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Resolution and Pro-resolving Lipid Mediators in *Leishmania* Infection

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ABSTRACT

The acute inflammatory response is the body's natural reaction to inciting stimuli, including trauma and pathogens. Well-known pro-inflammatory metabolites take control of this reaction to recruit the leukocytes into the inflamed tissue. These cells professionally ingest and kill the invading pathogens and clear the debris of dead or injured cells. This further signals the tissue regeneration and gain of function, another active process mediated by newly uncovered anti-inflammatory metabolites that downregulate the active inflammation. These molecules and their cognate receptors are novel targets for the treatment of chronic inflammatory diseases. Although not very well understood, these mediators are suspected of supporting intracellular parasite survival (as *Leishmania* parasite) and are worth further investigation for innovative therapeutic interventions.

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Leishmaniasis: the problem

Leishmaniasis is a vector-borne disease that remains an unresolved health problem of moderate to low-income countries around the world. According to the latest reports by WHO, an estimated 700,000 to 1 million new cases of leishmaniasis occur per year leading to 20,000 to 30,000 annual deaths (https://www.who.int/en/news-room/fact-sheets/detail/leishmaniasis). The causative agent is a unicellular parasite of *Leishmania* genus which is transmitted to the vertebrate host by infected female phlebotomine sand flies. The parasite species, host-parasite interaction, and saliva contents of each sandfly species are the main factors that together determine the outcome of the disease [1]. Major clinical manifestations of the disease are categorized as cutaneous leishmaniasis (CL), visceral leishmaniasis (VL) and mucocutaneous leishmaniasis (MCL) [2]. CL is the non-fatal form of the disease characterized by the local skin ulcers that heal spontaneously or after regular treatment, but after healing, the lesions turn to scars forever [3]. VL is the less common but more severe form of the disease characterized by the parasite dissemination into visceral organs and is fatal if left untreated [4]. MCL is the most severe form with irreversible disfiguring damage to the nasopharynx and sometimes laryngopharynx tissues. MCL is secondary to the CL infection in the new world and is characterized by destructive chronic inflammatory response and hyperactivity of the immune cells while the parasite is absent in the inflamed tissue [5]. Diffused Cutaneous Leishmaniasis (DCL) [6] and Post Kala-Azar Dermal Leishmaniasis (PKDL) [7] are the least common forms detected only in some endemic areas and are characterized by dispersed non-ulcerating parasite-full nodules all over the body due to a suppressed immune response.

The promising point about leishmaniasis is that most of the inhabitants in endemic areas can tolerate the infection without any signs and symptoms (asymptomatic), meaning that an effective prophylactic vaccine is a hope, not hype. However, the lack of knowledge around the details of the host-parasite interaction has delayed the prophylactic vaccine appearance in the market. In this respect, the present report reviews the inflammation and the resolution process with particular attention to the lipid mediators that direct both events and the critical role they might play in *Leishmania* infection.

Acute inflammation and *Leishmania* infection. Acute inflammation is a physiological protective reaction to trauma and pathogenic organisms. Obviously, without inflammation, the injured tissue will never heal, and the pathogens will go unchecked. In the acute inflammation scenario, pro-inflammatory cytokines (including TNF-α and IL-1β) and chemokines (such as CXCL-8 and CCL-2) collaborate with arachidonic acid (omega-6 polyunsaturated fatty acid) derived lipid mediators including prostaglandins (like PGE2), leukotrienes (like LTB4) and thromboxanes (like TXA2) for vasodilation and leukocyte recruitment. Polymorphonuclear (PMNs) and in particular neutrophils are the major cell types that are recruited early after inflammation and extravasate in

response to the aforementioned inflammatory mediators followed by the monocytes that mature into inflammatory macrophages.

In most cases, neutrophils kill the pathogens and macrophages clear the tissue debris and trigger proper resolution.

Neutrophils play a pivotal role in *Leishmania* inflammation. Typically, the lifespan of the neutrophils in the blood and in the steady state is relatively short but extends to a few days in inflammatory conditions [8]. During inflammation, they infiltrate the trauma or infection site within a few hours and return to basal levels 48-72 h after extravasation. However experimental models with *Leishmania major* has shown that parasite inoculation with saliva, not only augments the number of neutrophils recruited but also prolongs the presence of the neutrophils up to 7-8 days after infection showing persistent infiltration [9].

