Unfolded Protein Response (UPR) Pathway in cutaneous leishmaniasis: A review

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April 24, 2023

Abstract

Alteration in the physiological state of the endoplasmic reticulum (ER) leads to the specific response known as unfolded protein response (UPR) or ER stress response. The UPR is driven by three sensor proteins, namely: Inositol Requiring Enzyme 1 (IRE1), Protein Kinase RNA like ER kinase (PERK), and Activating Transcription Factor 6 (ATF6) to restore ER homeostasis. Pathogenic infection can initiate UPR activation; some pathogens can subvert the UPR to promote their survival and replication. Many intracellular pathogens, including *Leishmania,* can interact and hijack ER for their survival and replication, triggering ER stress and subsequently ER stress response. This review aims to provide a comprehensive overview of the ER stress response in infections with the *Leishmania* species .

Introduction to the UPR pathway

The endoplasmic reticulum (ER) is a vital cellular component and this dynamic tubular network has an important role in several essential cellular functions such as protein synthesis, folding, maturation, and translocation, along with regulating second messenger signaling, carbohydrate metabolism, maintenance of calcium storage and biogenesis of cholesterol, steroids, and lipids[1]–[3]. Alteration in physiological statuses such as oxidative stress, Ca^{2+}imbalance, drastic pH and temperature variations, and pathological conditions such as ischaemia, reperfusion and bacterial, viral, or protozoan infections can cause loss of ER homeostasis by disrupting the protein folding process[2], [4]. Interference with the protein folding process leads to the accumulation of unfolded and misfolded proteins in ER lumen subsequently triggering a cellular condition known as the "ER stress"[3].

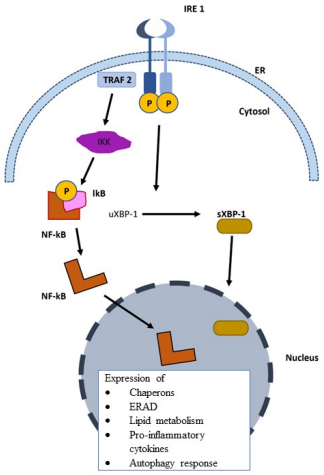
As a response to ER stress, ER induces an adaptive signaling cascade to restore protein homeostasis in ER and ensure cellular survival. This signaling cascade is collectively known as the Unfolded Protein Response (UPR), also known as the ER stress response[2], [4]. These adaptive signal transduction pathways help to increase the folding capacity of the proteins in the ER and get rid of the misfolded proteins accumulated in ER, thus helping the organelle to offload the proteins that are not properly folded[1], [3], [5]. During this process, genes that are responsible for the expression of cytokines and induction of resistance to oxidative stress are upregulated[1]. If these UPR mechanisms fail to restore the ER homeostasis, the cell will undergo apoptosis[6]. Traditionally, UPR is triggered by the accumulation of misfolded or unfolded proteins. But since UPR is significantly interconnected with inflammation and innate immune response pathways, pathogenic infections also can trigger the UPR signaling cascades[2], [7]. Intracellular pathogens can induce the UPR pathway as a response to a wide variety of cellular perturbations such as disruptions of the secretory pathways, accumulation of Reactive oxygen species (ROS), or increase of free fatty acids and nutrient depletion[8].

The stress sensors in UPR pathway

The UPR functions to maintain ER homeostasis in the presence of a high unfolded protein load. In this context, UPR reprograms and modulates several secretary pathway-related genes involved in protein entry to ER, folding, post-translational modifications, quality controlling, protein degradation, vascular trafficking, and lipid biogenesis[3]. There are some discrepancies between the numbers of sensor proteins that are involved in UPR in different eukaryotes. In higher eukaryotes, however, UPR is driven by three sensor proteins, namely; Inositol Requiring Enzyme 1 (IRE1), Protein Kinase RNA like ER kinase (PERK), and Activating Transcription Factor 6 (ATF6)[5]. They can sense abnormal conditions and regulate the expression of specific transcription factors and modulate downstream effectors/ signaling events associated with UPR, orchestrating the adaptations to ER stress[3], [5]. The IRE1 and PERK signaling cascade activate via oligomerization and autophosphorylation while ATP6 translocate to the Golgi apparatus and then activate through proteolytic cleavage[8].

