Chapter 6

Autophagy and Lipid Metabolism – A Cellular Platform where Molecular and Metabolic Pathways Converge to Explain Dengue Viral Infection

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Additional information is available at the end of the chapter

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Abstract

Dengue virus (DENV) is one of the most prevalent human pathogens worldwide. It causes a huge socioeconomic burden with approximately 400 million infections per year, but yet there is no vaccine or antiviral that is currently effective against the disease. DENV is spread by the mosquitoes *Aedes aegypti* and *Aedes albopictus*, and viral replication within the mosquito vector is required for transmission to human host. During its replication cycle, the virus cause significant changes to the host transcriptome profile, especially in the metabolic and trafficking pathways. Recent studies have shown a strong association between autophagy and lipid metabolism modulation.

For many years, biochemistry studies have been forgotten and replaced by the most advanced techniques and theories in molecular biology and their promises for solving the "life code"; however, after many years of strong molecular biology research, it had not found the key of many problems with which we have the elemental biosystems like viruses. Decades of molecular virology investigations did not give more light about several cellular processes that occurred into the host cells when the infections happen. The molecular virologists have cloned many viral genes, manipulating full viral genomes, and engineering chimeric constructs to study many details at the molecular level, but the host cell and the encrypted viruses do not want to reveal their secrets.

Only with the new perspective of complex diseases, a new approach has emerged: An integrative methodology wherein molecular cell biology is converging with the most pure and elegant biochemistry. In this way, more extensive research is necessary for future comparative analyses of the host and vector metabolic/signaling environments required for viral replication.

Keywords: Autophagy, Cellular Platform, Dengue Virus, Molecular and Metabolic Pathways

1. Introduction

1.1. Dengue Virus (DENV): Some clinic and basic issues

Emergent viruses with major impact in human health include several agents of Flavivirus gender, *Flaviviridae* family, the most important of these are DENV (Dengue Virus), YFV (Yellow Fever Virus), JEV (Japanese Encephalitis Virus) and WNV (West Nile Virus) [1]. There are nearly 3.6 billion people at risk of infection with DENV in tropical and subtropical countries [2]. In more than 100 endemic countries with an estimated nearly 390 million of DENV infections per year, approximately 100 million of dengue fever cases are estimated annually with over 2 millions cases of potentially fatal dengue hemorrhagic fever [3, 4]. In most cases, the symptoms of DF that include an acute febrile illness with retro-orbital pain, myalgia, arthralgia are self-limited [5]. However, in a proportion of people, the disease progresses to the severe clinical manifestations classified as dengue shock syndrome (DSS), which are characterized by the plasma leakage leading to hypovolemic shock and/or dengue hemorrhagic fever (DHF), which are characterized by massive bleeding, thrombocytopenia, evidence of plasma leakage such as pleural effusion and a rise of hematocrit, both of which has a high mortality rate [6-8].

DENV is a positive-single strand RNA virus surrounded by an icosahedral nucleocapsid (C) with approximately 10,700 bases, a unique open reading frame that codify to one polyprotein, which is post-translational cleaved by cellular and viral proteases. The 5′ end contains the region encoding the structural proteins in the following order: core protein (protein C), membrane precursor protein (protein M), and envelope protein (protein E). The remainder of it genome encode for seven non-structural proteins, xlink, NS2A, NS2B, NS3, NS4A, NS4A, and NS5 [9].

DENV exists as a four serotypes (DENV-1, DENV-2, DENV-3, and DENV-4). All of them have a same transmission cycle, which include vertebrate hosts (primate and human) and invertebrate vectors, mosquitoes of the following species: *Aedes aegypti, A. albopictus y A. polynesiensis* mosquitoes. An infection with one serotype provides lifelong protective immunity to that serotype. But, there is no cross-protective immunity between serotypes [10, 11]. Inside each one of these serotypes, there are several virus groups named genotypes.

2. Autophagy

This is defined in a general form like a catabolic selective process by means of which cytoplasmic material is transported to lysosomes for their degradation [12]. The autophagy is a remarkably conserved cellular process, from yeast to human, responsible for removing damaged organelles and misfolded proteins, and for maintaining cellular homeostasis under both normal and stress conditions [13-15]. Compartmentalization in eukaryotic systems brought numerous evolutionary advantages, but also great and new challenges with it, such as the selective removal of damaged organelles, controlled organelle number and quality, or

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the utilization of their components as potential energy source during times of starvation. In this way, autophagy represents an evolutionary answer to these challenges. It enables the recycling of intracellular components and allows cells to survive or death [16] (Figure 1).

