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Review

Leishmania molecules that mediate intracellular pathogenesis

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Abstract

Parasites of the *Leishmania* genus are the causative agents of a complex disease called leishmaniasis. Many activities of infected cells including their responses to a range of stimuli are modulated by *Leishmania* parasites. This review will profile some of the parasite molecules that target host cell processes for which there has been recent progress.

Keywords: Leishmania; Intracellular parasites; Pathogenesis; Tryparedoxin peroxidase; Casein kinase; Reactive oxygen

1. Introduction

Leishmania lesions are a milieu that is rich in immunologic activity: chemokines produced by infected or bystander cells induce cell recruitment to that site. Both pro-inflammatory and anti-inflammatory cytokines are produced in infection lesions where they activate and/or modulate immune cell responses. There is active research aimed at determining how *Leishmania* parasites contribute to the development of those immune responses. Several recent reviews have considered the impact of elicitation of cross regulatory mediators on *Leishmania* infections [1–3]. Going forward, it is of interest to determine whether elicitation of responses with counter regulatory effects occurs fortuitously or whether it is a *Leishmania* strategy for survival.

There is convincing evidence that intracellular signaling pathways that mediate responses to cytokines or to stimuli that result in cytokine production, are modulated by the infection. The reader is referred to several excellent recent reviews that have provided detailed summaries of those studies [4–6]. Examples of the signaling mediators and/or pathways that are modulated by infection include the MAP kinases [7,8]; signal transducers and activators of transcription (STATs) [9,10]; Src homology region 2 domain-containing phosphatase 1 (SHP-1) [11,12]; nuclear factor kappa B (NFκB) [13,14]; and phosphatidylinositol 3-kinase (PI3K)/AKT pathway [15,16]. Our understanding of the parasite mediators that target many of these host processes is still incomplete. Moreover, the signals and other mechanistic details that direct the trafficking of parasite molecules to their targets is presently not known. For parasite molecules with targets in the infected cell cytosol, they have to be exported across the parasitophorous vacuolar membrane (PVM); for parasite molecules whose targets are in the host cell nucleus, an additional membrane, the nuclear membrane has to be overcome; parasite molecules that have targets outside the infected cell have to be exported from the infected cell. Several recent studies have provided valuable insight into parasite mediators that are elaborated during infection. The identity of those parasite molecules and their targets in the host will be the focus of this review.

2. *Leishmania* mediators that detoxify the respiratory burst in phagocytes

Macrophages are the primary hosts of *Leishmania* parasites. The respiratory burst in macrophages is composed of several oxygen and nitrogen reactive compounds. Generation

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of these compounds occurs in response to distinct and varying stimuli. However, once the response is initiated, oxygen and nitrogen radicals can synergize to form more potent species. Reactive oxygen species (ROS), including superoxide radical anion and H_2O_2, are primarily produced by the NADPH oxidase (NOX) and dual-oxidase (DUOX) family of enzymes [17]. They are produced in response to innate stimuli such as phagocytosis. NOX-2 (out of 5 variants) is the most important in macrophages. This enzyme is composed of multi-subunits that assemble at the site of phagocytosis and subsequently on the limiting membrane of phagosomes that harbor internalized particles. In contrast to ROS, nitric oxide (NO), the initial reactive nitrogen species (RNS) is produced in response to immune stimuli, specifically TH1-type cytokines. NO production in macrophages is the product of the inducible nitric oxide synthetase (iNOS), which acts on arginine in the presence of oxygen to catalyze the release of NO. NO can be subsequently converted to nitrogen oxide or peroxynitrite, which are potent oxidative species. ROS and RNS can cause oxidative damage to biomolecules such as lipids, proteins and DNA, which leads to loss of membrane integrity, defective replication and eventually cell death.

Both the promastigote and amastigote forms of *Leishmania* are internalized by phagocytosis. It should be expected therefore that ROS is produced during this process. However, several studies with the amastigote forms of *Leishmania* have shown that uptake of amastigotes is silent [18–20]. The situation with promastigote forms is more controversial as species-dependent differences in superoxide production have been reported [21]. These observations imply that *Leishmania* amastigotes elaborate a mechanism to prevent superoxide production during parasite uptake. Details on the proposed mechanisms by which *Leishmania* parasites limit superoxide production have been discussed in previous reviews [6,22]. The molecules discussed below are elaborated by *Leishmania* parasites to counter the products of the respiratory burst.

