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Leishmania survival in the macrophage: where the ends justify the means

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Macrophages are cells of the immune system that mediate processes ranging from phagocytosis to tissue homeostasis. *Leishmania* has evolved ingenious ways to adapt to life in the macrophage. The GP63 metalloprotease, which disables key microbicidal pathways, has recently been found to disrupt processes ranging from antigen cross-presentation to nuclear pore dynamics. New studies have also revealed that *Leishmania* sabotages key metabolic and signaling pathways to fuel parasite growth. *Leishmania* has also been found to induce DNA methylation to turn off genes controlling microbicidal pathways. These novel findings highlight the multipronged attack employed by *Leishmania* to subvert macrophage function.

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Introduction

Macrophages: sentinels of the immune system

Macrophages are crucial to the immune response and their absence would allow infections to progress uncontrollably to the detriment and eventual demise of the host [1]. The importance of these phagocytic cells in antimicrobial defence and development was recognized and documented by Ilya Metchnikoff [2], an achievement that merited him the 1908 Nobel Prize in Physiology or Medicine. Macrophages are found in every tissue, and most develop from bone marrow myeloid precursor cells. Phagocytosis is at the helm of macrophage biology [3]. This process is essential for nutrient recycling, organismal homeostasis and defense from pathogens [4]. For example, macrophages phagocytose circulating erythrocytes in order to recycle iron back into circulation [5]. Resting macrophages can be chemoattracted to the site of infection, and can be activated by cytokines such as interferon gamma (IFN-γ) and by microbial compounds [6,7]. In their first-of-the-line role in infections, macrophages are some of the first cells to come in contact with, recognize and kill microbes. Macrophages can also present antigens to lymphocytes [8] via major histocompatibility complex (MHC) molecules at the macrophage's plasmalemma.

Leishmania parasites have evolved to conquer macrophages

Pathogens have evolved to circumvent and conquer many of the antimicrobial strategies mounted by macrophages [9•,10]. Living inside of the macrophage is an ideal way to escape the immune system, obtain nutrients, and proliferate. *Leishmania* parasites, which cause the leishmaniases, constitute one such example of a microorganism that has successfully adapted to life in the macrophage. The leishmaniases constitute a spectrum of diseases caused by flagellate protozoa. These intracellular parasites cause cutaneous, mucocutaneous and visceral pathologies in humans and other animals [11]. The leishamaniases are prevalent in 98 countries spread over 5 continents [12]. The parasite has a digenetic life cycle and is transmitted to humans by infected hematophagous female sand flies. Promastigotes, which are the extracellular forms of the parasite, are injected into the host via the proboscis of the sand fly [13]. Parasites in the skin are ingested primarily by macrophages, and also by neutrophils and dendritic cells. Although many promastigotes are destroyed by macrophages, some evade the microbicidal power of the phagolysosome, thereby transforming this powerful organelle into a parasitophorous vacuole (PV) that fosters parasite growth [14–16]. Within PVs, promastigotes transform into amastigotes that multiply via binary fission. When the host cell becomes overwhelmed by parasites, it may either rupture and release amastigotes, or become apoptotic and pass the amastigote cargo to surrounding macrophages [17]. Either way, amastigotes metastasize and cause pathology. The life cycle is completed when a previously uninfected sand fly takes a blood meal containing amastigotes or infected phagocytes.

Leishmania-macrophage interactions are multifaceted and involve a number of pathogenicity factors that allow parasites to use the phagolysosome to obtain nutrients, hijack antimicrobial pathways, and replicate [18•]. The zinc metalloprotease GP63 is the most abundant molecule on the promastigote surface. It is a pathogenicity factor that cleaves host proteins, enabling *Leishmania* to

subvert processes such as transcription and translation [16]. In the process of conquering the macrophage, *Leishmania* has also been known to hijack signaling pathways [19] that would otherwise kill the parasite in the phagolysosome and impede dissemination [14].

This review discusses recent studies (2013–2015) that cast light on how *Leishmania* sabotages macrophage functions for survival. It will focus on new discoveries concerning how the GP63 protease alters the macrophage's membrane fusion machinery to tamper with processes that are essential to macrophage physiology and function. Furthermore, it will discuss how *Leishmania* disrupts various host metabolic and signaling pathways, as well as DNA accessibility, in order to promote intracellular survival.

