Host Autophagy Response: Friend or Foe in Reproductive Tract Infections

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Abstract

Autophagy was discovered as a self-degradative process that is induced for balancing the nutrient levels in the cells during the times of starvation. It was found to play an additional housekeeping role in removing misfolded or aggregated proteins, clearing damaged organelles. Recent studies have established autophagy as an important mechanism of the innate immune defense of the host. It acts as a second line of defense for the clearance of the pathogen and is induced by various adaptor proteins like Pattern Recognition Receptors (PRRs). This role of autophagy has been chronicled in many microbial infections such as Group A Streptococcus (GAS), Mycobacterium tuberculosis, Salmonella typhimurium and Enterococcus faecalis. Infections of the reproductive tract form a significant part of the health problems faced by the world and are primarily transmitted through sexual mode. The role of autophagy during reproductive tract infections (RTIs) such as Chlamydia, Candidiasis, Trichomoniasis, and Viral Infections like Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV) and Human Papilloma Virus (HPV) have been studied using various in-vitro and in-vivo models. In this review, these findings on the relationships between autophagy and microorganisms causing RTI have been summarized to aid the understanding of the role of autophagy during these infections.

Keywords: Autophagy; RTIs; Innate immunity; Host-pathogen relationship

Autophagy

The term ‘autophagy’ is derived from Greek, which means ‘eating of self’. It is now used as a general term for the degradation of cytoplasmic and pathogenic components through the lysosome mediated pathway. The self-degradative nature of autophagy is essential in times of starvation as an amino acid source. Autophagy is constitutively involved in removing misfolded and aggregated proteins [1] along with selectively clearing damaged organelles, such as mitochondria [2], endoplasmic reticulum [3] and peroxisomes [4]. Autophagy is also involved in the elimination of microorganisms, cell death, tumor suppression [5-7] and antigen presentation [8].

The first step in the process of autophagy is autophagosome formation. Cytoplasmic constituents and foreign materials are sequestered by a unique membrane called phagophore or the isolation membrane. This membrane has a flat shape and is reminiscent of a Golgi cisterna. Some of the Atg proteins (autophagy-related proteins) gather at the phagophore formation site called “pre-autophagosomal structure” (PAS) [9]. Complete sequestration of the constituents occurs by elongation of the phagophore, results in the formation of autophagosome, which is a double-membrane organelle [10]. In the next step, autophagosomes fuse with lysosomes (in metazoan cells) or vacuoles (in yeast and plant cells). The inner membrane of the autophagosome and the sequestered materials are then degraded by lysosomal/vacular hydrolyases. These degrading structures are often called “autolysosomes” or “autophagolyosomes.” Once the macromolecules or pathogens have been degraded in the autophagolysosome, the resultant amino acids are exported to the cytosol for reuse [11] or the antigens are presented to the T cells to activate the adaptive immune response (Figure 1).

Regulation of autophagy is carried out by autophagy-related genes (ATG genes). Till date 32 ATG genes have been identified in yeast and 16 homologs of these have been characterized in humans [12]. A coordinated action of these genes is essential for the successful formation of an autophagosome. The function of each of these ATG genes is unique and is not complemented. Hence, knockdown or deletion of these genes will result in cell death through either apoptosis or necrosis.

Types of autophagy

There are three defined types of autophagy: macro-autophagy, micro-autophagy, and chaperone-mediated autophagy (CMA). Macro-autophagy is involved in organelle and protein recycling and pathogen clearance. It delivers the cytoplasmic cargo to the lysosomes through autophagosomes. Macroautophagy is further classified into “induced macroautophagy” and “basal macroautophagy”. The former is involved in amino acid recycling following starvation and during pathogen clearance, while the latter is involved in constitutive turnover of cytosolic components [13].

