Autophagy: For Better or for Worse, in Good Times or in Bad Times…

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Abstract: Autophagy is a bulk cytosolic degradative process which in the last few years has become a key pathway for the advancement of molecular medicine. Autophagy (cellular self-eating) has several implications in human disorders involving accumulation of cytosolic protein aggregates such as Alzheimer, Parkinson, Huntington diseases, as well as in myopathies caused by deficient lysosomal functions and in cancer. Moreover, autophagy affects intracellular microorganism lifespan, acting either as a cellular defense mechanism or, on the contrary, promoting pathogen replication. Furthermore, autophagy also participates in antigen presentation, as a part of the adaptive immune response. Therefore, autophagy association with cell survival or cell death would depend on cell nutrition conditions, presence of cell intruders, and alterations in oncogene or suppressor gene expression. In this review we will focus on the wide spectra of disease-related topics where autophagy is involved, particularly, in those processes concerning microorganism infections.

Keywords: Autophagosome, LC3, Atg, human diseases.

INTRODUCTION

Autophagy, a degradative process that takes place in all cell types, has been largely studied and was first described in the early 60s. It was in 1963 when Christian de Duve observed, through electron microscopy, single or double membrane vesicles containing parts of the cytoplasm and organelles. These vesicles were finally termed as "autophagosomes" and it was de Duve, who came up with the new term "autophagy" [1,2]. Autophagy is a cellular homeostatic regulated process that enables cells to get rid and recycle part of its own cytoplasm or organelles. This ancient process, conserved from yeast to humans, participates in protein turnover and takes place when an isolation membrane (also known as phagophore in mammals) sequesters cytoplasm portions, encloses it and forms an autophagosome. The engulfed material is finally degradated after autophagosome fusion with a lytic compartment; the whole process is known as macroautophagy, or simply "autophagy". The origin of the membrane is still unknown, but ER is pointed as one of the possible sources, although Golgi complex or mitochondria could also be contributing with membrane. Two other types of autophagy have been already described: microautophagy [3] and chaperone mediated autophagy (CMA) [4]. In the process of microautophagy, cytosolic components are directly incorporated into the lysosome lumen by membrane invagination and scission. CMA also takes place at the lysosomal membrane, but is based on the translocation of unfolded proteins across the lysosomal membrane, and requires the presence of the molecular chaperone Hsc70 and of a lysosome-associated membrane protein LAMP-2A [5]. There is enough evidence on the participation of the autophagic pathway in several physiological processes, like adaptation to starvation conditions, removal of damaged organelles and misfolded proteins, turn over of long lived proteins, elimination of potentially toxic aggregate-prone proteins and also in the immune defense against different intracellular pathogens. However, autophagy has also been associated to a great number of pathological processes, such as cancer, Huntington's disease, Parkinson's disease, and recently also Crohn's disease. Taking all together, autophagy contributes to homeostasis cellular regulation (Fig. **1**).

In this review we will focus on macroautophagy, hereafter referred as autophagy, and we will provide an introduction to its molecular mechanisms, how it can be regulated and its implications in several pathological processes with a special emphasis on its participation during infections with different microorganisms.

AUTOPHAGOSOME ASSEMBLY: A DESTRUCTION MACHINERY TO ENSURE INTEGRITY

Autophagosome formation is a complex process which is believed to take place *de novo*, involving a wide range of unique molecules. Autophagosome assembly results from the expansion of a membrane core of unknown origin (phagophore), apparently without the participation of a budding process. The extension of its membrane is postulated to be carried out by merging of intracellular vesicles. The current view is that the phagophore represents the nucleation membrane for autophagosome biogenesis. Taking into account the whole autophagic phenomena, the specific identified autophagic proteins participate almost exclusively in the first steps of autophagosome formation.

