ECRONICON OPEN ACCESS

EC MICROBIOLOGY
Review Article

Indispensible Biological Processes Offer Exceptional Cellular and Molecular Windows for Pharmacological Interventions in Protozoan Parasites

Saeed El-Ashram[1,2]*, Ibrahim Al Nasr[3,4], Shujian Huang[1]*, Haoji Zhang[1]*, Fathi Abouhajer[5,6], Gungor Cagdas Dincel[7] and Cheng He[8]*

1College of life science and Engineering, Foshan university, 18 Jiangwan street, Foshan 528231, Guangdong province, China
[2]Faculty of Science, Kafr El-Sheikh University, Kafr El-Sheikh, Egypt;
[3]College of Science and Arts in Unaizah, Qassim University, Unaizah, Saudi Arabia;
[4]College of Applied Health Sciences in Ar Rass, Qassim University, Ar Rass 51921, Saudi Arabia.
[5]Asmarya University for Islamic Sciences, Zliten, Libya;
[6]College of Animal Sciences and Technology, China Agricultural University (CAU), Beijing 100193, China;
[7]Laboratory and Veterinary Health Program, Siran Mustafa Beyaz Vocational School, University of Gumushane, Gumushane;
[8]Key Lab of Animal Epidemiology and Zoonosis of the Ministry of Agriculture, China. College of Veterinary Medicine, China Agricultural Beijing, 100193, China

***Corresponding Author**: Saeed El-Ashram (saeed_elashram@yahoo.com); Shujian Huang (sjhuang.foshan@163.com); Haoji Zhang (zhanghaoji@aliyun.com); Cheng He (chenghe@cau.edu.cn)

Received: May 24, 2017; **Published:** July 05, 2017

Abstract

Responses to stressful environmental conditions, endoplasmic reticulum stress, reactive species (e.g. free radicals, reactive oxygen and nitrogen species) and apicomplexan parasite sequesteration are crucial biological processes that deserve extensive review at a formative time for the development of our knowledge concerning the state-of-the-art data on their cellular and molecular mechanisms of action and the ways in which they can be manipulated in and by protozoan parasites. We have attempted to provide a comprehensive overview to reveal intervention points that could be exploited to discover novel therapies, vaccine strategies and prophylactic intervention points for broad-spectrum host-oriented inherent measures and eukaryotic parasite counter-measures, and to understand the parasitic disease progression and the infection consequence.

Keywords: *Endoplasmic Reticulum Stress; Reactive Species; Apicomplexan Parasite Sequesteration; Therapeutic and Prophylactic Intervention*

Introduction

Stressful environmental conditions, endoplasmic reticulum stress, reactive species and apicomplexan parasite sequesteration have currently emerged as critical areas of research within the context of protozoan infection. In this review article, we aim to summarize the present knowledge of biological processes that can be pharmacologically targeted in protozoan parasites. Along these lines, we will describe the responses of protozoan parasites to environmental stress, ER-stress, and reactive oxygen and nitrogen species (ROS/RNS). Furthermore, parasite sequestration is discussed as biological processes that can potentially be manipulated in and by protozoan parasites. The second aim of the article is to reveal specific intervention points that could be exploited to discover novel therapies, vaccine strategies and prophylactic measures.

Responses to stressful environmental conditions

The infective agents that are transmitted through vectors as in the case of *Plasmodium* and *Trypanosoma* come from different environments (i.e. poikilothermic invertebrate vector/cold-blooded animal vectors) and try to gain access into other environments (i.e. homeothermic mammalian host/worm-blooded vertebrates), consequently, both the parasite and the host become stressed. The epithelial cells upregulate the heat-shock proteins (highly conserved). Furthermore, the cytolytic activity of the innate NK and γδ T-cells after engage-

ment the heat-shock protein through their NKG2D receptor is augmented [1]. We hypothetically think that they can kill the parasites by the same manner: NK or γδ T cells can be activated by IL-12 and TNF-α from macrophage to release IFN-γ or can cause direct lysis of infected cells after enhancement by IFN-α from activated macrophage [2]. Responses to the stressful environmental conditions, for example, nutrient deficiency, hypoxia, oxidative stress, changing pH, and shifts in temperature are accounted for the adaptation and survival strategy developed by the *Leishmania* parasite. Once the encounter between *Leishmania* and a particular host occurs, the former expresses a myriad of molecular chaperones, among them heat shock proteins (Hsps), such as HSP100, HSP90, HSP70, HSP60/chaperonins, HSP40, Small HSPs (sHSPs), and Folding of cytoskeleton components CCT (TRiC) for cytoprotection, differentiation, stage development, as well as virulence [3]. The expression of the heat-shock protein 83 (HSP83) genes is temperature-dependent and may participate in the developmental program of *Leishmania infantum* [4]. The 75-kDa heat-shock protein (HSP70) was expressed in the sporozoite stage of rodent and human plasmodial species [5]. The protozoan *Leishmania* parasites cause serious pathology and lead to cutaneous and visceral lesions [6]. Two major heat-shock proteins, HSP70 and HSP83 were detected in protozoan *Leishmania* parasites [7,8]. HSP70 plays a vital target in the humoral immune response during infection with *Leishmania* [7,9]. Moreover, HSP70 leads to resistance to hydrogen peroxide in *Leishmania chagasi* promastigotes. It can thus be suggested that HSP70 can be involved in oxidative stress in *L. chagasi* promastigotes [8]. HSP70 could provide cytoprotection by binding to misfolded proteins under a variety of stresses, such as radiation, chemical/biochemical stress, heat, and microbial infection [10,11]. Heat-shock protein 90 (Hsp90) has been implicated to play a pivotal role in life cycle and growth of *Leishmania, Giardia, Toxoplasma* and *Plasmodium* [12-15]. Hsp90 is critical for growth and survival of *E. histolytica* and its attendance in regulation of phagocytosis and encystation [16,17]. *Plasmodium falciparum* Hsp70-z (PfHsp70-z), a member of the Hsp110 family of Hsp70-like proteins, is essential for malaria parasite survival [18,19]. Co-chaperones, such as PfHop, Pfp23, PfAha1, PfPP5 and PfFKBP35 are well-known regulators of Hsp90. The unity of Hsp70 and Hsp90 into a functional complex occurs through an adaptor protein called Hsp70-Hsp90 organising protein (Hop), which is essential for parasite growth [20,21]. Further studies need to be carried out in order to target the cooperation of these chaperones as potential antimalarial drug. Recently it was shown, that the mammalian pathogen *Trypanosoma brucei* has been able to respond to environmental stressors, such as temperature, arsenite, ethanol, puromycin or proteasome inhibitors by the increase in ZC3H11 level, an RNA binding protein, which stabilizes mRNAs that encode chaperones [22]. In a study conducted by Singh., *et al*. it was shown that Hsp90 inhibition has been led to the death of *Entamoeba* trophozoites [17]. Heat shock proteins are highly conserved molecules and have been shown to direct the immune system towards Th2 pattern and may be incapable of eliciting protective role in *L. major* infection [23]. Recent evidence suggests that the interaction of sphingosine-1-phosphate (S1P) with the heat shock-proteins (GRP94 and HSP90α) orchestrates critical stress-specific signals by triggering the key innate immune element, cathelicidin antimicrobial peptide (CAMP) production [24].

Endoplasmic reticulum stress

The endoplasmic reticulum (ER) is the cell's quality control site for precise folding of all transmembrane proteins and lipids for most of the cell's organelles. The ER translated proteins are sorted and trafficked by Golgi apparatus. Perturbing the cellular energy levels, the redox state, Ca^{2+} concentration and glucose homeostasis of ER owing to stresses reduce the ER protein folding capacity. Consequently, the unfolded proteins accumulated and aggregated resulting in ER stress. The cell responds by activating the unfolded protein response (UPR). The UPR ensures cellular repair and sustained survival by reducing the unfolded protein load either through protein synthesis global attenuation or chaperones, enzymes and structural components of the folding, trafficking and degradation machinery in the ER up-regulation. However, the targeted computational technique was employed by Gosline., *et al*. to evolutionarily characterize the UPR in eukaryotes. They reported the existence of a PRKR-like Endoplasmic Reticulum Kinase (PERK) translational control pathway, which reduces the load on the ER in *L. donovani* as well as an absence of UPR- mediated transcriptional or post-transcriptional control [25]. Similarly, Trypanosomes are unable to elicit the unfolded response [26]. This less evolved stress response could provide a new avenue for therapeutic treatment of parasitic infections. Cells switch from survival mode to apoptotic mode, when misfolded protein accumulation is overwhelming. Furthermore, there is a cross-talk between the ER and the mitochondrion. The release of Ca^{2+} from the ER lumen triggers changes in the mitochondrial membrane potential, which results in cytochrome release and apoptosis [27]. The processing of *Trypanosome* polycistronic mRNAs occurs via trans-splicing and polyadenylation. In the former, an exon known as the spliced leader (SL)

is added to all polycistronic mRNAs from a small RNA. The shutting off of Spliced Leader (SL) RNA transcription was induced by the ER stress response through perturbing the binding of the transcription factor tSNAP42 to the SL RNA promoter, changing the PH and silencing of relevant proteins such as the signal-recognition particle receptor, SEC63 (a protein participating in protein translocation across the ER membrane) or SEC61 (the translocation channel) [28-31]. A recent study by Inácio., *et al*. reported the activation of the ER-resident unfolded protein response (UPR) in *Plasmodium berghei* – hepatocytes [32]. In another major study, Chaubey., *et al*. suggested that the malaria parasite exploited the host ER stress during its growth in human erythrocytes to switch to the transmissible sexual stages; gametocytogenesis [33]. However, prolonged endoplasmic reticulum (ER) stress induces the spliced leader silencing (SLS) pathway in *Trypanosoma brucei*. During ER stress, the translocation of the serine-threonine kinase PK3 from the ER to the nucleus to phosphorylate the TATA-binding protein TRF4, leads to the transcription pre-initiation complex dissociation from the promoter of the SL RNA encoding gene [34]. This suggests that parasites employ apoptosis to ''altruistically'' self-regulate the infection intensity and lengthen the host/vector survival. The existence of SLS -mediated apoptosis trypanosomes offers the potential to abrogate them and develop novel therapeutic tools for trypanosomes that infect diverse hosts and cause a variety of diseases, including the fatal human diseases sleeping sickness and Chagas disease, caused by *Trypanosoma brucei* and *Trypanosoma cruzi* respectively (Figure1).