In micro-autophagy, cytosolic components are directly taken up by the lysosome itself through invagination of the lysosomal...
membrane. The maintenance of organelle size, membrane homeostasis, and cell survival under nitrogen restriction are the main functions of microautophagy. It complements and co-ordinates with macroautophagy, CMA and other self eating pathways to perform these functions [14]. In CMA, targeted proteins are translocated across the lysosomal membrane in complex with chaperone proteins that are recognized by the lysosomal-associated membrane protein 2A (LAMP-2A), resulting in their unfolding and degradation [15].

**Autophagy in Innate Immune Mechanisms:** Autophagy is rapidly developing into a new paradigm in innate immunology (Figure 2). Its roles in innate immunity have been shown through *in-vitro* and *in-vivo* models [16]. Autophagic adapters are capable of identifying Pathogen Associated Molecular Patterns (PAMPs) and Damage-Associated Molecular Patterns (DAMPs). Autophagy inducing PAMPs activate the different Pattern Recognition Receptors (PRRs) such as Toll-like receptors (TLRs), Nod-like receptors (NLRs), RIG-I-like receptors (RLRs) and Sequestosome-like receptors (SLRs) [17]. DAMPs, also called as alarmins, undergo a change in their intracellular localization or are released from damaged cells during cell or tissue injury under sterile or septic conditions [18]. These are identified by autophagic adaptors, thereby activating autophagy. Some examples of alarmins are High mobility group box-1 (HMGB1), Interleukin-1β (IL-1β), Adenosine Tri-Phosphate (ATP) and DNA complexes [19].

Autophagy possesses anti-inflammatory characteristics. Leaky or depolarized mitochondria release ROS that induce inflammasomes [20]. Basal autophagy prevents this activation of inflammasome by continuously removing the damaged mitochondria [21]. This process is called as mitophagy. If this basal autophagy is impaired, ROS and mitochondrial DNA cause unscheduled inflammasome activation [22]. However, Autophagy can target free pathogens in the cytosol as well as those not killed through the phagocytosis mediated degradation pathway [23]. A study by Nakagawa and colleagues [24] showed that Group A Streptococcus (GAS) that escapes from endosomes into the cytoplasm becomes enveloped by autophagosome-like compartments and is killed upon fusion of these compartments with lysosomes. Apart from GAS, autophagy has also been shown to play a role in the elimination of other bacteria like *M. tuberculosis*, *L. monocytogenes* and Salmonella species [25].

Recent studies have demonstrated that autophagy in response to an infection by opportunistically invasive commensals like *Enterococcus faecalis* and invasive intestinal pathogen like *Salmonella typhimurium* protects the host against invasion. A study from Hooper’s lab has shown that autophagy is activated following oral bacterial invasion of intestinal epithelial cells [26]. They have further shown that this autophagic response occurs via the innate immune adaptor protein, MyD88 that is intrinsically present in the epithelial cells. Additionally, they
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Figure 2: Autophagy in innate immunity. During infection, pathogen and their pathogen associated molecular patterns (PAMPs) are identified by various pattern recognition receptors (PRRs) such as Toll like receptors (TLRs), Nod like receptors (NLRs), RIG-1 like receptors (RLRs) and Sequestosome like receptors (SLRs). TLRs have been shown to activate autophagy through the MyD88 or TRIF mediated signaling. This activation of autophagy results in sequestering of the intracellular pathogens by autophagosomes, followed by clearance of the pathogen through lysosomal degradation.

showed that mice having the ATG-5 gene conditionally knocked out in intestinal epithelial cells exhibited increased pathogen load and greater dissemination of invasive bacteria to extra-intestinal sites. Thus, autophagy is an important intrinsic mechanism of the epithelial cells to exhibit defense against pathogens in in-vivo conditions [26].

Autophagy in Reproductive Tract Infections: Reproductive tract infections (RTIs) including sexually transmitted infections (STIs) represent a major public health problem especially in the developing countries [27]. The consequences of RTIs/STIs are numerous and potentially devastating. These include sepsis, ectopic pregnancy, fetal and perinatal death, cervical cancer, infertility, chronic physical pain, emotional distress, and social rejection of women [28]. Some of the most common STIs include Chlamydiolation, Candidiasis, Trichomoniasis, Gonorrhea, Syphilis and Viral Infections like Human Immunodeficiency Virus (HIV), Herpes Simplex Virus (HSV) and Human Papilloma Virus (HPV) infections.