The IRE1 Signaling

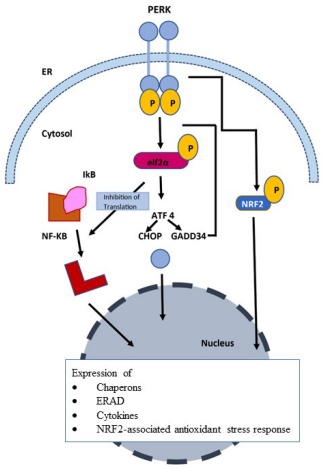
Class IRE1 sensor proteins have two isoforms in mammals: IRE1α and IRE1β which both are dual kinase/ endonucleases[9], [10]. Although IRE1α is universally expressed, IRE1β expression is limited to the respiratory, gastrointestinal tracks, and other mucosal surfaces[9], [11]. Upon oligomerization of IRE1, its carboxy-terminal endoribonuclease domain gets Autophagy response activated through autophosphorylation, which will subsequently splice out 26 bases from cytoplasmic unspliced X- Box Binding Protein-1 (uXBP-1) mRNA. This splicing event will lead to a shift in the open reading frame and allow the spliced XBP1 (sXBP1) translation. sXBP1 up-regulates the expression of several chaperones and proteins involved in ER-associated degradation (ERAD) and lipid metabolism. Translation of sXBP1 also mediates the expression of pro-inflammatory cytokines and autophagy response[3], [5]–[7], [11]. Activated IRE1 can also mediate the inflammatory pathways through inhibitor of NF-ϰB (IϰB) inhibition and nuclear factor-ϰB (NF-ϰB) activation[8]. Figure 1 shows a schematic model of IRE1 signaling pathway.



The PERK Signaling

PERK is an important component which is a type I ER transmembrane protein kinase in UPR pathways. Activated PERK subsequently phosphorylate α subunit of eukaryotic translation initiation factor 2 (eIF2α), thereby bringing about the global attenuation of protein translation. Because of the attenuation, all the proteins with a shorter half-life are degraded and cleared from the cell. Since IϰB has a much shorter half-life than NF-ϰB, NF-ϰB expression is promoted. In addition to that, eIF2α phosphorylation increase the translation of selective mRNA, which is containing inhibitory upstream open reading frames (uORFs) within their 5' untranslated region (UTR) that prevents translation in unstressed cells[3]. Simultaneously, activating transcription factor 4 (ATF4) escapes this inhibition and preferentially gets translated by altering

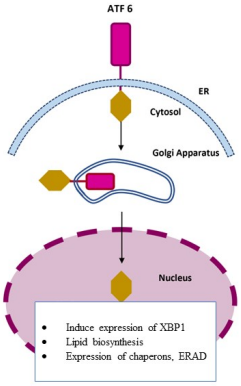
the translation initiation site. But according to PERK-dependent UPR target genes in mammalian cells, nearly half of the PERK-dependent targets are ATF4-independent. This suggests the existence of other PERK downstream effectors[3]. Subsequently, ATF4 induces the expression of CCAAT/enhancer-binding protein (C/EBP) homologous protein (CHOP/DDIT3) and growth arrest and DNA damage gene (GADD34). GADD34 is a regulatory subunit of protein phosphatase 1 (PP1) and it acts as a regulator of eIF2α phosphorylation. It directs the PP1 to dephosphorylate eIF2α, acting as an inhibitor[5], [7], [8], [12], [13]. PERK furthermore induces the expression of nuclear factor erythroid 2-related factor 2 (NRF2) which is associated with anti-oxidant stress responses[8], [12]. Figure 2 shows a schematic model of PERK signaling pathway.



