Microbial Pathogenesis 139 (2020) 103892

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

The role of Bax in the apoptosis of *Leishmania*-infected macrophages

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ARTICLE INFO

Keywords:
Apoptosis
Leishmania
Bax
Bcl-2
Macrophage
Transgenic
miRNA

ABSTRACT

Background: *Leishmania* is a protozoan parasite that nests in macrophages and is responsible for the Leishmaniasis disease. In spite of different defense pathways, last strategy of macrophage for killing parasite is apoptosis process. By permeableizing the mitochondrial outer membrane (MOM). As breaching MOM releases apoptogenic factors like cytochrome-*c* which activate caspases that result in the destruction of the cell. In this review, we summarized the appropriate manuscripts regarding the bax includes, its different types and the effect of bax on the apoptosis of *Leishmania* and parasite-infected macrophages.

Methods: Information about the role of BAX in the apoptosis of parasite-infected macrophage of recent articles were surveyed by searching computerized bibliographic database PubMed and Google Scholar entering the keywords BAX and leishmaniasis.

Results: The common studies revealed *Leishmania* use different survival strategies for inhibiting macrophage apoptosis. As *Leishmania* by preventing homooligomerization or upregulating the anti-apoptotic molecule Bcl-2 can prohibits proteins of host-cell apoptosis such as Bax that is required for mitochondrial permeabilisation during apoptosis.

Conclusion: With regard to the supportive role of bax in apoptosis and the preventive role of *Leishmania* in its function, it seems that expression of bax gene in parasite by technologies like transgenic or down regulating of anti-apoptotic molecule Bcl-2 by miRNA could be prompted the apoptosis process of infected-macrophages and inhibited extensive spread of *Leishmania* and the resulting lesions.

1. Introduction

Leishmaniasis is a group of diseases caused by *Leishmania* spp. that transmitted to animals and human by infected female sand fly bites of the *Phlebotomus* and *Lutzomyia* genera [1–3]. Clinical manifestations of leishmaniasis are ranging from severity skin lesions (cutaneous Leishmaniasis) to fatal systemic infection (visceral Leishmaniasis or kala-azar) [2]. Chemical methods and common drugs, such as miltefosin, pentavalant antimonials and etc. are used for treatment of leishmaniasis [1].

The life cycles of the parasite involves two stages of promastigote and amastigote forms. Promastigotes multiply in the alimentary tract of the female sandfly and transmit to the mammalian host during the blood meal. In the endosome area of infected endothelial macrophage of host, promastigote differentiate into amastigotes and replicate. After macrophage collapse, released amastigotes infect healthy macrophages and cause leishmaniasis lesions [4].

Macrophages, as a primary defense line, play key role against infection produced by *Leishmania*. As with entrance of *Leishmania,* macrophages attack parasites by oxidative burst, acidic enzymes of lysosome and etc [5]. In contrast *Leishmania* prevents oxidative bursts by producing acid phosphatases on itself surface, inhibits the function of lysosomal enzymes through lipophosphoglycan molecule and the proton pump on itself surface, increases the activity of Protein Kinase C and decreases the NADPH oxidase activity in macrophages [6].

Furthermore, during leishmanial infection, macrophage apoptosis

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https://doi.org/10.1016/j.micpath.2019.103892
Received 1 November 2018; Received in revised form 30 October 2019; Accepted 23 November 2019
Available online 25 November 2019

by a variety of immune factors that work alone or in combination inhibits the multiplication and the spread of the pathogen to lesion progression [7].

2. Apoptosis of *Leishmania*-infected macrophages

Macrophages, as sentinels of infection, are one of the first cell types to encounter pathogens. The mature phagosome of macrophages is a hostile environment for the vast majority of phagocytosed microbes (bacteria, parasite, fungus and virus). Interaction of macrophages with pathogens results in a whole variety of protective and stress responses including internalization or phagocytos, release of cytokines, secretion of defensins or production of oxygen radicals ([8]).