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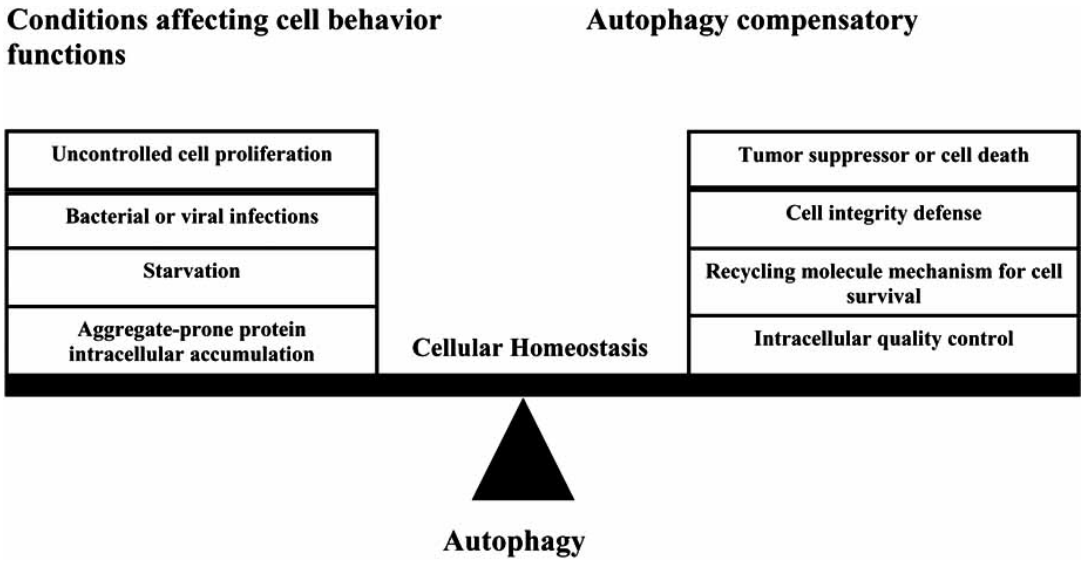

Fig. (1). Autophagy influence in cellular homeostasis. During cell lifespan autophagy has a pivotal role in homeostasis regulation upon different external factors such as bacterial or viral infections, nutrition deprivation or in response to internal factors such as uncontrolled cell cycle or unfolded protein accumulation.

In 1997, the first Atg (**a**u**t**opha**g**y-related gene), Atg1 was identified in yeast by Matsuura *et al* [6]. This discovery facilitated the genetic research in autophagy. Indeed, at present thirty Atg proteins, (from yeast) have been identified and almost all associate at least transiently with the preautophagosomal structure (PAS), which is thought to be the site for assembly of the autophagosome in yeast [7]. The Atg proteins may function in part by directing membrane to the phagophore as well as mediating the biogenesis of the three-dimensional double-membrane sphere that will become an autophagosome [8]. However, the study of autophagy components remains a complicated issue, because proteins that have a main function in the autophagic pathway also play an important role in other crucial cellular process. This is the case of Atg 5 which is involved in both autophagy and apoptosis [9].

One of the most prominent features of the autophagic machinery is the participation of more than one-quarter of the Atg proteins in two interconnected processes of protein conjugation [10]. Atg4 mediates the proteolytic removal of C-terminal residue(s) of Atg8 (LC3 in mammals), exposing a glycine as the ultimate amino acid, whereas Atg12 is synthesized with the glycine already exposed [11,12]. Both proteins are activated by Atg7, which is homolog to the ubiquitin-activating (E1) enzyme [13]. The activated intermediates are transferred to ubiquitin-conjugation (E2) analogs, Atg3 and Atg10, respectively [14]. Then Atg8/LC3 becomes membrane-associated when is covalently attached to phosphatidylethanolamine (LC3-II), whereas Atg12 is connected to Atg5 *via* an isopeptide linkage. Atg8/LC3 is the only Atg protein that remains associated to the autophagosome all along its maturation process in mammals cells, and thus serves as one of the few markers for autophagy [11]. Further details about autophagosome formation and the involved proteins have been recently reviewed by Kie and Klionsky [15].

Autophagosome, Many Roads But One Destiny

Autophagosomes have been reported to fuse with early [16] and late endosomes [17] as well as lysosomes [18,19]. These data indicate that autophagosomes maturation is a multi-step process in mammalian cells, including several fusion events with vesicles originating from the endo/lysosomal pathway. As shown in Fig. (**1**), autophagosome maturation is initiated by fusion with endosomes or multivesicular bodies, forming amphisomes. Next, amphisomes fuse with lysosomes to degrade the incorporated material. Alternatively, autophagosomes may fuse directly with the lysosomal compartment. Initially autophagosomes have the same pH as the surrounding cytoplasm, but during this maturation process the autophagic vacuoles become acidic [20].

A group of small GTPases called Rabs are involved in vesicle fusion, fission and transport events [21]. In our lab it was demonstrated by using a negative mutant of Rab7 that this protein is necessary at the amphisome/lysosomal fusion event [22]. This finding was also corroborated by using siRNA [23]. On the other hand, we have recently presented evidence of the role of Rab11 in the interaction between multivesicular bodies and the autophagic pathway to generate amphisomes [24]. Furthermore, Rab24 was also implicated in the autophagy pathway since upon autophagy induction *via* starvation [25], this protein changes its distribution colocalizing with the autophagosomal markers LC3 and monodansylcadaverine (MDC). As in other trafficking processes (e.g. phagocytosis), Rab proteins have determinant roles when it comes to a tight control of fusion events. The study of the molecular mechanism involved in fusion regulation is in high expansion but still many questions remain unanswered, like for instance, which are the v and t-SNARES (Soluble NSF Attachment Protein Receptor) that participates in the different fusion steps? Among all the 60 known