The onslaught of the GP63 protease: from membrane trafficking to nuclear pore dynamics

Manipulation of membrane trafficking to subvert antigen presentation and cytokine secretion

Phagocytosis is governed by sequential interactions with cell organelles. These interactions are spatiotemporally regulated by a complex molecular machinery involving proteins that mediate vesicle fusion [20]. Proteins regulating neurotransmitter release, notably those of the soluble *N*-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) family [21,22], are of salient importance in coordinating membrane trafficking in cells of the immune system. During vesicle fusion, SNAREs in vesicles and target membranes interact specifically to pull two membranes into close proximity [21,22]. SNAREs are selectively distributed in different organelles. VAMP8, which forms complexes with syntaxin-7, syntaxin-8 and Vti1b, participates in homotypic fusion of late endosomes and is recruited to phagosomes [23,24]. It also mediates the recruitment of $gp91^{phox}$ — a component of the NOX2 oxidase — to the phagosome [25••]. This in turn implies that VAMP8 plays an important role in modulating intraphagosomal function and host defence. Antigen cross-presentation is an important function of phagosomes [26,27]. Cross-presentation of microbial peptides on major histocompatibility (MHC) I molecules is essential to activate $CD8^+$ T cells. Using cells from *Vamp8*$^{-/-}$ mice, Matheoud *et al.* discovered that VAMP8 plays an essential role in antigen cross-presentation in both bone marrow-derived macrophages (BMM) and dendritic cells (BMDC) [25••]. Interestingly, infection of *Vamp8*$^{-/-}$ mice with *L. major* parasites results in larger footpad lesions [25••]. Bacteria have evolved numerous ways to hamper SNARE function. For instance, clostridial toxins, which block synaptic transmission in peripheral cholinergic synapses, cleave SNAREs [28]. Using *gp63*-KO *L. major* parasites, Matheoud and colleagues found that VAMP8 and VAMP3 were directly cleaved by GP63 [25••]. Given the importance of SNAREs in mediating trafficking to and from the phagosome, the authors sought to identify the consequences of SNARE cleavage on cross-presentation. Feeding *L. major*-infected macrophages with ovalbumin (OVA)-coated beads, the authors found that GP63 strongly inhibits OVA cross-presentation in both BMMs and BMDCs. Detailed analysis of these phagosomes revealed that cross-presentation is hindered due to decreased $gp91^{phox}$ recruitment, which leads to decreased intraphagosomal oxidation, increased proteolytic activity and altered pH (Figure 1a). In sum, by targeting SNAREs, *Leishmania* impairs crucial processes involved in phagolysosome biogenesis that are required for antigen cross-presentation.

Synaptotagmins (Syts) are membrane proteins that regulate vesicle docking and fusion in processes such as exocytosis [29,30] and phagocytosis [30–32]. They regulate SNARE activity by mediating membrane fusion in a Ca^{2+}-dependent manner [33]. All Syts possess two conserved tandem Ca^{2+}-binding C2 domains. However, Syt XI contains a conserved serine in its C2A domain that precludes this Syt from mediating vesicle fusion [34]. In macrophages, Syt XI is a recycling endosome-associated and lysosome-associated protein that negatively regulates the secretion of tumour necrosis factor (TNF) and IL-6 [1,22,30]. The roles of Syts in vesicle trafficking make these proteins great targets for attack by intracellular pathogens. For instance, the parasite *Trypanosoma cruzi* uses Syt VII, a Ca^{2+}-dependent regulator of lysosome exocytosis, to invade target cells [35]. It had previously been observed that *Leishmania* promastigotes of certain species are able to trigger TNF and IL-6 secretion post-infection [36–40]. Although the parasite signal that induces TNF and IL-6 secretion is not known, synthesis and secretion of these cytokines may be initially triggered by engagement of TLR2 and TLR3 with parasite molecules [41]. Given the role of Syt XI in cytokine secretion, it was hypothesized that *Leishmania* could affect Syt XI function to deregulate cytokine release. Using *L. major* strains that express or lack GP63, Arango Duque *et al.* found that the GP63 protease degrades Syt XI and positively regulates the post-infection release of TNF and IL-6 [42••]. Using RNA interference, it was shown that cytokine release was induced by GP63-mediated degradation of Syt XI. At the forefront of these findings is the observation that, early during infection, GP63 induces the release of TNF and IL-6 *in vivo*. Importantly, injection of GP63-expressing parasites induces the influx of neutrophils and inflammatory monocytes to the inoculation site. This is likely to be a consequence of increased TNF and IL-6 release. Infection of recruited inflammatory monocytes [43•] can trigger IL-10 secretion [44,45], and induce their differentiation into immunosuppressive, arginase-expressing alternatively activated macrophages [46]. The role of Syt XI in the recruitment of these phagocyte populations is to be elucidated. This new role for GP63 implicates this protease in the manipulation of