagy contributes to the breakdown of these lipid stores or lipid droplets thus

providing free fatty acids (FFA) that, upon leaving the lysosomal lumen, can be utilized to generate energy through mitochondrial β -oxidation (Singh et al. 2009) (Fig. 2)

This new type of autophagy, named macrolipophagy, is constitutively active at low levels in most cell types and it contributes to control the size and number of lipid droplets under basal conditions. Many cells activate this pathway when nutrients are scarce to obtain energy through the mobilization of intracellular lipid stores (Singh et al. 2009). Activation of macrolipophagy also occurs in cells when exposed to lipid challenges to prevent massive accumulation of intracellular lipids. For example, liver macrolipophagy is upregulated during starvation to accommodate the affluence of lipids into the blood stream from the lipolysis that takes place in the adipose tissue (Singh et al. 2009). Likewise, acute exposure to a high dietary lipid load (i.e. cholesterol or high-fat enriched diets) also activates macrolipophagy (Singh et al. 2009). However, when the lipid challenge persists for a long time or the intracellular lipid content reaches a particular threshold, macrolipophagy activity becomes compromised (Koga et al. 2010) (Fig. 2). The increase in the cholesterol content of the membranes of autophagosomes and lysosomes under these conditions decreases their fusion efficiency, slowing down the autophagic flux (Koga et al. 2010). The failure to control lipid content by mobilization through macrolipophagy could underlie the basis for lipotoxicity in common metabolic disorders such as the metabolic syndrome. Furthermore, changes in the lipid composition of the lysosomal membrane as a result of high intracellular lipid content would also have a negative impact on other forms of autophagy. For example, lateral mobility at the lysosomal membrane is essential for proper CMA function, since the formation of the translocation complex occurs through dynamic assembling of monomeric forms of LAMP-2A at the lysosomal membrane (Bandyopadhyay et al. 2008). An increase in membrane cholesterol through experimental manipulations has proven inhibitory on CMA (Kaushik et al. 2006). It is anticipated that lipid challenges caused by high-fat diets could have a similar inhibitory effect on CMA *in vivo*.

The discovery of macrolipophagy and the sensitivity of this process to circulating lipids confer FFA a new regulatory role in cellular catabolism. In fact, recent studies support that the stimulatory effect of circulating FFA on autophagy underlies the basis of nutrient sensing and appetite control by hypothalamic neurons (Kaushik et al. 2011b). The increase in circulating FFA during starvation stimulates macroautophagy in the hypothalamic neurons that secrete the orexigenic agouti-related peptide (AgRP). Mobilization of lipid stores by macroautophagy in these neurons stimulates production and secretion of this “hunger” peptide that elicits food-seeking behaviors to restore the cellular energetic balance.

After the discovery that autophagy can contribute metabolites other than amino acids through the catabolism of cytosolic components, new studies have begun addressing the possible involvement of this pathway in the mobilization of other energy stores. In this respect, macroautophagy and microautophagy have been shown capable of breaking down glycogen (Kotoulas et al. 2006; Raben et al. 2008) (Fig. 2). Macro- and micro-glycophagy provides glucose during nutrient deprivation, but determining the extent of its contribution compared to the traditional cytosolic degradation of glycogen will require future investigation.

The regulatory roles that circulating macromolecules (FFA, amino acids and sugars) exert on macroautophagy, underlines the importance that nutrients could have in the control of the multiple cellular processes depending on this catabolic pathway. In the following sections, we focus on one of these functions, the regulation of the immune response, and discuss the possibility that autophagy could become an important downstream effector in the growing field of nutritional immunology.

4. Autophagy and the immune response

Helped by the recent advances in the molecular characterization of the different autophagic pathways, research developed in the last few years has revealed that autophagy also plays crucial roles in the regulation of the immune system. Specific forms of autophagy control key aspects of the innate and adaptive responses, from pathogen destruction to antigen presentation and lymphocyte activation (Fig. 3).