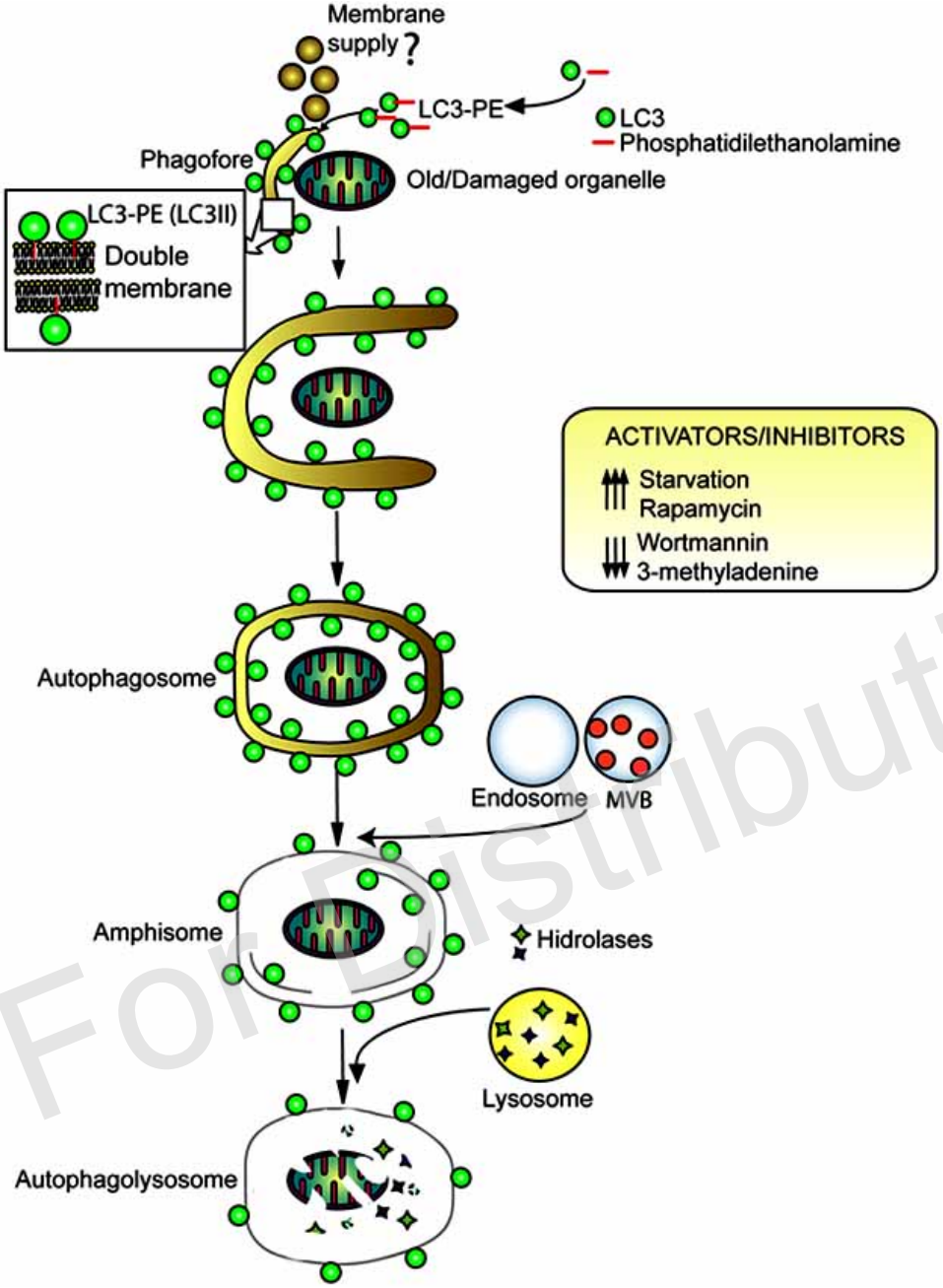

Fig. (2). The autophagic pathway: Autophagy is a degradative process characterized by the formation of double membrane vacuoles known as autophagosomes. The process begins by the formation of the isolation membrane (phagophore), a step that depends on the activity of the complex Vps34/Beclin1, among other Atg proteins, and also on the lipidation of LC3. The phagophore sequesters cytoplasmic portions and aged organelles such as mitochondria. Then, the autophagosome fuses with endocytic compartments and finally with the lysosomes to degrade and recycle the incorporated material.