The ATF6 Signaling

ATF6 is a type II transmembrane protein with an N-terminal bZip transcription factor domain. In response to ER stress, ATF6 translocate to the Golgi apparatus, where it is subjected to proteolysis by site-1 and site-2 protease and subsequently releases the transcriptionally active amino acid terminal domain to the membrane. TR [5], [8]. ATF6 too has two isoforms: ATF6α and ATF6β. These isoforms are found to be having opposite roles in UPR[8], [14]. ATF6α is a strong and rapidly degraded transcriptional activator whilst ATF6β is a weak and slowly degraded transcriptional activator[8]. Additionally, ATF6β acts as a transcriptional repressor of the ATF6α signal, thereby acting as a negative regulator of the ATF6 branch of the UPR[14], [15].

Several studies have reported results that ATF6 is a vital transcription regulator in ER stress response[16]–[19]. Wang *et al.*2000 identified that ATF6 directly binds on to a consensus DNA binding sequence under *in vitro* conditions. This site is found to be activated in ATF6 overexpression. When placed upstream of a reporter gene, this ATF6 site was activated by the ER stress response. Furthermore, they suggested that endogenous ATF6 seems to mediate ER stress response since dominant negative forms of ATF6 blocked the induction of this response[18]. ER stress-induced proteolysis is an essential step in UPR. This proteolysis of ATF is carried out by the subsequent involvement of site 1 protease (S1P) and site 2 protease (S2P). According to Yu *et al.* 2000, cells lacking S2P failed to induce GRP78, an ATF6 target, in response to ER stress suggesting that proteolysis of ATF is a crucial step in UPR[17]. Figure 3 shows a schematic model of ATF6 signaling pathway.



The Crosslinks between the Sensor Proteins

Although the three branches of the UPR pathway have been investigated extensively, the interconnections between these three branches are yet to be studied more thoroughly. But it has been found that IRE1α and ATF6 pathways pose a close relationship. In attempts of analyzing the UPR pathway using *cis* -acting elements and *trans* -acting elements involved with genes associated with the UPR pathway, Yoshida*et al.* 2000 has reported that overexpression of soluble ATF6 activates transcription of the CHOP, XBP1 genes, and ER chaperone genes constitutively, whereas overexpression of a dominant negative mutant of ATF6 blocks the induction by ER stress[19]. Yoshida *et al.* 2001 proposed a mechanism for ER stress response activation through ATF6, with their findings. They proposed that in response to ER stress ATF6 initiates and induces the expression of ER chaperones and the XBP1 gene by directly binding to ER stress response elements. Then the spliced XBP1 produced by the activation of IRE1 induces the transcription of ER chaperons[20].

The *cis* -acting unfolded protein response elements (UPRE) is playing a significant role in UPR. Yamamoto *et al.* 2007 demonstrated that ER stress-mediated transactivation through UPRE and expression of some of the ER quality control proteins diminish in ATF6α knockout cells even in the presence of XBP1. They further reported that ATF6 cannot directly bind with UPRE to execute the UPR, suggesting that ATF6 and XBP1 form a heterodimerized ATF6-XBP1 complex to bind with UPREs and this complex has shown an eight-fold higher affinity to UPRE than the XBP1 homodimer, indicating the importance of the crosstalk between the two branches. Additionally, they demonstrated that ATF6 plays a crucial role in ER quality control process as EDEM and HRD1, two proteins involved in the degradation branch of ER quality control, both depend on XBP1 and ATF6[21].

Upon the induction of ER stress, splicing of XBP1 is induced. Because of this splicing, the C-terminal region of XBP1 is switched, resulting in the production of both unspliced and spliced mRNA forms, which will then lead to the production of pXBP1(U) and pXBP1(S) respectively. pXBP1(S) functions as the transcription factor with its specific C terminal region while pXBP1(U) acts as a shuttle between the cytoplasm and the nucleus[16], [22], [23]. The pXBP1(U) and pXBP1(S) get together and form the pXBP1(U) - pXBP1(S) complex which is subjected to the proteasome, because of the presence of degradation domain on the C-terminal of pXBP1(U)[16], [24]. Furthermore, it has been reported by Yoshida *et al.* 2009 that pXBP1(U) prefers to bind with pATF6α(N), making it susceptible to the proteasome**,**suggesting that pXBP1(U) has a negative effect on ATF6[24].