Similar to its well characterized role in organelle homeostasis, macroautophagy can also capture and kill pathogens (Deretic 2011). This form of autophagy is well suited to target intracellular pathogens for degradation. The ability of phagocytic cells to use macroautophagy to kill intracellular pathogens has been amply documented, including not only bacteria, such as group A streptococcus or mycobacteria, but also viruses and parasites (Andrade et al. 2006; Nakagawa et al. 2004; Singh et al. 2006). Macroautophagy may provide the cells with alternative ways to overcome some of the mechanisms that pathogens develop to evade killing. For instance, in macrophages infected with *Mycobacterium*, macroautophagy-mediated killing would allow the infected cell to bypass the inhibition of phagosome fusion to the lysosome that is imposed by this pathogen (Gutierrez et al. 2004). Macroautophagy can, however, not only trap pathogens that have escaped phagocytic or endocytic vesicles but also contribute to the degradation and killing of pathogens still contained in membrane vesicles (Andrade et al. 2006). It is only in the last couple of years that we have begun to learn how pathogens can be specifically targeted for degradation through macroautophagy. Although differences may exist between the regulation of this form of autophagy, which has been termed xenophagy, and other target-specific forms of autophagy, some of the adaptors that direct specific delivery of organelles also appear to be involved in the sequestration of pathogens into autophagic vacuoles. Three of these adaptor proteins, p62, NDP52 and optineurin have recently been shown to bind ubiquitinated residues in the surface of *Salmonella typhimurium* leading to elimination of this bacteria through autophagy (Thurston et al. 2009; Wild et al. 2011; Zheng et al. 2009). Little is known, however, about how vesicle-contained pathogens might also be selectively delivered to autophagosomes. The role of those adaptor proteins does not seem to be restricted to cargo targeting. Thus, p62 can also deliver ubiquitinated proteins directly into the autolysosome, which are then processed into bactericidal peptides, increasing the microbicidal capacity of this compartment (Alonso et al. 2007; Ponpuak et al. 2010).

As discussed above, although classically macroautophagy was characterized as a response to starvation, the signals that regulate the activation of this pathway when it is used to kill pathogens are different. Cells of the immune system normally sense the presence of pathogens through the engagement of pattern recognition receptors (PRR) that interact with

pathogen-associated molecular patterns (PAMP), including toll-like receptors (TLR) and NOD-like receptors (NLR). It is also signaling through these receptors that appears to mediate the activation of macroautophagy in cells of the immune system that capture or are infected by pathogens (Munz 2011) (Fig. 3). Engagement of different TLRs, including TLR4 and TLR7, have been implicated in the activation of macroautophagy in different cell types including macrophages and dendritic cells (Delgado et al. 2008; Sanjuan et al. 2007; Xu et al. 2007). Canonical TLR-downstream signaling appears to be involved in this process, as activation of autophagy in response to TLR engagement is dependent on MyD88 (Delgado et al. 2008; Shi and Kehrl 2008). However, how those signaling pathways intersect with the regulatory pathways that control macroautophagy remains to be fully elucidated. Recently, it has been shown, though, that TLR4-induced TRAF6-mediated ubiquitination of Beclin-1 interferes with the stability of the Beclin-1/Bcl-2 complex and leads to the activation of macroautophagy in a murine macrophage cell line (Shi and Kehrl 2010). Similar to TLRs, members of the NLR family of intracellular PRRs have also been shown to upregulate autophagic activity. Engagement of both NOD1 and NOD2 has been recently linked to the macroautophagy activation in dendritic cells and macrophages (Cooney et al. 2010; Travassos et al. 2010), where they directly interact with Atg16L at the site of bacterial entry (Travassos et al. 2010). Interestingly, the functional interaction between PRRs and autophagy appears to function both ways. Not only do these receptors activate macroautophagy, but it appears that in certain situations, delivery of ligands to intracellular PRRs also depends on autophagy. For instance, plasmacytoid dendritic macroautophagy mediates the delivery of Herpes simplex virus replication intermediates to intracellular TLR7, allowing viral recognition and the initiation of antiviral responses (Lee et al. 2007).