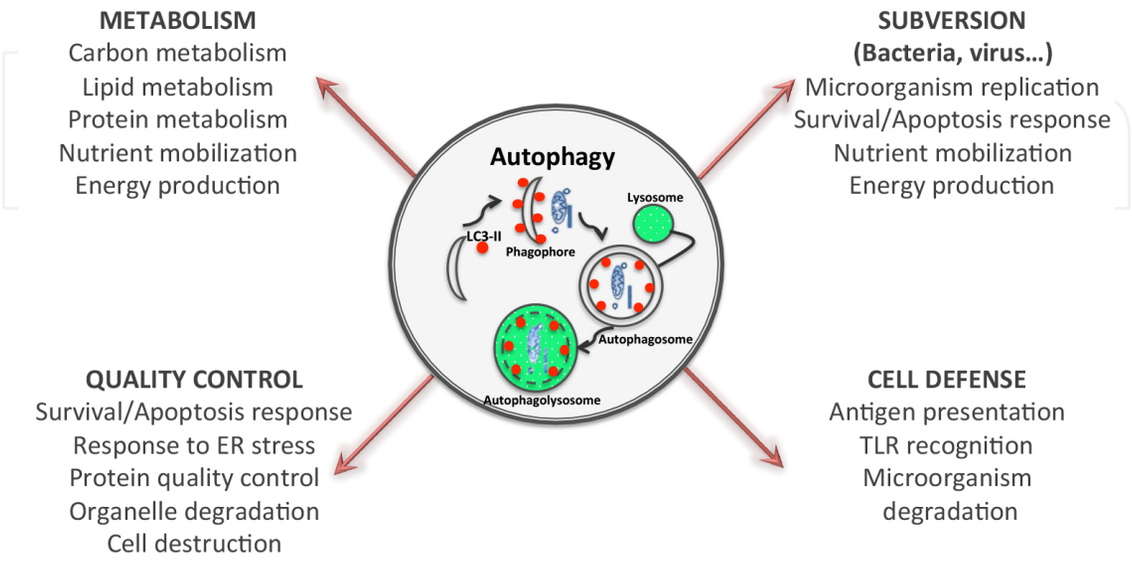

Figure 1. Functions of autophagy

The primary role of autophagy is to protect cells under stressful conditions. Under this viewpoint, both autophagy and the vertebrate immune system play essential roles to maintain cellular homeostasis in the face of external perturbations [17]. Indeed, several studies have revealed the narrow relationship between autophagy and the vertebrate immune system [18]. Besides, the crosstalk has become evident between autophagy and apoptosis [19-22] because the induction of autophagy has often been linked to inhibition of apoptosis [23].

More than 30 genes have been identified as crucial in the autophagy regulation process in yeast, which are known as ATG (autophagy-related genes). Many of these genes have homologs in mammals and are grouped according to expression and participation in the different stages of the autophagic route [12]. The activation of this pathway depends on the kinase mTOR (mammalian Target of Rapamycin) identified as the main negative regulator when the cell is in the presence of growth factors and abundance of nutrients. Under starvation, mTOR activity is inhibited. And consequently, the autophagy is activated allowing the recruitment of complexes inducers of the route [24, 25]. There are three mechanisms identified for autophagic degradation: macroautophagy, microautophagy, and chaperone-mediated autophagy (CMA).

Macroautophagy imply the formation of double membrane vesicles recognized as autophagosomes. It engulfs cytoplasmic components and then are fused to lysosomes, carrying the cytosolic material until the lysosomal lumen where a hydrolases, lipases, and cathepsins degrade it [26, 27]. Therefore, we can divide the pathway into 4 basic steps: initiation, elongation, termination, and fusion. During initiation, the recruitment of protein complexes, such as

phosphatidylinositol-3-kinase Class III (PI3K), Vps15, Vps34, and beclin-1, that are critical to the autophagosome formation is given [28]. During the elongation, the assembly occurs. In this stage, the related protein complex ATG5-Atg12-Atg16, the lipid conjugation complex LC3-II- phosphatidylethanolamine (PE), and the respective conjugating enzymes, which act similarly to ubiquitin ligase system, link lipid that allows the growth of the double membrane due to the transformation that undergone LC3-I to LC3-II, which has a PE-binding domain, the main lipid component of autophagosomes. Later, in the termination stage, the double membrane vesicle is closed with the intracytoplasmic content therein, which is possible because the cut that performs Atg-4 enzyme on the binding LC3-II-PE permits the release of the complex into the cytosol preventing the continuation of joining new lipid molecules [28].

Subsequently, the fusion process occurs between autophagosomes and lysosomes, which generate a vesicular structure called autophagolysosome or autolysosome. This process is mediated by the cell membrane fusion proteins, such as integral proteins SNARE (soluble N-Ethylmaleimide-sensitive factor-attachment protein receptor) [29], the Rab family proteins, especially Rab7 and Rab9, that are involved in the transport of the vesicles and fusion with target membranes [30]. And besides, the lysosomal membrane receptor LAMP2 allows the attachment between vesicular membranes and autophagosome contents discharge into the lysosome forming the structure known as autophagolysosome, where protein degradation occurs [31, 32] (Figure 2).