But many pathogens like bacteria upon infection induce macrophage cell death pathways (such as apoptosis, necrosis, pyroptosis and autophagy and etc) by a variety of direct and indirect mechanisms and secreting an array of toxins and virulence factors that is triggered potent anti-microbial responses [9].Also bacteria are able to activate several pro-apoptotic proteins, e.g. caspases, to inactivate anti-apoptotic proteins, e.g. NF(kappa)B or MAP-kinases, or to upregulate endogenous receptor/ligand systems, that induce apoptosis on the surface of the infected cell [10]. In the fact the induction of apoptosis upon infection results from a complex interaction of bacterial proteins with cellular proteins finally mediating apoptosis.Apoptosis plays two defensive roles in infection include eliminating pathogens at the early stage of infection without emitting alarm signals and engulfing apoptotic bodies containing infected microbes by dendritic cells (DCs), which allows extracellular antigens to access MHC I molecules and subsequently induce a protective immune response [11]. However, in some cases, inducing cell death, as a strategy of pathogenesis, can eliminate key immune cells and consequently, evade host defenses and allows the bacteria to efficiently exit the host cell, spread to neighboring cells, and/or gain nutrients ([12]).

About *leishmania* parasite, macrophages are definitive cellular host that phagocyte parasite at the infection site. Macrophage apoptosis is induced by activation of caspases-3, 8 and 9 and correlates with chronic infection and regardless of its severity [13]. As Fas expression in *leishmania* -infected macrophages occur in response to the Th1cytokine (IFN-γ) and TNF-α. *L. major* induces cellular stress response in macrophages and stress actives JNK, C-JUN/AP-1 signaling pathway. Then the expression of Fas ligand is increased through this pathway and leads to delete infected macrophages through external pathway and reduces parasite load [14].

3. Mechanisms of delaying macrophage apoptosis in leishmaniasis

Leishmania in order to differentiate into the intracellular amastigotes form in the phagolysosome, buys time by inhibiting the spontaneous apoptosis of short-lived macrophages (independent on the host cell genetic) so that maintains its niche [15]. Although the mechanism by which the parasite inhibits macrophages apoptosis is not well elucidated, many studies showed that *Leishmania* promastigotes utilize multiple signalling to promote anti-apoptotic effects in human macrophages. For example total surface phosphoglycans of promastigotes are important in delaying apoptosis in *L. infantum, L. major*, and *L. donovani*-infected macrophages at the early phase of the infection [16]. As the researches have shown that treating macrophages with the LPG (lipophosphoglycan) protects *L.major* or *L. donovani*-infected BMMs of apoptosis through PKC-d (protein kinase C delta) signalling inhibition [17]. Another inhibitor pathway of macrophage apoptosis is the signaling pathway Akt3/PI3 K which prevents secretion of mitochondrial cytochrome C and caspase 3 activity through phosphorylation and Bad inactivation and *Leishmania* blocks macrophages apoptosis by activating this pathway [15]. *Leishmania* can also delay macrophage apoptosis by preventing procaspases 3 and 7 processing [16]. Also *Leishmania* induces cytokine signaling proteins (SOCS) inhibitors and prevents apoptosis with thioredoxin [18]. Furthermore at the time of injury or stress, ATP triggers macrophage apoptosis by attaching with purinergic receptors of X2 P family and *Leishmania* prevents induced cytolysis by secreting nucleoside diphosphate kinase (Ndk) and reducing ATP [19–22]. In addition *Leishmania* leads to prevent macrophage apoptosis with the factor Lm1740MIF (human MIF homologue) and ERK1/2 phosphorylation [23]. *Leishmania* infection in macrophages also leads to cleavage and inhibition of the mTOR (mammalian/mechanistic Target of rapamycin) serine/threonine kinase resulting in a global repression of host translation and autophagy induction as an alternative anti-apoptotic strategy [24].