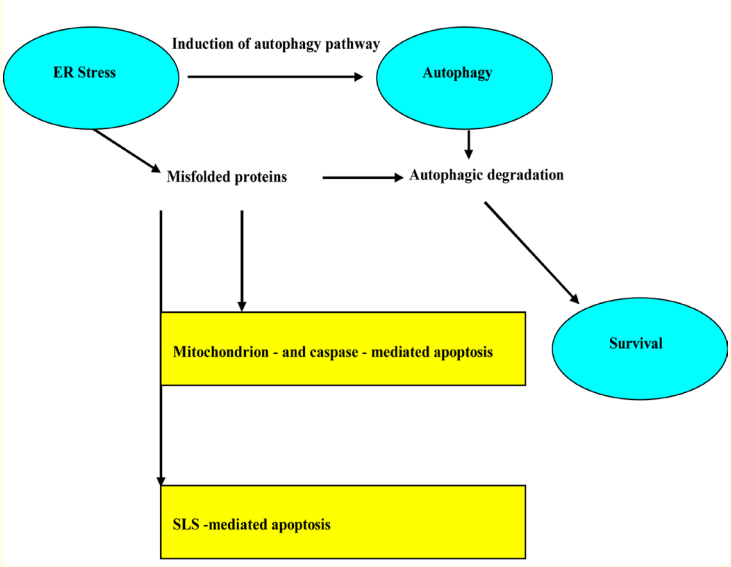

Figure 1: *Cross-talk among ER stress, autophagy and apoptosis.*

Abbreviations: SLS: Spliced Leader Silencing Pathway; ER: Endoplasmic Reticulum Stress

Reactive species (free radicals, reactive oxygen and nitrogen species [ROS/RNS])

It is necessary here to clarify exactly what is meant by reactive species, which refers to any existed independently chemical species that has one or more unpaired electrons, for example, the superoxide (O2.-). The superscript dot and minus sign signify the unpaired electron and the negatively charged free radical. Free radicals are generally reactive species, but reactive species are not needed to be free radicals (e.g. non-radical ROS, including hydrogen peroxide and peroxynitrite). ROS/RNS are produced from diverse endogenous sources, including NAD (P) H oxidases and mitochondria and a variety of exogenous sources, such as protozoan parasites. They are able to deteriorate various cellular ingredients, including proteins, lipids, and nucleic acids; however, well-restrained ROS/RNS production fulfils essential physiological roles, such as anti-bacterial, anti-viral, anti-parasital and anti-tumoral activity and contribution in programmed cell deaths (signaling species) [35-39].

Reactive nitrogen species (RNS)

Similarly, the term reactive nitrogen species (RNS) will be used solely when referring to nitric oxide (.NO), peroxynitrite, nitrogen dioxide (.NO_2), and other oxides of nitrogen or nitrogen-containing reactive species. Peroxynitrite can be loosely described as both an ROS and an RNS in the literature. Analogous to oxidative stress, the term nitrative stress is associated to higher level of RNS. For example, RNS peroxynitrite adds a nitro (-NO_2) group (nitration process) to a biomolecule. Similarly, nitrosative stress involves the addition of a nitroso (-NO) group to a thiol group cysteine protein (S-nitrosylation).

Innate or innate-like cells, including NK, NK T and TCRγδ+ cells, and the adaptive T cells, such as $CD4^+$ and CD8+ TCRβ+ lymphocytes contributed to the IFN-γ production (originally called macrophage-activating factor) in response to infection challenge. The increased IFN-γ production is concident with increased expression of important effector molecules, such as interferon-inducible GTPases and nitric oxide synthase (NOS) for controlling intracellular parasite growth [40]. NOSs have been implicated in nitric oxide (NO) production, which has been characterized to have beneficial anti-bacterial, anti-viral, anti-fungal, anti-parasital, immuno-modulatory, and anti-tumoral effects [41]. It was reported that NO synthesis (parasite control)/arginase activation (parasite survival) influenced by the cytokines secreted from the T-helper 1 /T-helper 2 respectively [42-44]. Additionally, different pro-inflammatory cytokines (e.g., IL-1, IL-2, TNFα, IFNα) have significant effects on iNOS induction (e.g., in resistance to infection and mediation of inflammation), albeit counter-regulatory factors (e.g., IL-4, IL-10, TGFβ) appear to engage contradictory effects avoiding the detrimental consequences of aberrant iNOS induction. Importantly, in a study conducted by Oliveira., *et al*. it was shown that TLR4 signaling is required for IFN-gamma, TNF-alpha and nitric oxide (NO) optimal production in the spleen of *T. Cruzi* -infected mice [45]. The capacity for iNOS upregulation extends far beyond the immune cells (e.g. macrophage and neutrophil) to include a broad spectrum of cell types (e.g. hepatocytes, astrocytes, and neuronal cells) [46,47] (Figure 2). It is no surprise, therefore, that nearly three different NOS isoforms (neuronal/brain [bNOS], inducible [iNOS], and endothelial [eNOS]) have been characterized [42,48,49].

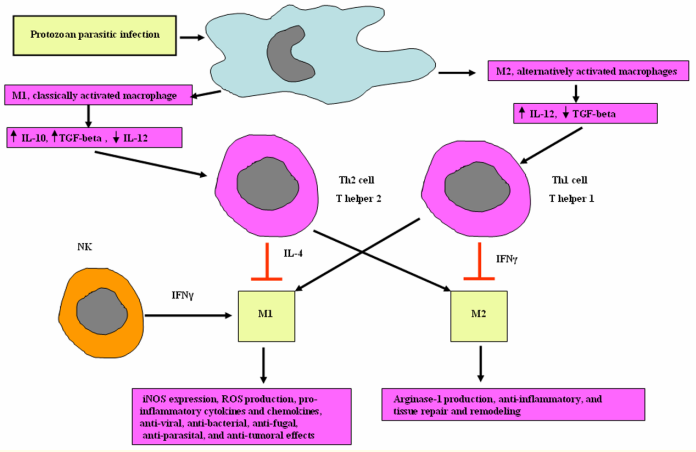

Figure 2: *Cytokine regulation of NO production.*

Activated macrophage synthesizes nitric oxide (NO) from L-arginine under the influence of inducible nitric oxide synthase (iNOS/NOS II) or alternatively L-arginine can be cleaved by arginase (two forms, arginase I, a cytosolic enzyme, and arginase II, a mitochondrial enzyme is widely distributed throughout the body) to ornithine and urea. L-ornithine is the precursor for the parasite growth factors, such as L-glutamine, L-proline, and putrescine (Figure 3).

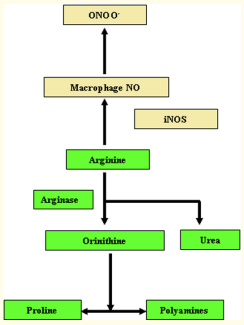

Figure 3: *A much simplified illustration of arginine metabolic pathways.*

The NOS-L-arginine metabolic fate generates a series of compounds, such as nitric oxide ($NO^{\cdot}$), $ONOO^{-}$, superoxide ($O^{\cdot}_{2}{}^{-}$) and hydrogen peroxide (H_2O_2) collectively known as the reactive nitrogen species, which plays an essential role in efficient immune response and cytotoxicity of host cells (RNS) [50]. It is well-established that M1 (NOS expression/NO production)/M2 (arginase expression/ornithine production) macrophage polarization can be directed by protozoan parasitic infection or innate danger signals. T helper (Th) 1 (cytotoxic)) and Th2 (antibody-mediated) lymphocyte products down-regulate M2 and M1 macrophage respectively [51,52]. For a full review of the macrophage heterogeneity and plasticity, please refer to the reviews by Gordon., *et al.* [53], De Paoli., *et al.* [54], Rojas., *et al.* [55], Novoselov., *et al.* [56], Ivanova., *et al.* [57] and Ghosh., *et al* [58]. Once activated, macrophages, as well other cells, including cardiomyocytes produce various effector molecules, such as RNSs, which kill *T. cruzi* intracellular stages by chemically modifying protein structural properties and inactivating enzyme catalytic sites. IL-12 and TNF-α, and IL-1β stimulates NK cell activation, resulting in IFN-γ production to further improve parasite killing. Furthermore, Self-destruction and tissue damage are dampened by IL-10 and TGF-β. Relatedly, chemokines, including JE/MCP-1, RANTES, MIP-1α, MIP-1β, MIP-2 and CRG-2 are also positively elicited NO synthesis. The anti-inflammatory effect attributed to the non-opsonic interactions between *Plasmodium*-infected RBC (iRBC) and macrophages is mediated by *P. falciparum* membrane protein-1 (PfEMP-1) - CD36 receptor binding. However, the same engagement enhances oxidative burst activity (i.e. higher level of generated superoxide anions) involving protein kinase C (PKC) pathway activation in the absence of phagocytosis of iRBC. Additionally, Glycosylphosphatidylinositol (GPI)-anchored proteins recognized by both Toll like receptor (TLR) 2 and TLR 4 results in the production of inflammatory cytokines and nitric oxide by macrophages [59-62]. More recently, reactive nitrogen species (RNS), including nitric oxide (NO) targeted Falcipain-2 (FP2), *Plasmodium falciparum* hemoglobinase, appears to mediate anti-plasmodial growth and host protection [63]. In this context, Weinberg., *et al.* demonstrated that the mouse innate resistance to certain human pathogens, such as *Mycobacterium tuberculosis* is appeared to be associated with the production of more NO and expression higher levels of NOS2 than human cells do (i.e. species differences) [64]. Moreover, an array of effector mechanisms is possessed by macrophages and other specific types of cells to limit *T. gondii* intracellular replication that function either by generating toxins (e.g., RNS, p47 immunity-related GTPases (IRGs) and ROS) that are detrimental for *T. gondii*, or by depleting nutrient stores (e.g. iron deprivation and tryptophan degradation) that play a central role for *T. gondii* growth in a cell autonomous manner. An astonishingly broad range of intracellular protozoan parasites, including *Toxoplasma* and *Leishmania*, are killed or established persistent subclinical latent infection owing to NO involvement. NO-mediated mechanisms result in killing of intracellular *Leishmania*, extracellular *Trichomonas vaginalis* and *Naegleria fowleri*, both intracellular and extracellular morphotypes of *Trypanosoma cruzi* by activated macrophages. Interestingly, *C. parvum* infection induced nitric oxide (NO) production in host epithelial cells in a TLR4/NF-κB-dependent manner, which are crucial for host's defense against cryptosporidial infection [65]. NO dependent mechanisms are responsible for killing of *N. caninum* tachyzoites inside macrophages [66]. Based on several studies in which both RNS/ROS have been examined, the parasite species, strain and developmental stage as well as on the host species, tissue origin, and phagocyte activation states are important for determining the infection outcome and explaining the discrepancy between both studies. The phagocyte NADPH oxidase plays an indispensable Role for splenic *L. major* control; however, dispensible for splenic *L. braziliensis* and hepatic *L. donovani* killing. Furthermore, the association between NO production and disease protection has