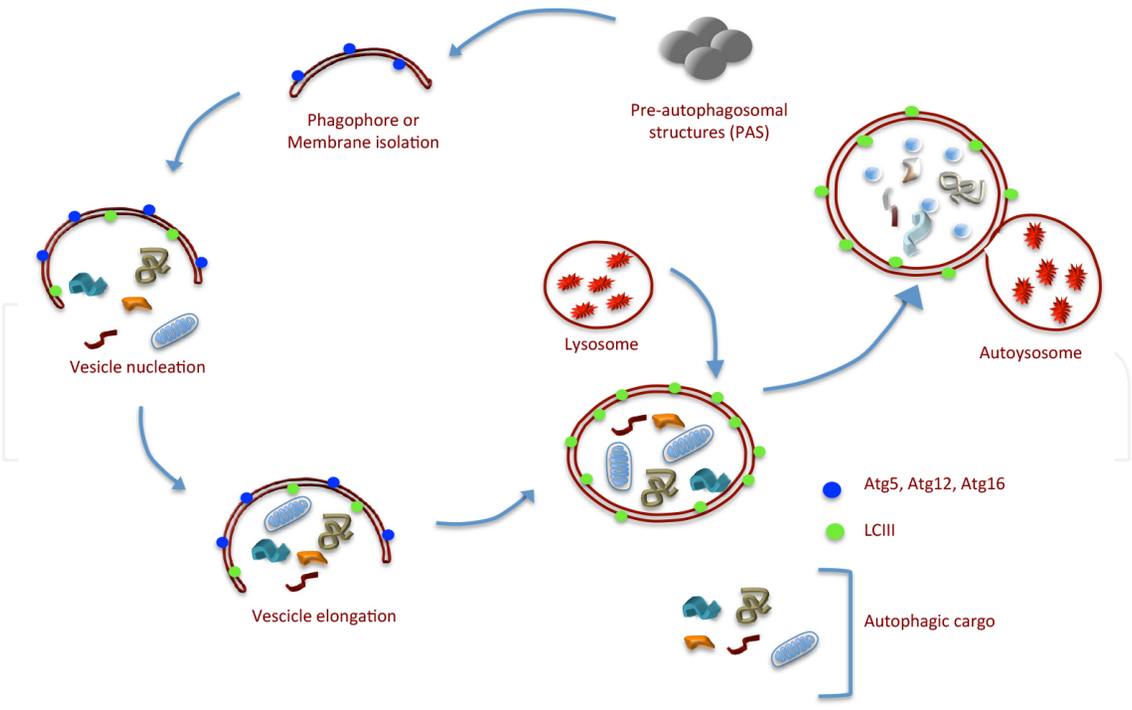

Figure 2. The Process of Macroautophagy. A portion of cytoplasm (including organelles) is enclosed by a phagophore or isolation membrane to form an autophagosome. The outer membrane of the autophagosome subsequently fuses with the lysosome, and the internal material is degraded in the autolysosome.

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The second possible mechanism is microautophagy. It involves direct lysosomal membrane invaginations production to generate vesicles or tubules capturing surrounding cytoplasm. Microautophagy studies has mainly been developed in yeast, wherein several genes have been found sharing the macroautophagy and microautophagy pathways, but the components and regulation systems are still not well understood [33]. For the CMA mechanism, the cargo is specifically selected by the unique pentapeptide motif present in the amino acid sequence (KFERQ), which is recognized by the chaperone proteins specially Hsc70, in where the membrane receptor LAMP2 (Lysosome associated membrane protein 2] carrying the load into the lysosome lumen for degradation [34].

In the past years, autophagy has emerged as a critical player in the control of viral infection and immunity [35-39]. On one hand, autophagy can serve as a host defense mechanism for some pathogens by clearing them out of the cells [40-42]. On the other hand, many positive-stranded RNA viruses have been reported to subvert this cellular machinery to favor their own replication and release [23]. This issue will be discussed below.

3. Cellular metabolism in viral infections: Rediscovering the other side of the coin

Metabolism is broadly defined as the sum of biochemical processes in living organisms that either produce or consume energy [43]. In the "Golden Age of Biochemistry" (1920s to 1960s), most of the metabolic network in humans and other organisms, which included routes like glycolysis (Embden, Meyerhof, and Parnas), respiration (Warburg), the tricarboxylic acid (TCA) and urea cycles (Krebs), glycogen catabolism (Cori and Cori), oxidative phosphorylation (Mitchell), and the supremacy of ATP in energy transfer reactions (Lippmann) was defined. This research was awarded with about 15 Nobel Prizes in Physiology, Medicine, or Chemistry. All of them were related to energy balance or core metabolic pathways [43].

Richard W. Hanson wrote "By 1970, the writing was on the wall for metabolism; it was largely considered a "mature area", lacking excitement; molecular biology was the area of the future" [44].

"A sure sign of this was that graduate students in biochemistry almost never selected their thesis research in metabolism. The course in intermediary metabolism that I taught was dropped from the curriculum of our graduate education program; our students were expected to learn all they needed to know about metabolism as undergraduates before they attended graduate school. After all, as a graduate student once said to me, "the great problems in metabolic research have been solved". As long as diseases like diabetes, obesity, and atherosclerosis, remain to be cured, there will be no shortage of interest in metabolism" [44].

In this way, the understanding of diseases in light of alterations in metabolic status was dropped and shifted by the search of an explanation based on the nascent era of molecular biology. However, the ongoing exploration of molecular biology and disease complexity has stimulated a revival of interest in intermediary metabolism [45]. In this view, several works

propose a new way to arrive the disease: cell metabolism, because it affects cell signaling and modulate protein trafficking, localization, and enzyme activity [43]. For example, Acetyl-CoA plays a central role in intermediary metabolism (carbohydrate, fatty acid, and amino acid oxidation,) and at the same time have tremendous influence on cell signaling and gene expression [46-48]. Recently, it has been demonstrated that some biomarkers of metabolic syndrome are related with any infection, acute or chronic in patients [49].