2.1. Thioredoxins and peroxiredoxins

Are members of a family of proteins that are characterized by their active site motifs that contain either one or two cysteinyl residues. These thiol groups are essential for (1) the reduction of protein disulfides (thioredoxins) (2), protein deglutathionylation (glutaredoxins), or (3) the reduction of H_2O_2 (peroxiredoxins). Peroxiredoxins (PRXs) use a redox active cysteine residue (peroxidatic Cys) to reduce a broad spectrum of substrates, including H_2O_2, organic hydroperoxides and peroxynitrite ($ONOO^-$) [23]. Upon reduction of the peroxide, the peroxidatic Cys-SH is oxidized to sulfenic acid (Cys-SOH). Peroxiredoxins return to their reduced state upon reduction of the disulfide by an appropriate electron donor. *Leishmania* lack catalase and selenium–glutathione peroxidases. Instead they have a unique oxidoreductase of the thioredoxin superfamily, known as tryparedoxin (TXN). The PRXs of *Leishmania* and other trypanosomatids are commonly referred to as tryparedoxin peroxidase (TXNPx) isoforms [24]. Two TXNPx isoforms have been described in *Leishmania* that are localized in the parasite mitochondrion and cytosol [25–27]. Overexpression and other approaches have revealed some substrate differences between these two variants: cTXNPx preferentially inactivate H_2O_2 and the organic hydroperoxide *t*-bOOH [28]; in contrast, overexpression of *Li*mTXNPx in promastigotes did not ensure any significant resistance to exogenously added H_2O_2, but sheltered parasites when exposed to *t*-bOOH. The functions for the TXNPx molecules have thus far been linked to their localization within the parasite. Current views are that the mitochondrial variant detoxifies oxidative radicals that are produced primarily during electron transport [29]. The cytosolic variant functions in response to parasite stress from changes in other factors including the temperature increase in the mammalian host, the environment within PVs or the sand fly gut [30].

The generation of superoxide in phagosomal compartments occurs at the limiting membrane from where oxygen radicals diffuse. ROS diffuse poorly across membranes; translocation across membranes occurs through channels and transporters [31]. NO that is produced by the cytosolic enzyme, iNOS, reacts with oxygen to form peroxynitrite and other species that diffuse more readily across membranes [32]. In light of the fact that the mitochondrial and cytosolic TXNPx isoforms are mostly located within the parasite, it would appear that the parasite surface is still vulnerable to the effects of oxygen radicals unless there are other molecules that are exported to the parasite surface or secreted from the parasite. Based on an inability to recover peroxidase activity in the supernatant fluid from infective promastigotes [30], it was concluded that it is unlikely that cTXNPx is secreted either from promastigotes or amastigotes. In studies to identify parasite molecules that are exported from PVs. A novel TXNPx variant that is predominantly expressed in amastigote forms (aTXNPx) and appears to be released from intracellular parasites and exported from PVs in the host cell was recently identified [33]. Ongoing studies have implicated the response to iNOS as the trigger for aTXNPx release, which is consistent with a likely role for this molecule beyond the parasite [34]. A secreted peroxidase activity was also recently described [35] and was proposed to target host molecules in the infected cell cytosol. Although the peroxidase activity of the secreted peroxidase in the later report has not been evaluated sufficiently, there is a strong likelihood that parasite molecules are released to detoxify oxygen and nitrogen radicals within the PV and in the host cell cytosol.

In addition to their activity on oxygen radicals, peroxiredoxins have been shown to exert other functions that might be just as important in parasite pathogenesis. It was determined in studies where the mTXNPx gene was deleted, that loss of the mitochondrial peroxidase variant had no impact on the parasite's capacity to resist oxidants, which given the localization of this mitochondrial variant, was assumed to be its primary function [36]. Instead, complementation with a peroxidase defective but otherwise identical mutant restored wild-type infectivity to the genetically ablated parasites. They deduced that the protein conferred thermotolerance to the parasite by stabilizing other critical parasite molecules,