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Review

# *Leishmania*: manipulation of signaling pathways to inhibit host cell apoptosis

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***Abstract*:** The maintenance of homeostasis in living systems requires the elimination of unwanted cells which is performed, among other mechanisms, by type I cell death or apoptosis. This type of programmed cell death involves several morphological changes such as cytoplasm shrinkage, chromatin condensation (pyknosis), nuclear fragmentation (karyorrhexis), and plasma membrane blebbing that culminate with the formation of apoptotic bodies. In addition to the maintenance of homeostasis, apoptosis also represents an important defense mechanism for cells against intracellular microorganisms. In counterpart, diverse intracellular pathogens have developed a wide array of strategies to evade apoptosis and persist inside cells. These strategies include the manipulation of signaling pathways involved in the inhibition of apoptosis where mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K) play a key role. *Leishmania* is an intracellular protozoan parasite that causes a wide spectrum of diseases known as leishmaniasis. This parasite displays different strategies, including apoptosis inhibition, to down-regulate host cell defense mechanisms in order to perpetuate infection.

***Keywords:*** Akt, apoptosis, ERK 1/2, inhibition, JNK, MAPK, p38, pathways, protozoan parasites, signaling PI3K

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## Introduction

### *Leishmaniasis and its causative agent,* Leishmania

The term leishmaniasis refers to a group of parasitic diseases caused by different species of an intracellular protozoan of the genus *Leishmania*. It is a health problem worldwide, affecting more than 12 million people. This disease develops in different clinical manifestations comprising from cutaneous lesions (cutaneous leishmaniasis) and damage to the oral, nasal, and pharyngeal mucosae (mucocutaneous leishmaniasis) to life-threatening systemic infections that affect internal organs, mainly the liver, spleen, and bone marrow (visceral leishmaniasis). These clinical forms derive from complex interactions between the host immune response and the infecting *Leishmania* species[1,2] that are transmitted to human beings and other mammals through the bite of sandflies that belong to the genus *Phlebotomus* (in Europe, Asia, and Africa) and *Lutzomyia* (in America).[3]

*Leishmania* presents with two developmental stages during its life cycle: the promastigote and the amastigote. The promastigote is the flagellated, mobile, and extracellular form of the parasite that develops and multiplies in the digestive tract of the sandfly vector.[4] On the other hand, the amastigote is the immobile and intracellular form of the parasite that resides in the parasitophorous vacuole of phagocytic cells such as macrophages and dendritic cells.[5,6]

Within macrophages, promastigotes differentiate into amastigotes inside the phagolysosome and divide intensely by binary fission until they lyse the cell. Once released, amastigotes infect

adjacent macrophages in which they once again actively divide until they lyse the cell and subsequently infect more macrophages. Interestingly, the rapid phagocytosis of *Leishmania* promastigotes by macrophages, in particular through complement receptor 3 (CR3),[7] prevents the activation of macrophage microbicidal mechanisms and prevents immune recognition of the parasite.

As a response to infection, host cells initiate different processes to counteract it, where apoptosis plays a predominant role. Apoptosis is important for the host to eliminate infected cells and to activate the immune system. In turn, different viruses, intracellular bacteria, and protozoa have evolved multiple mechanisms to overcome apoptosis of the infected cell, survive, and perpetuate infection.[8]

## Generalities of apoptosis

Apoptosis is a crucial process that occurs in many organisms under normal conditions. It is involved in the maturation, remodeling, growth, and the development of tissues, as well as in pathological situations that involve tissue damage. Apoptosis is a finely-tuned process which is regulated through stimulatory and inhibitory mechanisms that control apoptosis to maintain tissue homeostasis.