To reach a deep and elegant comprehension of the role of metabolism in all levels of the human being, it is better to take the exact quotation of DeBerardinis and Thompson: "...the metabolism pervades every aspect of biology from the single-cell to whole organism level. No cellular functions occur independently of metabolism, and a metabolic perturbation at one node has ripple effects that can extend throughout the network and out into other systems. Thus, metabolic disturbances have an extremely long reach, and this extends to disease phenotypes..." [43].

The Warburg effect is a concept used to link metabolism and cancer wherein a disturbance of cellular metabolic activity is at the root of tumor formation and growth [43]. Thereby, dysregulated cellular metabolism is a key feature of cancer [50-53]. This concept could be adapted perfectly to viral infections, because viruses are biological entities that depend on cell metabolism to replicate and spread. Therefore, it would be expected that the success of viruses inside the cell will be dependent on their ability to subrogate the metabolism and put it in his favor.

It had been shown in this sense that tumor cells display increased metabolic autonomy in comparison to non-transformed cells [51]. In the case of viral infections, this "metabolic autonomy" may be triggered by a viral entity in normal cells. Thinking about it, it is not absurd if we take into account that many genes implicated in several signaling/metabolic pathways have also been reported to be modulated and altered in viral infections [54-57]. How these metabolic pathways are regulated in infected cells, including if they fluctuate according to infection stage or at the cell cycle, remains to be a question. It will be important to determine whether viral infection can regulate all aspects of the metabolic dynamics or if any special metabolic pathway implicated in their replication or pathogenicity exists.

Many pathogens have developed sophisticated molecular machinery, which interferes with host cell signaling. Thereby, effector molecules are introduced or released by the pathogens during the invasion of the host [55, 58, 59]. Autophagy is a evolutionarily refined and sophisticated process wherein molecular cell signaling and cellular metabolism regulation converge to regulate the intermediary metabolism (Figure 3) including the lipid metabolism through a process called lipophagy, which modulate the degradation of lipid droplets in triglycerides and free fatty acid that can be used as a fuel to elevate the rate of β-oxidation and consequently of energy production [60-62], which recently has been demonstrated that some pathogenic agents can subvert this cell process to ensure their own survival.

Recent investigations using genetic, cell biology, and biochemical approaches have led to a better understanding of mechanistic interaction between pathogens and hosts. Based on this, a resource that permit integrate terms of ViralZone, UniProtKB, and GO, has been created, which provide a global view of viral biology and their complex host interactions, based in evasive adaptations and inactivation of antiviral effectors [63]. Advancements in research are

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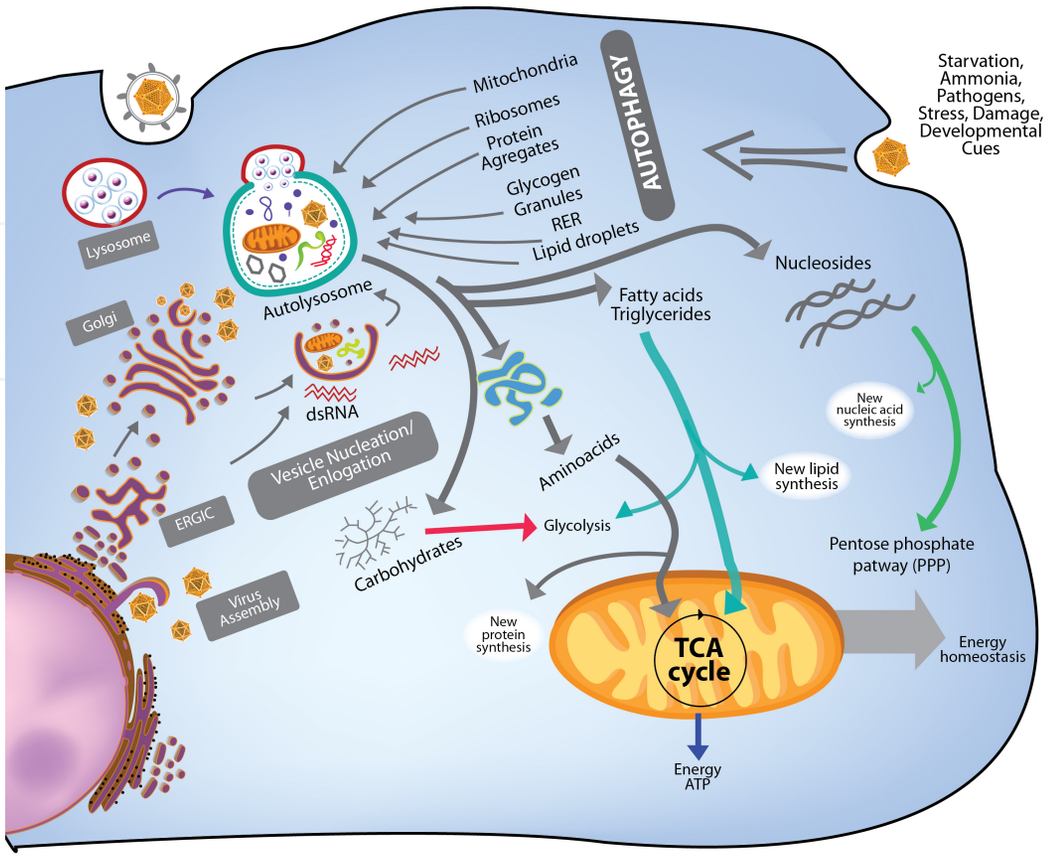

