Leishmania donovani promastigote Lipophosphoglycan interaction with macrophage Toll-like receptor 4 modulates parasite internalization and macrophage dysfunction

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CHAPTER- VII

CONCLUSION AND FUTURE PROSPECTS

7.1 Conclusion

Based on this study, we concluded following points that relates with the underlying molecular mechanism in *L. donovani* promastigote internalization through LPG-TLR4 interaction, (i) the identification of specific molecular patterns which facilitates this process, (ii) the role of TLR4 receptor in the establishment of *L. donovani* promastigote infection, and (iii) association of parasite entry in macrophages with its functional status. Moreover, our study unveiled the importance of organ specific role of TLR4 particularly focusing its association with hepatic AHSG levels and the establishment of *L. donovani* infection in BALB/c mice model. Further, we investigated the anti-leishmanial efficacy of imidazo[1,2-α] pyridine derivatives, particularly IMPA-2 and IMPA-12, for their anti-proliferative and pro-apoptotic properties against *L. donovani* promastigotes.

1. In this study, we identified macrophage toll-like receptor 4 (TLR4) as a novel receptor for the recognition of lipophosphoglycan (LPG) of *L. donovani* promastigotes and such interaction play a pivotal role in parasite burden in macrophages. We also discovered the specific molecular patterns in TLR4 that facilitates this process. To investigate the mechanism of *L. donovani* entry inside the macrophages, we have found that the parasites LPG interacted with the macrophage TLR4 leading to parasite uptake without any significant alteration of macrophage cell viability. Increased burden of parasites within the macrophages strikingly inhibits LPS-mediated NF-κB activation and pro-inflammatory cytokines gene activation. Silencing of macrophage TLR4 or blocking of parasite LPG markedly prevents parasite load within the macrophages. Interestingly, we observed a significant enhancement of macrophage migration, and generation of reactive oxygen species (ROS) in the parasite-infected TLR4 silenced macrophages, whereas, parasite infection in TLR4 overexpressed macrophages exhibited a notable reduction of macrophage migration and ROS generation. Moreover, mutations at the leucine-rich repeats (LRRs), particularly LRR5 and LRR6, notably prevent TLR4 interaction with LPG thus inhibiting intra-macrophagic parasite load. Collectively, our study revealed that *L. donovani* through its LPG interacted with the LRR5/LRR6 of

macrophage TLR4 that facilitates parasite entry and favors parasite burden in macrophages, along with the impairment of macrophage functions.