been reported in the skin and draining lymph node of *L. major* and *L. mexicana* infected mice. The leishmanial parasite control via RNS is either very debilitated or dependent on the mouse genetic background in splenic and hepatic (visceral organs) *L. major* and *L. donovani* respectively [67,68]. The isoforms; eNOS, iNOS and nNOS derived NO, produced by microglia/macrophages, astrocytes and neurons contribute to the development of tissue cysts, and protective immunity [69,70]. The participation of NO in trypomastigote differentiation to amastigotes has been reported by Soares., *et al.* in *T. cruzi.* Moreover, protective and pathogenic role of NO has documented in *Plasmodium falciparum* [71]. NO derived from nNOS is effective in the elimination of *Giardia lamblia* "hiker's diarrhea" infection in mice. It has been reported that reactive oxygen species (ROS), nitric oxide and IFN-γ play a protective role against *Giardia.* Furthermore, mucosal IgA and serum IgG have a role as well [72]. NO inhibition causes excystation and encystation of *G. lamblia* cysts, thereby establishing and continuing the infection process in the small intestine [73]. Additionally, *Giardia* has evolved strategies to escape NO-mediated host defenses. For example, epithelial NO production was depleted because of consuming arginine by *Giardia* [73,74]. Cruzipain (major cysteine proteinase) of *Trypanosoma cruzi*, an intracellular parasitic protozoon, favours Th2 arm of immunity; therefore, evade NOS pathway resulting in regulation of the parasite survival and differentiation through arginase activation [75]. Interestingly, *T. cruzi* - infected macrophage behaves either as host and primary effector cells. Regardless of the mobilization of innate and adaptive immune responses by the parasite that elicits the macrophage activation and keeps the parasite under control, the *Trypanosoma* can persist chronically in the host, well beyond the initial acute phase of *Trypanosoma* infection. The chronic persistence of *Trypanosoma* is associated with continual lymphocyte apoptosis and phagocytosis of apoptotic cells by macrophage. In this context, *T. cruzi* exploits macrophage-mediated apoptotic clearance and triggers TGF-β release and disable signals from IFN-γ avoiding the interaction of the infected macrophages with parasite-specific Th1 T cells, which trigger IFN-γ production, and induction of iNOS activity [76]. Clearly, this biochemical cascade will ultimately impact on the promising target for therapies, for example cyclo-oxygenase inhibitors or Z-VAD-FMK (predominantly Caspase-1 and -3 inhibitor) have potential therapeutic application in *Trypanosoma* control. Additionally, targeting polyamine production is another potential avenue for chemo-therapic intervention against parasitic protozoa [76-78]. Arginase induction during *Leishmania major* infection leads to promastigote–amastigote transformation and amastigote replication within the phagolysosome of macrophage [79]. *T. gondii* survives in the activated macrophages, and avoids nitric oxide-killing mechanism. A possible explanation for this might be that a *T. gondii* patatin-like protein (TgPL1) protects *T. gondii* from nitric oxide degradation in the activated macrophages [80]. Recently it was shown, that *T. gondii*-infected macrophages degrade inducible nitric oxide synthase (iNOS) by the Ubiquitin Proteasome Pathway (UPP) [81].

Having considered what is meant by the inducible nitric oxide synthase (NOS2) and the macrophage-secreted NO, we will now move on to briefly shed the light on IFN-γ-inducible GTPases, the immunity-related GTPases (IRGs) and the guanylate-binding proteins (GBPs), target intracellular vacuolar protozoan parasites and limit their replication by destroying PV [82-85]. Intriguingly, pseudokinase (ROP5) and kinase (ROP18) are secreted by certain *T. gondii* strains resulting in the formation of ROP5/ROP18 kinase complex, which phosphorylates and inactivates IRG proteins. However, some strains of mice are ROP5/ROP18 kinase complex-resistant [84,86]. It is worth mentioning that RabGDIα as a negative regulator of IFN-γ–inducible GTPase-dependent orchestrates vacuolar disruption of *T. gondii* has been reported by reported by Meunier., *et al.* [85]. Of the six discrete typing units (DTUs): TcI, TcII, TcIII, TcIV, TcV, and TcVI, the trypomastigote of TcI strains Colombiana (COL), SylvioX10/4 (SYL) exhibit cytosolic and mitochondrial tryparedoxin peroxidases and their substrate (tryparedoxin) and iron superoxide dismutase that contribute significantly to the resistance to the exogenous treatment with stable oxidants (H_2O_2 and $ONOO^-$) and dampen the intracellular superoxide and nitric oxide response in macrophages [87]. This study may eventually help to clarify the role of the parasite-specific antioxidant network in COL and SYL trypomastigote sylvatic transmission cycle and human disease. Relatedly, the myocardial activity of catalase and superoxide dismutase in cardiac tissue, which provides protective adaptations to the host against chagas-associated cardiomyopathy, is improved in response to exercise training [88]. *T. cruzi* trypomastigote is equipped with antioxidant (cytosolic and mitochondrial peroxiredoxins and trypanothione synthetase) - based host oxidative assault (e.g. $O_2^{\cdot-}$, H_2O_2, and $ONOO^-$) intervention to play a role in parasite virulence and persistence [89].

NO-releasing compounds prevent the enzymatic activity of the highly conserved cysteine proteases (CP) of *P. falciparum*, *T. cruzi* and *L. infantum* in a dose-dependent manner [90]. Insufficient NO production are harmful and can cause tissue cyst reactivation [91-95]; however, excess NO elicits apoptosis in various cell types, including macrophages, microglia, and pancreatic β-cells by the DNA damage-p53 pathway, and its lower concentration leads to apoptotic cell death by the ER stress-CHOP (a C/EBP family transcription factor) pathway [96]. Importantly, Encephalitogenic *T. brucei* -infected wild-type mice (C57BL/6 background) upregulates TNF and IFN-γ by macrophages and T cells respectively. NF-κB p65 nitrosylation might lead to diminished TNF release and signaling. However, $iNOS^{-/-}$ mice exhibited high level of TNF levels resulting in an intensified MMP9 expression that mediates inflammatory tissue damage [97]. Among the causes of central nervous system (CNS) pathologies in *Toxoplasma* encephalitis (TE) are *T. gondii* –induced apoptosis either directly by *T. gondii* itself and/or cytotoxins (e.g. NO) expressed due to the infection. As can be seen from the figure below both extrinsic (TNFR1, caspase 8, caspase 9 and caspase 3 up-regulation) and intrinsic apoptotic (eNOS), iNOS and nNOS up-regulation) pathways are triggered, and the period of time essential for the development of neuropathologies are the first 30 days after infection. NO inhibits the formation of apoptosome complex by inhibiting the cytochrome c release from mitochondria double membranes at physiological concentrations. Thus, it prevents the intrinsic apoptotic pathway. However, NO induces mitochondrial membrane potential loss, which in turn causes cytochrome c release from cytosol, and the intrinsic apoptosis pathway is activated at pathological levels. Thereby, they induce severe neurodegeneration and cerebral pathology in Toxoplasmic encephalitis (TE) (Figures 4-7). Recently, the increased levels of NO and apoptosis might contribute to oxidative stress (OS) - related pathogenesis of TE [98]. It is worth mentioning that exaggerated NO leads to programmed cell death through DNA damage (p53-mediated apoptotic and poly (ADP-ribose) polymerase (PARP)-mediated necroptotic pathway). However, the low amount of NO causes ER stress and ER Ca^{2+} depletion, which results in unfolded protein accumulation leading to CHOP, Jun N-terminal kinases (JNK) and caspase-12 mediated apoptosis [99].

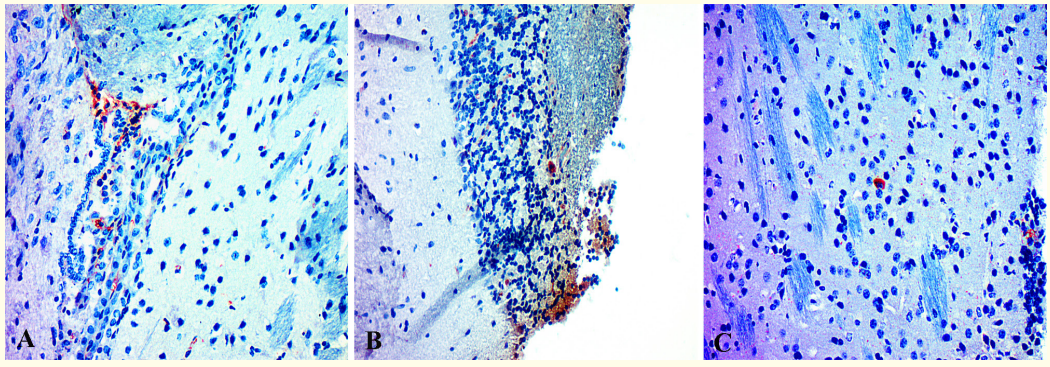

Figure 4: *Apoptosis in healthy brain tissues (Control groups).*
ABC method (anti-eNOS/ anti-iNOS/ anti-nNOS), counterstaining with Mayer's hematoxylin

A) Slight expression of eNOS in endothelial and some glial cells.
B) Slight expression of iNOS in some glial cells.
C) Slight expression of nNOS in neuron.

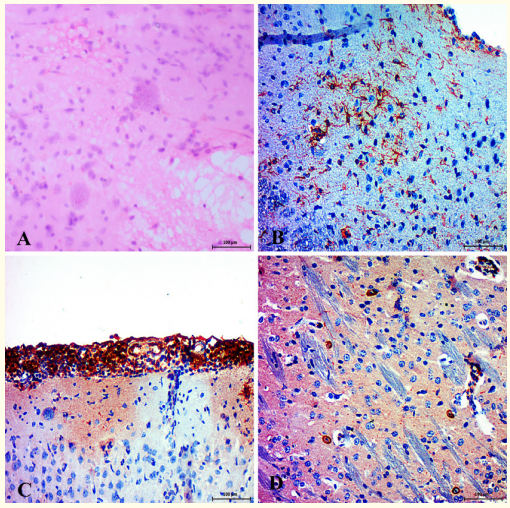

Figure 5: *High nitric oxide expressions in TE and tissue cyst in brain T. gondii tissue cysts.*

A) Squash-smear technique (HandE)

B) Severe eNOS expression in glial cells and endothelial cells.