However, signals other than PRR-mediated signals have been also shown to regulate pathogen-induced activation of autophagy, including some cytokines and membrane co-receptors. IFN γ -R signaling and the downstream immune-related GTPase IRGM are positive regulators of autophagy in macrophages (Singh et al. 2006), whereas Th2-like cytokines, such as IL-4 and IL-13, have the opposite effect (Harris et al. 2007). On the other hand, engagement of CD40 in macrophages has been reported to increase anti-toxoplasma responses by potentiating macroautophagy-mediated vacuole-lysosome fusion (Andrade et al. 2006).

Our understanding of the relevance of autophagy to the responses against microorganisms does not result only from the fact that cells or mice deficient in key macroautophagy proteins show defects in their ability to recognize and kill microorganisms, but it is also underscored by the development in pathogens of strategies to block autophagy activation as mechanisms of immune evasion (Blanchet et al. 2010), and by the recently discovered relationship between genes involved in the regulation of autophagy and Crohn's disease. Several genome-wide association studies have reported association of this inflammatory disease with polymorphisms in genes that regulate macroautophagy, including Atg16L, IRGM and NOD2 (Fisher et al. 2008; Parkes et al. 2007). The pathogenic mechanisms that may underlie these associations is not completely understood yet, but mutations in ATg16L associated with Crohn's disease appear to affect the secretory machinery of Paneth cells (Cadwell et al. 2008), which could possibly affect the ability of these cells to secrete antimicrobial peptides and alter the normal interaction of the intestinal mucosa with the gut

bacterial flora. However, a direct alteration in the processing of intestinal bacteria by dendritic cells defective in autophagy has also been proposed as a possible immunopathological mechanisms for Crohn's disease (Cooney et al. 2010)

Although classically presentation of antigens by antigen presenting cells in MHC class I or class II proteins has been described as being dependent on the activity of the proteasome or the endocytic/phagocytic system, respectively, recent evidence has begun to uncover an important role for different forms of autophagy in these processes (Munz 2010) (Fig. 3). Autophagy has provided a new mechanism to account for previous observations that had shown that endogenous proteins could be presented on MHC class II complexes. In fact, macroautophagy has been shown to modulate the presentation of self proteins and antigens from intracellular pathogens, including viruses and intracellular bacteria (Dengjel et al. 2005; Lee et al. 2011; Schmid et al. 2007). Macroautophagy thus, is positioned to modulate two crucial aspects of the adaptive immune response: it can enhance priming of CD4+ T cell responses against intracellular pathogens, but at the same time, by allowing the presentation of self peptides, it may also regulate the establishment of peripheral T cell tolerance. In this regard, macroautophagic activity in thymic epithelial cells has been found to regulate positive and negative selection of thymocyte in certain T cell receptor transgenic mouse strains, suggesting that autophagy-dependent presentation of self peptides in the thymus may help shape the T cell repertoire and contribute to the maintenance of central tolerance (Nedjic et al. 2008).

Macroautophagy is not the only form of autophagy that has been implicated in antigen presentation. Selective transport into the lysosome of cytosolic proteins through CMA has also been reported to contribute to the presentation of intracellular antigens in class II molecules (Zhou et al. 2005). Very recently, a new mechanism for delivery of cytosolic proteins into class II compartments in dendritic cells has been described. This process, as is also the case in CMA, requires hsc70 but involves the selective internalization of cytosolic cargo into late endosomes through a process similar to canonical microautophagy (Sahu et al. 2011). Whether or not this microautophagy-like mechanisms could also be utilized for self-antigen presentation remains to be elucidated.

Interestingly, recently published data indicates that macroautophagy can also contribute to the presentation of exogenous antigens in MHC class II molecules, as NOD2-mediated induction of macroautophagy in dendritic cells appears to be required for the generation of specific CD4+ T cell responses to extracellular bacteria (Cooney et al. 2010) (Fig. 3).

Although the mechanisms behind it are less well characterized, autophagy has also been shown to contribute to the loading of viral antigens into MHC class I molecules and to the increased efficiency of cross-presentation of tumor antigens onto class I complexes (English et al. 2009; Li et al. 2008) (Fig. 3).

