Editorial

Manipulation of autophagy by sexually transmitted infections: new opportunities for intervention

Autophagy, from the Greek words meaning "self-eating", is an evolutionarily conserved multistep process preserved amongst all eukaryotes to maintain cellular homeostasis[1]. It is an intracellular, catabolic process whereby dysfunctional organelles such as mitochondria and inflammasomes, aggregated or unneeded proteins as well as intracellular bacteria and viruses and their components are degraded[2,3]. The entities marked for destruction become surrounded by a double membrane structure called an autophagosome. The autophagosome fuses with a lysosome and the sequestered components are catabolized by lysosomal enzymes[4-6]. The resulting amino acids, fatty acids, carbohydrates and nucleic acid components are returned to the cytoplasm to provide additional nutrients for various metabolic processes.

Under physiological conditions, autophagy is maintained at a low basal level in most cells[7]. Its induction is primarily inhibited by a compound called mammalian inhibitor of rapamycin (mTOR) that senses the availability of nutrients, oxidative stress as well as other deviations from intracellular homeostasis[8-10]. When non-physiological conditions that increase cellular stress develop mTOR is inhibited and the pathway to autophagy is activated[11]. More specifically, the pathway of autophagy is induced by nutrient and oxygen deprivation, decreased levels of growth factors, the presence of a cytotoxic environment, oxidative stress due to defective mitochondria, and intracellular changes induced by infection or malignancy[12-14].

In addition to its central role in the clearance of intracellular pathogens autophagy has an additional role in the regulation of both innate and adaptive immunity. Peptides produced by the lysosome-mediated degradation of microorganisms that have been sequestered in autophagosomes combine with major histocompatibility complex molecules and migrate to the cell surface. Their subsequent binding to receptors on the surface of T lymphocytes results in the activation of peptide-specific acquired immunity[15]. Activated T cells as well as antibody production by activated B lymphocytes require additional energy and this is provided by activation of autophagy within these immune cells[16]. Autophagy also prevents the induction of excessive inflammation that can harm healthy cells. It inhibits the activation of NFκB, the transcription factor that turns on genes coding for pro-inflammatory cytokines[13,17]. In addition, it down-regulates production of the primary pro-inflammatory cytokine, interleukin (IL)1β, by sequestering and degrading an intracellular structure called an inflammasome that is responsible for production of biologically active IL-1β[18-20].

The contribution of autophagy in eliminating sexually transmitted viruses and bacteria that infect the female genital tract has received only limited attention. This editorial highlights the role of autophagy in the defense against these infections and the mechanism utilized by sexually transmitted pathogens to circumvent autophagy-mediated destruction. Novel studies are proposed, based on these interactions to facilitate autophagy-mediated destruction of female genital tract pathogens.

CHLAMYDIA TRACHOMATIS

C. trachomatis is the most common sexually transmitted bacterial pathogen, a major cause of infertility and ectopic pregnancy as well as the leading cause of preventable blindness worldwide[21]. According to the Centers for Disease Control in the United States there was a 5.9% increase in the rate of chlamydial infections from 2014 to 2015, resulting in 478.8 cases per 100,000 people[22]. *C. trachomatis* has a unique biphasic developmental cycle. The extracellular elementary body infects epithelial cells by binding to a heparin-containing surface component and is subsequently internalized[23]. It then converts into an intracellular form, the reticulate body, which replicates within inclusion bodies in the host cell cytoplasm. Autophagy-related proteins do not assemble into autophagosomes in response to the inclusion body and so *C. trachomatis* replication is resistant to autophagy[24].

The role of interferon (IFN)-γ is pivotal in combating chlamydial infections; its introduction eliminates this bacterium[25]. IFN-γ depletes intracellular tryptophan and iron, induces production of nitric oxide, and activates host autophagy[8,13,26]. Recent data have shown that IFN-γ induces the fusion of autophagosomes with chlamydial inclusion bodies via a mechanism involving induction of guanylate binding proteins[27].

There remains a still largely unmet need to identify the role of autophagy in a chlamydial genital tract infection. What is the mechanism by which an intracellular chlamydial infection prevents autophagy induction and how can this be reversed? Are women with an inherited or acquired deficit in autophagy induction at increased susceptibility to this infection and its sequelae? Can we identify compounds that can safely induce autophagy and/or IFN-γ production in women newly infected with *C. trachomatis* to prevent harmful consequences of this infection?