Apoptosis is classified, along with autophagy, as a type of programmed cell death and is distinguished from necrosis, which is a type of non-programmed cell death.[9] The term apoptosis was coined in 1972 by Kerr to describe a type of cell death characterized by morphological and molecular features different from other types of cell death. Such morphological characteristics include progressive cell rounding, pseudopod retraction, a reduction in cell and nuclear volume (pyknosis), nuclear fragmentation (karyorrhexis), the structural modification of organelles, and vesicle formation due to blistering of the plasma membrane.[9,10] Recently, the Committee for Nomenclature of Cell Death has pointed out the importance of including quantifiable biochemical parameters in the characterization of apoptosis (along with other types of cell death).[11]

## Apoptosis induction pathways

Apoptosis can be initiated by three pathways: the extrinsic pathway, the intrinsic pathway (subdivided into mitochondrial-induced apoptosis and endoplasmic reticulum-induced apoptosis), and the caspase (aspartate-specific peptidases, dependent on cysteine)-independent pathway.[12–14]

The extrinsic pathway is activated by extracellular processes through soluble ligands that bind to their respective receptors encoded in genes of the tumor necrosis factor receptor (TNFR) superfamily; they are characterized by the presence of an intracellular domain of approximately 80 amino acid residues called the death domain (DD).[15–17] Some of the known members of this family include the Fas receptor and its ligand FasL (FasR/FasL), the tumor necrosis factor α1 (TNFα1) and its ligand TNF-α (TNF-α/TNFR1), Apo3L/DR3, and TNF-related apoptosis-inducing ligand (TRAIL) and its receptor (TRAILR). The extrinsic pathway initiates when one of these receptors, for example, FasR, found in the cell surface as a labile homotrimer, is activated by the binding of its ligand. This stabilizes it and induces conformational changes in the DD that recruits proapoptotic proteins such as the DD union protein associated with FAS (FADD),[18] the protein kinase that interacts with receptors [receptor-interacting serine/threonine-protein kinase 1 (RIPK1)], and the initiation procaspases (2, 8, 9 and 10), mainly 8 and 10. DD also recruits antiapoptotic proteins such as the cellular proteins inhibitors of apoptosis (IAP), the ubiquitin ligase E3, and the protein cFLIP that interfere with caspase activation, among others. All these proteins bind to the FasR trimer to form the death inducer signaling complex (DISC), which constitutes a platform for the autocatalytically activation of procaspase 8 or 10. Once caspase 8 or 10 is activated, the executioner phase of apoptosis starts. Interestingly, caspase 8 can degrade BH3 interacting-domain death agonist (Bid) and form a truncated Bid (tBid), initiating the intrinsic pathway of apoptosis.[12]

## The intrinsic pathway of apoptosis

The mitochondrial or intrinsic pathway is initiated by cellular stress, which originates from different sources, including DNA damage, oxidative stress, radiation, hypoxia, nutrient deprivation, and high concentrations of calcium ($Ca^{+}$) in the cytoplasm, among others.[18] Regardless of the origin of the inducer, this pathway inevitably leads to the permeabilization of the mitochondrial external membrane (MOMP). This event is so crucial in apoptosis that it is finely regulated by proteins that belong to the B-cell lymphoma 2 (Bcl-2) family.

These are characterized by the presence of homology domains called BH, ranging from 1 to 4. The Bcl-2 family is subdivided into three groups: (1) antiapoptotic proteins that possess four BH homology domains, for example Bcl-2, Bcl-xL, induced myeloid leukemia cell differentiation protein (Mcl-1), and A1. (2) Proapoptotic proteins that have homology domains BH1 to BH3, such as Bcl-2-associated X protein (Bax) and Bcl-2 homologous antagonist killer protein (Bak). (3) Proapoptotic proteins that solely possess the homology domain BH3, such as Bcl-2-associated agonist of cell death (Bad), BH3 interacting-domain death agonist (Bid), Bcl-2-interacting killer (Bik), Bcl-2-modifying factor (Bmf), p53 upregulated modulator of apoptosis (PUMA), phorbol-12-myristrate-13-acetate-induced protein 1 (NOXA), and activator of apoptosis harakiri (Hrk),