Rab proteins, which other Rabs are involved in the autophagy pathway?

Regulation: Who has the Control?

A precise knowledge of autophagy regulation is required to avoid undesirable consequences (excessive autophagy stimulation could lead to cell death). At present, numerous *in vivo* studies are being performed in order to use the modulation of the autophagy pathway as a tool for future therapeutic purposes. Autophagy regulation has been extensively reviewed [26, 27], and our only intention is to briefly introduce this topic. Autophagy is subjected to control by a range of nutrients including nitrogen and carbon in yeast, and by amino acids starvation and certain hormones such as insulin and glucagon in mammals. The autophagy-inhibitory effect of amino acids and insulin has been established in cell culture and organ perfusion experiments [28]. Nonetheless during short-term starvation, the blood amino acid concentration remains virtually unchanged [29].

A key point in autophagy regulation is the mTor (**t**arget **o**f **r**apamycin) kinase complex 1, which negatively regulates autophagy. Nutrient depletion results in mTor inhibition and therefore activation of autophagy [30]. Likewise rapamycin also inhibits mTor activity and thus enhances autophagy. The induction process also requires a class III phosphatidylinositol 3-kinase (PI3K III) and its associated proteins like beclin-1 [31]. The use of PI3K III inhibitors (e.g. wortmannin) is widely exploited in autophagy investigation. There are many additional factors involved in autophagy control summarized by Mizushima *et al*. [32].

AUTOPHAGY IN PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES

At present, autophagy is not only considered an adaptation response (self-eating) upon transient nutrient deprivation. In fact, in the last few years it has been demonstrated that autophagy induction is also a potent defense mechanism and a protein quality control in protein conformational disorders. In addition, it was recently discovered that functional deficiency in the Ambra1 gene product (a positive regulator for Beclin1) in mouse embryos, leads to severe neural tube defects, indicating that autophagy has a relevant role during embryonic vertebrate development [33]. Therefore, the vast implications in molecular medicine make autophagy attractive for basic scientist research.

I) HUMAN DISEASES INVOLVING AUTOPHAGY

In the last decade, an increasing number of research studies have emerged relating the autophagic pathway with the etiology of several human diseases. Autophagy can have either positive or negative effects depending on the specific disease and its level of progression [34]. Here we will review only a few of the most important diseases in which this pathway has been implicated.

Protein Conformational Disorders

In general, the intracellular accumulation at high levels of a mutant protein hinders its normal function. In addition, mutations in amino acid sequence likely affect protein conformation leading to impropriate protein-protein interaction. Therefore, autophagy is essential for the elimination of these large aggregates, but if this bulk protein degradative process is inefficient or fails, cellular homeostasis and viability are severely compromised.

The study of alpha-1-antitrypsin deficiency, which produces liver inflammation and carcinogenesis, was one of the first evidences connecting the autophagic pathway with diseases associated with aggregate-prone proteins in the endoplasmic reticulum [35].

Considering diseases associated with cytosolic aggregate-prone proteins, and specifically in the case of neurodegenerative diseases, such as some forms of Parkinson's, Huntington's and Alzheimer's disease, autophagosome accumulation is observed in brain samples of affected patients, and also in cell lines or mouse models of these diseases. This is likely due to an increase in autophagy induction and to a deficient autophagosome-lysosome fusion [36,37]. Thus, the normal "autophagic flow" is reduced or blocked and consequently, autophagosome accumulation is detected.

Alzheimer's disease (AD), the most prevalent human neurodegenerative disorder, involves a progressive loss of neurons leading to profoundly impaired memory and declines in other intellectual functions. This disorder is characterized by two neuropathological lesions, the extracellular aggregates of β–amyloid peptide (Aβ) in association with degenerating dendrites or axon, mainly composed of intra-neuronal neurofibrillary tangle containing aggregated forms of the microtubule-associated protein, tau and the neuritic plaque [38]. Using a transgenic mouse model of AD it was observed that autophagy (LC3 marker) was induced in vulnerable populations of neurons, at early stages of AD, in normal adult brain. Therefore, the fact that in these mice this process begins before β-amyloid pathology and the development indicates that autophagy induction is an early response in the disease and not only a consequence of amyloid deposition [39]. Aβ peptide is believed to be generated at several sites within neurons, including endosomes, Golgi, and ER [40,41] and each of these organelles could contribute to the involvement of autophagy in Aβ generation. In conclusion, autophagy as a pathogenically relevant pathway for Aβ generation and as a mediator of both cell survival and degeneration phenomena, represents a new direction for research into the pathogenesis and possible therapy of AD.