C) Severe iNOS expression in infiltrating macrophages in the meningitis area and glial cells.

D) A great number of nNOS expressed neurons and endothelial cells. Bar, 100 µm.

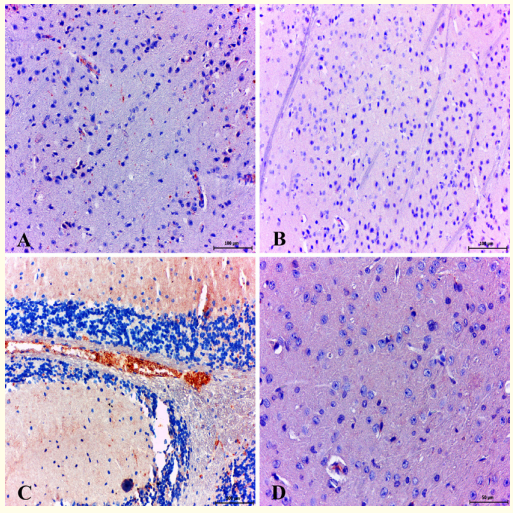

Figure 6: *Nitric oxide expressions in healthy brain tissues (Control groups).*

ABC technique (anti-TNF-R1/ anti-Caspase 8/ anti- caspase 9/ anti- caspase 3), Mayer's hematoxylin counterstain

A) Slight expression of TNF-R1 in endothelial and some glial cells.

B) Slight expression of caspase 8 in endothelial and some glial cells.

C) Slight expression of caspase 9 in endothelial and some glial cells.

D) Slight expression of caspase 3 in some glial cells. Bar, 50 µm.

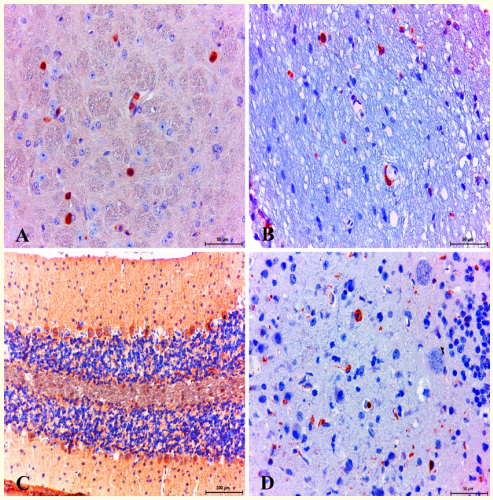

Figure 7: *Neuronal apoptosis in TE. Avidin–biotin peroxidase complex (ABC) (anti-TNFR1/ anti-caspase 8/ anti-caspase 9/ anti-caspase 3) and Mayer's hematoxylin counterstain techniques*

A) Substantial expression of TNFR1 in glial cells.

B) Substantial expression of caspase 8 in glial and microglia/macrophage cells.

C) Substantial expression of caspase 9 in purkinje and glial cells.

D) Substantial expression of caspase 3 in neuronal and microglia/macrophage cells.

Scale Bar: 50 µm

Owing to the instability and inconvenient handling of NO aqueous solutions, the NO-donor S-nitroso-N-acetilpenicillamine (SNAP), which is capable of creating NO in situ, has been used to kill *T. cruzi* epimastigotes and *L. infantum* promastigotes in culture. The development of new NO donors, such as NO-drug hybrids (e.g. metronidazole bearing a NO-releasing group) and site-specific delivery of NO (e.g. NO-producing cream) have been formulated to significantly enhance killing of protozoan parasites [90]. A triatomine insect vector-derived lysophosphatidylcholine (LPC) recruits the inflammatory cells, such as macrophages to the insect bite site, inhibits nitric oxide production by *T. cruzi*-stimulated macrophages and enhances parasite invasion [100]. Given the urgent need for productive prevention strategies, information derived from this study will likely participate in the development of an effective *T. cruzi* vaccine and improvement of immunotherapies.

Therefore, a compound that can release the precise amount of NO in the exact place and time under specific conditions can be used therapeutically to palliate the deleterious effects of NO over- or under- production. More importantly, employing nitric oxide- releasing nanoparticle (NO NPs) for accelerating the efficacy of the available NO, is a promising strategy [101]. The pharmacological challenge will be to discriminately abrogate enormous NO generation or to convey NO equivalents to the infection sites in the relevant clinical settings.

Reactive oxygen species (ROS)

The term refers to oxygen-containing reactive species, including superoxide ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\cdot}OH$), singlet oxygen ($^1O_2$), peroxyl radical ($LOO^{\cdot}$), alkoxyl radical ($LO^{\cdot}$), lipid hydroperoxide (LOOH), peroxynitrite ($ONOO^-$), hypochlorous acid (HOCl), and ozone (O_3). NADPH oxidase (phox), a NADPH: O_2 –oxidoreductase is the key enzyme for generation of ROS. The term oxidative burst in granulocytes and macrophages refers to a condition where the active phox enzyme is involved in the electron transfer from NADPH to molecular oxygen to produce superoxide anion upon exposure to a multitude of stimuli.

Interestingly, higher level of ROS is associated with oxidative biomolecule (e.g. proteins, lipids, and nucleic acids) damage. *T. Cruzi* parasite is constantly exposed to ROS in the triatomine gut, which turns on epimastigote proliferation while antioxidants, such as N-

acetyl Cysteine (NAC) switch the cycle towards metacyclogenesis (trypomastigotes) [102]. Intriguingly, Caspase-1/ASC Inflammasome-Mediated Activation of IL-1b–ROS–NF-kB Pathway is unessential and reimbursed for by ROS-mediated for *T. cruzi* replication and survival control in macrophages [103].

C-type lectin receptors (CLRs), including Dectin-1 and mannose receptor (MR) of M2b macrophage-mediated the activation of Syk-p47phox and arachidonic acid (AA)-NADPH oxidase signaling pathways, respectively, which enhance the ROS production and also provoked Syk-coupled signaling for caspase-1-induced IL-1β production. However, the CLR (e.g. DC-SIGN homolog SIGNR3 expression) favored parasite resilience via the (5-(S), 12-(R)-dihydroxy-6, 14-cis-8, 10-trans-eicosatetraenoic acid (LTB4)-IL-1β axis inhibition [104].

The host innate immune cells such as macrophages, eosinophils, neutrophils, and platelets release reactive oxygen species (ROS) resulting in harsh environmental condition for parasite survival. Protozoa developed countermeasures to avoid oxygen-mediated killing. They produce antioxidant enzymes, e.g. superoxide dismutase (SOD) which is responsible for the dismutation of the superoxide anion to hydrogen peroxide (H_2O_2), Catalase and Glutathione-peroxidase (GPX), which detoxify the hydrogen peroxide, and glutathione-S-transferase (GST) and peroxiredoxin which eliminate the hydrogen peroxide. The anti-oxidants avert the toxic action of the secreted ROS [105,106].

Spin traps, especially α-phenyl-tert-butylnitrone (PBN) are chemical agents, which can scavenge free radicals to form a stable end product suitable for measurement and possess other pivotal biological activities, including endogenous anti-oxidant enzyme up-regulation, inflammatory response down-regulation. They can be detected by electron paramagnetic resonance (EPR) techniques, called also electron spin resonance (ESR) [107]. Strikingly, spin-trap (PBN) has been found to oppose the heme polymer or β-hematin, which causes oxidative stress-mediated apoptosis of macrophages [108]. This technique will then also lay the foundation for therapeutic agents to harness the enormous power of ROS.

Cross-talk of RNS and ROS

Interactions between RNS and ROS increase the potential range of reactive species produced, which results in oxidative stress (oxidative signalling), and frequently stimulate anti-microbial potency [109-113]. Relatedly, RNS and ROS synthesis can act either synergistically or independently to trigger PCD [114]. Relatedly, oxidative stress (OS) condition is associated with oxidative damage to cellular biomolecules, including proteins, lipids, and mirochondrial and nuclear (mt/N) DNA and elicited by RNS and reactive nitrogen oxide (RNO), which augment the pro-inflammatory response resulting in oxidative/inflammatory stress. Due to the detrimental effects of ROS/RNS, the endogenous antioxidants, such as copper, zinc superoxide dismutase (Cu, ZnSOD or SOD1) and glutathione reductase (GR) have been evolved to counteract ROS/RNS-mediated biological damage. As shown in Figure 8, the expression of SOD1 is seriously down-regulated in the *Toxoplasma*-infected mice brain compared to the healthy control one owing to RNS and RNO or *T. gondii* evasion strategy.

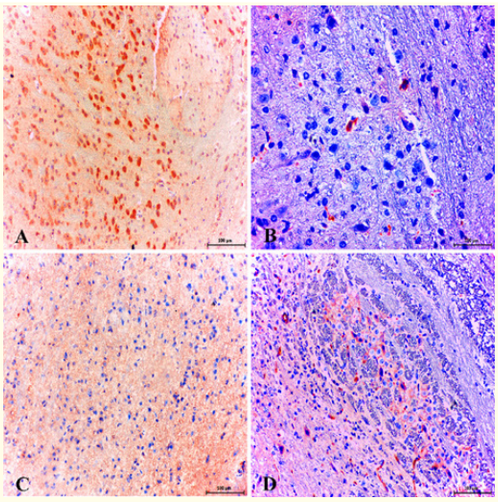

Figure 8: *SOD1 and GR immunohistochemistry. ABC technique (anti-SOD1/ anti-GR), Mayer's hematoxylin counterstain, bar, 100 µm.*

Healthy control group:

A) Severe expression of SOD1 in neuronal and glial cells

B) Slight expression of GR in some glial cells.

Toxoplasma - infected mouse brain tissue:

C) Very slight expression of SOD1 in neuronal and glial cells.

D) Strong expression of GR in neuronal and glial cells.

GR could be implicated as a powerful defense mechanism upon the *T. gondii*-related oxidative damage owing to its increased activity and its ability to catalyze GSH formation from glutathione disulfide (GSSG) upon reaction with ROS/RNS.