The MOMP originates by the action of the pro-apoptotic proteins Bak and Bax, which polymerize in the mitochondrial external membrane to form pores. Once settled, they constitute a point of no return in the apoptotic process, as three lethal processes for the cell occur: (1) dissipation of the membrane potential with the subsequent disappearance of ATP synthesis and active transport systems, (2) release from the mitochondrial intermembrane space to the cytoplasm of toxic proteins such as cytochrome C, apoptosis-inducing factor (AIF), endonuclease G (EndoG), direct union protein to IAP with low pI (DIABLO or SMAC), high temperature requiring protein A2 (HTRA2), and (3) inhibition of the respiratory chain. In the cytoplasm, cytochrome C binds to the adaptor protein apoptotic protease activating factor 1 (APAF1) and activates it, which induces the recruitment of procaspase 9 and the formation of a multiproteic complex named apoptosome, through which procaspase 9 is activated by autoproteolysis, which in turn activates procaspase 3 and initiates the executioner phase of apoptosis.[19,20] These key downstream effector caspases can process at least 1000 proteins triggering cellular changes that result in apoptosis.[21]

## Endoplasmic reticulum-induced apoptosis

Some authors have established a subdivision of the intrinsic pathway as the intrinsic pathway *via* endoplasmic reticulum stress-induced apoptosis, which occurs when the inducing stimulus is the misfolding of proteins and their subsequent accumulation in the endoplasmic reticulum (ER). When this accumulation reaches a critical point, some ER membrane sensors such as protein kinase RNA-like endoplasmic reticulum kinase (PERK), inositol-requiring protein 1 (IRE1a), and activating transcription factor 6 (ATF6) are activated. When these sensor mechanisms are not able to compensate for the misfolded protein concentration, apoptosis is induced, mainly through the association of IRE1 with Bax and Bak, and at the same time, members of the mitogen-activated protein kinase (MAPK) family, mainly MAPK p38 and jun N-terminal kinases (JNK), are activated.[14,22]

## Caspase independent pathway

The caspase-independent pathway is characterized by the fact that mitochondrial damage induces the release of various molecules with pro-apoptotic capacities, such as the AIF, EndoG and the serine protease HTRA2. These three molecules alone can induce apoptosis, without the participation of caspases as intermediaries. AIF and EndoG can enzymatically attack DNA, while HtrA2 exerts a proteolytic effect on the cytoskeleton.[14,23]

## Phases of activation of apoptosis

Regardless of the initiation pathway, apoptosis transits through three phases. These are: initiation or activation, execution, and cell demolition.[14] The initiation phase is characterized by the turning on of genes and signaling pathways required for apoptosis. The executioner phase is characterized by the participation of the executioner caspases 3, 6, and 7, which in turn are activated by the initiation caspases 2, 8, 9, and 10. The executioner caspases are the enzymes responsible for the dismantling of the cell and provoke the morphological changes characteristic of apoptosis.[24] They exert proteolytic activity on multiple substrates of vital importance to the cell, for example, cytoskeletal proteins damaging both cell and nuclear membrane integrity. In addition, they activate endonucleases, which promote DNA degradation and fragmentation.[24–31]

## Signaling pathways that participate in apoptosis

As mentioned before, apoptosis is crucial for the maintenance of homeostasis. This silent type of cell death requires fine regulation which is

achieved through a complex genetically-encoded machinery that regulates an extensive circuit of different intracellular signaling pathways. These pathways activate both cell death signals (pro-apoptotic) and regulatory signals for apoptosis or survival (antiapoptotic signals). Every step in the initiation and executioner phases is regulated at one level by different proteins, among them caspase inhibitory proteins and proteins of the Bcl-2 family, and to another level by signal transduction pathways. Among the different signaling pathways that intervene in apoptosis, the MAPKs are key participants.