Moreover, Tsuru *et al.* 2016, reported a novel mechanism that explains the interconnection between IRE1α expression and PERK-ATF4, under ER stress. Their experiments showed that the splicing ratio of XBP1 mRNA in PERK knockout cells was increased by treatment with tunicamycin but decreased thereafter,

whereas PERK-expressing cells maintained the ratio for several hours. Therefore, they suggested that PERK affects on IRE1α -XBP1 pathway under ER stress. Additionally, they demonstrated that the effect of PERK on the IRE1α -XBP1 pathway occurs in a different manner to that of ATF6 on IRE1α -XBP1 pathway[25].

UPR in intracellular parasite-infected cells

Although a large number of studies have been done to characterize the UPR and its effects in metabolic syndromes and cells infected by bacteria, there is a shortage of investigations on its role in shaping the outcome of intracellular parasitic infections. Many pathogens induce ER stress and UPR via interacting with the ER functions[26], [27] yet several pathogens can subvert the UPR to promote their survival and replication[8]. Parasites-triggered ER stress response pathways have been investigated and reported to a certain extent in infections by Apicomplexan and Trypanosomatid protozoan parasites that are responsible for malaria, toxoplasmosis, cryptosporidiosis, and leishmaniasis[8].

Plasmodium species is the causative agent of malaria and are obligate intracellular parasites belonging to the phylum*Apicomplexa* [28]. During the initial stages of infection,*Plasmodium* sporozoites migrate to the liver and infect hepatocytes[29]. Inácio *et al.* demonstrated that upon infecting mice with *Plasmodium berghei* the UPR pathways of host hepatocytes are activated. They showed that as a result of the UPR triggered by *Plasmodium berghei* infection, the expression of spliced XBP1, which is the downstream effector of the IRE1 branch and liver-specific branch of the UPR mediated by the cAMP-responsive element binding protein-hepatocyte (CREBH) are induced, favoring the liver stage infection of *Plasmodium* [30]. Experimental mouse models of cerebral malaria have demonstrated the presence of all three main sensor proteins of UPR, indicating the activation of three main arms of UPR[31].

Toxoplasma gondii is another obligate intracellular protozoan parasite that belongs to the phylum *Apicomplexa.* These parasites can invade any nucleated cell from a wide range of warm-blooded animals[32]. Upon invasion of the host cell, *Toxoplasma gondii* forms a unique parasitophorous vacuole (PV) that does not fuse with the endolysosomal system and acts as a protective niche.ROP18 kinase, a key virulence factor that is secreted into host cells during the invasion by *T. gondii* has been found to target ATF6β· a member of the ATF6 family which operates UPR. Experiments done by Yamamoto *et al.* demonstrated that ATF6β deficient mice were susceptible to infection, indicating that tATF6β has a role in resistance against *Toxoplasma gondii* infection[8], [33]. Studies done by two individual study groups; Wang *et al.* and Zhou*et al.* reported that *Toxoplasma gondii* induces apoptosis in neural stem cells (NCS) by up-regulation of CHOP, caspase-12, and JNK which are associated with ER stress signal pathways[32], [34]. A recent study done by Augusto *et al.* reported that*Toxoplasma* triggers the UPR in host cells through the release of calcium from ER. Furthermore, they have shown that IRE1 is activated in the host during the infection and involved in a non-canonical role in the cytoskeletal remodeling of infected cells, thus enhancing cell migration[35].

Cryptosporidium parvum yet another intracellular protozoan parasite belonging to phylum *Apicomplexa,* that is identified as using the host UPR for its survival *Cryptosporidium parvum* is partially dependent on the host for its polyamine requirement. It poses a retro-conventional pathway that can produce spermidine and spermine, utilizing spermidine/spermine N^1-acetyltransferase (SSAT)[36], [37] and it has been demonstrated that upon the cell invasion, *Cryptosporidium parvum* triggers host UPR pathways that cause expression of SSAT in the human host which will then lead to overproduction and excretion of N^1-acetylspermine and N^1-acetylspermidine[37].