4. Bcl-2 family members

The Bcl-2 family as major regulators of apoptosis in mammalian cells plays a key role in the control of apoptosis via mitochondrial pathway. They have been localized to membranes of various organelles including mitochondria, the nucleus and the endoplasmic reticulum and comprises at least 18 members that are categorized into pro-apoptotic and anti-apoptotic regulatory proteins [25]. Bcl-2 family members have one or more of the four characteristic domains of homology entitled the Bcl-2 homology (BH) domains (BH1, BH2, BH3 and BH4), and can form hetero- or homodimers. These domains include nine α-helical fragments, along with a hydrophobic α-helix core covered by amphipathic helices and a transmembrane C-terminal α-helix anchored to the mitochondrial outer membrane (MOM) [26]. The main structural target of the Bcl-2 proteins is the outer mitochondrial membrane (OMM) and the outer (MOMP)the ratio and interaction As Anti–apoptotic proteins inhibit MMP, whereas pro-apoptotic Bcl-2 homologues enhance MMP [27]. The characteristics of Bcl-2 family members (anti-apoptotic and pro-apoptotic proteins) are showing in Table 1 [25,28,29].

Pro-apoptotic effector proteins (such as Bak, Bax and Bok) act downstream of both the BH3-only and prosurvival Bcl-2 members to induce MOMP [22,25]. As mitochondrial alterations including changes in mitochondrial morphology by pro-apoptotic proteins such as Bax (which directly interact with mitochondria), disruption of electron transport, oxidative phosphorylation and ATP production, release of other proteins such as Htr2/Omi, Smac/Diablo that trigger caspase activation also contribute to the intrinsic PCD pathway.

5. Bax gene

The BAX gene is the first known pro-apoptotic member [30]. In the 19q13.3-q13.4. The BAX gene, with 6,939 bases in length, consists of 6 exons and 5 intervening intronsAccording to available complete genome data, orthologs of the BAX gene have been identified in most mammals [31] expressed in a p53-regulated pathway for induction of apoptosis [32].

The *BAX* gene encodes several variants of the prototypical Baxα to several alternatively spliced isoforms such as Baxβ, ω, ψ, γ, σ, and δ that expressed in a wide variety of tissues [31]. BAX protein has been reported to be localized in the mitochondria, mitochondrial permeability transition pore complex, mitochondrial outer membrane, endoplasmic reticulum membrane, and cytoplasm. Also Bax proteins can be found as monomers in the cytosol (beta and gamma) or associated with the outer mitochondrial membrane (Baxα) when not activated [30]. The characteristics of BAX isoforms are showing in Table 2.

6. BAX and leishmaniasis

Apoptosis results when the pro-survival proteins are overwhelmed and Bax/Bak undergo a conformational change at the mitochondrial membrane. As multiple cellular stresses, including heat, hydrogen peroxide, low or high pH, mitochondrial membrane remodeling, DNA damage, energy starvation, hypoxia, disruption of the 14-3-3σ gene

Table 1
The characteristics of Bcl-2 family proteins.

kinds	Type		Structure	Function
Antiapoptotic	Bcl-2 Bcl-XL (B-cell lymphoma-extra-large) Mcl-1 (myeloid cell leukaemia 1) Bcl-W Ced-9 A1/Bfl-1		They conserved sequence in three to four BH domains (BH1-4)	They inhibit apoptosis by directly binding either BH3-only proteins or Bax/Bak. As form competing heterodimers with Bax which are unable to promote apoptosis. Also maintains BAX in the cytosol by inducing constant retrotranslocation Furthermore prevent apoptosis by sequestering proforms of caspases (a complex called the apoptosome) or by preventing the release of cytochrome *c* and AIF (apoptosis-inducing factor) into the cytoplasm.
Proapoptotic	**Apoptotic sensors**	HRK Bik Bak (Bcl-2 homologous antagonist killer 1) Bax (Bcl-2-associated X protein) BOK (also known as Mtd) Bcl-Xs	They have three conserved domains (BH1-3)	They sense cellular stress and initiate apoptosis. Conversely, BAX can become inactivated by interacting with VDAC2, Pin1, and IBRDC2.
	BH3-only proteins(BOPs)	Bad (Bcl-2 antagonist of cell death) Noxa PUMA (p53- upregulated modulator of apoptosis) BNIP3 BIM	They have sequence and structural homology only to the α-helical BH3 domain	In response to stress or damage signals, BH3-only proteins are upregulated either transcriptionally or post-translationally or both and promote mitochondrial permeability. Also promote apoptosis by inhibiting the BCL2 inhibitors of apoptosis.