During a blood meal uptake and for a successful blood sucking, the sandfly inoculates saliva, which encompasses different mediators to deregulate blood coagulation, hemostasis, and immune response activation [10]. Simultaneously, the saliva transmits the metacyclic promastigotes of the parasite into the epidermis of the vertebrate host. Early after injection and within a few hours, the obligatory intracellular parasites appear inside the neutrophils which are the sentinels of the innate immune response [11, 12] and are massively recruited to the bite site mainly because of the chemoattractants of the host, saliva [13] and Promastigote Secreted Gel (PSG) [14]. Neutrophils are specialized to kill the invaders using an array of mechanisms; however, some *Leishmania* species have evolved to skip these killing mechanisms and to resist the hostile environment inside the neutrophils until the monocytes enter the site [15, 16]. Neutrophils undergo apoptosis within a few days after extravasation then the newly arrived macrophages and dendritic cells uptake the apoptotic bodies with the parasites inside [17]. This "Trojan Horse" entry known as efferocytosis downmodulates all anti-parasitic activities within the cells, polarizes macrophages toward M2 [18] subtypes and eventually ends in Th2 [19] response which favors further disease progression. Macrophages are the only cell types serving as the host for *Leishmania* where the promastigotes are able to transform into amastigotes and to start proliferation. Paralyzed macrophages finally burst and released amastigotes start a new cycle by infecting nearby macrophages or recycle back into the sand fly midgut by the next blood meal.

Resolution of inflammation and tissue regeneration: Active and not passive. The clearance of the initial stimuli (like pathogens) signals tissue regeneration, which is no longer considered a simple passive event following the degradation of the inciting inflammatory signals [20]. Instead, newly introduced lipid metabolites, known as pro-resolving mediators, actively take over the resolution phase [21-23]. Apoptosis of neutrophils is the central event in this respect that signals resolution [24, 25]. The "find me eat me" signals on the surface of apoptotic neutrophils are recognized by receptors on macrophages [26, 27]. Transmitted signals then promote phagocytosis, enhance the clearance of apoptotic bodies (efferocytosis), and increase the production of IL-10 and TGF-β by converting the phenotype of macrophages to pro-resolving [28]. Apoptotic neutrophils are the source of lipoxin A4 (LXA4) generated from arachidonic acid under the resolving condition, which means a class-switch in lipid profile from pro-inflammatory to pro-resolving [29, 30]. LXA4, generally decreases neutrophil activity, ROS formation, NF-kβ related gene expression and synthesis of pro-inflammatory mediators especially neutrophil recruiting cytokines (such as CXCL8) and promotes phagocytosis of apoptotic neutrophils and IL-10 production [31, 32]. Moreover, LXA4 stimulates the production of other pro-resolving lipid mediators from omega-3 polyunsaturated fatty acids. Docosahexaenoic acid (DHA) gives rise to D-series of resolvins (like RvD1), protectins/neuroptotectins (like PD1 or NPD1) and maresins (like Mar1) while Eicosapentaenoic acid (EPA) gives rise to E-series of resolvins (Like RvE2) [33].

The most crucial role of pro-resolving mediators is the induction of efferocytosis which leads to the reprogramming of macrophages from pro-inflammatory M1 cells to anti-inflammatory M2 cells [34, 35], tissue regeneration and regulation of the subsequent adaptive immune response [36, 37]. Any failure in the timely production of pro-resolving lipid mediators and regulation of acute inflammation leads to the chronicity of inflammation and persistent neutrophil influx. Therefore, the fate of the acute inflammation could be either "complete resolution" with full regeneration of the tissue and gain of function in the presence of active lipid mediators or "chronic inflammation" followed by loss of function and tissue damage in the absence of lipid mediators [38]. The scar formation, typically induced by *Leishmania*, falls between full resolution-tissue regeneration and chronic inflammation. The underlying reason is not very well known but might be attributable to the persistent stimuli at the injured site [39].

Pro-resolving lipid mediators in *Leishmania* infection. The pro-inflammatory lipid mediators and their pivotal role in *Leishmania* infection have been under intensive investigation for years [40]. In contrast, the role of pro-resolving lipid mediators in *Leishmania* infection is not yet fully uncovered because the discovery of these mediators dates back to the year 2000 after lipid metabolomics advancement [41]. For the moment, our understanding is similar to an incomplete puzzle that more pieces should be put together for completion.