## The role of MAPK in apoptosis

The MAPKs are a family of kinases that specifically phosphorylate serine/threonine residues and is composed of at least three groups of kinases: ERK, JNK, and p38, each one with different isoforms. One of the main actions of MAPK is the activation of transcription factors which regulate gene expression and lead to crucial molecular events in the cell. Affected are growth, proliferation, the production of inflammatory cytokines, and apoptotic cell death.[14,32] In particular, JNK phosphorylate proapoptotic and antiapoptotic proteins, causing their activation or inhibition. JNK translocates to the nucleus and activates c-Jun and other transcription factors that promote the expression of pro-apoptotic genes, through mechanisms dependent on p53/73 or c-Jun/activator protein 1 (AP-1).[33,34] In addition, the activation of JNK and its translocation to the mitochondria promotes the phosphorylation of the inhibitory protein 14-3-3. When the protein 14-3-3 is phosphorylated, Bax is released and translocated to the mitochondria, inducing the formation of pores in the mitochondrial membrane. This facilitates the subsequent release of cytochrome C and the induction of apoptosis through the intrinsic pathway. Furthermore, JNK phosphorylates Bcl-2 and Bcl-Xl, and causes its inactivation; in addition, the phosphorylation of BAD by JNK induces its dissociation from Bcl-xL, which ultimately favors apoptosis.[35,36] Another MAPK that plays an important role in apoptosis is p38, which can be activated simultaneously with JNK.[37] Similarly to JNK, p38 phosphorylates proteins both for its activation and inactivation. As an example, p38 phosphorylates Bad, Bax, and Bim, as well as extra-long Bim (BimEL), which results in its activation that drives apoptosis.[35,36] (pro-apoptotic proteins),[38–42] and at the same time inhibits the extracellular single-regulated kinase (ERK) and protein kinase B (Akt) pathways (antiapoptotic proteins).[41,42] On the other hand, ERK mainly participates through the activation of signaling pathways that favor cell survival.[43]

## The PI3K/Akt signaling pathway in apoptosis

The PI3K/Akt signaling pathway is a key participant in cellular survival and is involved in differentiation, proliferation, and metabolism; despite this, it also participates in apoptosis down-regulation. PI3K is a heterodimer formed by a p85 regulatory subunit and a p110 catalytic subunit responsible for phosphate transfer. Akt is a kinase that phosphorylates serine or threonine residues and in mammals is present in three isoforms: Akt1, Akt2, and Akt3. The signaling pathway initiated by these kinases is activated by different stimuli; of particular note are growth factors. When the ligand binds to receptors coupled to tyrosine kinases (RTK) or G proteins (GPCR), an insulin receptor substrate (IRS) adaptor protein is activated. This, in turn, activates the regulatory PI3K subunit and results in a conformational change that allows for the binding of the catalytic subunit. The result is the assembly of the active molecule which catalyzes the conversion of phosphatidylinositol 4,5-biphosphate ($PIP_2$) into phosphatidylinositol 3,4,5-trisphosphate ($PIP_3$).[44,45] $PIP_3$ binds to and recruits Akt through the pleckstrin homology domain present in Akt. This facilitates the complete phosphorylation of Akt by phosphoinositide-dependent protein kinase 1 (PDK1) at threonine 308 and by mTOR complex 2 (mTORC2) at serine 473.[46,47]

The PI3K/Akt signaling pathway modulates apoptosis primarily through the inactivation of proapoptotic proteins and the induction of antiapoptotic genes. The phosphorylation of Bad by Akt facilitates its binding with protein 14-3-3; this, in turn, promotes the degradation of Bad in the proteasome. This pathway also activates the transcription factors cAMP response element-binding protein (CREB) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB), which regulate the expression of antiapoptotic genes such as those found in the Bcl-2 family or IAPs. Active Akt also induces the inactivation of glycogen synthase kinase-3 (GSK3), a kinase that is constitutively active in the absence of exogenous signals and which phosphorylates

different substrates for their degradation by the proteasome. The inactivation of GSK3 by Akt stabilizes the intracellular concentration of antiapoptotic molecules such as induced myeloid leukemia cell differentiation protein 1 (Mcl-1) or transcription factors such as c-Myc or forkhead box protein O (FOXO). C-Myc promotes the expression of antiapoptoitc genes, while FOXO the intracellular concentration of antiapoptotic proteins such as Mcl-1, together with transcription factors such as c-Myc which intervene in the expression of antiapoptotic genes. FOXO is a transcription factor that, in the absence of an exogenous stimulus, is found in the nucleus, inducing the synthesis of pro-apoptotic genes such as PUMA, Bim, TRAIL, FasL, among others. However, when Akt is activated, it phosphorylates FOXO, which induces it to leave the nucleus into the cytosol; here, it is recognised by the 14-3-3 protein and is degraded by the proteasome pathway.[35,36,46,47] In this way, Akt allows the balance within the Bcl-2 protein family to remain in favor of the antiapoptotic side, inducing the degradation of pro-apoptotic proteins and inducing the expression of antiapoptotic genes.