The roles of autophagy in the adaptive immune response are not restricted to antigen presentation. Active macroautophagy has also been detected in B and T lymphocytes (Li et al. 2006; Miller and Krijnse-Locker 2008). In T cells, where it has been more thoroughly characterized, macroautophagy appears to regulate organelle homeostasis, but it also plays

key roles in the regulation of cell survival and expansion (Bell et al. 2008; Hubbard et al. 2010; Jia et al. 2011; Pua et al. 2007) (Fig. 3). As discussed above, autophagy is an essential mechanism that regulates cell response to stress, including nutritional stress. By allowing the degradation of non-essential components it can provide the energy and the building blocks required to overcome an extracellular or intracellular stress, which would otherwise cause cell damage. Antigen recognition poses a great metabolic burden on T cells, which need to respond by dividing rapidly and secreting large quantities of soluble mediators. Through mechanisms yet to be characterized, engagement of the TCR also upregulates macroautophagy, which becomes essential to support the increased bioenergetic demand required to ensure cell proliferation and cytokine production (Hubbard et al. 2010)

It has become increasingly evident that nutritional factors have an important regulatory role in modulating the function of the immune system. Obesity, metabolic syndrome, nutritional restriction, among other circumstances, all have a tremendous impact in the development of innate and adaptive immune responses (Dorshkind and Swain 2009; Olefsky and Glass 2010). The mechanisms that underlie these effects are still not fully understood. Changes in secretion of immune mediators by the adipose and other tissues, alterations in the composition of resident populations of T cells or macrophages, direct effects of metabolites such as fatty acids on immune cells, intrinsic defects in signaling pathways or membrane structure in cells of the immune system, among others, have all been proposed to account for some of the modulatory effects on immune responses of nutritional factors. Given the important role of the different forms of autophagy in the regulation of processes that range from pathogen killing to T cell activation, and the fact that autophagy is tightly regulated by nutritional and metabolic cues, it would be very interesting to explore if changes in autophagic activity of different cell populations may also contribute to the changes in the quality of the immune responses that occur in response to nutritional stress or nutritional intervention. Providing the first link between nutritional stress, autophagy and inflammation, it has recently been shown that in the context of a high fat diet, palmitate leads to increase IL-1 β and IL-8 production by macrophages through a process that involves palmitate-induced downregulation of macroautophagy, which results in the activation of the NLRP3 inflammasome and the production of pro-inflammatory cytokines (Wen et al. 2011).

5. Autophagy, aging and immunosenescence

Both macroautophagy and CMA undergo a gradual decrease in activity with age, becoming defective in aging organisms (Cuervo 2008). The initial biochemical observations of the compromise of macroautophagy with age revealed problems both in the regulation of the induction of this process as well as in the late steps of macroautophagy, namely autophagosome clearance. In old livers the stimulatory effect of glucagon was shown to be neutralized by constitutive insulin-mediated repression of macroautophagy (Del Roso et al. 2003; Donati et al. 2001). Furthermore, the few autophagosomes that still form encounter limitations in their ability to fuse with lysosomes and undergo degradation (Terman 1995). Although the specific reasons for these defects remain unknown, it has been proposed that the accumulation of undigested products in the form of lipofuscin in the lumen of lysosomes interferes both with their capability to fuse with autophagosomes and also with their ability to degrade the sequestered cargo.

Later studies have provided genetic evidence in support of the importance of autophagy in longevity. Blockage of macroautophagy reduces the life extension attained in worms through genetic manipulations in different essential cellular pathways such as the insulin signaling pathway, mTOR, p53 or sirtuin-1, as well as in models of caloric restriction that also display lifespan extension (Hansen et al. 2008; Melendez et al. 2003; Morselli et al. 2010; Tavernarakis et al. 2008). Furthermore, upregulation of macroautophagy has been shown to occur during the period of life extension in almost all these models. Similar findings have been reported in flies where age-related conditions such as neurodegeneration can be prevented by overexpressing specific autophagy effectors shown to decrease with age (Simonsen et al. 2008).