Further complexity is introduced by studies showing that the antioxidant effect NO (nitrosative stress) can provide cytoprotective effects against ROS-dependent processes (oxidative stress) required for the microbial destruction [115,116]. The RNO/reactive nitrogen intermediate (RNI) - parasitic protozoan reaction is likely to illuminate important mechanisms of host defense and pathogenesis in parasitic protozoan diseases. Moreover, understanding the mechanisms by which parasitic protozoans evade RNO/RNI toxicity and identifying parasitic protozoan molecules with which RNO/RNI react can develop new prophylactic, therapeutic, and vaccine strategies. Moreover, The ROS/RNS contributing role in diverse protozoan parasitic diseases makes it compulsory to develop antioxidant-based intervention to control these reactive species-mediated pathophysiological processes.

Apicomplexan parasite sequesteration

Parasitophorous vacuoles (PVs), which are formed within the host cells as part of the apicomplexan infection process, are the residence and developmental microhabitat of apicomplexan parasites. The PV is a nonfusogenic vacuole, which allows the parasite to develop and egress while being protected from the lysosome-destroying mechanism apart from being merged with acidic organelles from the different exo- and endo- cytic pathways and is therefore distinguishable from phagosomes [80,117-120]. It is also worth noting that the acidification of parasitophorous vacuole-containing *Toxoplasma gondii* by hydroxyurea results in NO oxidative attack of the parasite [121]. Figure 9 (A and B) presents an overview of the division of *T. gondii* RH strain parasites within the parasitophorous vacuole of human foreskin fibroblast (HFF) cells.

The secretory organelles, the rhoptries are engaged in the formation of the parasitophorous vacuole membrane (PVM) of *T. gondii*. The PVM, which is the interface between host and parasite, plays a role in parasite nutrient uptake, cellular function manipulation and the cyst wall formation in the latent infection of tissue cyst-forming coccidian [122]. Additionally, the PV is a metabolically active compartment in the infected host cells and indispensable for parasite survival far from harsh extracellular conditions [123-125]. The apical complex of apicomplexan parasites is a cone-shaped projection of the cell membrane and consists of micronemes (rod-like structure), rhoptries (club-shaped organelle), dense granules (spherical organelles) and conoid. They are associated with host cell attachment, penetration (conoid and microneme secretion) and establishing a parasite-friendly environment (rhoptry secretion) suitable for parasite growth, development, and exit from the host cell. Several rhoptry (ROP) proteins incorporate kinase-like domains, which play key roles in signal transduction, for example; the active rhoptry kinases ROP16 alters STAT 3/6 phosphorylation in the infected cell, albeit the ROP18 is of crucial importance for the virulence determinant [126-128]. Parasite ROP38 is a good illustration of the down-regulation of host genes associated with MAPK signaling and the apoptosis and proliferation management [129]. Recently, the Rhoptry Associated Protein 2/3 (RAP2/3) is secreted into the PV during infection of hepatocytes by *P. falciparum* and *P. yoelii* sporozoites (yet unknown ligands) and associated with the host tetraspanin CD81-regulated protein complex receptor [130]. There are two basic approaches currently being adopted in research into the strategies exploited by the *Plasmodium* sporozoites to evade host cell defense and establish an intracellular microhabitat for replication. One is the non-productive invasion (perforin-like protein 1 (PLP1)-dependent/ moving junction (MJ)-independent process) approach, including, and the other is the productive invasion (CD81- dependent/ MJ-dependent process). The earlier approaches are used by Risco-Castillo., *et al.* to refer to *Plasmodium* sporozoites migration through cells using pH sensing and PLP1 to exit the transient vacuoles and avoid the fusigenic events by host lysosomes before establishing a replicative parasitophorous vacuole inside hepatocytes in CD81- dependent/ MJ-dependent processes in a corresponding manner [131]. A reasonable approach to avert infection of the liver by *Plasmodium* sporozoites could be to interfer with these interactions. Apicomplexan parasites develop a tubulovesicular membranous network (TMN) within PV. Additionally, some of the TMN membranes are connected to the PVM [132-135]. After parasites active entry into the host cells, *T. gondii* and *N. caninum* tachyzoites, *Encephalitozoon cuniculi* spores, *Plasmodium falciparum* merozoites, which cause malaria, *T. cruzi* and *L. amazonensis* amastigotes hide inside the PV and rescue host humoral recognition and killing [80,136]. Surprisingly, the movement of small molecules between the Host and the PV is mediated by the synergistic action of *Toxoplasma* Dense

Granule Proteins GRA17 and GRA23. Moreover, GRA17 is conserved across PV- residing apicomplexan parasite and related to the putative *Plasmodium* translocon protein EXP2. GRA17-deficient parasites are avirulent in mice and proliferate slowly [137].

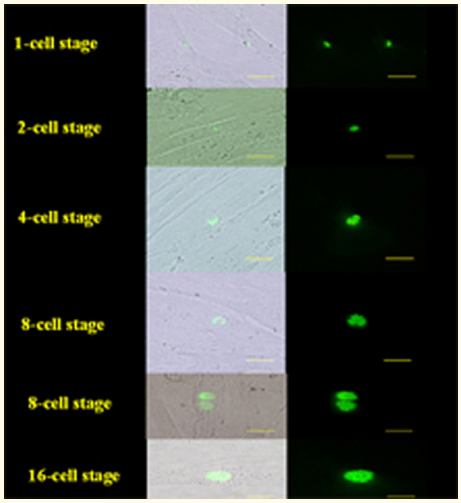

Figure 9a

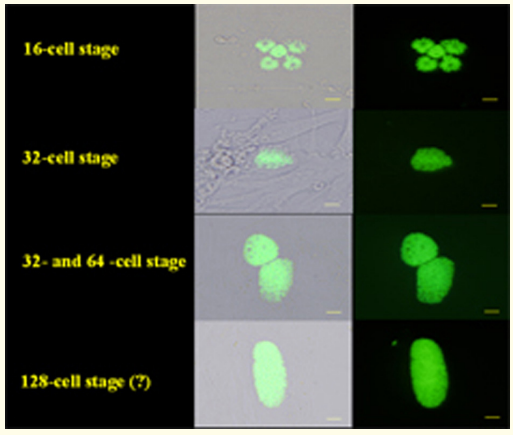

Figure 9b

Figure 9 (A and B): *Endodiogeny of green fluorescent protein (GFP) expressing T. gondii RH strain within the PV. Bar, 20 µm.*

The interaction between *Leishmania* spp. and macrophage membrane receptors results in the internalization of the flagellated, motile *Leishmania* promastigote metacyclic form within PV, which differentiates into non-motile, metacyclic amastigote form. The PV expansion and the intracellular proliferation of the *Leishmania* amastigote forms take place through fusing with recycling endosomes, late endosomes and the endoplasmic reticulum [138]. On the other hand, it has been shown that lysosomal trafficking regulator (LYST)/Beige plays an important role at the innate level [139]. They also illustrated that increased expression of LYST/Beige functions to protect the host cells via restricting *Leishmania* amastigote growth because of struggling PV enlargement [140]. This would be a fruitful area for novel therapeutic strategies by specifically blocking *Leishmania* PV enlargement. Interestingly, the cross-priming of CD8 + T cells directly correlates with the retrotranslocation of the host endoplasmic reticulum (hER) to the PV in *T. gondii* - infected dendritic cells (DCs) and no association between hER and phagosomes or Ag resentation activity was observed in DCs containing phagocytosed live or dead parasites.

Importantly, the hER serves as a channel for the transport of *T. gondii* - derived antigens between the PV and host cytosol to be processed and presented within the context of the major histocompatibility complex (MHC) class I to CD8 + T cells [140]. In the same vein, the interaction between *T. gondii* - specific CD4 + T cell and the infected macrophages through CD40-CD154 binding result in the activation of down stream signalling pathway. Consequently, the non-fusogenic nature of the PV changes in a process called CD40-induced autophagy (lysosomal degradation pathway), which leads to lysosomal - dependent *T. gondii* killing [141]. This information can be exploited to manipulate CD40 and autophagy signaling for apicomplexan parasite eradication.

The CD40 receptors are expressed on the surface of different cells, such as B cells, macrophages and dendritic cells (DCs) and engage CD40L on the surface of T-cells. The duality of CD40-CD40L (activation versus suppression) is due to the avidity of binding between the receptor and its ligand; the higher the avidity of binding, the more the bias of the immune response to type 1 immune response (i.e. resolving the infection) and vice versa. The upstream avidity will direct the downstream activation, and the outcome will be suppression (ERK1 and ERK2- mediated IL-10 production) versus activation (i.e. P38MAP kinase- mediated IL-12 release) with CD40 -CD40L weak and strong signaling respectively. *Leishmania* can evade the CD40-CD40L regulation mechanism by P38MAP kinase- mediated IL-12 release and activates ERK1 and ERK2- mediated IL-10 production that favors parasite survival and progression of the disease [142]. What follows is the ability of a cleaved soluble derivative (sCD40L) from cell membrane retains its capacity to bind and activate CD40 on professional antigen presenting cells (APC) in individual sera disclosed to *L. infantum* endorsing both the microbicidal and inflammatory cytokine response of *L. infantum*- infected macrophages [143]. Interferon-γ (IFN-γ) secretion in the presence of the essential autophagy gene Atg5 (autophagy related 5) leads to the induction of effector molecules, for example, the immunity-related p47 GTPases (IRGs) and guanylate - binding proteins (GBPs) to target and accumulate on the PVM, thus disrupting PVM and subsequent parasite death instead of forming cytoplasmic aggregates [82,144-147]. Relatedly, the ubiquitin-like conjugation machinery, including E1 Atg7, E2, Atg3, and E3 Atg12-Atg5-Atg16L1 of the autophagy pathway, are required for the IFN-γ effector proper targeting onto the PVM [148]. The secreted effectors of dense-granule and rhoptry organelles rewire host gene expression [149]. On the other hand, *Sarcocystis* spp. are intracellular apicomplexan parasites, which locate in the host cytoplasm without PV formation. Additionally, many secreted effectors of dense-granule and rhoptry kinase, which are responsible for manipulating the host transcriptome in *Toxoplasma* and *Neospora* are absent from *S. neurona* [150]. Any *Toxoplasma*, *Neospora* and *sarcocystis* non-conserved secreted effectors could offer a real opportunity for new prophylactic and therapeutic intervention.