As already mentioned, apoptosis is an important defense mechanism against intracellular microorganisms. In response, pathogens employ a range of strategies to inhibit apoptosis and, therefore, survive, reproduce, and develop.[14] An excellent example of an intracellular microorganism that utilizes numerous strategies to counteract host cell defense mechanisms is *Leishmania.*

## *Leishmania* induces inhibition of cell death by apoptosis

The intracellular parasite *Leishmania* has the capacity to invade a variety of cell types, including neutrophils, macrophages, and dendritic cells. As a strategy to evade the immune response, as well as to achieve intracellular survival, it has been well demonstrated that *Leishmania* inhibits the apoptosis of these cells, although the molecular mechanisms underlying this inhibition are not completely understood. (Table 1).

The first demonstration of the capacity of *Leishmania* to inhibit the apoptosis of host cells was carried out in 1994 by Moore and Matlashewski. They showed that bone marrow-derived macrophages (BMM) infected with *Leishmania donovani* (*L. donovani*) promastigotes or stimulated with LPG (glycolipid lipophosphoglycan) inhibited apoptosis induced by macrophage colony-stimulating factor (M-CSF) deprivation.

They observed that the inhibition observed was probably due to soluble mediators, since the culture supernatant of infected BMM was able to inhibit apoptosis.[56]

Later, it was shown that macrophages infected with promastigotes of a different *Leishmania* species, *Leishmania major* (*L. major*), and cultured in the absence of M-CSF or the presence of ailuropodine, showed a decrease in MOMP, inhibition of mitochondrial cytochrome c release, and inhibition of caspase-3 activation, which caused a delay in the initiation of apoptosis.[57] Other studies conducted with cell lines revealed similar results. The infection of the monocyte cell line U937 with *Leishmania infantum* (*L. infantum*) inhibited apoptosis mediated by actinomycin D[58] and the infection of the macrophage RAW 264.7 cell line with *L. major* decreased the frequency of apoptosis, even in the presence of cycloheximide.[59]

Although macrophages are considered the principal host cells for *Leishmania*, the parasite has the capacity to invade other cells, and its survival inside them has important repercussions during its life cycle. Neutrophils are the first phagocytic cells to infiltrate the inoculation site. They are the first cells that phagocytose *Leishmania* promastigotes and constitute a temporal refuge for the parasite, where apoptosis inhibition is an important requisite to achieve this goal. It has been shown the infection of neutrophils with *L. major* promastigotes decreases caspase-3 activity and thus inhibits the spontaneous apoptosis of these cells.[60]

*Leishmania* also has the capacity to invade dendritic cells, which play a predominant role in the development of the disease. Our group has demonstrated that the infection of monocyte-derived dendritic cells with *Leishmania mexicana* (*L. mexicana*) amastigotes or promastigotes inhibits camptothecin-induced apoptosis of these cells.[61]

## Signaling pathways involved in the inhibition of apoptosis by *Leishmania*

During the process of inhibition of apoptosis induced by *Leishmania*, various molecular mechanisms are altered, both on behalf of the host cell and the parasite. These mechanisms involve

**Table 1.** The main proteins thus far described which participate in the inhibition of apoptosis induced by different species of *Leishmania*.