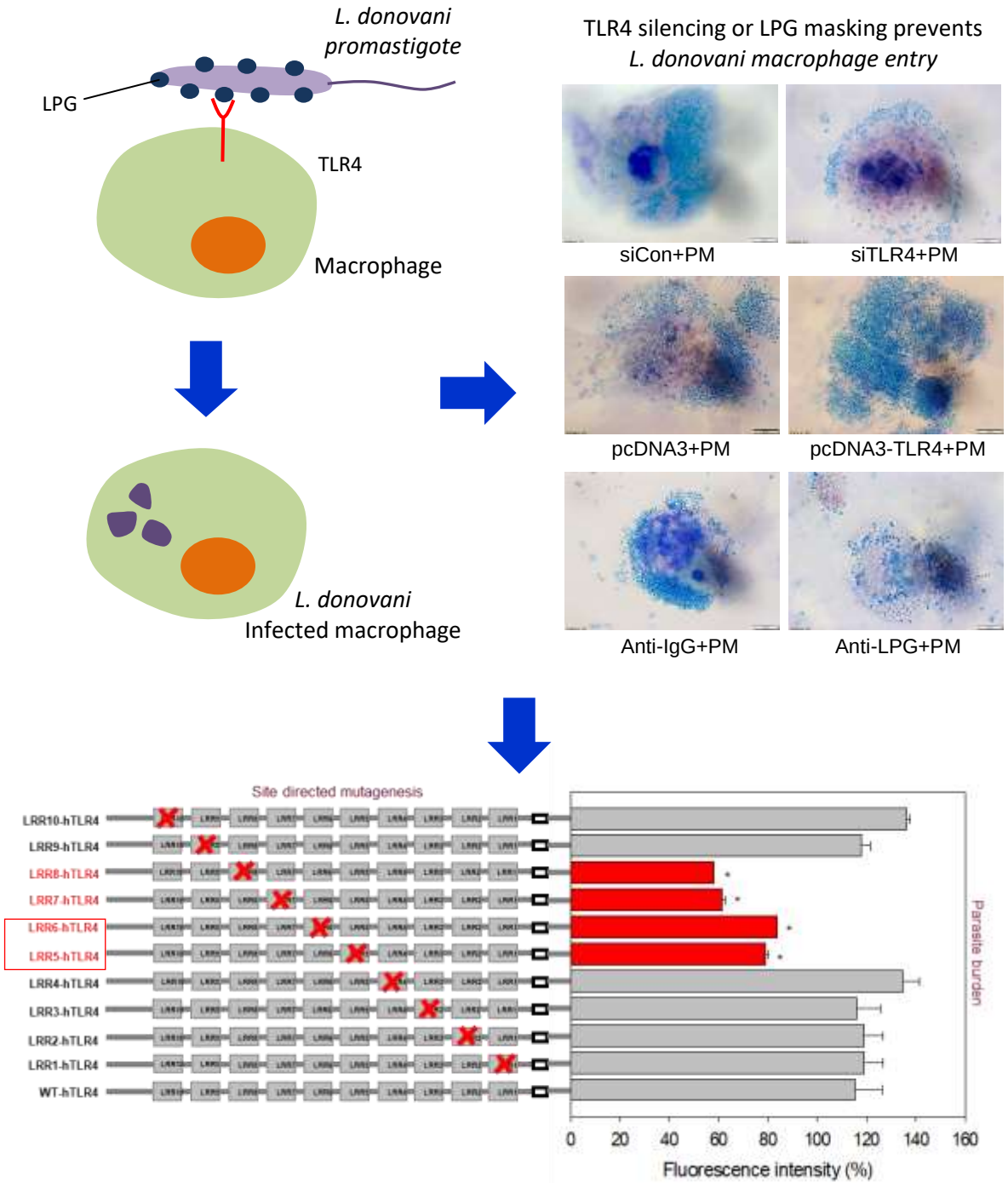

Fig 7.1 Schematic model of *L. donovani* LPG-TLR4 interaction-mediated parasite internalization.

2. We created a BALB/c mouse model to evaluate experimental data regarding the organ-specific role of TLR4 on the establishment of *L. donovani* infection in the liver. We discovered that LPS stimulation significantly increased parasitic burden in the liver, whereas TLR4 antisense oligonucleotide (ASO) and Cli-095 significantly modulate parasite burden in the liver, critically associated with alteration of inflammatory cytokine expression and signaling pathways. We discovered that *L. donovani* infection enhanced the expression of AHSG in the liver. While systemic TLR4 silencing or the inhibition of TLR4 activation significantly decreased AHSG expression, and the stimulation of TLR4 by LPS significantly boosted AHSG levels in the liver. The finding shows that the development of *L.donovani* infection may be associated to hepatic AHSG expression. To further investigate in *in-vitro* macrophage cell culture model, we found that AHSG knockdown in macrophages drastically reduced parasite numbers while considerably increased macrophage ROS levels. All of these findings suggest that macrophage AHSG could able to influence *L. donovani* survival in macrophages.

3. We investigated the efficacy of different imidazo[1,2-] pyridine (IMPA) derivatives and found that IMPA-2 and IMPA-12 have notable anti-leishmanial properties. Moreover, our investigation showed that IMPA-2 and IMPA-12 were effective against *L. donovani* promastigotes due to their anti-proliferative and pro-apoptotic properties against the parasite. Promastigote proliferation inside host macrophage cells is hampered by oxidative stress and apoptosis, both of which were considerably enhanced by IMPA-2 and IMPA-12. Evidence also suggested that IMPA-2 is more effective in preventing the proliferation of parasites, the generation of ROS, the malfunction of mitochondria, the suppression of the cell cycle at the G0/G1 phase, and the promotion of apoptosis as compared to IMPA-12.