Autophagy and Chlamydiasis: Chlamydia trachomatis (CT) is a human pathogen associated with common STIs. These bacteria enter the host cell and survive within a membrane-bound vacuole, termed as inclusion body, in which they ensure their successful propagation by avoiding fusion with lysosomes [29]. Non-fusogenity with lysosomes is controlled by the mode of cellular uptake and chlamydial protein factors [30]. Autophagy and its related proteins interact with CT infection in the following ways (Figure 3).

Al-Younes et al. [31] have reported that autophagosome specific stain – Mono Dansyl Cadaverine (MDC) didn’t co-localize with chlamydia containing vacuoles. However, autophagy related proteins - MAP-LC3 and calreticulin showed increased accumulation around the pathogen containing vacuoles at an MOI (Multiplicity of Infection) ~1. Thus, autophagosome mediated clearance of pathogen may not occur but autophagy related proteins are modulated during infection by CT. The role of autophagy during CT infection has been further established using autophagy inhibitors such as 3-Methyl Adenine (3-MA) and synthetic amino acids such as Asparagine, Lysine, and Valine etc. This resulted in smaller CT inclusion bodies and development of defective CT. Hence, inhibition of autophagy appears to affect the proliferation of CT. Pachikara et al. [32] observed that autophagy is induced during chlamydia infection after 24 hrs, and hypothesized that unlike the previous observation where autophagy related proteins interacted with the pathogen, autophagy may be induced as a response to excessive nutrient depletion, due to proliferation of CT within the host cells, and not for pathogen clearance. This hypothesis was confirmed by their observation that autophagosome marker protein – Microtubule
Figure 3: Chlamydia infection and autophagy: Various studies have shown that autophagy plays an important role during Chlamydia trachomatis (CT) infection. However, the interaction with CT depends on the multiplicity of infection (MOI) of the pathogen. It was observed that during an infection of MOI ~ 1, autophagy mediated degradation of the pathogen was brought about when the cells were treated with IFNγ. However, at the same MOI, when Bafilomycin A (BafA) was added to the cells, CT survived as BafA inhibited the lysosomal enzyme vATPase. At higher MOI, it was observed that autophagy was induced but the autophagosomes did not co-localize with the pathogen. Hence, it was thought that autophagy is induced in response to the depletion of nutrients in the host due to the proliferating pathogen.

Associated Protein Light Chain 3 (MAP-LC3 or LC3) did not co-localize with chlamydia containing vacuoles. However, these authors observed that autophagy deficient cells were unable to prevent the growth of pathogen. This suggested that autophagy mediated nutrient recycling was not involved in the growth of pathogens.

vATPase is an enzyme in the lysosome which regulates the function of other lysosomal hydrolases. This would affect the degradation of pathogen through autophagolysosome. When the cells are treated with vATPase inhibitor, Bafilomycin A (BafA), lysosomal activity is inhibited. Yasir et al. [33] have shown that post infection by CT of wild type Murine Embryonic Fibroblasts (MEFs), treatment with BafA results in the proliferation of CT. This proliferation is due to the inhibition of autophagosome mediated pathogen clearance. Thus, autophagy deficient MEFs should show proliferation of CT. However, it was observed that autophagy deficient ATG5-/- MEFs when treated with BafA results in inhibition of CT. This opposite effect is because BafA affects cells having proficient and deficient autophagy in different ways.

Chlamydia infection of the host cell is known to induce interferon gamma (IFNγ) mediated immune response. Al-Zeer et al.[34] have shown that Irga6, Irgd, Irgm2 and Irgm3 proteins accumulate at bacterial inclusion bodies in MEF’s upon stimulation with IFNγ and this accumulation triggers a rerouting of bacterial inclusions to autophagosomes. These autophagosomes subsequently fuse to lysosomes for the elimination of chlamydia. They have also demonstrated that autophagy-deficient Atg5-/- MEFs were unable to clear the chlamydial pathogens and succumbed to infection even in the presence of IFNγ. Thus, autophagy may be essential in the clearance of C. trachomatis during infection.