Leishmania **species and the disease**

leishmaniasis

Leishmaniasis is one of the neglected tropical, vector-borne diseases which is widely spread in tropical and certain subtropical areas[38], [39]. The poorest people with malnutrition, population displacement, weak immune system, poor housing, and lack of financial resources are affected more by this disease. According to the world health organization, around 700 000 to 1 million new cases occur annually. Leishmaniasis is caused by an intracellular flagellated Trypanosomatid protozoan parasite that belongs to the genus*Leishmania* [40],

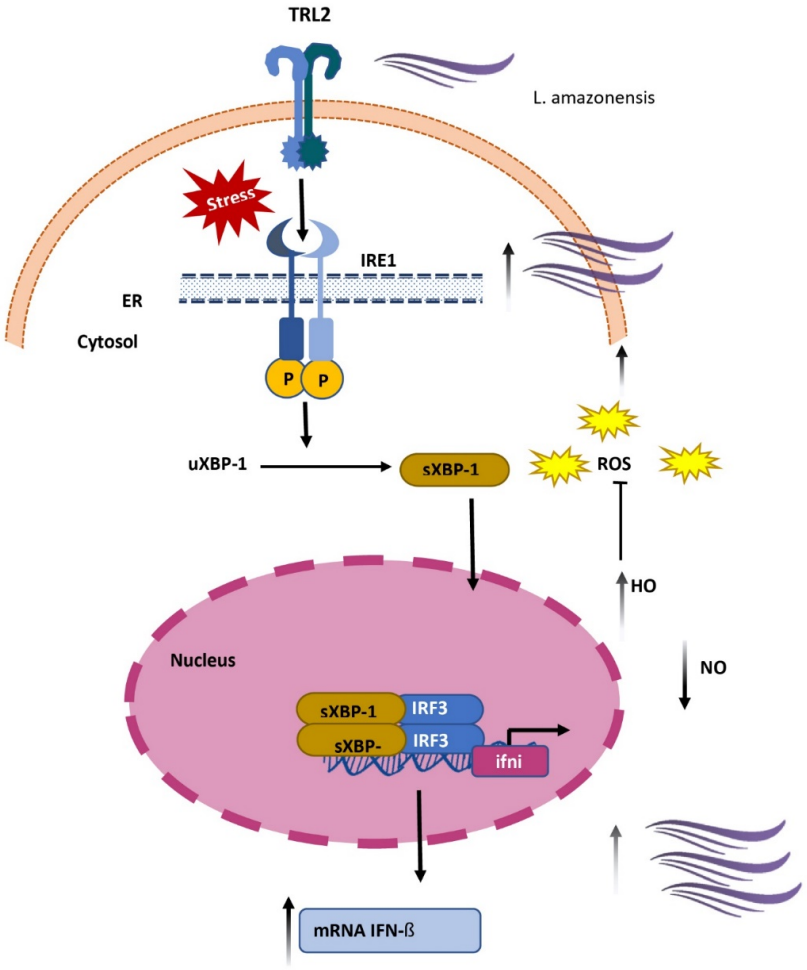
[41]. These parasites are transmitted by infected phlebotomine female sand fly bites[40]. Around 20 *Leishmania* species are identified as pathogenic to humans which are transmitted by around 30 species of phlebotomine sandflies[40], [41]. *Leishmania* parasites have a digenetic lifecycle where they spend the flagellated promastigote form inside the sand fly which is the vector, and the non-flagellated amastigote form inside the mammalian host[42], [43]. Inside the mammalian host, *Leishmania* mainly infects host macrophages[44]. Upon entering into macrophages, to ensure its stabilization and survival against macrophage's defense responses such as immune activation, oxidative stress, and apoptosis, the*Leishmania* parasite modulates the host macrophage in such a way that it can overcome the host immune response against the infection[8], [13], [44].

Leishmaniasis is clinically classified into three forms, namely; cutaneous leishmaniasis (CL), visceral leishmaniasis (VL), and mucocutaneous leishmaniasis (MCL) which is caused by the replication of the infected parasite in macrophages in the dermis, reticuloendothelial system, dermis, and naso-oropharyngeal mucosa, respectively [40], [41], [45]. When the macrophage is full of parasites within its hold, the host cell bursts and releases the parasites that will go and infect the neighboring macrophages[40]. Untreated VL can cause life-threatening systemic infection while CL can cause chronic skin sores. Facial disfigurement and life-threatening destruction of nasopharyngeal mucosa can be caused by the MCL[46]. Multiple species of the genus Leishmania cause CL: *L. tropica, L. major, L. aethiopica, L. infantum, and L. donovani* in the old world (Africa, Asia, and Europe), and *L. mexicana, L. amazonensis and L. braziliensis* in the new world [41], [46]. Moreover, many intracellular pathogens like *Leishmania* can interact and hijack cellular organelles like endoplasmic reticulum (ER) for their survival and replication, triggering the ER stress and subsequently ER stress response [7], [8]