Figure 3. Autophagy and intermediary metabolism relationship

now fueled by increasing interests aimed at the discovery of novel therapeutic interventions against major infectious diseases [64]. The cell biology of microbial pathogenesis has opened many doors for future research into the role of lipids in host-pathogen interactions because lipids of both host and pathogen play critical roles in the pathogen stability to replicate and persist in host cells, and these interactions are very complex and dynamic [58].

The metabolic host cost and contribution of lipids (biosynthesis, catabolism, and trafficking) to the formation of replication factories is in the early stages of investigation [65-69], and yet is need to know pathogen and host lipid profiles as a starting point for tests of functional relevance and comparative profiling in several physiological conditions (status before/after infection) to better understand the details of the metabolic role in the different conditions of the disease and dissect the complicated signaling during host-pathogen interactions for developing drugs and disease biomarkers pathways identification [64].

4. Role of autophagy in infectious disease

The intracellular invaders, after million years of evolution, have developed several sophisticated strategies for evading the host defenses like the immune system. In this regard, autoph-

agy is a complex cellular process that can have a dual role in viral infections depending on the pathogenic agent and host [70, 71]. Although it has been extensively cited and reviewed, the role of autophagy in maintaining the cellular homeostasis [12, 13] still remains to be elucidated in terms of what is their precise role in viral infection.

Considering several infectious agents, there are a number of important findings. For example, macrophages can eliminate *Legionella pneumophila* infection through cholesterol or lipid-raft-rich induction of autophagy [72]. Mycobacteria usurp the host lipid stores for energy production via β- oxidation of fatty acyls, using the glyoxylate cycle enzymes isocitrate lyases for survival and persistence in its human host [73, 74]. *Helicobacter pylori* have been related with elevated cholesterol levels and metabolic syndrome alterations. However, it remains controversial [75, 76]. In HSV-1 (Herpes Simplex Virus), the virulence factor ICP34.5 inhibits autophagy via inhibition of Beclin 1 and PKR [77], and Us3 acts as a viral Akt surrogate to activate mTORC1 inhibiting host autophagy [78]. Curiously, additional members of the herpes virus family employ similar strategies to inhibit autophagy. Gamma herpes virus 68 (gHV68) encodes a virulence factor vbcl2 (M11), which inhibits host autophagy via interaction with Beclin 1 [79]. Kaposi's Sarcoma Herpes Viruses (KSHV) interact with ATG3 and inhibit autophagy [80]. Human Cytomegalovirus (HCMV) inhibits autophagy via upstream activation of mTOR signaling [81]. Autophagy functions as an antiviral host defense of central nervous system against Sindbis Virus (SIN) infection [40, 41].

Hepatitis C virus (HCV) infection has a controversial role in lipid metabolism and autophagy. It has shown that this infection is associated with enhanced lipogenesis, reduced β-oxidation, decreased lipoprotein secretion, and increased autophagy counteracting the alterations in lipid metabolism induced by HCV. In this way, a disruption of autophagic process might contribute to develop steatosis (occurs in about 50% or more of patients) in patients with HCV [82, 83].

It has also been described that the infection of human cells with Poliovirus and Rhinovirus induces autophagosome formation, which are used as sites of viral RNA replication [84]. Autophagosome is required for the formation of Coronavirus replication complexes with the formation of the double membrane vesicles significantly enhancing viral replication efficiency [85]. The use of small interfering RNAs against LC3 or Atg12 has shown to reduce both the intracellular and extracellular yields of poliovirus (+ss) [84]. Reduction in the intracellular concentration of Atg7 reduces the amount of viral capsid protein synthesized in Coxsackievirus B3 [86]. Hepatitis C Virus (HCV) infection was found to activate autophagy, and it extends cell survival for the establishment of a successful viral infection [87].

It should be noted that not only RNA virus (poliovirus, etc.) but also DNA virus (Epstein-Barr virus) infection can induce autophagic machinery, and whether the activation of autophagic machinery can enhance viral replication (poliovirus and mouse hepatitis virus) or not (Vaccinia virus and Herpes Simplex Virus type 1 etc.) depends on the type of viruses [84, 85, 88-90] and on cell type infected [91]. Thus, for some areas for research, the development of the specific inducers of autophagy will offer a promise as a novel class of antiviral therapeutics [16], while for others, the design of specific inhibitors of autophagy could provide new therapeutic strategies [92]. Either will serve as a powerful tool to dissect the autophagic process.