Huntington's disease is an autosomal dominant disorder caused by mutations of huntingtin, a cytosolic protein enriched in striatal and cortical neurons. Huntingtin has a polyglutamine (polyQ) tract in its N-terminus. In this illness, abnormal expansion of polyQ, originated by repetitions of the codon CAG in exon 1 of the Huntington gene, produces mutated huntingtin with expanded polyQ repeats. The mutant huntingtin cleaved by caspases accumulate in the cell forming insoluble aggregates, which lead to cell toxicity and cell death. The mutant huntingtin protein is frequently associated with autophagic vacuoles, with or without sequestered components, dense lysosomes and multilamellar and tubulovesicular structures. A recent report by Ravikumar *et al.* has demonstrated that rapamycin treatment *in vitro* and *in vivo* models enhances the clearance of mutant huntingtin [42]. This finding really envisions the "autophagy solution" for Huntington disease.

Myopathies and Lysosomal Storage Diseases

The lysosome is the major site in the cell where organelles and long-lived proteins are degraded. However, when the normal function of the lysosomal sys-

tem is reduced, excessive levels of undigested materials accumulate inside the lysosomes affecting cell viability. Certain lysosomal storage diseases are commonly manifested by cardio- and other myopathies [43,44]. The autophagic vacuolar myopathies have distinct morphological characteristics of the sarcolemma, which allow the differentiation of this group of diseases. However, major reports in this field are based on morphological observation, and it is not well understood what is the mechanism for the formation of these distinct autophagic vacuoles, or even whether autophagy has a beneficial or a detrimental contribution to the disease.

Danon's disease is an X-chromosome-linked myopathy and cardiomyopathy caused by mutations in the LAMP-2 gene. LAMP-2B is a major lysosomal membrane protein, which is highly glycosylated and expressed mainly in skeletal muscles and heart. This disorder is characterized by massive accumulation of autophagy vacuoles present in cardiac and skeletal muscle cells of the patients [45].

Pompe's disease is another example of autophagic vacuolar myopathy caused by a lysosomal acid α–glucosidase-deficiency and storage of glycogen in the lysosome in multiple tissues, but clinical manifestations are mainly due to skeletal and cardiac muscle involvement [46].

To date, the limitations in animal or cell culture models available to study the pathophysiology turn the investigation into a hard task by making difficult the determination of the autophagy role in lysosomal storage diseases and myopathies.

Cancer

The relationship between autophagy and cancer was presented many years ago, but a major progress on the elucidation of the possible molecular mechanism was achieved only recently [47-50]. Cancer is a consequence of an accumulation of mutations in the DNA that deregulates cell growth and results in the subsequent tumor formation. The partial loss of the autophagic related gene Beclin1, frequently results in human breast, ovarian, and prostate tumors. These observations were made using a model of beclin +/- mice [51-53]. These data propose a role for beclin-1 and autophagy in tumor suppression; nevertheless, the molecular mechanisms beyond this process are still uncertain. In order to understand how autophagy can function as a tumor suppression process it has been proposed that autophagy reduces the production of reactive oxygen species by degrading specifically damaged mitochondria and the subsequent raising of the DNA mutation rate, which is the basic cause of most cancers [54]. However, other studies have shown that autophagy can also contribute to tumor survival, mainly by regulating nutrition of the cells. For instance, HeLa cells, with Atg knockout genes incubated in nutrient deprivation conditions undergo apoptosis, suggesting a cell survival role of autophagy [55].

In conclusion, the dual role of autophagy in cell protection and programmed cell death suggests that this process can have a double function to protect or to get rid of cancer cells, depending on the particular class of cancer or the evolution step of the disease. Thus, the modulation of autophagy with therapeutic purposes might be in the future a useful tool, although all these factors should be taken into consideration for each particular case [56].

II) AUTOPHAGY IN BACTERIAL AND PARASITE INFECTIONS

Different species of bacteria and parasites have been threatening world human health for centuries, causing infections that lead to serious disease, or even death. The entry of bacteria into the cell can be promoted by the bacteria itself, by an active bacteria-dependent mechanism (i.e. *Salmonella*, *Shigella*. or *Trypanosome cruzi*), or enter the cell *via* a conventional phagocytosis pathway mediated by the host cell (i.e. *Coxiella burnetii* or *Mycobacteria*). In order to face those infections, the immune system counts on two branches: innate and acquired immunity. Innate immunity constitutes the first line of host defense against pathogens and is mediated by professional phagocytes such as macrophages and dendritic cells. Acquired immunity is involved in the elimination of pathogens in late phases of infections and also in the generation of immunological memory. As part of the innate immunity, phagocytes count on a battery of degradative enzymes mainly located in lysosomes, which degrades and kills internalized pathogens. Autophagy is an intracellular pathway that also ends in these degradative compartments. However, as a mechanism to evade the host cell defenses, certain bacteria have developed different strategies. For example some are able to get away from the phagosome by puncturing the phagosomal membrane to escape to the cytoplasm, where they usually undergo replication. This is the case of *Shigella flexneri*, *Listeria monocytogenes*, *Francisella tularensis*, Group A *Streptococcus*, as well as the intracellular parasite *Trypanosoma cruzi* [57,58]. Other group of bacteria modifies the phagosome to prevent fusion with the lysosomes, which allows bacteria to replicate within a non lytic phagosomal compartment. This is the case of *Mycobacterium tuberculosis* and *Salmonella* enterica serovar *Typhimurium.* On the other hand, the host immune system reacts against these escapes, and activates other defense mechanisms. Such is the case of autophagy, which can target certain intracellular pathogens, enwrap the microorganisms within an autophagosome and finally deliver the intruders to the lysosomes where they are eliminated [59-61].