dephosphorylate, cleave bax protein and binding BCL-2, as well as non-BCL-2 proteins such as p53 and Bif-1 leading to its activation and translocation to the mitochondria and consequent apoptosis [40,41].

Bax upon activation interacts with an activator such as BID, which induces a conformational change in BAX, resulting in oligomerization and insertion into the MOM to open the mitochondrial voltage-dependent anion channel (VDAC) and also form an oligomeric pore, MAC, in the MOM, which leads to the loss in membrane potential and release apoptogenic factors such as cytochrome *c* and ROS from the mitochondrial intra membrane space into the cytosol. Cytochrome *c* binds to apoptotic peptidase activating factor 1 (APAF1) that leads to the assembly of a heptameric protein ring called the apoptosome, which binds and activates caspase 9. Caspase 9 stimulates the caspase cascade, committing the cell to apoptosis [42].

Different pathogens use multiple mechanisms to suppress their host apoptosis. As despite of increased translation of pro-apoptotic proteins such as apoptosis regulator BAX and caspase 3 by tumor suppressor p53 in cells infected with *Leishmania* during 24 h, researches show that *Leishmania* blocks apoptosis at the mitochondrial permeability step by neutralizing of BAX ([43]). Also Researches have suggested however, Bcl-2 and Bax are both able to regulate apoptosis independently, apoptosis inhibition depends partly on the balance between *Bcl-2* and *Bax* gene expression. As *Leishmania* counteracts the apoptotic machinery of macrophage by upregulating the anti-apoptotic molecule Bcl-2 and its relative BCL-Xl and downregulating the apoptosis-related proteins including BAX, caspase-3, caspase-8 and caspase-9. For example, Cianciulli showed *L. infantum* was able to increase the expression of Bcl-2 levels and at the same time to reduce Bax expression in actinomycin D-treated cells, in comparison with what observed in macrophages submitted to actinomycin D treatment alone [44]. Wei-Xing Zong showed that the anti-apoptotic members of the Bcl-2-family block apoptosis very likely by binding either BH3-only proteins or Bax/Bak [45]. In agreement with Wei-Xing Zong study, Yan et al. demonstrated that *H. pylori* stimulate PI3K-dependent activation of the anti-apoptotic factor Akt through phosphorylation of EGFR (epidermal growth factor receptor) that lead to increased expression of the anti-apoptotic factor Bcl-2, and decreased expression of the pro-apoptotic factor Bax [46]. Also in *Mycobacterium tuberculosis* infection, Arnett et al. showed PPARγ effector and 15-lipoxygenase (15-LOX) are critical regulators of apoptosis, as PPARγ limits apoptosis of human macrophages through regulating Mcl-1 and Bax expression [47].

About structural conformation during apoptosis, Giri study revealed *L. donovani* with using Myeloid Cell Leukaemia 1 (MCL-1) inhibits of apoptotic machinery of the host cell by preventing homooligomerization of both BAK and BAX during *Leishmania* infection because of its impaired translocation to mitochondria during infection [48]. Also about *Anaplasma* bacterium Niu et al. showed, T4SS effector Ats-1 (the causative agent of human granulocytic anaplasmosis) can target the mitochondria and prevent Bax from relocalizing from the cytoplasm into the mitochondria and inhibit cytochrome *c* release in neutrophils, because of contain an N-terminal mitochondria-targeting sequence [49].