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In summary, many different viruses and other pathogens can induce the cellular process, such as apoptosis and autophagy, and on the other hand, host cells can also activate the same pathways when they participate in clearance of infectious agent (Figure 4). Thus, although some viruses may encode one or more inhibitors of both these processes, others have been shown to induce autophagosome-like structures and to benefit from their formation, which may be critical for the viral spread within the infected tissues [35]. Although numerous studies support the beneficial role of autophagy in +ssRNA virus replication [23], the induction of this process is not always favorable for them. And drawing the path and explaining this behavior have shown interesting findings for some researchers.

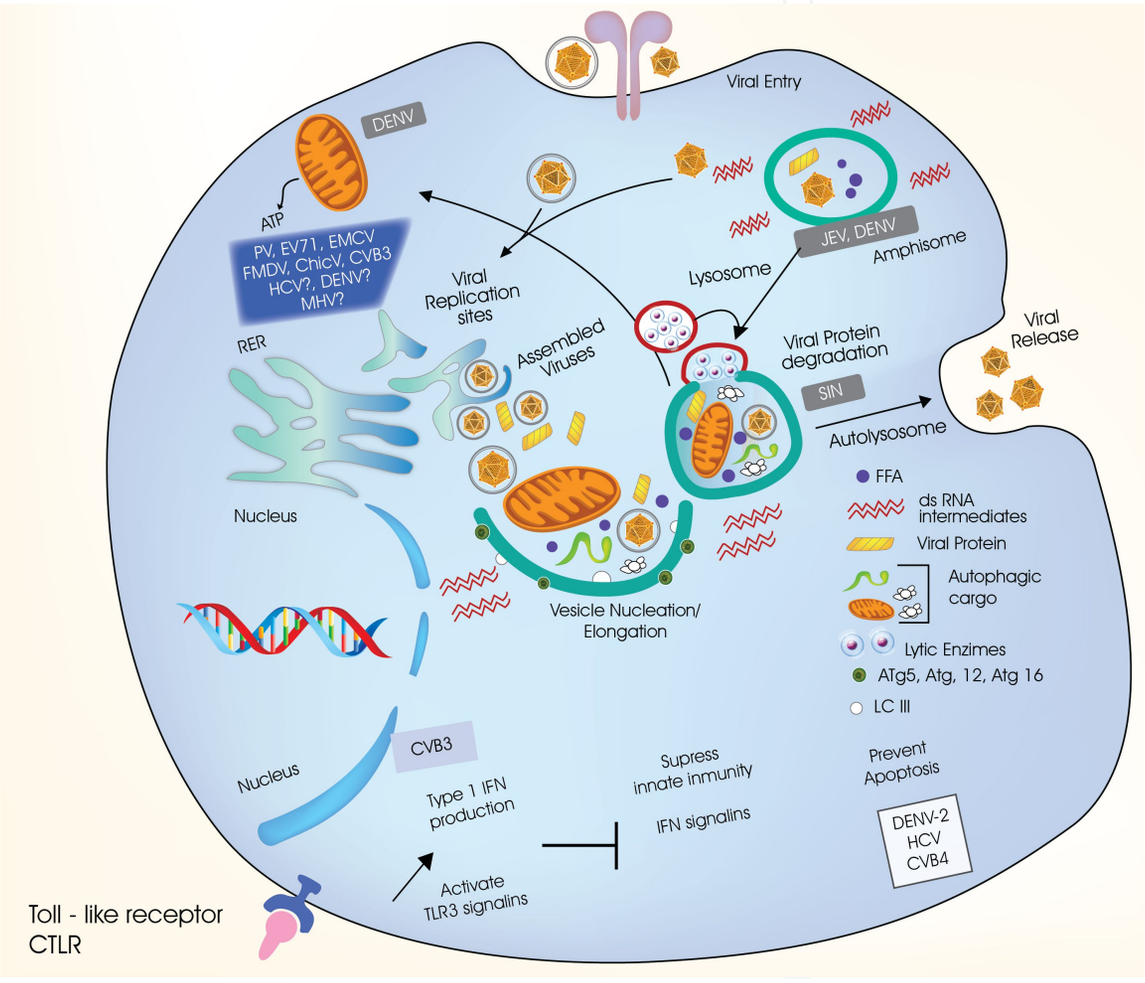

Figure 4. Viral modulation of autophagy. Several viruses have been shown to block or activate various stages of autophagy process.

5. DENV infection and autophagy: Molecular and metabolic convergences

DENV is a major but neglected global public health problem, and despite many efforts, they are made to understand the mechanisms by which it usurps the host cells and this research

field has grown dramatically during the last years with multiple studies in molecular and evolutionary biology [93-96], genome sequencing [97-99], construction of infectious clones [100], and use of these to attempt to dissect the specific role of each viral protein [101, 102], and immunological approaches [103, 104]. All of these have failed to produce results that allow the design of vaccines or drugs effective to cure this disease [105-107], and the secrets of DENV and its pathogenesis remain unclear. However, a recent viewpoint of the disease highlights the relevance of the relationship between both hosts and vector systems with the viruses and assign a key role to energy metabolism alterations during the infection, indicating that the virus reprogram the central carbon metabolism (lipid, glucose, TCA cycle and others) [108-112] in order to facilitate their own replication.