UPR triggered by leishmaniasis

Numerous studies have been done to assess the activated UPR in leishmaniasis caused by different *Leishmania* species, mainly,*Leishmania braziliensis* , *Leishmania infantum, Leishmania donovani,* and *Leishmania amazonensis* [1], [7], [47], [48].

The role of IRE1/XBP1-splicing arms of the UPR is well documented in viral and bacterial infections but poorly reported in protozoan parasitic infections. In an attempt to investigate the role of ER stress during *Leishmania amazonensis* infection, Dias-Teixeira *et al* . group demonstrated that, upon infection by *Leishmania amazonensis* , the IRE1-XBP1 branch of UPR gets activated to overcome the cellular oxidative stress and leads to up-regulation of IFN-1β which helps in establishment of infection and its pathogenesis [47]. Induction of IFN-1β expression in *Leishmania amazonensis*infection is dependent on Toll-Like Receptor 2 (TLR2) activation, which is a pattern recognizing receptor and IFN-1β appears to favor*Leishmania amazonensis* parasite growth[49]. Further studies by this group have shown that ER-stress response enhances*Leishmania amazonensis* infection in IFN-1 dependent manner.*Leishmania amazonensis* activates ER stress response by inducing the IRE1-XBP1 branch which subsequently leads to the splicing of XBP1 and nuclear translocation. By knocking down the expression of XBP1, they observed a reduction in infection mediated by the reduction of IFN-1β. Furthermore, down-regulation of heme oxygenase (HO) and an increase in cellular Nitric Oxide (NO) concentration were also reported when the XBP1 was knocked down. These experimental data collectively suggest that XBP1 is critical for *Leishmania amazonensis* growth in infected macrophages and the promotion of *Leishmania amazonensis* infection by XBP1 is dependent on IFN-1β[47]. Ωιτη αλλ τηε δατα αιλαβλε υπ το δατε, τηε στυδψ γρουπ ηας προποσεδ α μοδελ ωηιςη δεςιπηερς τηε ρολε οφ ΞΒΠ1 δυριν γ *Λειςημανια αμαζονενσις* ινφεςτιον ας σηοων ιν φιγυρε 4 ανδ 5.



Dias-Teixeira *et al.* group has investigated the importance of the PERK/p-eIF2α signaling branch in *Leishmania amazonensis*infection. The western blotting results of the total extracts of the infected cells showed an increased level of PERK, 8 hours post-infection. The observed levels were similar to that of thapsigangin-treated cells which are used as positive controls. To determine the importance of the PERK/p-eIF2α/ATF4 signaling branch, mouse macrophages transduced with lentiviral short hairpin RNA expression vectors that target PERK (shPERK), were used as test samples and, mouse macrophages transduced with scrambled shRNA (shSCR) were used as controls. Significant down-regulation of p-eIF2α, which is the downstream effector of PERK was detected in PERK knockout cells compared to that of cells transduced with shSCR. In addition to that, a decrease in the infection index was also observed in PERK knockdown cells. These results were further confirmed using *Leishmania amazonensis*infected cells treated with PERK inhibitors. The cells treated with the inhibitor were reported to have a negative effect on infection. However, no effect of PERK was observed on the parasite load. Collectively with these results, they concluded that *Leishmania amazonensis*infection indeed activates the PERK/eIF2α signaling cascade and that PERK/eIF2α signaling favors parasite infection[48]. ATF4 is the immediate downstream effector of the PERK/eIF2α signaling axis. To determine whether *Leishmania amazonensis* infection induced the expression of ATF4 through the PERK/eIF2α branch, whole cell lysates were subjected to western blotting at specific time intervals and an increase in ATF4 was observed in infected cells compared to uninfected cells. Using shATF4 transduced infected cells, the importance of ATF4 in*Leishmania amazonensis* infection was determined. The

ATF4 knockout infected cells showed a reduced parasite burden compared to shSCR transinfected cells which are the controls. These observations decipher that upon infection with *Leishmania amazonensis* , PERK/eIF2α/ATF4 axis is induced and plays a critical role in shaping the*Leishmania amazonensis* infection[48].