The manner by which autophagy encounters the pathogens differs depending on the bacteria itself. Autophagy can target bacteria that either reside within an immature phagosome (*M. tuberculosis*) [62], bacteria that have already escaped from the phagosomal compartment and that reside in the cytosol (*Streptococcus pyogenes*) [63], or bacteria residing in a dam-

aged phagosome-like vacuole (*Salmonella* enterica serovar *Typhimurium*) [64]. Other autophagy mediated process that can also work as a host defense mechanism, is the autophagy-dependent host cell death like in *Salmonella* infected macrophages [65], where the bacteria induces autophagy-dependent cell death. This response could also take part of the host cell defense by restricting bacterial growth and limiting further infections of neighboring cells.

Pathogens that are Victims of Autophagic Surveillance

In the case of *Listeria monocytogenes*, the behavior of the bacteria towards autophagy differs depending on the time of infection. After being trapped in the phagosome early during infection, *Listeria* disrupts the phagosomal membrane and escapes to the cytosol, where it can be targeted by autophagy. The later only occurs before the development of actin tails by the bacteria, which allows them to evade the autophagic recognition during the late infection [66,67]. *Shigella* spp. faces a similar fate as *Listeria*. *Shigella* is also able to lyse the phagosome and escape into the cytosol, where it can be captured by autophagy. As all action leads to a reaction, once in the cytosol, these bacteria developed mechanisms to hide from the autophagic pathway. In the case of *Shigella*, it secretes a factor capable of interacting, and blocking a bacterial surface protein that normally leads to autophagy by binding to the host Atg5 protein, thus inhibiting pathogen recognition by the autophagic machinery [68].

A similar situation of elimination *via* autophagy faces *Streptococcus* Group A, which once inside the phagosomal compartment secretes the enzyme streptolysin A. This leads to the rupture of the phagosomal membrane and subsequent escape of the bacteria to the cytosol where they are finally trapped and sequestered into autophagosomes, that eventually target bacteria to the lysosomes, resulting in the elimination of the pathogen [63]. This constitutes another example of autophagy acting as an innate immune defense system against intracellular pathogens that escapes to the cytosol.

When it comes to *S. Typhimurium*, early after infection, a subset of the infecting bacteria is able to escape from the phagosome. Recent studies have demonstrated that part of the population of bacteria residing in the cytosol colocalizes with the autophagic marker LC3, but this only occurs at early times of infection [69,70].

M. tuberculosis has been considered for years as one of the most successful pathogens when it comes to evading host immune system, which is carried out by arresting normal phagosomal maturation, blocking the fusion with the lysosomes and hence, degradation. However, we have demonstrated that this blockage can be overcome through the activation of the autophagic pathway [62]. The survival of the bacteria is impaired when autophagy is induced, either by starvation conditions or pharmacologically, when the inhibitor of mTOR rapamycin is used. Gutierrez *et al*. also demonstrated that activation of macrophages with IFN-γ stimulates the autophagic pathway and decreases bacterial load. Altogether these data suggest that autophagy could be in charge of re-routing the bacterium to a degradative compartment where it will be finally eliminated. These initial findings were supported by other studies and the list of intracellular pathogens targeted by autophagy was expanded (*Francisella tularensis* and *Toxoplasma gondii*) [71,72]. We have recently published a work where we demonstrated that autophagy has also a role during infection with extracellular pathogens [73]. Work from our laboratory with the hemolytic exotoxin *Vibrio cholerae* cytolysin (VCC) demonstrates that this toxin has the ability to induce an important vacuolization when incubated in culture cells like CHO or even the human intestine cell line Caco-2. These large vacuoles observed when VCC was used at high doses, these were shown to have autophagic features by electron microscopy and were strikingly labeled with LC3. At low doses, VCC induced the targeting of LC3 to punctuate structures that resemble autophagosomes. Moreover, autophagy inhibition, either by 3-methyladenine or wortmannin led to a decrease in the survival of the cells exposed to the toxin. Likewise, similar results were accomplished using cells that lacked essential autophagy proteins (Atg5 knock out mouse embryonic fibroblasts), where VCC not only failed to induce vacuolization, but also cell survival decreased dramatically when incubated with the toxin. These results showed for the first time that autophagy works as a defense mechanism against secreted bacterial toxins, produced by extracellular pathogens. More recently, in our laboratory we have obtained similar observations using other pathogen toxins (unpublished results) indicating that the autophagic response to secreted toxins is a previously underestimated common phenomenon.