Understanding how DENV can differently infect mammals and insect cells is a very interesting issue. In the last years, there has been a notable increase in the research for mosquito DENV infection, revealing the importance of identifying this dual behavior between the host and vector in order to know the cell biology of viral infection. In the enveloped positive-sense RNA viruses such as DENV, a cytoplasmic replication of its are associated with a dramatic rearrangement of host cellular membranes, the merging of viral and target cell membranes, and endosomal trafficking routes are essential to carry out a successful replication cycle [113], and these virus-induced changes the result in induction of vesicular structures that envelope the virus replication complex [114].

A few years ago, it was postulated that autophagosomes might play a structural role in the replication complex formation, and numerous investigations about the role of autophagy in DENV infection were conduced. In 2008, a researcher group from the National Cheng University in Taiwan was the first to demonstrate that DENV can activate autophagic machinery and induce autophagosome formation to promote viral replication, and ATG5 is directly implicated in this activation process [6]. Also, it has been demonstrated that DENV2 induce autophagy and prevent premature cell death, thus, an inhibition of autophagy abolishes its protective role against cell death providing an unfavorable environment for the viral propagation leading to a reduced viral replication [115]. There were experiments to compare single-cycle infections of murine embryonic fibroblasts derived from autophagy-proficient and autophagy-deficient mice showing clear reductions in the yield of extracellular virus in the absence of a functional autophagy pathway [6]. But in 2009, it was demonstrated that DENV replicates on endoplasmic reticulum (ER) cisternae invaginations and not on classical autophagosomes [116]. From this discovery, scientists kept researching the role of autophagy induction in DENV infection.

In the same year, it was shown that the DENV Capsid structural protein contained determinants for lipid droplets targeting. This association was a determinant for reach DENV yield [117], and this discovery was associated with previous findings that reported liver vacuolization and steatosis in DENV infected mice and fatal human cases of DHF [118-120], suggesting a possible role for lipid metabolism in DENV pathogenesis. That was when researchers reported that autophagy process induced by DENV infection plays an indirect role in DENV replication by the modulation of cellular lipid metabolism. Furthermore, it stimulated a cellular triglycerides depletion that are stored in the lipid droplets, leading to release free fatty acids,

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increased β-oxidation, and energy production to raise the virus yield [121-125] (Figure 5). Subsequently, it was shown that autophagy is mediated in a cell type specific manner, given that autophagy does not have a significant role in DENV replication in monocytic cells [91]. More recent studies in suckling mice demonstrated that DENV infection induce autophagy mechanism *in vivo,* and it played an important role in viral replication, clinical symptoms development, and survival rate [126]. Although it has been widely supported that the autophagy role in DENV infection is more related to a metabolic requirement, it also has been shown that the autophagy pathway plays a determinant role in viral maturation [124], which conduce to think that autophagy does not have a unique function in the viral replication cycle.

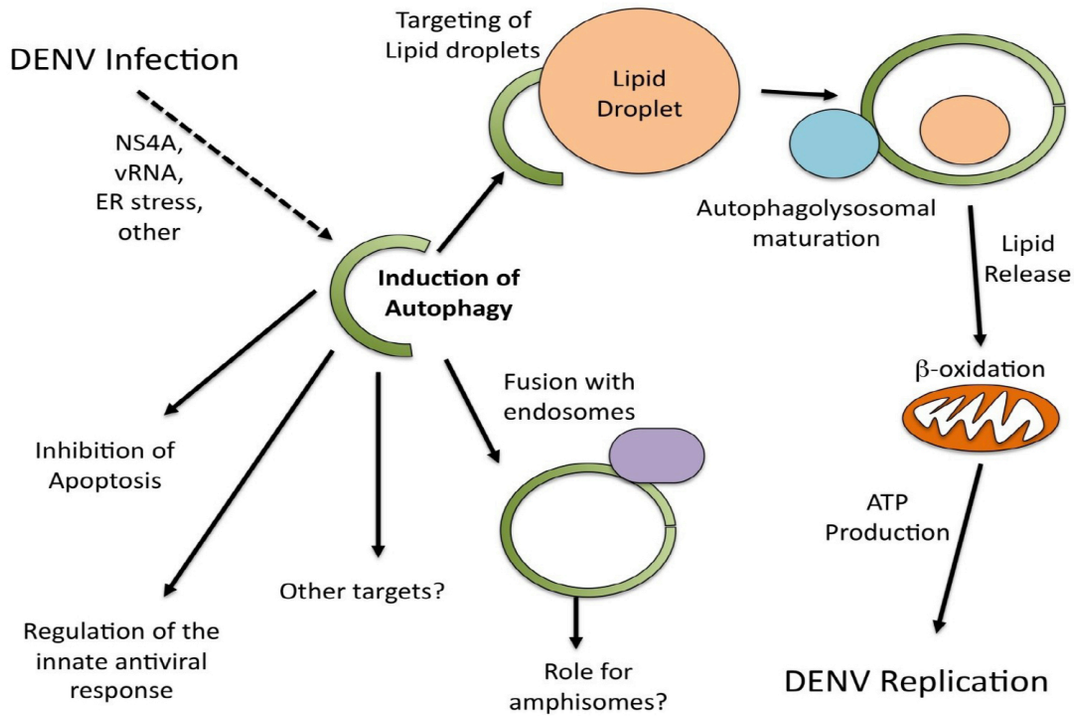

Figure 5. Roles for autophagy during DENV infection From [122].