PERK protein sensor triggered branch can phosphorylate and activate the NRF2 transcription factor which is responsible for inducing the expressions of genes involved in anti-oxidative response[8]. In a study done to evaluate the effect of ATF4 on oxidative stress defense in*Leishmania amazonensis* infection, researchers observed an increase in (NO) production and nitric oxide synthase (iNOS) in shATF4 transduced *Leishmania amazonensis* infected cells. Heme oxygenase-1 (HO-1) is a key enzyme triggered by cellular stress and is a target of NRF2[48], [50]. Heme Oxygenase-1 can diminish the reactive oxygen species, thereby ensuring parasite survival[50]. The luciferase assay data of infected cells transduced with shATF4 showed a down-regulation in NRF2, concluding that the activation PERK/eIF2α/ATF4 signaling protects *Leishmania amazonensis* infected macrophages from oxidative stress[48].

Galluzzi *et al.* demonstrated that *Leishmania infantum*infection induces a mild UPR. The main aim of their study was to investigate the ER stress responses in macrophages infected with*Leishmania infantum* and uncover underlying molecular mechanisms which lead to anti-apoptotic properties in infected cells, using tunicamycin-treated cells as positive controls. Tunicamycin triggers apoptosis via the induction of ER stress. The gene expression analysis studies showed significant induction of several ER stress markers, namely, DDIT3/CHOP, ATF3, ATF4, and CEBPB at 6-hour and 24-hour post-infection in infected cells, but the expressions were much lower compared to positive controls. The western blot analyses of infected U937-derived macrophages showed the induction of sXBP1 and GRP78/HSPA5 proteins. However, *Leishmania infantum* infection did not appear to induce the ER stress markers phospho-eIF2α and DDIT3/CHOP. These results concluded that there is a mild but significant induction in ER stress markers tested in infected cells, but the magnitude of induction is significantly higher in positive controls compared to the infected cells. Furthermore, they have pointed out that different kinetic properties and activation of main UPR branches or the cells not being infected simultaneously might be the reason for uneven induction between the tested UPR markers. (GRP78/HSPA5 and sXBP1 being induced and lack of induction of phospho-eIF2α and DDIT3/CHOP)[7] Additionally, they assessed the ability of *Leishmania infantum* to modulate the UPR to shape the infection. After 4 hours of treatment with tunicamycin, a significant reduction in both phospho-eIF2α and DDIT3/CHOP protein levels was detected in infected cells compared to non-infected cells. These results suggested that *Leishmania infantum* infection delays or attenuates the effects of the host UPR[7]. Prolonged ER stress is reported to cause cell death, but mild ER stress is reported as a possible adaptive mechanism for the cell to build up resistance to subsequent ER stress. This is known as hormesis. Mild ER stress has been shown to have a protective role in neurodegenerative diseases, diabetes, cancer, and in certain cell lines[51]–[53]. The mechanism underlying hormesis is shown in figure 6.

Additionally, their findings may suggest the induction of ER stress as a mechanism by which *Leishmania* triggers host antioxidant enzymes that act as scavengers of superoxide anions generated during infection. The qPCR results showed a significant induction in XBP1 both in human and mouse cells confirming the observations of Dias-Teixeira *et al* . 2016, which suggest a common pathogenic mechanism with minor alterations between cutaneous and visceral species[7]. Their study also shows an increase in ATF3 and CHOP expression, indicating potential involvement of the PERK-ATF4 arm but this needs further investigation[7], [8].