| ***Leishmania* species** | **Apoptosis protein/pathway affected** | **Cell type** | **Reference** |
|---|---|---|---|
| *Leishmania donovani* | MAPK P38, JNK and ERK<br>Akt<br>FOXO-1<br>MOMP<br>Caspase 3<br>PD-1<br>Bad<br>CREB<br>MCL-1<br>SOCS<br>Caspase 7 | Bone marrow macrophages<br>RAW 264.7 | Privé and Descoteaux[48]; Gupta *et al.*[49]; Roy *et al.*[50]; Giri *et al.*[51]; Pandey *et al.*[52]; Srivastav *et al.*[53] |
| *Leishmania major* | MAPK ERK 1/2<br>Bcl-2, Bfl-1<br>Cytochrome c<br>Caspase 6<br>Fas | Neutrophils | Sarkar *et al.*[54] |
| *Leishmania major*<br>*Leishmania pifanoi* | PI3K/Akt<br>Bad<br>Bcl-2 | Bone marrow macrophages | Ruhland *et al.*[55] |

MAPK (mitogen-activated protein kinase), JNK (c-Jun N-terminal kinases), ERK, extracellular signal-regulated kinases), Akt (serine/threonine-specific protein kinase from AKR mouse), FOXO-1 (Forkhead box protein O1), MOMP (Mitochondrial outer membrane permeabilization), Caspase (cysteine-aspartic proteases), PD-1 (Programmed cell death protein 1), Bad (BCL2 associated agonist of cell death), CREB (cAMP response element binding protein), MCL-1 (Myeloid cell leukemia 1), SOCS (Suppressor of cytokine signaling), Bcl-2 (B cell Lymphoma), Bfl-1 (Bcl-2-related gene from human fetal liver), Fas (FS-7-associated surface antigen).

both survival and cell death signals, whose balance contributes to the resolution or progression of the disease. Recent research has tried to decipher the pathways that are manipulated by the parasite to permit its survival inside the host. Among the main actors participating in the inhibition of apoptosis induced by *Leishmania* are members of the MAPK family, as well as PI3K/Akt (Figure 1).[14,43,62,63]

The participation of MAPK in the inhibition of apoptosis by *Leishmania* has been demonstrated in several cells infected with different *Leishmania* species. Our group has demonstrated that amastigotes and promastigotes of *L. mexicana* significantly reduced the phosphorylation of MAPK, JNK, and p38 in monocyte-derived dendritic cells.[64–67] The inhibitory effect on MAPK activation has been reported to be only observable in immature dendritic cells, since LPS stimulation-driven maturation did not suppress the phosphorylation of MAPK, particularly JNK.[66] It has been shown that not only does the parasite inhibit pro-apoptotic signaling of MAPK p38, but that this phenomenon has been observed with certain soluble structural components of the protozoan, such as the surface protein gp63.[68]

Similar results reflecting the participation of MAPK in the inhibition of apoptosis by *Leishmania* have been observed in bone marrow macrophages (BMM) stimulated with interferon-gamma (IFN- $\gamma$) and infected with *L. donovani* promastigotes, where the inhibition of p38, JNK, and ERK ensured the survival of the parasite.[48] Inhibition of p38 has also been shown to be associated with an increase in the number of infected macrophages and parasite survival.[69]

## Participation of the PI3K/Akt pathway in the inhibition of apoptosis by *Leishmania*

Interestingly, in addition to *Leishmania's* capacity to down-regulate proapoptotic signaling pathways such as MAPK, it has been shown that the parasite is capable of activating signaling pathways