LC3B exists in two forms LC3B type 1 that is cytosolic and LC3B type 2 that is incorporated into the autophagosomal membrane. Al-Younes et al., [35] have showed that LC3B that lines the chlamydia vacuole [31] is LC3B type 1 and favors the growth of pathogen. Also, ATG5 knockout results in increased proliferation of C. trachomatis. Hence, this function of LC3B type 1 is independent of autophagy and the autophagy process itself is inhibitory to pathogen proliferation and mediates pathogen killing at lower MOIs.

Autophagy and Candidiasis: Candida albicans (CA) is the most pathogenic species of Candida and is found to be the causative agent in most cases of candidiasis [36]. The most frequent manifestations of genitourinary candidiasis include vulvovaginal candidiasis (VVC) in women, balanitis and balanoposthitis in men, and candiduria in both sexes [37]. Autophagy has been shown to be upregulated in CA infection in multiple studies. Nicola et al., [38] showed that LC3 is localized to vacuoles containing CA during infection of murine macrophages.
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by CA. They also showed that autophagy is essential in the clearance of pathogen. Disruption of host autophagy in vitro by RNA interference against ATG5 decreased the phagocytosis of CA. Thus, the fungistic activity of J774.16 macrophage-like cells also decreased. Moreover, mice with a conditionally knocked out ATG5 gene in myeloid cells showed increased susceptibility to intravenous CT infection.

Studies have also implicated the role of autophagy adaptor proteins in the antifungal response of the host cell. Rao et al., [39] have demonstrated that terpenoid phenols like carvacrol exhibit anti-fungal activity against CA by creating downstream effects similar to the known autophagic inducer, rapamycin that inhibits the activity of major regulator of autophagy, mTOR (mammalian target of rapamycin). The effects include Ca²⁺ bursts, intracellular pH changes and increased expression of autophagy related genes like ATG1, ATG5, ATG6, etc.

Autophagy and Trichomoniasis: Trichomoniasis is the most common non-viral STI caused by the parasitic protozoan Trichomonas vaginalis (TV), with more than 170 million cases annually worldwide. Trichomoniasis leads to serious health outcomes for women, including vaginitis, preterm delivery, infertility, low birth weight, susceptibility to human papilloma virus (HPV) that also leads to cervical cancer and susceptibility to Herpes Simplex Virus (HSV) [40]. In males, TV infection is usually asymptomatic, although urethritis and chronic prostatitis are reported [41]. It has been shown that TV infection is correlated with increased risk of human immunodeficiency virus (HIV) transmission [42] and, lethal prostate cancer [43].

During the time of infection, TV faces competition for nutrients from the commensal flora of the vagina. Huang et al. [44] demonstrated that during conditions of glucose restriction, punctate structures of Atg8, a hallmark indication of autophagy were observed in TV cells. However, these structures were not observed in conditions of glucose abundance. Hence, TV employs autophagy as a survival mechanism during the time of establishing the infection. Once it has access to the host’s nutrients, autophagy levels return to the basal state.

Autophagy and viral infections:

Human Immuno-deficiency Virus (HIV): HIV is the causative agent of Acquired Immuno Deficiency Syndrome (AIDS), and cross-talk between autophagy and HIV occurs at various stages in a host (Figure 4). Kyei et al. [45] have shown that autophagy augments HIV1 yield in macrophages. The Gag protein of HIV1 interacts with LC3B and these are localized on autophagosomes in conditions of basal autophagy. This localization is followed by the proliferation of HIV1 virions within the autophagosome. The involvement of basal levels of autophagy in viral proliferation was further proved by knockdown of basal level autophagy. This knockdown resulted in a decrease in viral load. Therefore, basal level autophagy plays a key role in the proliferation of the virus within the cell. Rapamycin induced autophagy also resulted in increased viral load. Thus, both basal and induced autophagy may support viral replication in macrophages. However, this support is because HIV1 inhibits the degradation capacity of autophagy through Negative Regulatory Factor (Nef) gene. This Nef protein interacts with Beclin1 and inhibits autophagy. Knockout of Nef gene lead to effective autophagy and resulted in decreased viral load.