Bacteria that Takes Advantage of the Autophagic Pathway

Even when the first approaches pointed out that autophagy could serve as a defense mechanism against intracellular pathogens, there is some evidence that it is a pathway that can be positively used by bacteria. At least in certain cases, induction of autophagy can turn into something beneficial towards pathogens. In this review we will summarize some examples of them.

Work from our laboratory has indicated that *Coxiella burnetii* is a pathogen that benefits from autophagy [74]. *C. burnetii* is an obligate intracellular gram negative bacterium that resides and multiplies in large acidic, hydrolase-rich vacuoles with clear autophagic features. The later relies on the association of the *Coxiella* containing vacuole (CCV) with autophagic markers such as LC3 or Rab24, and also labeling with MDC. Interestingly, this interaction with LC3 is dependent upon bacterial protein synthesis, given the fact that LC3 recruitment is inhibited when chloramphenicol is

used [75]. The later suggests that this bacterium is actively modifying or modulating its interaction with the autophagic pathway. Far from being detrimental to the bacteria, induction of autophagy prior to infection with *C. burnetii* enhances the number of infected cells and increases the size and bacteria load of the CCV. Moreover, overexpression of the autophagic related proteins LC3 and Rab24, accelerates the development of the CCV soon after infection. Hence, our work demonstrates that autophagy has beneficial effects on the development of the replicative niche of *C. burnetii*, and turns it into a more favorable place where the bacteria can survive and replicate properly.

Legionella pneumophila can activates the autophagic pathway in a macrophage model through soluble factors released *via* a type IV secretion system, and resides in a vacuole that resembles an autophagic compartment [76]. The secreted proteins not only are able to activate autophagy but can also define the bacterium fate, since vacuoles containing bacteria mature much slower than those autophagosomes induced either by rapamycin or starvation. Moreover, when autophagy inhibitors like 3 methyladenine were used, an increase in the degradation of bacteria was observed [77,78]. However, using the soil amoeba *Dictyostelium discoideum*, autophagy did not seem to be play a critical role for *Legionella*'s replication [79]. In this case, several autophagy mutant strains of *D. discoideum* were infected with *L. pneumophila*, but the bacterium replication rate was not affected. Apparently, these pathogens are able to bypass the requirement of some autophagic genes, and even when it has been demonstrated that autophagy favors Legionella's intracellular development and survival, autophagy seems to be dispensable, at least in this particular host model.

*Porphyromonas gingivali*s is another good example of how bacteria can benefit from autophagy. Once inside the cell, this bacterium resides in intracellular compartments labeled by the autophagic protein Atg7 [80,81]. In this case, researchers also demonstrated that the survival of the bacteria was impaired when autophagy is inhibited, either by using wortmannin or 3-methyladenine, which again leads to the idea that autophagy is not always a threat to all pathogens, but in contrast, it may favor their development. So is the case of other pathogens like *Brucella abortus* and *Staphylococcus aureus* [82,83], which similarly to *Porphyromonas*, reside in autophagic-like compartments but prevent their fusion with the lysosomes. It is believed that these groups of bacteria reside within autophagic compartments in order to gain access to host nutrient sources (cytoplasmic materials sequestered by autophagosomes) and thereby to survive intracellularly.

III) AUTOPHAGY DURING VIRAL INFECTIONS

Similarly like bacteria and parasites, some virus can also interact with the autophagic machinery and this interaction is, in some cases, beneficial and in others detrimental to the replication of the virus inside the host cell. Newly assembled virions can be targeted and captured by the autophagic pathway; therefore the latter again behaves as a defense barrier of the innate immunity system. This autophagy-mediated antiviral response has already been demonstrated with Herpes simplex virus, which is a DNA virus that replicates inside the nucleus of the host cell. Viral nucleocapsids are trapped and degraded by autophagosomes in their way out of the nucleus to the cytoplasm [84,85]. In addition, overexpression of the autophagy-related protein Beclin 1 has been shown to decrease Sindbis virus replication, thus preventing mice to suffer from lethal virus induced encephalitis [86].