The RT-qPCR assays performed on RNA samples obtained from skin lesions of *Leishmania braziliensis* infected human CL patients showed that samples from patients displayed elevated ATF4 expression compared to healthy individuals, indicating that patients with CL have high ATF4 expression in infected tissue. With their previous findings[47], these data suggest that *Leishmania* parasites activate at least two branches of the ER stress response during human infection; namely, the IRE1/XBP1 branch and PERK/ eIF2α/ATF4 branch[48]. Overall, data demonstrates that *Leishmania* parasites activate the PERK/eIF2α/ATF4 pathway and that this pathway is important for parasite survival and favors pathogenesis[48].

A study done on *Leishmania donovani* in the Indian subcontinent by Abhishek *et al.* 2018 showed that *Leishmania donovani*infection induces the UPR in PERK dependent manner and it has an important role in delaying apoptosis of infected macrophages. They demonstrated that *Leishmania donovani* infection induces UPR and ER stress inducers that enhance parasite infection. Western blotting data showed that host PERK phosphorylation gets induced by*Leishmania donovani* . Moreover, real-time PCR data shows an up-regulation in mammalian inhibitor of apoptosis (IAP) proteins cIAP1 and cIAP2 in infected cells, whereas no such induction is detected in normal cells.

Conclusion

The unfolded protein response is an essential cellular mechanism that promotes the survival of a cell during conditions of cellular stress, including intracellular parasitic infections. Pathogens are known to induce ER stress and UPR via interacting with the ER functions, while some are recognized to subvert the UPR to promote their survival and replication. *Leishmania amozonensis* activated the IRE1-XBP1 branch of the UPR to promote their growth in an IFN-1β depended manner, while activation of the PERK/eIF2α/ATF4 signaling cascade protected infected macrophages from oxidative stress. A mild ER stress response induced in *Leishmania infantum* infections may act as a possible adaptive mechanism for the cell to build up resistance to subsequent ER stress. *Leishmania donovani* infection induces the UPR in PERK dependent manner and has shown to delay apoptosis of infected macrophages. Thus, it is important to characterize the nature of the ER stress response to target the UPR pathway as a potential treatment option.

This review is intended to provide a comprehensive view of the available knowledge on the ER stress response markers and the UPR pathways characterized in intracellular parasitic infection by Trypanasomatids and particularly during infection *Leishmania* spp.infections.

Acknowledgements

This work was supported by the National Institute of Allergy and Infectious Diseases of the National Institutes of Health, USA, Grant/ Award Number: U01A|136033 to N.K.D. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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Glossary

Allosterically: being a change in the shape and activity of a protein (such as an enzyme) that results from combination with another substance at a point other than the chemically active site

Anti-oxidant: substances that protect the body by neutralizing unstable molecules

Apoptosis: a form of programmed cell death that occurs in multicellular organisms

Autophagy response: the natural, conserved degradation of the cell that removes unnecessary or dysfunctional components through a lysosome-dependent regulated mechanism

Autophosphorylation: the phosphorylation by a protein of one or more of its own amino acid residues

Biogenesis: the synthesis of substances by living organisms

Chaperones: intracellular proteins that assist in the correct folding of other proteins by means of hydrophobic surfaces that recognize and bind to exposed hydrophobic surfaces on misfolded proteins

Endogenous: Originating or produced within an organism, tissue, or cell

Homeostasis: state of steady internal, physical, and chemical conditions maintained by living systems

Isoforms: is a member of a set of highly similar proteins that originate from a single gene or gene family and are the result of genetic differences

Oligomerization: the process of converting a monomer or a mixture of monomers into an oligomer which is a molecule that consists of a few repeating units.

Oxidative stress: an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability

Post-translational modifications: the covalent and generally enzymatic modification of proteins following protein biosynthesis

Protozoan: a group of single-celled eukaryotes also known as "one-celled animals" because their animal-like behaviors, such as motility and predation, and lack a cell wall, either free-living or parasitic that feed on organic matter such as other microorganisms or organic tissues and debris

Reactive oxygen species (ROS): chemically reactive chemical species containing oxygen

Transcription factors: a protein that controls the rate of transcription of genetic information from DNA to messenger RNA, by binding to a specific DNA sequence

Translation: the process in which ribosomes in the cytoplasm or endoplasmic reticulum synthesize proteins after the process of transcription of DNA to RNA in the cell's nucleus

Translocation: the movement of substances from one part to another