Conclusion and Future Directions

The molecular battle between the host and protozoan parasites has led to diverse measure/counter-measure responses. The reader of this review may be perplexed by the paradoxical data, and the multitude of mechanisms involved in this two-way interaction and may ask the crucial question of what, as a concise blueprint, the plausible approach actually is. A reasonable approach to exploit these cellular and molecular mechanisms could be to discover contemporary therapeutic and prophylactic intervention points for broad-spectrum host- oriented measures and parasite counter- measures, and to determine the infection outcome. Furthermore, more data are needed to investigate to what extent the natural selection of protozoan parasite strategies may re-shape the host phenotype as though host evolution can itself be adapted.

Acknowledgments

This research was supported by Guandong Science and Technology Plan Project (2012A020100001) , and the Start-up Research Grant Program provided by Foshan University, Foshan city, Guangdong province for distinguished researchers.. Additionally, the funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests

The authors declare that they have no competing interests.

Bibliography

1. Pardoll DM. "Immunology. Stress, NK receptors, and immune surveillance". *Science* 294.5542 (2001): 534-536.
2. Scharton-Kersten TM and Sher A. "Role of natural killer cells in innate resistance to protozoan infections". *Current Opinion in Immunology* 9.1 (1997): 44-51.
3. Requena JM., *et al.* "Molecular Chaperones of Leishmania: Central Players in Many Stress-Related and -Unrelated Physiological Processes". *BioMed Research International* 2015: 301326.
4. Larreta R., *et al.* "The expression of HSP83 genes in Leishmania infantum is affected by temperature and by stage-differentiation and is regulated at the levels of mRNA stability and translation". *BMC Molecular Biology* 5 (2004): 3.
5. Tsuji M., *et al.* "Demonstration of heat-shock protein 70 in the sporozoite stage of malaria parasites". *Parasitology Research* 80.1 (1994): 16-21.
6. Herwaldt BL. "Leishmaniasis". *Lancet* 354.9185 (1999): 1191-1199.
7. Quijada L., *et al.* "Analysis of the antigenic properties of the L. infantum Hsp70: design of synthetic peptides for specific serodiagnosis of human leishmaniasis". *Immunology Letters* 63.3 (1998): 169-174.
8. Miller MA., *et al.* "Inducible resistance to oxidant stress in the protozoan Leishmania chagasi". *The Journal of Biological Chemistry* 275.43 (2000): 33883-33889.
9. Quijada L., *et al.* "Analysis of post-transcriptional regulation operating on transcription products of the tandemly linked Leishmania infantum hsp70 genes". *The Journal of Biological Chemistry* 272.7 (1997): 4493-4499.
10. Morimoto RI and Santoro MG. "Stress-inducible responses and heat shock proteins: new pharmacologic targets for cytoprotection". *Nature Biotechnology* 16.9 (1998): 833-848.
11. Hartl FU and Hayer-Hartl M. "Molecular chaperones in the cytosol: from nascent chain to folded protein". *Science* 295.5561 (2002): 1852-1858.
12. Wiesgigl M and Clos J. "Heat shock protein 90 homeostasis controls stage differentiation in Leishmania donovani". *Molecular Biology of the Cell* 12.11 (2001): 3307-3316.
13. Banumathy G., *et al.* "Heat shock protein 90 function is essential for Plasmodium falciparum growth in human erythrocytes". *The Journal of Biological Chemistry* 278.20 (2003): 18336-18345.
14. Echeverria PC., *et al.* "Toxoplasma gondii Hsp90 is a potential drug target whose expression and subcellular localization are developmentally regulated". *Journal of Molecular Biology* 350.4 (2005): 723-734.
15. Nageshan RK., *et al.* "Trans-spliced heat shock protein 90 modulates en cystation in Giardia lamblia". *PLOS Neglected Tropical Diseases* 8.5 (2014): e2829.
16. Debnath A., *et al.* "Hsp90 inhibitors as new leads to target parasitic diarrhea diseases". *Antimicrobial Agents and Chemotherapy* 58.7 (2014): 4138-4144.
17. Singh M, Shah V. "Tatu UA novel C-terminal homologue of Aha co-chaperone binds to heat shock protein 90 and stimulates its ATPase activity in Entamoeba histolytica". *Journal of Molecular Biology* 426.8 (2014): 1786-1798.

18. Zininga T., *et al.* "Overexpression, Purification and Characterisation of the Plasmodium falciparum Hsp70-z (PfHsp70-z) Protein". *PLoS One* 10.6 (2015): e0129445.

19. Zininga T., *et al.* "Plasmodium falciparum Hsp70-z, an Hsp110 homologue, exhibits independent chaperone activity and interacts with Hsp70-1 in a nucleotide-dependent fashion". *Cell Stress and Chaperones* 21.3 (2016): 499-513.

20. Chua CS., *et al.* "Co-chaperones of Hsp90 in Plasmodium falciparum and their concerted roles in cellular regulation". *Parasitology* 141.9 (2014): 1177-1191.

21. Zininga T., *et al.* "Plasmodium falciparum Hop (PfHop) Interacts with the Hsp70 Chaperone in a Nucleotide-Dependent Fashion and Exhibits Ligand Selectivity". *Plos ONE* 10.8 (2015): e0135326.

22. Minia I and Clayton C. "Regulating a Post- Transcriptional Regulator: Protein Phosphorylation, Degradation and Translational Blockage inControl of the Trypanosome Stress-Response RNA-Binding Protein ZC3H11". *PLOS Pathogens* 12.3 (2016): e1005514.

23. Holakuyee M., *et al.* "Heat shock proteins enriched-promastigotes of Leishmania major inducing Th2 immune response in BALB/cmice". *Iranian Biomedical Journal* 16.4(2012): 209-217.

24. Park K., *et al.* "ER stress stimulates production of the key antimicrobial peptide, cathelicidin, by forming a previously unidentified intracellular S1P signaling complex". *Proceedings of the National Academy of Sciences of the United States of America* 113.10 (2016): E1334-1342.

25. Gosline SJC., *et al.* "Intracellular Eukaryotic Parasites Have a Distinct Unfolded Protein Response". PLoS ONE 6.4 (2011): e19118.

26. Schroder M. "Endoplasmic reticulum stress responses". *Cellular and Molecular Life Sciences* 65.6 (2008): 862-894.

27. Malhi H and Kaufman RJ. "Endoplasmic reticulum stress in liver disease". *Journal of Hepatology* 54.4 (2011): 795-809.

28. Smirlis D., *et al.* "Targeting essential pathways in trypanosomatids gives insights into protozoan mechanisms of cell death". *Parasites and Vectors* 3 (2010): 107.

29. Goldshmidt H., *et al.* "Persistent ER stress induces the spliced leader RNA silencing pathway (SLS), leading to programmed cell death in Trypanosoma brucei". *PLoS Pathogens* 6.1 (2010): e1000731.

30. Michaeli S. "Spliced leader RNA silencing (SLS) - a programmed cell death pathway in Trypanosoma brucei that is induced upon ER stress". *Parasites and Vectors* 5 (2012): 107.

31. Michaeli S. "The response of trypanosomes and other eukaryotes to ER stress and the spliced leader RNA silencing (SLS) pathway in Trypanosoma brucei". *Critical Reviews in Biochemistry and Molecular Biology* 50.3 (2015): 256-267.

32. Inácio P., *et al.* "Parasite-induced ER stress response in hepatocytes facilitates Plasmodium liver stage infection". 16.8 (2015): 955-964.

33. Chaubey S., *et al.* "Endoplasmic reticulum stress triggers gametocytogenesis in the malaria parasite". *The Journal of Biological Chemistry* 289.24 (2014): 16662-74.

34. Hope R., *et al.* "Phosphorylation of the TATA-binding protein activates the spliced leader silencing pathway in Trypanosoma brucei". *Science Signaling* 7.341 (2014): ra85.

35. Fridovich I. "Superoxide anion radical (O 2 -.), superoxide dismutases, and related matters". *The Journal of Biological Chemistry* 272.30 (1997): 18515-18517.

36. Pacher P., *et al.* "Nitric oxide and peroxynitrite in health and disease". *Physiological Reviews* 87.1 (2007): 315-424.

37. Petruccelli LA., *et al.* "Vorinostat Induces Reactive Oxygen Species and DNA Damage in Acute Myeloid Leukemia Cells". *PLoS ONE* 6.6 (2011): e20987.

38. Prasad S., *et al.* "Reactive oxygen species (ROS) and cancer: Role of antioxidative nutraceuticals". *Cancer Letters* 387 (2016): 95-105

39. Somasundaram V., *et al.* "Nitric oxide and reactive oxygen species: Clues to target oxidative damage repair defective breast cancers". *Critical Reviews in Oncology/Hematology* 101 (2016):184-92.

40. Teixeira L., *et al.* "Enrichment of IFN-γ producing cells in different murine adipose tissue depots upon infection with anapicomplexan parasite". *Scientific Reports* 6 (2016): 23475.

41. Kleinert H., *et al.* "Regulation of the expression of inducible nitric oxide synthase". *Biological Chemistry* 384(10-11) (2003): 1343-1364.

42. Vincendeau P., *et al.* "Arginases in parasitic diseases". *Trends in Parasitology* 19.1 (2003): 9-12.

43. Musaya J., *et al.* "Role of cytokines in Trypanosoma brucei-induced anaemia: A review of the literature". *Malawi Medical Journal* 27.2 (2014): 45-50.

44. Jankovic D and Feng CG. "CD4 (+) T Cell Differentiation in Infection: Amendments to the Th1/Th2 Axiom". *Frontiers in Immunology* 6 (2015): 198.

45. Oliveira AC., *et al.* "Impaired innate immunity in Tlr4(-/-) mice but preserved CD8+ T cell responses against Trypanosoma cruzi in Tlr4-, Tlr2-, Tlr9- or Myd88-deficient mice". *PLoS Pathogens* 6.4 (2010): e1000870.

46. Kim YJ., *et al.* "IL-1beta, an immediate early protein secreted by activated microglia, induces iNOS/NO in C6 astrocytoma cells through p38 MAPK and NF-kappaB pathways". *Journal of Neuroscience Research* 84.5 (2006): 1037-1046.

47. Kumar S., *et al.* "Functional and molecular characterization of NOS isoforms in rat neutrophil precursor cells". *Cytometry Part A* 77.5 (2010): 467-77.

48. Förstermann U and Kleinert H. "Nitric oxide synthase: expression and expressional control of the three isoforms". *Naunyn-Schmiedeberg's Archives of Pharmacology* 352.4 (1995): 351-364.