Autophagy also plays a role in the induction of type two Programmed Cell Death in HIV infected CD4 T cells. Espert et al. [46] have demonstrated that HIV1 envelope proteins X4 and R5 are able to induce autophagy mediated cell death in T-cell cell line MOLT-4. However, this effect was not observed in primary monocytes, which were differentiated into macrophages. Further, these authors observed that autophagy mediated cell death in both T cells and macrophages when they were infected with X4 and R5 HIV1 tropic strains. Hence, envelope proteins are capable of inducing autophagy mediated cell death even in uninfected T-cells.

Autophagy also plays an important role in lentivirus affected patients, who acquire neurodegenerative diseases. Alirezaei et al. [47] evaluated the number and location of autophagosomal vacuoles in primary monkey and human neurons in response to infection by Simian Immunodeficiency Virus (SIV). They observed that autophagy was inhibited in the neurons on SIV infection. These results were confirmed through the decline in LC3 type 2 and Atg5-Atg12 complex expression levels. However, they found that rapamycin treatment to such SIV infected neurons enhanced autophagic activity and conferred significant protection to the treated neurons. The authors further showed that uninfected neurons when exposed to culture supernatant fluid from SIV-infected microglia resulted in autophagy inhibition in the uninfected neurons.

This effect of SIV infected cells on uninfected cells was further characterized by Van Grol et al. [48]. They have shown that even components of the HIV1 virus inhibits autophagy in uninfected cells through Src-Akt signaling, which is an autophagy regulator. This inhibition also decreases the overall pathogen clearance ability of the uninfected cells and is not overcome even by using autophagy stimulator- rapamycin. Further, TransActivator of Transcription (Tat) protein of HIV1, receptors like CXCR4, VEGF1 and β-integrins, cytosolic adapters like STAT3 and anti-inflammatory cytokines like IL-10 also play a role in autophagy inhibition in infected cells.

Herpes Simplex Virus (HSV): HSV-1 infection results in varied manifestations such as mild mucocutaneous disease to life-threatening viral encephalitis. Post infection, HSV-1 induces the production of Infected Cell Protein (ICP) 34.5, which plays an important role in the neurovirulence ability of HSV-1 [49]. ICP34.5 inhibits the autophagic response to HSV-1 infection in murine embryonic fibroblasts [50]. This inhibition of autophagy is through dephosphorylation of eukaryotic transcription initiation factor – eIF2α and physical interaction with autophagy regulatory protein - Beclin-1 [51]. During infection by ICP34.5-lacking HSV-1 mutants, the autophagic response depends on the host Protein Kinase R (PKR) signaling pathway and its downstream transcription factor – eIF2α [52].

Ovedahl et al. [53] have demonstrated that levels of autophagy are affected on infection with wild type or ICP34.5 mutant HSV-1 virus even in Bone Marrow derived Dendritic
Cells (BM-DCs). They also found that induction of autophagy is not carried out by conventional viral PRRs like TLR2 and TLR9, instead cytoplasmic viral nucleic acid is identified by the cytoplasmic protein-STimulator of INterferon Genes (STING). Viral nucleic acid separates from the viral capsid between 2 to 4 hrs post infection and induction of autophagy is observed around the same time. Further, autophagy is not induced by viral replication and viral entry and hence, cytoplasmic viral nucleic acid is the only viral component that induces autophagy during HSV-1 infection in Bone Marrow derived Dendritic Cells (BM-DCs). Inhibition of autophagy results in decreased production of Interferon-β (IFN-β) and affects the type I interferon (IFN) response against viral infection.