About other obligate intracellular pathogens, including Chlamydiae and Coxiella burnetii, Fan and hrmann showed that in the early invasive stages of infection these pathogens prevent infected cells apoptosis presumably by blocking cytochrome *c* release from mitochondria [50].

Furthermore, about other parasites researches showed that parasite-derived molecules can directly interfere with cytochrome c-induced caspase activation. As recent study showed that *T. gondii* is not dependent on the transcription machinery of the host cell to inhibit apoptosis and may directly prevents the activation and mitochondrial targeting of Bax without changes in the total levels of Bax, Bak or the anti-apoptotic Bcl-2 [51].

Moreover, Quan et al. study showed that pro-apoptotic protein BAD activated through PI3K/AKT pathway during *T. gondii* infection prevents host cell apoptosis via inhibition of mitochondrial translocation of BAX. So indirect suppression of the anti-apoptotic protein of Bad in the mitochondria and endoplasmic reticulum can cause apoptosis [52].

In addition to multicellular organisms, apoptotic death has been described in *Leishmania* with stress stimuli such as NO or ROS produced by host immune response and etc [53]. As apoptosis of the unicellular eukaryote *Leishmania* major involves cysteine proteinase activation and mitochondrion permeability. For example, Arnoult et al. mixed human recombinant Bax a Bcl-2 family member with *L. major* intact mitochondria and results showed that *L. major* mitochondria released cytochrome *c* and cysteine proteinases with nuclear pro-apoptotic activity, even when Bax was deleted of its transmembrane domain

Table 2
The characteristics of BAX isoforms.

Isoform	Mass (Da)	Length (AA)	structure	function	Tissue	Ref.
α	21,184	192	It contains the BH1, BH2 and BH3 domains, a shorter and different C terminus, lackes of the first α-helix	It is in the cytoplasm and activated upon apoptosis induction through conformational changes	Spleen, breast, ovary, testis, colon, brain and at low levels in skin and lung	[33] [34],
β	24,220	218	It contains the BH1, BH2 and BH3 domains	Spontaneously integrates into mitochondrial membrane and potently induces cytochrome *c* release from mitochondria		[35]
γ	4678	41	It misses exon 2 and terminates in exon 3 due to a translational frame shift			[36].
Σ	15,772	143	It misses exon 3 but retains the same translational frame, the BH1, BH2 and the transmembrane domains	It interacts with BOP/C22 or f29, BCL2A1 and BCL2L1 isoform Bcl-X (L) and RNF144B (as regulator of the ubiquitin-dependent stability of BAX under stress conditions) that cause a conformation change leading to BAX oligomerization and association with mitochondria and etc	Breast, colon, prostate, ovary, lung and skin.	[33]
Δ	19,718	179	It lacks exon 3 and truncated from of BAX- αthat has a shorter and different C terminu and retains the C-terminal membrane anchor region as well as BH1 and BH2 domains		Spleen, breast, ovary, testis, lung, colon, brain and at low levels in skin.	[31], [37]
Ψ			It is natural variant of Bax α without the first 20 amino acids	Because of deletion its CT, is structurally associated with mitochondria.		[38]
Ω			It is without a putative transmembrane domain	It is antiapoptotic after overexpression, as in primary β-cells might represents one of the factors that protects β-cells against the rapid onset of apoptosis	Breast, colon, prostate, ovary, lung and skin.	[39]
€	18	164	It misses last 69 amino acids of Baxalpha, contains an extra fragment within the coding region, as well as a distinct 3′ coding region and 3′ UTR, resulting in a isoform with a shorter and distinct C terminus			[31]

required for insertion in mitochondrial outermembranes, indicating that *L. major* mitochondrion may express proteins able to interact with Bax [54].

