PUBLICATIONS

A. List of publication (from thesis work):

1. **Mazumder, S.**, Sinha, A., Ghosh, S., Sharma, G.C., Prusty, B.M., Manna, D., Pal, D., Pal, C. and Dasgupta, S., 2023. Leishmania LPG interacts with LRR5/LRR6 of macrophage TLR4 for parasite invasion and impairs the macrophage functions. ***Pathogens and Disease***, 81: 1-14, ftad019.

2. **Sayani Mazumder**, Archana Sinha, Soumyajit Roy, Monalisa Ray, Mousumi Das, Deepronil Roy, Durba Pal, Chiranjib Pal, Suman Dasgupta. Modulation of TLR4 expression and its activity govern L. donovani infection in liver by regulating AHSG expression (Manuscript Under preparation).

3. **Sayani Mazumder**, Kumari Bhavya, Manohar Mantipally, Rambabu Gundla, Durba Pal, Suman Dasgupta. Investigating the therapeutic efficacy of Imidazo[1,2-α] pyridine derivatives on the growth inhibition and apoptotic cell death in L. donovani promastigotes (Manuscript Under preparation).

B. Other publication in peer-reviewed international journal:

1. Choudhary, S.A., Patra, D., Sinha, A., **Mazumder, S.**, Pant, R., Chouhan, R., Jha, A.N., Prusty, B.M., Manna, D., Das, S.K. and Tikoo, K., 2023. A small molecule potent IRAK4 inhibitor abrogates lipopolysaccharide-induced macrophage inflammation in-vitro and in vivo. ***European Journal of Pharmacology***, 944: 175593.

C. Conferences and workshops attended:

1. Presented a **poster** on "*L. donovani* LPG interacts with macrophage TLR4 for parasite invasion and impairment of macrophages functions" in **EMBO Workshop** on **"Pathogen Immunity and Signalling"** held at Saint-Malo, France during 4th-8th April, 2022.

2. Participated in **"2 days Workshop cum Training Program on Ribosome and Translational Techniques"** organized by Department of Molecular Biology and Biotechnology, Tezpur University on 25-26 November 2017.

3. Presented a poster on the topic "*L. donovani* LPG interacts with macrophage TLR4 for parasite invasion and impairment of macrophages functions" at **48th Annual**

Conference of Indian Immunology Society on **"Infections, Vaccines & Immuno-innovations for Human Health"** conducted by Department of Molecular and Human Genetics, Banaras Hindu University, Varanasi from 8th-9th July, 2022 **(Virtual)**.

4. Participated in **Virtual Workshop** entitled as **"Introduction to Pathogen Data"** conducted by **NCBI** on August 4, 2022.

5. Participated in **Virtual Workshop** entitled as **"An NCBI Guide to Finding and Analyzing Metagenomic Data"** conducted by **NCBI-NLM** on October 25, 2022.

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Research Article

Leishmania LPG interacts with LRR5/LRR6 of macrophage TLR4 for parasite invasion and impairs the macrophage functions

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Abstract

Visceral leishmaniasis (VL) is a severe form of leishmaniasis, primarily affecting the poor in developing countries. Although several studies have highlighted the importance of toll-like receptors (TLRs) in the pathophysiology of leishmaniasis, the role of specific TLRs and their binding partners involved in *Leishmania donovani* uptake are still elusive. To investigate the mechanism of *L. donovani* entry inside the macrophages, we found that the parasite lipophosphoglycan (LPG) interacted with the macrophage TLR4, leading to parasite uptake without any significant alteration of macrophage cell viability. Increased parasite numbers within macrophages markedly inhibited lipopolysaccharide-induced pro-inflammatory cytokines gene expression. Silencing of macrophage-TLR4, or inhibition of parasite-LPG, significantly stemmed parasite infection in 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 in the leucine-rich repeats (LRRs), particularly LRR5 and LRR6, significantly prevented TLR4 interaction with LPG, thus inhibiting cellular parasite entry. All these results suggest that parasite LPG recognition by the LRR5 and LRR6 of macrophage-TLR4 facilitated parasite entry, and impaired macrophage functions. Therefore, targeting LRR5/LRR6 interactions with LPG could provide a novel option to prevent VL.

Keywords: lipophosphoglycan, macrophage, toll-like receptor 4, leucine-rich repeats, visceral leishmaniasis

Introduction

Leishmaniasis is a vector-borne infectious disease, caused by an unicellular protozoan parasite of genus *Leishmania* (Burza et al. 2018), underpinning three major clinical manifestations of cutaneous, mucocutaneous and visceral forms (Ghosh et al. 2023). Predominantly found in tropical and subtropical regions, leishmaniasis affects millions of people, with more than 90% of the visceral leishmaniasis cases found in the Indian subcontinent and East Africa (Alvar et al. 2012, Kal et al. 2017, Cunze et al. 2019). *Leishmania* exists in two different forms in the course of its life cycle, the extracellular flagellated promastigote form, and the intracellular amastigote form (Burza et al. 2018). The bite of an infected female phlebotomine sandfly transmits the promastigotes into the vertebrate host cells macrophages, where they differentiate into amastigote forms and replicate inside the parasitophorous vacuole (Bates 2018). The emerging incidence of leishmaniasis and the growing concern of drug resistance against the available therapeutics (Mukherjee et al. 2020) demand the development of novel therapeutic strategies for countering this insidious disease.

During the course of infection, recognition and internalization of parasites by the macrophages are the first and most crucial steps for the development of leishmaniasis (Goto and Mizobuchi 2023). As a principal host cell for *Leishmania* infection, macrophages play a critical role in post-infection outcomes that either facilitate the killing or survival of parasites. While macrophages have countered parasite infection by employing different antimicrobial mechanisms from their cellular arsenal, the parasites have evolved various strategies to circumvent and evade the host antimicrobial response (Duque and Descoteaux 2015, Goto and Mizobuchi 2023). Parasites entry inside the macrophage is a complex and multistep process that involves interactions of membrane-associated molecules between promastigotes and macrophages (Gurung and Kanneganti 2015). It has been shown that several cell surface receptors on the macrophage plasma membrane could recognize the cognate ligands of the parasite (Dens and Wilson 2012). The involvement of macrophage toll-like receptors (TLRs) in pathogen recognition and contribution to immune response are well established (Akira and Takeda 2004, Sefat El-Zayat et al. 2019, Fitzgerald and Kagan 2020). In this context, it has been demonstrated that *Leishmania major* parasites are primarily recognized by the macrophages TLR2, TLR4 and TLR9 (Tuon et al. 2010). Studies on the involvement of TLRs in

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