In contrast, a positive role of autophagy in viral replication inside the host cell has also been proposed, as autophagic vacuoles serve to provide viruses with a membrane support for virus replication. This is the case of poliovirus, where recent studies demonstrated that autophagy provides a membranous scaffold to the replicating virions, in the poliovirus replicating complex [87]. Similar results have been obtained with coronavirus mouse hepatitis virus [88].

Altogether these studies demonstrate how autophagy is also involved in viral infections, in some cases favoring viral development and, in others as a part of the innate immune system. Further investigations will be needed to identify which are the specific viral proteins that lead autophagy to target and recognize the different viruses.

ANTIGEN PRESENTATION: AUTOPHAGY IMPLICATIONS

The job of the autophagic pathway in immunity goes beyond the simple elimination of intracellular pathogens *via* sequestration of the intruders and their delivery to the lysosomes. Evidences show that autophagy also participates in the MHC II-mediated antigen presentation as part of the adaptive immune response. [89-94]. In adaptive immunity, antigen presentation can be carried out through major histocompatibility complex (MHC) class I or II. MHC class I is in charge of the presentation of endogenous antigens, which are previously processed by the proteasome, antigenic peptides are then translocated to the ER where they meet and assemble with MHC class I. The antigenic-MHC class I complex is next transported to the cell surface, where it will be finally presented to $CD8^+$ cytotoxic T cells. On the other hand, exogenously-derived antigens are presented to $CD4^+$ helper T cells and this is performed by the MHC class II complex. Internalized pathogens, either by phagocytosis or endocytosis, constitute the source of these exogenous antigens and in most cases; these intruders are delivered to the lysosomal system where antigenic peptides are generated to be subsequently presented on the cell surface in conjunction to MHC class II [95]. However, these two systems are not completely separated from each other, as some cross talk between both pathways has been shown to take place [96]. The autophagic role in adaptive immunity, and specifically in antigen presentation, was impli-

cated for the first time ten years ago when it was shown that the autophagy inhibitor 3-methyladenine, impairs MHC class II presentation of the endogenous protein C5 (complement component 5) [97]. A few years later, other researchers demonstrated that MHC class II presentation of bacterial peptides was impaired by 3-methyladenine [98], while a similar situation was reported in the context of antigen presentation of viral proteins derived from Epstein Barr [99]. Paludan *et al*. provided for the first time genetic evidence of autophagy implication during MHC class II antigen presentation. On the other hand, other publications demonstrated that when autophagy is induced using starvation conditions, MHC class II presentation of intracellular antigens is remarkably enhanced [100]. In addition, a recent publication by Schmid *et al*. working with an Influenza virus antigen, showed a direct evidence of autophagy interplay with class II MHC antigen presentation, through the observation that autophagosomes are able to fuse with MHC class II loading compartments, which are labeled with the autophagic marker LC3 [101]. Another finding with enormous implications in vaccine development is that antigen presentation of influenza virus antigen can be improved if they are targeted to the autophagosome.

The participation of the autophagic pathway in antigen presentation was also suggested using animal models. Observations of thymic epithelial cells, belonging to newborn mice transgenically expressing GFP-LC3, show high levels of autophagic activity in the thymic epithelial cells [102]. The latter would suggest that perhaps; autophagy enables the presentation of endogenous antigens to lymphocytes during positive or negative selection [94].

In conclusion, all these studies have demonstrated a role of autophagy beyond the innate immunity. However, a large number of questions remain unanswered, and further work will be needed to completely understand as to which extend autophagy is important in antigen presentation and in adaptive immunity, so as to take advantage or this pathway in the field not only innate but also of adaptive immunity.

CONCLUDING REMARKS AND FUTURE PERSPECTIVES

All along this report, we have intended to briefly travel across the implications of the autophagic pathway and its importance in both physiological and pathological processes. It is evident that autophagy goes beyond a simple intracellular degradation pathway as it was originally described many years ago. The critical intervention of autophagy in the immune system opens another chapter in the field of immunology, and hence represents hereafter a new challenge for immunologists all over the world. On the other hand, autophagy has been clearly associated with neurodegenerative diseases, myopathies and cancer; which nowadays are very frequent diseases in older people. Thus, targeting autophagy will have an important role in the discovery and improvement of clinical therapies for modern society diseases. Even though there are numerous unanswered questions, we expect to move forward and make progress in our knowledge of the role of autophagy in innate and adaptive immunity response, so that in the future, we can take advantages of manipulating the autophagy machinery in the treatment of different pathogen infections, as well as in other non infectious processes.

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ABBREVIATIONS

LC3 = Microtubule associated protein light chain 3

MDC = Monodansyl cadaverine

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