In the case of CMA, the age-related decline of this form of autophagy seems to be mainly a consequence of the reduction in the total levels of the CMA receptor, LAMP-2A, in the membrane of lysosomes from old organisms (Cuervo and Dice 2000a). This decrease in LAMP-2A does not occur at the transcriptional level but rather it is due to reduced stability of this protein at the lysosomal membrane (Kiffin et al. 2007). The contribution of the functional decrease in CMA to the phenotype of aging is currently supported by studies in a transgenic mouse model in which normal levels of LAMP-2A in the liver can be maintained until late in life (Zhang and Cuervo 2008). These animals present improved cellular homeostasis, better response to stress and preserved functionality when compared to old wild type littermates.

The age dependent decrease in autophagic function could be, in part, responsible for the negative energetic balance characteristic of old organism (Fig. 4). Inability to upregulate macroautophagy in response to nutritional cues, such as starvation, would reduce mobilization of intracellular stores of energy as well as the capability to sustain protein synthesis through amino acid recycling. In addition, low autophagic function would render cells more susceptible to toxicity when exposed to lipid challenges. High intracellular lipid content, as the one found in many aging tissues, has been already demonstrated to have a negative impact in both macroautophagy and CMA, mainly through changes in the lipid composition of the vesicles and organelles involved in these processes (Kaushik et al. 2006; Koga et al. 2010). In fact, it is possible that the reduced stability of LAMP-2A at the lysosomal membrane observed in old organism is, at least in part, consequence of age-related changes in the lipid composition of lysosomes.

Those same defects in the autophagic function with age may also negatively impact innate and adaptive immune responses (Fig. 4). Alterations in the innate and adaptive immune system have been described in the elderly and contribute to the immunosenescent phenotype in old organisms (Haynes and Maue 2009; Shaw et al. 2010). One could speculate that inefficient T cell responses and defective functioning in macrophages and dendritic cells might be, at least in part, due to an inability of these cells to mobilize stores to obtain sufficient energy to maintain their function, or to a direct interference with specific functions that may be regulated by autophagy, such as vesicular trafficking and lysosomal fusion, cytokine secretion or antigen processing and loading (Fig. 4). Furthermore, the so called “inflammaging” phenotype could also be exacerbated by defective macroautophagy, since a direct relation between macroautophagy and NLRP3-dependent secretion of proinflammatory

cytokines has recently been discovered (Wen et al. 2011). More research is still needed to understand how age-related defects in different autophagic pathways may influence the function of immune cell populations and contribute to immunosenescence.

6. Concluding remarks

The multiplicity of cellular functions attributed now to autophagy and the recently established roles for this pathway both in the maintenance of the energetic balance and in cellular defense make this catabolic process very attractive for the young field of nutritional immunology. Well characterized immunoregulatory molecules such as free fatty acids can originate, in part, from the mobilization of intracellular stores through autophagy. This confers changes in autophagic activity a previously unknown modulatory role on the immune system by determining the availability of these regulatory molecules. In addition, autophagy provides part of the energy required to engage and maintain the immune response. Consequently, in addition to the previously known negative effect on cellular quality control, failure of the autophagic system can also compromise the ability of cells to orchestrate a proper immune response.

Many questions are now waiting to be addressed regarding this novel connection between nutrition and the immune system through their autophagic function. For example, what are the determinants that favor autophagic mobilization of one type of energy store or another? How does an excess of different nutrients exert an inhibitory effect on autophagy? Is nutrient-induced autophagy regulated in a completely independent manner than basal quality control autophagy? Is this regulation cell-type specific when considering the different cellular types participating in the immune response? Particularly interesting will be to further explore whether the impact that nutritional manipulations have on the autophagic system could be utilized to preserve or restore an adequate immune function in a condition such as aging.

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Vitae

Ana Maria Cuervo is a professor of Developmental and Molecular Biology and co-director of the Institute for Aging Studies. She received her MD and PhD from University of Valencia, Spain and did her postdoctoral training at Tufts University under the latest Fred Dice. She established her independent group at Albert Einstein College of Medicine in 2001. Her group is interested in the molecular characterization of different autophagic pathways and the contribution of changes in autophagy to aging and age-related disorders.

Fernando Macian is an associate professor of Pathology. He completed his MD and PhD at University of Valencia, Spain. After a postdoctoral stay at the Center for Blood Research at Harvard University he became faculty at Albert Einstein College of Medicine in 2003.