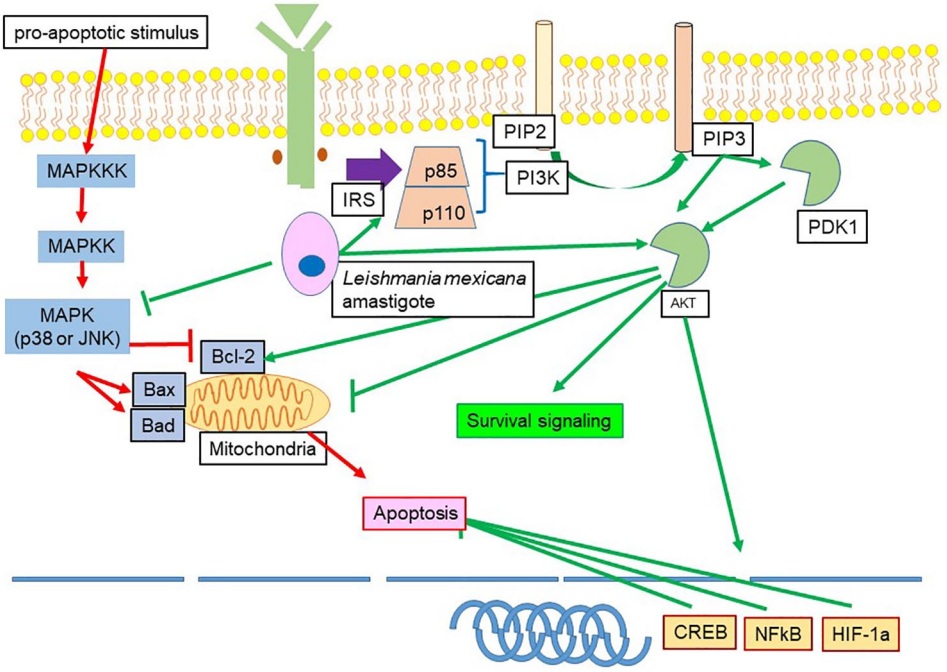


**Figure 1.** Signaling pathways involved in the inhibition of apoptosis by *Leishmania*. Schematic representation of the proposed apoptosis inhibition mechanisms of *Leishmania mexicana* (*L. mexicana*) amastigotes in human monocyte-derived dendritic cells (mdDC) by Vázquez-López and colleagues. In this model, *L. mexicana* inhibits mitogen-activated protein kinase (MAPK), Jun N-terminal kinase (JNK) and p38 phosphorylation (proapoptotic mechanisms), while activating phosphatidylinositol 3-kinase (PI3K)/Akt (antiapoptotic mechanisms).
CREB, cyclic AMP response element binding protein; HIF-1, hypoxia-inducible factor 1-alpha; IRS, insulin receptor substrate; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PDK1, phosphoinositide dependent kinase 1; PIP2, phosphatidylinositol 4,5-biphosphate; PIP3, phosphatidylinositol 3,4,5-triphosphate.

involved in cell survival. A demonstration of this has been performed in neutrophils infected with *L. major*; here, a delay in apoptosis was observed as a result of activation of the MAPK ERK 1/2, activation of anti-apoptotic proteins Bcl-2 and Bfl-1, inhibition of mitochondrial cytochrome c release, inhibition of caspase-6, and low expression of FAS.[54] We have also shown that monocyte-derived dendritic cells infected with *L. mexicana*, with or without a pro-apoptotic stimulus (camptothecin), activate the PI3K/Akt survival pathway.[64] In addition, the infection of BMM with *L. major* or *Leishmania pifanoi* has also been shown to activate the PI3K/Akt survival pathway and phosphorylate Bad downstream; this, in turn, inhibits the 14-3-3 protein, enhancing the antiapoptotic action of Bcl-2.[55]

Furthermore, pharmacological inhibition of the Akt pathway has been observed to inhibit its antiapoptotic signal in BMM or RAW 264.7 infected with *L. donovani*. Likewise, it was shown that downstream in this pathway, Akt phosphorylates and inhibits FOXO-1, a transcriptional regulator of pro-apoptotic proteins. Using a constitutively active construct of FOXO-1 that cannot be inhibited by Akt, it was possible to reduce the antiapoptotic effect of Akt. In addition, within the Akt pathway, the inhibition of glycogen synthase kinase 3 β (GSK-3β) also occurs downstream; as a consequence, the release of β-catenin to initiate the transcription of anti-apoptotic proteins is enhanced. Employing constitutively active construction GSK-3β, it was possible to inhibit β-catenin, leading to the inhibition of the transcription of anti-apoptotic proteins with the consequent loss of MOMP together with the activation of caspase-3. In addition, the inhibition of the anti-apoptotic pathway of Akt leads to increased interleukin-12 (IL-12) production and decreased interleukin-10 (IL-10) production, which correlates with a higher death rate of the parasite.[49]