49. Tobin C., *et al.* "Toxoplasma gondii cyst wall formation in activated bone marrow-derived macrophages and bradyzoite conditions". *Journal of Visualized Experiments* 442 (2010): 2091.

50. Patel R P., *et al.* "Biological aspects of reactive nitrogen species". *Biochimica et Biophysica Acta* 1411 (1999): 385-400.

51. Rath M., *et al.* „Metabolism via Arginase or Nitric Oxide Synthase: Two Competing Arginine Pathways in Macrophages". *Frontiers in Immunology* 5 (2014): 532.

52. Mills CD. "Anatomy of a discovery: m1 and m2 macrophages". *Frontiers in Immunology* 6 (2015): 212.

53. Gordon S and Taylor PR. "Monocyte and macrophage heterogeneity". *Nature Reviews Immunology* 5.12 (2005): 953-964.

54. De Paoli F., *et al.* "Macrophage phenotypes and their modulation in atherosclerosis". *Circulation Journal* 78.8 (2014): 1775-1781.

55. Rojas J., *et al.* "Macrophage Heterogeneity and Plasticity: Impact of Macrophage Biomarkers on Atherosclerosis". *Scientifica (Cairo)* 2015: 851252.

56. Novoselov VV., *et al.* "Study of the activated macrophage transcriptome". *Experimental and Molecular Pathology* 99.3 (2015): 575-580.

57. Ivanova EA and Orekhov AN. "Monocyte Activation in Immunopathology: Cellular Test for Development of Diagnostics and Therapy". *Journal of Immunology Research* (2016): 4789279.

58. Ghosh M., *et al.* "Cyclic AMP is a key regulator of M1 to M2a phenotypic conversion of microglia in the presence of Th2 cytokines". *Journal of Neuroinflammation* 13.1 (2016): 9.

59. Artavanis-Tsakonas K., *et al.* "The war between the malaria parasite and the immune system: immunity, immunoregulation andimmunopathology". *Clinical and Experimental Immunology* 133.2 (2003): 145-152.

60. Patel SN., *et al.* "CD36 mediates the phagocytosis of Plasmodium falciparum-infected erythrocytes by rodent macrophages". *The Journal of Infectious Diseases* 189.2 (2004): 204-213.

61. Krishnegowda G., *et al.* "Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of Plasmodium falciparum: Cell signaling receptors, glycosylphosphatidylinositol (GPI) structural requirement, and regulation of GPI activity". *The Journal of Biological Chemistry* 280.9 (2005): 8606-8616.

62. Zhu J., *et al.* "Induction of proinflammatory responses in macrophages by the glycosylphosphatidylinositols of Plasmodium falciparum: The requirement of extracellular signal-regulated kinase, p38, c-Jun N-terminal kinase and NF-kappaB pathways for the expression of pro-inflammatory cytokines and nitric oxide". *The Journal of Biological Chemistry* 280.9 (2005): 8617-8627.

63. Bertoldo JB and Terenzi H. "Oxidation and tyrosine nitration induce structural changes and inhibits Plasmodium falciparum falcipain-2 activity *in vitro*". *Protein and Peptide Letters* 23.6 (2016): 562-572.

64. Weinberg JB., *et al.* "Human mononuclear phagocyte inducible nitric oxide synthase (iNOS): analysis of iNOS mRNA, iNOS protein, biopterin, and nitric oxide production by blood monocytes and peritoneal macrophages". *Blood* 86.3 (1995): 1184-1195.

65. Zhou R., *et al.* "miR-27b targets KSRP to coordinate TLR4-mediated epithelial defense against Cryptosporidium parvum infection". *PLoS Pathogens* 8.5 (2012): e1002702.

66. Tanaka T., *et al.* "Growth-inhibitory effects of interferon-gamma on Neospora caninum in murine macrophages by a nitric oxide". 86.9 (2000):768-771.

67. Linares E., *et al.* "Role of peroxynitrite in macrophagemicrobicidal mechanisms *in vivo* revealed by protein nitrationand hydroxylation". *Free Radical Biology and Medicine* 30.11 (2001): 1234-1242.

68. Lopes MF., *et al.* "Innate immunity to Leishmania infection: within phagocytes". *Mediators of Inflammation* (2014): 754965.

69. Dincel GC and Atmaca HT. "Nitric oxide production increases during Toxoplasma gondii encephalitis in mice". *Experimental Parasitology* 156 (2015): 104-112.

70. Dincel GC and Atmaca HT. "Increased expressions of ADAMTS-13 and apoptosis contribute to neuropathology during Toxoplasma gondii encephalitis in mice". *Neuropathology* 36.3 (2016): 211-226.

71. Soares CO., *et al.* "Nitric oxide affects trypomastigote to amastigote differentiation in Trypanosoma cruzi". Free Radic Biol Med 2015: 1: S50.

72. O'Handley RM., *et al.* "Passive immunity and serological immune response in dairy calves associated with natural Giardia duodenalis infections". *Veterinary Parasitology* 113.2 (2003): 89-98.

73. Eckmann L., *et al.* "Nitric oxide production by human intestinal epithelial cells and competition for arginine as potential determinants of host defense against the lumen-dwelling pathogen Giardia lamblia". *Journal of Immunology* 164.3 (2000): 1478-1487.

74. Pavanelli WR., *et al.* "The effects of nitric oxide on the immune response during giardiasis". *Brazilian Journal of Infectious Diseases* 14.6 (2010): 606-612.

75. Aoki MP., *et al.* "Cruzipain, a major Trypanosoma cruzi antigen, promotes arginase-2 expression and survival of neonatal mouse cardiomyocytes". *The American Journal of Physiology - Cell Physiology* 286.2 (2004): 206-212.

76. Freire-de-Lima CG., *et al.* "Uptake of apoptotic cells drives the growth of a pathogenic trypanosome in macrophages". *Nature* 403.6766 (2000): 199-203.

77. Müller S, *et al.* "Targeting polyamines of parasitic protozoa in chemotherapy". *Trends in Parasitology* 17.5 (2001): 242-249.

78. Yang B., *et al.* "Inhibitors directed towards caspase-1 and -3 are less effective than pan caspase inhibition in preventing renal proximal tubular cell apoptosis". *Nephron Experimental Nephrology* 96.2 (2004): 39-51.

79. Iniesta V., *et al.* "Arginase I induction during Leishmania major infection mediates the development of disease". *Infection and Immunity* 73.9 (2005): 6085-6090.

80. Mordue DG and Sibley LD. "Intracellular fate of vacuoles containing Toxoplasma gondii is determined at the time of formation and depends on the mechanism of entry". *Journal of Immunology* 159.9 (1997): 4452-4459.

81. Padrão Jda C., *et al.* "Toxoplasma gondii infection of activated J774-A1 macrophages causes inducible nitric oxide synthase degradation by the proteasome pathway". *Parasitology International* 63.5 (2014): 659-663.

82. Howard JC., *et al.* "The IRG protein-based resistance mechanism in mice and its relation to virulence in Toxoplasma gondii". *Current Opinion in Microbiology* 14.4 (2011): 414-421.

83. Hunter CA and Sibley LD. "Modulation of innate immunity by Toxoplasma gondii virulence effectors". *Nature Reviews Microbiology* 10.11 (2012): 766-778.

84. Gazzinelli RT., *et al.* "Innate resistance against Toxoplasma gondii: an evolutionary tale of mice, cats, and men". *Cell Host and Microbe* 15.2 (2014): 132-138.

85. Meunier E and Broz P. "Interferon-inducible GTPases in cell autonomous and innate immunity". *Cell Microbiology* 18.2 (2016): 168-80.

86. Lilue J., *et al.* "Reciprocal virulence and resistance polymorphism in the relationship between Toxoplasma gondii and the house mouse". *Elife* 2 (2013): e01298.

87. Zago MP., *et al.* "TcI isolates of Trypanosoma cruzi exploit antioxidant network for enhanced intracellular survival in macrophages and virulence in mice". *Infection and Immunity* 84.6 (2016):1842-1856.

88. Novaes RD., *et al.* "Modulation of inflammatory and oxidative status by exercise attenuates cardiac morphofunctional remodeling in experimental Chagas cardiomyopathy". *Life Science* 152 (2016): 210-219.

89. Piacenza L., *et al.* "Trypanosoma cruzi antioxidant enzymes as virulence factors in Chagas disease". *Antioxidants and Redox Signaling* 19.7 (2013): 723-734.

90. Gradoni L and Ascenzi P. "Nitric oxide and anti-protozoan chemotherapy". *Parassitologia* 2004; 46(1-2): 101-103.

91. Gazzinelli RT., *et al.* "Acute cerebral toxoplasmosis is induced by *in vivo* neutralization and correlates with the down-regulated expression of inducible nitric oxide synthase and other markers of macrophage activation". *Journal of Immunology* 151.7 (1993): 3672-3681.

92. Bohne W., *et al.* "Reduced replication of Toxoplasma gondii is necessary for induction of bradyzoite-specific antigens: a possible role for nitric oxide in triggering stage conversion". *Infection and Immunity* 62.5 (1994): 1761-1767.

93. Khan IA., *et al.* "A dichotomous role of nitric oxide during acute Toxoplasma gondii infection in mice". *Proceedings of the National Academy of Sciences of the United States of America* 94.25 (1997): 13955-13960.

94. Schlüter D., *et al.* "Inhibition of inducible nitric oxide synthase exacerbates chronic cerebral toxoplasmosis in Toxoplasma gondii-susceptible C57BL/6 mice but does not reactivate the latent disease in T. gondii-resistant BALB/c mice". *Journal of Immunology* 162.6 (1999): 3512-3518.

95. Ibrahim HM., *et al.* "Toxoplasma gondii cyclophilin 18-mediated production of nitric oxide induces bradyzoite conversion in a CCR5-dependent manner". *Infection and Immunity* 77.9 (2009): 3686-3695.

96. Mori M. "Regulation of nitric oxide synthesis and apoptosis by arginase and arginine recycling". *The Journal of Nutrition* 137 (2007): 1616-1620.

97. Olivera GC., *et al.* "Nitric Oxide Protects against Infection-Induced Neuroinflammation by Preserving the Stability of the Blood-Brain Barrier". PLoS Pathog 12.2 (2016): e1005442.

98. Dincel GC and Atmaca HT. "Role of oxidative stress in the pathophysiology of Toxoplasma gondii infection". *International Journal of Immunopathology and Pharmacology* 29.2 (2016):226-240.

99. Oyadomari S and Mori M. "Roles of CHOP/GADD153 in endoplasmic reticulum stress". *Cell Death and Differentiation* 11.4 (2014): 381-389.