The discovery of vector factors altered during DENV infection of mosquito may help to identify conserved protein families and pathways that represent both anti-viral mechanisms and requirements for viral life cycle in the vector, and understanding these effects in mosquito vector and correlating it with conserved mammal pathways could help to comprehend the host interactions and development of methods to treat and prevent viral infection and spread. In this way, the mosquito vector, as well as the cell lines, derived from it, was transformed in novel and interesting study models. It has been described in mosquito MAL04 and C6/36 cells that DENV ensures its fusion in late endosomes exploiting cell-controlled differences between lipid compositions of different organelles through interactions between virus and endosomes rich in anionic lipids, protecting against premature release, viral inactivation, or endosome fusion pore opening [127].

The *fat body* plays a major role in intermediary metabolism, and it is the central storage depot of nutrients and energy reserves essential for the holometabolous insects' life, which must accumulate at least a minimal amount of nutrients in larval stages to survive during starvation and metamorphosis. Lipids, mainly triglycerides, represent the major component of *fat body* and are the main source of metabolic fuel, it are stored in the core of lipid droplets, which are mobilized for several purposes as energy provision to flight muscles, ovaries lipids provision and overall maintenance of metabolic activity [128]. The lipolytic machinery identified in insects includes two lipases: TGL and Brummer lipase, and two evolutionarily conserved lipid droplet proteins, Lsd1 and Lsd2 [129]. Current information indicates that insects share with mammals and other organisms, several aspects of the mechanisms of deposition and mobilization of triglycerides. This information validates the use of insect models to investigate basic questions related to the processes of lipid storage and mobilization [130].

DENV drastically alters the lipid profile of mosquito-infected cells, increasing the expression of lipids that have the capacity to change the physical properties of the bilayer such as: bilayer curvature, permeability, and recruitment/assembly of protein complexes in the membrane. Several of the identified molecules also function as bioactive messengers that control signaling and membrane trafficking pathways in the cells [131]. These observations shed light on the emerging role of lipids in shaping the membrane and protein environments during viral infections and suggest membrane organizing principles that may influence virus-induced intracellular membrane architecture [131]. Later, a transcriptome study in *Aedes aegypti* infected with several flaviviruses (WNV, DENV and YFV) was described and an expression profile was observed with 20 significantly upregulated genes and 15 downregulated genes quite similar among them. Something of these genes were related with the regulation of genic expression (juvenile hormone-inducible protein, core histone H3), genes related with antiviral response were downregulated (Jak-STAT pathway downregulated, Toll pathway) and other genes related to ion binding, ion transport, several *metabolic processes* and peptidase activity [132].

In [133], a mosquito protein interaction network based on large-scale protein interaction datasets was developed, and 714 putative dengue-associated mosquito proteins (physical interaction assays, RNAi and microarray) were identified and predicted. Subsequent analysis of these proteins highlighted a sub-network, four regions of highly interconnected proteins with closely related functions (replication/transcription/translation (RTT), immunity, transport, and metabolism). 15 out of 23 proteins (65.2%) were highly interconnected in metabolism region. Consequently, the host infected by the virus can experiment dramatic metabolic alterations. These results support the presence of some common host requirement of DENV in humans and mosquitoes.

6. Concluding remarks and perspectives

After reviewing the historical issues about biochemistry with emphases on metabolism, together with the remarkable findings in cell molecular biology of autophagy pathways, it is

clear that right now, we have a great open field for research. Curiously, the animal viruses, during several decades, had been studied, but under the viewpoint of virus-host cell interactions wherein the cell and the viruses have been considered isolated entities. Only in the last decade where the Cell Biology of Virus Infection emerged [134] was considered the cell as the structural and functional unit of infection. Therefore, now, the animal viruses play a role in the physiology of the cell, mimicry and using the metabolic and autophagic cell pathways for the completion of their viral cycles.

An overview was shown here for understanding the viral disease and other human pathologies from an integrative perspective including the theoretical framework and methodology of biochemistry fused to the molecular biology in a cellular compartment (autophagosome), which is triggered for several injuries and/or diseases.

It is important to establish the differences in mechanisms of infection. Therefore, in the basic requirements for this process in both vector (mosquito) and host (mammal), it is important to determine whether it alters in a similar way the metabolism in both models, although the molecular signaling through which these metabolic changes are induced to be different for everyone. It is very interesting that all of these recent researches in mosquitoes suggest alterations in JACK / STAT signaling, toll-like receptors, and metabolism (especially lipid). But knowing that autophagy is conserved from yeast to mammals, the role of autophagy has not been reported in DENV infection in mosquitoes. Moreover, there is a recent research which supports that autophagy is not decisive in the infection in monocytes. It appears that the autophagy is dependent on the cell type.

Together the ideas exposed here with the remarkable findings of several researchers give us a whole landscape where it is possible to find some cellular processes or events, which can be modulated by drugs trying to discover new therapeutical tools.

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