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Short Review: Special Edition

Apicomplexan autophagy and modulation of autophagy in parasite-infected host cells

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ABSTRACT

Apicomplexan parasites are responsible for a number of important human pathologies. Obviously, as Eukaryotes they share a number of cellular features and pathways with their respective host cells. One of them is autophagy, a process involved in the degradation of the cell's own components. These intracellular parasites nonetheless seem to present a number of original features compared to their very evolutionarily distant host cells. In mammals and other metazoans, autophagy has been identified as an important contributor to the defence against microbial pathogens. Thus, host autophagy also likely plays a key role in the control of apicomplexan parasites, although its potential manipulation and subversion by intracellular parasites creates a complex interplay in the regulation of host and parasite autophagy. In this mini-review, we summarise current knowledge on autophagy in both parasites and their host cells, in the context of infection by three *Apicomplexa*: *Plasmodium*, *Toxoplasma*, and *Theileria*.

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Autophagy in *Apicomplexa*

The core machinery for autophagy is evolutionarily conserved in most of the eukaryotic phyla, however *Plasmodium*, *Toxoplasma* and *Theileria* possess a reduced repertoire of recognizable autophagy-related proteins. Except in *Toxoplasma*, they noticeably lack clear orthologues of the initiating kinase ATG1/ULK1/2, and all lack proteins involved the nucleation of autophagosomes [Table 1]. Apicomplexan parasites also lack the equivalent of mammalian lysosomes, so they rather resemble fungi and plants by degrading autophagosome cargo in vacuoles with a proteolytic function. For example, in *Plasmodium*-infected red blood cells, autophagosomes fuse with the digestive food vacuole that is better known for degrading haemoglobin that the parasite imports from the erythrocyte cytosol [1]. *Plasmodium* sporozoites and merozoites are the developmental stages invasive for hepatocytes and erythrocytes, respectively, but they do not possess a food vacuole. However, it has been proposed that post-invasion of hepatocytes, *Plasmodium berghei* ATG8-decorated micronemes (an invasion-related organelle) are expelled from the parasite and degraded by enzymes present in the parasitophorous vacuole (PV) lumen [2]. Proposing the PV as a degradative compartment is an interesting concept, as invasive *Toxoplasma* tachyzoites leave behind a residual body of unused material after their division by endodyogeny, which vanishes quite rapidly as parasites develop in the vacuole. Therefore, the PV might be an important interface between the parasite and its host cell for nutrient acquisition, where import of autophagosome-recycled parasite material from the lumen back into the parasite might be facilitated. One should point out that post-invasion of leukocytes or erythrocytes, *Theileria* parasites reside only very transiently within a PV that is rapidly degraded, leaving the parasites exposed to the host cell cytosol [3]. If secretory autophagy occurs, then lysosomes in the host cell cytosol could be the digestive compartment for *Theileria*-derived autophagosome cargo.

Autophagy in *Plasmodium* parasites

A better understanding of autophagy regulation in malaria-causing *Plasmodium* species has taken on renewed urgency due to the recent description of artemisinin-resistance mutations occurring in *Plasmodium falciparum Atg18 (PfAtg18)* [4]. In addition, previously, resistance to another anti-malaria drug (chloroquine) was associated with alterations in PfATG8 distribution [5]. Although PfATG18 has not yet been characterised, studies on PfATG8 are well documented (14 papers in PubMed). Particularly, a surprisingly common observation was the localisation of PfATG8 on a non-photosynthetic plastid, present in most apicomplexan parasites, called the apicoplast [6,7]. This led to the proposition that PfATG8 has a non-canonical function in apicoplast biogenesis [1] or, since apicoplasts also bind Phosphatidylinositol 3-phosphate (PI3P) produced by Vps34, its membrane might be the site of phagophore formation [2]. Once formed, the maturation of autophagosomes is associated with them becoming decorated with PfRab7, and then fusing with the food vacuole for degradation of their cargo [1]. PI3P binds to FYVE-domains [8] and the single parasite FYVE domain-containing protein also locates to the food vacuole [9], where it might participate in fusion of the autophagosome with the food vacuole membrane. The function of autophagy in *Plasmodium* blood stages is largely unexplored, but one proteomic study suggested that PfATG8 could be involved in parasite ribophagy and piecemeal microautophagy of the nucleus [10].

In the absence of a recognizable ATG1 orthologue (see Table 1, [11]) it's intriguing as to how malaria parasites regulate the initiation of autophagy and one can only hypothesize that another unidentified parasite kinase activity might play an ATG1-like role. Clearly, little is known and one possibility is that post-translational modifications of ATG proteins play a dominant role in regulating autophagy. In Table 1 we have indicated the phosphorylation status collated from PlasmoDB (http://plasmodb.org) of the different PfATG proteins and most are phosphorylated at more than one site. One can see that only PfATG5, PfATG7 and PfATG12 are not phosphorylated in infected red blood cells. cAMP-dependent protein kinase A (PKA) likely plays an important regulatory role, as PfATG4 (T625), PfATG8 (T83), PfATG11 (S243, S465), PfVps34 (T47, S90, S1036, S1362), PfVps15 (S250) and PfRab7 (S72) are all phosphorylated *in vivo* at typical PKA sites. Other phospho-sites and the two in PfATG18 (S42, S375) are not typical of PKA suggesting that additional parasite kinases must be responsible. Clearly then, kinases and phosphatases are likely key players in the regulation of parasite autophagy.

Autophagy in *Plasmodium*-infected host cells

Although autophagy is well studied as reticulocytes develop into normocytes, a process during which organelles including the nucleus are eliminated during erythropoiesis [12], little is known about host cell autophagy in *Plasmodium*-infected mature erythrocytes. However, in *P. berghei*-infected hepatocytes the PV membrane (PVM) is decorated with LC3 ("microtubule-associated protein 1A/1B-light chain 3", the mammalian orthologue of ATG8), ubiquitin, SQSTM1/p62 and lysosomes in a process resembling selective autophagy [13]. As *P. berghei* development is dampened in host hepatocytes deficient in autophagy, it gave rise to the proposition that host cell autophagy was occurring at the PVM to supply the parasite with nutrients necessary for optimal growth [13,14]. Moreover, in human hepatocytes infected with *Plasmodium vivax,* interferon-gamma (IFN-γ) stimulation also enhances LC3 and lysosome recruitment to the PVM [15]. However, this IFN-γ mediated induction of autophagy seemed detrimental to liver-stage *P. vivax* infection, in contrast to the role described promoting *P. berghei* development [13,14]. Moreover, IFN-γ mediated induction of autophagy appeared non-canonical, as it did not involve activation of the mammalian ATG1 orthologue ULK1. Thus, during liver stage infection the parasite provokes hepatocyte autophagy to help it grow, while the host appears to respond to infection by IFN-γ stimulated autophagy to eliminate the parasite.