In addition, Akt can be inhibited by the activation of the programmed death receptor 1 (PD-1). It has been shown that BMM and RAW 264.7

infected with *L. donovani* and experiencing induced apoptosis with $H_2O_2$, results in the reversal of activation effect of PD-1. In addition, phosphorylation and inhibition of the pro-apoptotic protein BAD occurs. Furthermore, it was demonstrated that *L. donovani* induces the negative regulation of nuclear factor of activated T cells (NFATc1), which regulates PD-1 expression in T cells, thus promoting the survival of the parasite within macrophages.[50]

### *Leishmania* modulation of proteins of the Bcl-2 family

As another strategy to modulate host cell apoptosis, it has been shown that *Leishmania* has the capacity to interact with members of the Bcl-2 family. It has been shown that *L. donovani* promastigotes activate the transcriptional factor CREB and the synthesis of the Mcl-1 protein in murine macrophages, as a survival and inhibition mechanism of apoptosis. The inhibition of CREB, Mcl-1, or both, reduces their anti-apoptotic effects; as a result, apoptosis is induced.[51]

In addition, it has been shown that the infection of different macrophage populations with *L donovani* activates the expression of Bcl-2 as an anti-apoptotic mechanism. This activation leads to the inhibition of nitric oxide (NO) production and enhances the survival of the parasite. When specific Bcl-2 inhibitors were used, the anti-apoptotic effect was reversed, NO levels increased and the parasite load diminished. Interestingly, this has been shown to occur in patients with visceral leishmaniasis.[52]

Finally, in a model of infection of monocyte-derived dendritic cells with *L. mexicana*, we have demonstrated an increase in the protein presence of Bcl-xL as a strategy to inhibit apoptosis.[70]

### Other survival mechanisms employed by *Leishmania*

As already mentioned, reactive oxygen species (ROS) are important inducers of apoptosis. In basal conditions, the protein thioredoxin participates in the ROS uptake system; therefore, it plays an important role in cell protection from ROS-induced apoptosis. Along with thioredoxin, members of the suppressors of cytokine signaling (SOCS) family also participate in this mechanism *via* inhibition of ROS-mediated apoptotic signaling cascade. This mechanism is utilized by *L. donovani* to survive in RAW 264.7 macrophages treated with $H_2O_2$. It has been suggested that the parasite activates the SOCS pathway in addition to thioredoxin and tyrosine phosphatase activity; as a result, caspase three and seven activity is inhibited.[53]

It is evident that *Leishmania* employs diverse strategies to achieve intracellular survival, evade recognition and the immune response, obtain sustenance, grow and develop within the infected cell, and even more favorable is infecting the cells responsible for its elimination.

## Conclusion

The process of cell death due to apoptosis and its regulation involves a complex circuit of signaling pathways, both for the induction and inhibition of cell death. Apoptosis plays a vital role in many biological processes, including growth, development, and cellular and tissue remodeling which are crucial for the homeostatic balance of an organism. Due to this, apoptosis is a highly-regulated process, achieved through a complex network of signaling pathways. In addition, apoptosis represents an important defense mechanism against intracellular microorganisms. The initiation of cell suicide in infected cells is promoted to limit infection. As a counterpart to this strategy, through the course of evolution intracellular pathogens have developed a wide array of mechanisms to counteract apoptosis and promote their own survival inside host cells.

Recent research has tried to elucidate the complex signaling pathways involved in apoptosis and its regulation. Knowledge generated through this work will provide crucial information to aid our understanding of the molecular mechanisms involved in the pathophysiology of the disease. In the future, this will provide the basis for the development of new drugs for the treatment and prevention of leishmaniasis.

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## Author contributions

R.V.-L. contributed to the conception and design of this project, general supervision of the research group, and gave final approval of this manuscript. D.-A.A.-H. contributed to the conception and

design of this project, general supervision of the research group, and gave final approval of this manuscript. L.G-K. contributed to the conception and design of this project, general supervision of the research group, and gave final approval of this manuscript. S.G.S.-G. contributed substantially to the drafting of the manuscript. All authors revised and approved the final version of the paper.

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