Also Esseiva found that ectopic expression of Bax in *T. brucei* induced loss of ΔΨm and release of cytochrome *c*. So he suggested that functional homologues of Bcl-2 family members can exist in trypanosomatids and apoptosis initiated from the mitochondrion in trypanosomatids, by certain death signals [55]. Also Crausaz Esseiva observed three events of (i) the release of cytochrome *c* and the loss of oxidative phosphorylation, (ii) the depolarization of the membrane potential and a decline of the intracellular ATP concentration, and (iii) mitochondrial fission through mammalian Bax expression exogenously in *T. brucei*. These events induced by Bax alone and do not require other apoptotic factors. Also in contrast to mammalian cells all three Bax-induced events were temporally separated and reversible in *T. brucei* [55].

Eventually, though *Leishmania* delay macrophage death, it cannot prevent apoptosis. *Leishmania* exerts strategies for using apoptosis to its own benefit. As when an infected macrophages die, exposing PS on their surface being phagocytized by vicinal healthy macrophages, propagating the infection. For example recent study of Real et al. have showed by multidimensional live cell imaging that, *in vitro*, that *L. amazonensis* amastigotes are transferred from cell to cell when the donor host macrophage delivers warning signs of apoptosis. This transfer happens without full exposure of the parasite to the extracellular milieu. As the parasites are ejected from the host macrophages within membrane blebs rich in phagolysosomal membrane components, which are in turn phagocytized by nearby macrophages that will then secrete the infection promoting cytokine IL-10 [56].

7. Conclusion

During the last decades, several chemical drugs like miltefosin or pentavalant antimonials have been developed in order to control and treatment of leishmaniasis. The main purpose of these drugs is apoptosis of the parasite, but unfortunately their applications are after incidence of lesion and also have side effects such as toxicity to other cells. Since one of the strategies of *Leishmania* to escape the host's immune system is preventing or delaying the macrophage apoptosis, so targeting critical regulators of *Leishmania* infected macrophage apoptosis may represent a useful strategy to eliminate *Leishmania* infection. Since propaoptotic proteins such as Bax can exert natural apoptosis process to macrophages, it seems that the expression of these proteins by parasites, without any side effects on other cells, could been advanced the macrophage apoptosis process naturally. Therefore, the effect of bax on the apoptosis of *Leishmania* and *Leishmania*-infected macrophages should be more studied until biological pathways involved in *Leishmania* or macrophage apoptosis could introduce new methods for the treatment or prevention of the disease in susceptible individuals. For example, the entrance of expressional cassettes from *bax* gene in *Leishmania* by transgenic technology or down regulating of anti-apoptotic molecule Bcl-2 by miRNA could be accelerated the process of infected macrophage apoptosis, limited the possibility of differentiation and more proliferation of *Leishmania*, the spread of the disease and the appearance of lesion. Therefore, there is a need for more studies in order to obtain more crucial results about the therapeutic or preventive effects of this transgenic strain as an effective protective agent or vaccine, in the endemic areas in the future.

Author contribution

MA contributed in the conception of the work, conducting the study, drafting and revising the draft and approval of the final version of the manuscript. H KhA contributed in the conducting the study and revising the draft and approval of the final version of the manuscript. SHA contributed in the conception of the work, conducting the study, drafting and revising the draft and approval of the final version of the

manuscript. MAN contributed in the drafting and revising the draft. MAM contributed in the conducting the study and drafting and revising the draft. SHA contributed in the drafting and revising the draft and analyzing of data.

Compliance with ethical standards

All procedures performed in studies were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Funding

This research received no specific grant from any funding agency in the public, commercial, or not for profit sectors.

Financial support and sponsorship

Nil.

Declaration of competing interest

There are no conflicts of interest.

Acknowledgments

Authors would like to Acknowledge Vice-chancellor of Skin Diseases and Leishmaniasis Research Centre, Isfahan University of Medical Sciences for approval of the present study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.micpath.2019.103892.

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