Research in his group is aimed at characterizing the molecular mechanisms that regulate T cell activation and tolerance, with a special focus on the role that autophagy may play and the contribution that age-associated changes in the different autophagic pathways may have on the altered function of T cells with age.

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Figure 1. Types of autophagy in mammalian cells

Three types of autophagy co-exist in all mammalian cells. Macroautophagy involves the sequestration of regions of the cytosol inside double membrane vesicles that become degradative compartments upon fusion with the lysosomes. Both in bulk and selective macroautophagy can take place. Specific examples of selective macroautophagy are highlighted on the left. Microautophagy occurs when cytosolic components are directly engulfed by lysosomes through invaginations of the lysosomal membrane. Recent studies support that microautophagy occurs in late endosomes both in bulk and in a selective manner depending on the interaction of the substrates with a chaperone. Chaperone-mediated autophagy, the third type of mammalian autophagy, initiates through the recognition by a chaperone of a targeting motif in the cytosolic protein to be degraded. The chaperone/substrate complex reaches then the lysosome surface and the substrate is internalized through a translocation complex in the lysosomal membrane.

Figure 2. Interplay between autophagy, nutrients and the cellular energetic balance

Autophagy contributes to the catabolism of different essential molecules provided by the diet. In the absence of nutrients, autophagy facilitates breakdown of intracellular proteins to facilitate the amino acids required to maintain protein synthesis under those conditions. New affluence of amino acids with the diet represses autophagic function. Cells can also utilize macroautophagy to mobilize intracellular lipid stores (left) and glycogen (right) to generate energy when nutrients are scarce. Breakdown of intracellular stores by macroautophagy can also occur in response to a high affluence of lipids or glucose. This mechanism is used by cells to control the size of intracellular stores and prevent their massive accumulation.

Figure 3. Autophagy and the immune system. Pathogen Killing

Engagement of plasma membrane or intracellular pattern recognition receptors (PRR), such as toll like receptors can activate macroautophagy to induce the degradation and killing of intracellular pathogens. Bacteria that escape from the phagosomes into the cytosol can be targeted by macroautophagy adaptor proteins (AP), such as p62 or optonerin, and incorporated into autophagosomes. Phagosomes containing pathogens can also be incorporated into nascent autophagosomes. In both cases, autophagosomes fuse with lysosomes to degrade microorganisms contained in them. Furthermore, macroautophagy helps ensure pathogen killing through the delivery of proteins with microbicidal activity into the autolysosomes. Contributing to the overall regulation of the phagocytic response, macroautophagy can also delivered PRR ligands (PPRL) to intracellular PRR-containing endosomes. **Antigen Presentation:** Cytosolic proteins can be delivered into late endosomes for processing and subsequent loading of peptides into MHC class II molecules, which will eventually reach the plasma membrane to present those peptides to

CD4⁺ T cells. Three forms of autophagy, macroautophagy, microautophagy and chaperone-mediated autophagy (CMA) contribute to the delivery of those proteins into the class II compartment in antigen presenting cells. **T lymphocytes function:** Macroautophagy allows the presentation of self-peptides in MCH class II molecules by epithelial cells in the thymus and regulates the positive and negative selection of thymocytes, contributing to the shaping of the T cell repertoire. Furthermore, in peripheral T cells basal macroautophagic activity is crucial to maintain proper organelle homeostasis, whereas activation-induced macroautophagy regulates the energy metabolism and controls cell proliferation and survival of T cells.

Direct effect – Age related changes in autophagies

Indirect effect – Reduced availability of energy sources

Figure 4. Autophagy, aging and immunosenescence

Both macroautophagy and CMA activity decrease with age in most cell types and could contribute directly or indirectly to immunosenescence. **Direct effect:** Reduced macroautophagic activity can compromise the cellular response to pathogens and self-recognition by the immune system by interfering with antigen presentation. The age-dependent decline in CMA could also contribute to inadequate antigen presentation in elders. **Indirect effect:** Compromised autophagic function with age may contribute to deficient maintenance of the cellular energetic balance and inability to adapt to the high energetic demands of the immune response.