CANDIDA ALBICANS

C. albicans is a dimorphic fungus that can exist in either a yeast-like or a filamentous form. It is a harmless commensal organism in about 20% of healthy reproductive age women. Under conditions of a transient local immune suppression or the administration of antibiotics that disrupt the vaginal microbiome, endogenous *C. albicans* proliferates, converts from the yeast to the filamentous form, becomes invasive and causes a symptomatic infection. The mechanism that this organism utilizes to resist immune destruction and to persist in the female genital tract involves autophagy. In response to *Candida* proliferation in the genital tract, the Th17 class of CD4+ T lymphocytes is activated and the cytokine interleukin (IL)-17a is released. IL-17a recruits neutrophils and macrophages to the genital tract to engulf and destroy the fungal invader[28]. However, *Candida* has a cell surface receptor that binds IL-17a and induces autophagy in the yeast[29]. This results in conversion to the filamentous form and formation of a biofilm that resists engulfment. Once the extracellular infection is resolved and phagocytic cells are no longer present the yeast forms are again

produced and recolonize the vagina. Thus, activation of autophagy in *C. albicans* in response to anti-candidal immunity ensures its survival.

Recurrent vulvovaginal candidiasis (RVVC) is a frustrating consequence of a *C. albicans* infection in about 5% of infected women. A symptomatic episode is usually resolved with anti-fungal treatment, but the infection returns shortly after treatment cessation. It remains untested whether the interference with autophagy in *Candida* has the potential to allow the immune system to totally eliminate this organism from the genital tract, and, thus, prevent recurrent infections. An antibody that blocks the IL-17a receptor on yeast and/or introduction of an exogenous compound that activates yeast mTOR and prevents autophagy induction are attractive options that remain to be tested.

HERPESVIRUS

Type I and type 2 herpes simplex virus (HSV) are double stranded DNA viruses that infect the female genital tract. They can cause painful lesions and, in pregnancy, are major inducers of neonatal morbidity and mortality. Their intracellular replication should be inhibited and the virus destroyed by autophagy. However, both HSV 1 and HSV 2 produce a protein, infected cell protein 34.5 (ICP34.5), that inhibits formation of the autophagosome and, thereby, prevents viral elimination[30]. Human cytomegalovirus (CMV) produces an analogous protein, TRS1, that also inhibits autophagy in CMV-infected cells[31]. Thus, herpesviruses persist within host cells by inhibiting autophagy. The introduction of exogenous agents capable of inducing autophagy in herpesvirus-infected cells appears to be a logical option worthy of evaluation.

HUMAN PAPILLOMAVIRUS

HPV, a double stranded DNA virus, is the etiological agent of cervical carcinoma as well as a causative factor for anal cancer and head and neck malignancies[32]. HPV infects terminally differentiated epithelial cells in the female genital tract. The induction of autophagy in these infected cells is essential for the production of amino acids needed for HPV replication as well as for the continued survival of the host cell. The HPV E7 protein has been shown to induce autophagy in epithelial cells. Uninfected epithelial cells that have been transfected with the E7 protein undergo autophagy[33].

The inhibition of autophagy in HPV-infected genital tract epithelial cells to limit the consequences of this infection is an attractive but unexplored area of research. Development of protocols to target HPV-infected cells with agents that inhibit autophagy and/or activate mTOR might be useful adjuncts to conventional treatments. In addition, contrary to the situation with *C. trachomatis*, we predict that women with a reduced capacity for autophagy would be relatively resistant to an HPV infection or development of a cervical malignancy. This possibility is easily amenable to testing.

HUMAN IMMUNODEFICIENCY VIRUS

HIV is a retrovirus that infects monocytes/macrophages and $CD4^+$ T lymphocytes in the female genital tract. Like herpesviruses, the intracellular replication of HIV should be disrupted by autophagy. However, unlike herpesviruses, HIV uses autophagy to facilitate its replication in monocytes/macrophages. The HIV gag protein binds to an essential autophagy component, LC3, and facilitates the assembly of HIV within autophagosomes[34]. A second HIV protein, nef, binds to another autophagy component, Beclin 1, and inhibits the autophagosome from interacting with a lysosome[34]. A third HIV protein, tat, prevents IFN-γ from inducing autophagy in HIV-infected cells[35]. When HIV infects $CD4^+$ T cells the HIV env protein is released into the extracellular milieu where it binds to uninfected $CD4^+$ T cells. This results in the induction of autophagy in these cells and their subsequent destruction by apoptosis[36]. This elimination of uninfected T cells down-regulates anti-HIV immunity and facilitates viral persistence.

Thus, HIV has evolved an amazing repertoire of mechanisms that target autophagy. This suggests that autophagy has a major role in HIV biology. However, focusing on the development of mechanisms to promote autophagy to eliminate HIV in infected monocytes/macrophages and to prevent autophagy in uninfected T cells has received scant research attention.

CONCLUSIONS

Autophagy is a major mechanism to eliminate microorganisms that reside within the cytoplasm of infected cells and to promote effective anti-microbial immune responses. Microorganisms that successfully invade the female genital tract have evolved mechanisms to prevent their autophagy-mediated destruction, utilize autophagy to promote their survival and/or promote their ability to replicate within host cells. The testing of novel protocols to prevent or reverse the microorganism-directed subversion of autophagy remains an under-appreciated area of research. It is our hope that this editorial will facilitate laboratory investigations that result in development of novel means to combat the initiation, development and consequences of sexually transmitted infections.

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