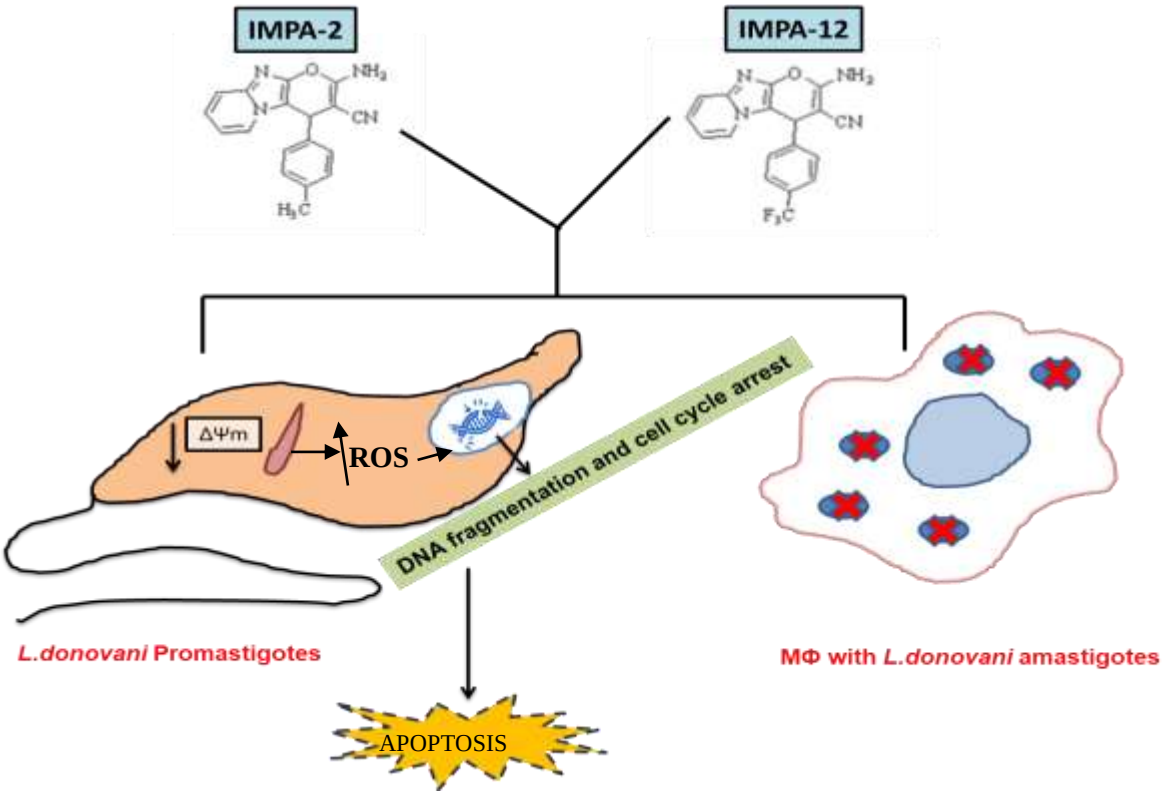

Fig 7.2 Illustration of anti-leishmanial efficacy of IMPA derivatives on *L. donovani* promastigotes.

7.2 Future prospects

1. Present study provides a novel insight about the specific relevance of TLR4 in macrophage infection (recognition and entry) by *L. donovani* as well as in the downregulation of macrophage function. Therefore, targeting LPG-TLR4 pathway could provide a novel therapeutic option for the management of *L. donovani* infection.

2. Moreover, identification of specific molecular motifs in *L. donovani* LPG that involved in the recognition of LRR5 and LRR6 of TLR4 could also provide us a better insight about developing targeted therapeutic interventions in coming days to combat visceral leishmaniasis.

3. From both *in-vivo* and *in-vitro* experimental evidences, we found that *L. donovani* infection increased the expression of AHSG in the liver. While, systemic TLR4 silencing or inhibition of TLR4 activation significantly decreased AHSG expression, and the stimulation of TLR4 by LPS significantly elevated AHSG levels in the liver. From these findings, we concluded that there is a significant role of AHSG protein in the establishment of infection mediated by *L. donovani*. However, the exact molecular mechanism and the participation of AHSG protein in the establishment by *L. donovani* infection is currently unknown and therefore future investigations in this direction is needed to explore the role of macrophage AHSG protein in the regulation in *L. donovani* mediated VL.

4. The effectiveness of IMPA derivatives, especially IMPA-2 and IMPA-12, for their anti-proliferative and pro-apoptotic activities against *L. donovani* promastigotes was emphasized in the current study. Both IMPA-2 and IMPA-12 are effective in preventing the proliferation of parasites, the generation of ROS, the malfunction of mitochondria, the suppression of the cell cycle at the G0/G1 phase, and the promotion of apoptosis. In order to combat VL, IMPA-2 and IMPA-12 may be employed as viable treatments. Further studies both in-vitro and in-vivo needed for the identification of specific molecular targets of IMPA-2 and IMPA-12 in the management of VL.