Wenzel *et al.* first indicated that the human neutrophils pretreated with LXA4 and then infected with *L. major* are more permissive for *Leishmania* growth and survival. As previously explained, LXA4 actively deregulates the inflammatory functions of neutrophils, which is the pre-requisite for parasite survival and further promotes efferocytic phagocytosis and disease establishment [42]. Recently, Laskay *et al.* have demonstrated an early lipid profile change within the first 6 hours after *in vitro* infection of primary human neutrophils with *L. major*. During this early time point, neutrophils release more LTB4 and less LXA4 in concordance with the requirements of *Leishmania* infection. LTB4 is a potent lipid mediator that recruits

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neutrophils to the inflammation site. In contrast, LXA4 restricts neutrophil transmigration and reduces ROS metabolites. Thus at the early time points after infection, the parasite takes advantage of the augmented recruitment of neutrophils disregarding the killing activity [43].

From this evidence, one can infer that the parasite potentially modulates the lipid profile inside the neutrophils respecting the time post infection. Whether the parasite-derived polyunsaturated fatty acid metabolites (PUFAs) are directly responsible for the host lipidome modulation or indirectly impact host PUFA production, is a matter of further investigation. A recent study by Paloque *et al.* on the biosynthesis of polyunsaturated lipid metabolites by *Leishmania infantum* parasite indicated that the concentration of the pro-resolving lipid mediators' precursors was significantly higher in infectious stages of the *L. infantum* promastigotes compared to the noninfectious stage parasite. Macrophages treated with the lipid extracts of the infectious stage, differentiated into M2 instead of M1 cells producing more IL-10/TGF-β than IFN-γ, respectively besides elevated production of pro-resolving lipid mediators [44]. Furthermore, *L. infantum* is among the parasite species that can induce NETosis of neutrophils by inducing LXA4 receptors [45] while is naturally resistant to this killing mechanism of neutrophils [46]. It is hypothesized that the *Leishmania*-induced anti-inflammatory LXA4 receptor signaling augments efficient phagocytosis and promotes intracellular survival of promastigotes [42]. Other *Trypanosoma* species form Trypanosomatida family such as *Trypanosoma cruzi* have been proved to produce RvD1, RvD5, and RvE2 [47]. So production of pro-resolving lipid mediators could be an inbuilt ability within the whole family (including *Leishmania* species) which remains to be adequately addressed.

Another piece of evidence regarding the role of pro-resolving lipid mediators in *Leishmania* infection comes from the study of Malta-Santos *et al.* in an endemic area for tegumentary leishmaniasis in Brazil. This study indicates an increased level of RvD1 metabolite in plasma samples of LCL (Localized Cutaneous Leishmaniasis) patients versus DCL suffering individuals (~100 folds). The number of lesions in DCL patients well correlated with the plasma levels of the RvD1 molecule. Infection of the peripheral blood monocyte-derived human macrophages with *Leishmania amazonensis* in the presence of RvD1 showed further improved parasite persistence. They have postulated that the parasite modulates the endogenous RvD1 production by macrophages early after infection [48].

CONCLUSION

Pro-resolving lipid mediators are a group of newly unveiled polyunsaturated fatty acid derivatives with plenty of anti-inflammatory and resolution-promoting functions. Although they have attracted much attention in different inflammatory diseases, their specific role in the chronicity of infectious diseases like leishmaniasis remains to be adequately addressed. As explained so far, during natural hemostasis, specialized pro-resolving lipid mediators potentially downmodulate the acute inflammation and initiate the resolution process until functional tissue regeneration. The sandfly-transmitted *Leishmania* parasites are likely evolved to benefit the natural hemostasis process for chronic disease establishment in the absence of inflammation. We still need to validate the data presented in this review with different parasite species and even relevant transmitting sandflies and to correlate the results with the different manifestations of the disease including asymptomatic, LCL, MCL, VL, etc. Lipidomics analysis using LC-MS (liquid chromatography-mass spectrometry) technology can shed light on the role of these metabolites in different stages of the infection in respect to host/parasite/sandfly saliva interactions and modulation of the immune response. This could hopefully pave the way toward novel therapeutic interventions in the future.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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