Yordy and colleagues [54] have shown that viral replication during vaginal infection of ICP34.5 mutant of HSV-1 in wild type and autophagy knockout mice mice is not significantly different. Hence, autophagy in the vaginal epithelial cells of mice may not be involved in limiting the replication of the virus. HSV-1 spreads to the Dorsal Root Ganglionic (DRG) neurons from the vagina. The authors further observed that autophagy played a very important role in the clearance of the pathogen in these neurons. Rasmussen et al., [55] have demonstrated that induction of autophagy differs during HSV1 infection of non-permissive cell types like dendritic cells. They observed that HSV1 infection of BM-DCs led to an up-regulation of autophagy even in the presence of ICP34.5. Also, the autophagy response was not through the phosphorylation of eIF2α but through the expression of IFN genes, which are generally involved in anti-viral response of the cell. They also observed that only viral entry was sufficient to induce this autophagy response and viral gene expression was not required for induction of autophagy.

**Human Papilloma Virus:** Human papilloma virus (HPV) is a sexually transmitted infection and is the major pathogen in the development of cervical cancer in women [56]. Cervical cancer can be fatal if not identified in the early stages, and prevalence rate of HPV ranges from 7% to 22% worldwide [57].

Beclin-1 overexpression results in apoptotic and autophagic death of cervical cancer cell line - HeLa[58]. Zhu et al., [59] have showed that there was a significant down regulation in the expression of Beclin-1 as well as LC3 between normal cervical cells and carcinoma cells. The authors postulated that expression of Beclin-1 and LC3 may have prognostic significance in early stage cervical squamous cell carcinoma. Hence altered Beclin-1 expression levels have been investigated in cervical cancer, cervical intraepithelial neoplasia (CIN) and normal cervical tissues by Cheng et al., [60]. They observed that Beclin-1 expression was significantly different between the three groups, but Beclin-1 expression was negatively correlated with cervical cancer differentiation, lymph node metastasis, recurrence and death.

Potential anti-cancer therapeutics interacts with autophagy in different ways. Metformin [61], Resveratrol [62], Etoposide
[63], Carboplatin [65] and Paclitaxel [66] induce autophagy and result in the death of cervical cancer cells through apoptosis. On the other hand, autophagy induction through Cisplatin resulted in prevention of apoptosis mediated death of HeLa cells [64]. The association of HPV infection with the expression of ATPase family AAA domain containing 3A (ATAD3A), which is an autophagy inhibitor was investigated by Chen et al., [67]. The authors found that HPV infection correlated with increased ATAD3A expression and increased drug resistance in cervical cancer patients. They hypothesize that persistent HPV infection may stabilize ATAD3A expression in the cervical carcinoma cells, resulting into inhibition of programmed cell death processes and increased drug resistance.

**Conclusion and Future Perspective**

The roles of autophagy have been revealed in the host’s innate immune responses and autophagy has been shown to be a critical cellular process that strongly influences inflammation, immunity, and barrier function. Recent *in vitro* and *in vivo*, and molecular studies have provided evidence of selective autophagic degradation of bacteria and viruses during infection, also referred to as xenophagy. In this review, the role of autophagy during a number of (RTI’s) such as chlamydiiasis, candidiasis, trichomoniasis, HIV, HPV and HSV has been discussed. Autophagy response is generally detrimental to the proliferation of pathogens. However, various pathogens such as HIV have developed ways to inhibit the autophagy pathway and thereby prevent their clearance from the cell. Hence, future studies should be directed towards delineating the molecular details of the autophagy response to therapeutics targeted against these infections. Each pathogen interacts with the autophagy pathway in different ways in different cell types. Therefore, analyzing the molecular details of the autophagy response during other infections such as gonorrhea, syphilis, chancroid and Lymphogranuloma Venereum (LGV) need to be explored.

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**Contribution to authorship**

KVR and AS formulated the idea for the review. AS, KA and DS conducted the literature review and wrote the first draft of the article. KVR edited the article and all authors contributed to the final version.

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