100. Silva-Neto MA., *et al.* "Here, There, and Everywhere: The Ubiquitous Distribution of the Immunosignaling Molecule Lysophosphatidylcholine and Its Role on Chagas Disease. *Frontiers in Immunology* 7 (2016): 62.

101. Pelgrift RY and Friedman AJ. "Nanotechnology as a therapeutic tool to combat microbial resistance". *Advanced Drug Delivery Reviews* 65.13-14 (2013): 1803-1815.

102. Nogueira N P., *et al*. "Proliferation and differentiation of Trypanosoma cruzi inside its vector have a new trigger: redox status". *PLoS One* 10.2 (2015): e0116712.

103. Dey N., *et al*. "Caspase-1/ASC inflammasome-mediated activation of IL-1β-ROS-NF-κB pathway for control of Trypanosoma cruzi replication and survival is dispensable in NLRP3-/- macrophages". *PLoS One* 9.11 (2014): e111539.

104. Lefèvre L., *et al*. "The C-type lectin receptors dectin-1, MR, and SIGNR3 contribute both positively and negatively to the macrophage response to Leishmania infantum". *Immunity* 38.5 (2013): 1038-1049.

105. McGonigle S., *et al*. "Peroxidoxins: A new antioxidant family". *Parasitology Today* 14.4 (1998): 139-145.

106. Mohamed AM., *et al*. "Sativa seeds against Schistosoma mansoni different stages". *Memorias do Instituto Oswaldo Cruz* 100.2 (2005): 205-211.

107. Barriga-González G., *et al*. "Spin trapping: an essential tool for the study of diseases caused by oxidative stress". *Current Topics in Medicinal Chemistry* 15.5 (2015): 484-495.

108. Deshmukh R and Trivedi V. "Phagocytic Uptake of Oxidized Heme Polymer Is Highly Cytotoxic to Macrophages". *PLoS ONE* 9.7 (2014): e103706.

109. Pacelli R., *et al*. "Nitric oxide potentiates hydrogen peroxide-induced killing of Escherichia coli". *Journal of Experimental Medicine* 182.5 (1995): 1469-1479.

110. DeGroote MA., *et al*. "Periplasmic superoxide dismutase protects Salmonella from products of phagocyte NADPH-oxidase and nitric oxide synthase". *Proceedings of the National Academy of Sciences of the United States of America* 94.25 (1997): 13997-14001.

111. Umezawa K., *et al*. "Induction of nitric oxide synthesis and xanthine oxidase and their roles in the antimicrobial mechanism against Salmonella typhimurium infection in mice". *Infection and Immunity* 65.7 (1997): 2932-2940.

112. Joseph LE., *et al*. "Are oxidative stress activated signaling pathways mediators of insulin resistance and cell dysfunction?" *Diabetes* 52.1 (2003): 1-8.

113. Schieber M and Chandel NS. "ROS function in redox signaling and oxidative stress". *Current Biology* 24.10 (2014): R453-R462.

114. Wang Y., *et al*. "Cross-talk of nitric oxide and reactive oxygen species in plant programed cell death". *Frontiers in Plant Science* 4 (2013): 314.

115. Gupta MP., *et al*. "Nitric oxide attenuates hydrogen peroxide-mediated injury to porcine pulmonary artery endothelial cells". *American Journal of Physiology* 272 (1997): 1133-1141.

116. Habib S and Ali A. "Biochemistry of Nitric Oxide". *Indian Journal of Clinical Biochemistry* 26.1 (2011): 3-17.

117. Jones TC and Hirsch JG. "The interaction between Toxoplasma gondii and mammalian cells. II. The absence of lysosomal fusion with phagocytic vacuoles containing living parasite". *Journal of Experimental Medicine* 136.5 (1972): 1173-1194.

118. Sinai AP and Joiner KA. «Safe haven: the cell biology of nonfusogenic pathogen vacuoles". *Annual Review of Microbiology* 51 (1997): 415-462.

119. Akerman SE and Muller S. "Peroxiredoxin-linked detoxification of hydroperoxides in Toxoplasma gondii". *Journal of Biological Chemistry* 280.1 (2005): 564-570.

120. Carruthers V and Boothroyd JC. "Pulling together: an integrated model of Toxoplasma cell invasion". *Current Opinion in Microbiology* 10.1 (2007): 83-89.

121. Carvalho CS and Melo EJ. "Acidification of the parasitophorous vacuole containing Toxoplasma gondii in the presence of hydroxyurea". *Anais da Academia Brasileira de Ciências* 78.3 (2006): 475-484.

122. Sinai AP. "Biogenesis of and activities at the Toxoplasma gondii parasitophorous vacuole membrane". *Subcellular Biochemistry* 47 (2008): 155-164.

123. Beckers CJ., *et al*. "The Toxoplasma gondii rhoptry protein ROP 2 is inserted into the parasitophorous vacuole membrane, surrounding the intracellular parasite, and is exposed to the host cell cytoplasm". *Journal of Cell Biology* 127.4 (1994): 947-961.

124. Schwab JC., *et al*. "The parasitophorous vacuole membrane surrounding intracellular Toxoplasma gondii functions as a molecular sieve". *Proceedings of the National Academy of Sciences of the United States of America* 91.2 (1994): 509-513.

125. Beĭer TV., *et al*. "Formation and diversity of parasitophorous vacuoles in parasitic protozoa. The Coccidia (Sporozoa, Apicomplexa)". *Tsitologiia* 45.4 (2003): 339-356.

126. Saeij JP., *et al*. "Polymorphic secreted kinases are key virulence factors in toxoplasmosis". *Science* 314.5806 (2006): 1780-1783.

127. Taylor S., *et al*. "A secreted serine-threonine kinase determines virulence in the eukaryotic pathogen Toxoplasma gondii". *Science* 314.5806 (2006): 1776-1780.

128. Saeij JP., *et al*. "Toxoplasma co-opts host gene expression by injection of a polymorphic kinase homologue". *Nature* 445.7125 (2007): 324-327.

129. Peixoto L., *et al*. "Integrative genomic approaches highlight a family of parasite-specific kinases that regulate host responses". *Cell Host Microbe* 8.2 (2010): 208-218.

130. Risco-Castillo V., *et al*. "CD81 is required for rhoptry discharge during host cell invasion by Plasmodium yoelii sporozoites". *Cellular Microbiology* 16.10 (2014): 1533-1548.

131. Risco-Castillo V., *et al*. "Malaria Sporozoites Traverse Host Cells within Transient Vacuoles". *Cell Host Microbe* 18.5 (2015): 593-603.

132. Sheffield HG and Melton ML. "The fine structure and reproduction of Toxoplasma gondii". *Journal for Parasitology* 54.2 (1968): 209-226.

133. Sibley LD., *et al*. "Phagosome acidification blocked by intracellular Toxoplasma gondii". *Nature* 315.6018 (1985): 416-419.

134. Sibley LD., *et al*. "Toxoplasma modifies macrophage phagosomes by secretion of a vesicular network rich in surface proteins". *Journal of Cell Biology* 103.3 (1986): 867-874.

135. Sibley LD., *et al*. "Regulated secretion of multi-lamellar vesicles leads to formation of a tubulo-vesicular network in host-cell vacuoles occupied by Toxoplasma gondii". *Journal of Cell Science* 108.4 (1995): 1669-1677.

136. Moreira-Souza AC., *et al*. "Pyrimidinergic Receptor Activation Controls Toxoplasma gondii Infection in Macrophages". *PLoS One* 10.7 (2015): e0133502.

137. Gold DA., *et al*. "The Toxoplasma Dense Granule Proteins GRA17 and GRA23 Mediate the Movement of Small Molecules between the Host and the Parasitophorous Vacuole". *Cell Host Microbe* 17.5 (2015): 642-652.

138. Liévin-Le Moal V and Loiseau PM. «Leishmania hijacking of the macrophage intracellular compartments". *FEBS* Journal 283.4 (2016): 598-607.

139. Wilson J., *et al*. "Control of parasitophorous vacuole expansion by LYST/Beige restricts the intracellular growth of Leishmania amazonensis". *PLoS Pathogens* 4.10 (2008): e1000179.

140. Goldszmid RS., *et al*. "Host ER-parasitophorous vacuole interaction provides a route of entry for antigen cross-presentation in Toxoplasma gondii-infected dendritic cells". *Journal of Experimental Medicine* 206.2 (2009): 399-410.

141. Subauste CS. "CD40, autophagy and Toxoplasma gondii". *Memórias do Instituto Oswaldo Cruz. Rio de Janeiro* 104.2 (2009): 267-272.

142. Mathur RK., *et al*. "The conundrum of CD40 function: host protection or disease promotion?" *Trends in Parasitology* 22.3 (2006): 117-122.

143. Oliveira FA., *et al*. "Soluble CD40 Ligand in Sera of Subjects Exposed to Leishmania infantum Infection Reduces the Parasite Load in Macrophages". *PLoS ONE* 10.10 (2015): e0141265.

144. Zhao Z., *et al*. "Autophagosome independent essential function for the autophagy protein Atg5 in cellular immunity to intracellular pathogens". *Cell Host Microbe* 4.5 (2008): 458-469.

145. Zhao YO., *et al*. "Disruption of the Toxoplasma gondii parasitophorous vacuole by IFNgamma-inducible immunity-related GTPases (IRG proteins) triggers necrotic cell death". *PLoS Pathogen* 5.2 (2009): e1000288.

146. Kim BH., *et al*. "IFN-inducible GTPases in host cell defense". *Cell Host Microbe* 12.4 (2012): 432-444.

147. Selleck EM., *et al*. "Guanylate-binding protein 1 (Gbp1) contributes to cell-autonomous immunity against Toxoplasma gondii". *PLoS Pathogens* 9.4 (2013): e1003320.

148. Choi J., *et al*. "The parasitophorous vacuole membrane of Toxoplasma gondii is targeted for disruption by ubiquitin-like conjugation systems of autophagy". *Immunity* 40.6 (2014): 924-935.

149. Hakimi MA and Bougdour A. "Toxoplasma's ways of manipulating the host transcriptome via secreted effectors". *Current Opinion in Microbiology* 26 (2015): 24-31.

150. Blazejewski T., *et al*. "Systems-based analysis of the Sarcocystis neurona genome identifies pathways that contribute to aheteroxenous life cycle". *MBio* 6.1 (2015): e02445.

Volume 9 Issue 2 July 2017