REPORT

Report on participation of the ICMR International Fellow (ICMR-IF) in Training/Research abroad.

1. Name and designation of ICMR- IF : **DR. SOMADITYA DEY, ASSISTANT PROFESSOR IN ZOOLOGY, WEST BENGAL EDUCATION SERVICE**.

2. Address : **BARASAT GOVT. COLLEGE, 10, K.N.C. ROAD, BARASAT, NORTH 24 PGNS., KOLKATA-700124, WEST BENGAL**.

3. Frontline area of research in which training/research was carried out : The frontline area of research was focused on the establishment of the Sand Fly- bite initiated experimental murine Visceral Leishmaniasis (VL) and, Cutaneous Leishmaniasis (CL) models, and utilising these systems to explore the kinetics of parasite tropism at the bite site and the visceral organs and the effect of vector-derived and/or host factors affecting the establishment and severity of the disease. We have explored the mechanistic relationship between sand fly (SF) challenge, visceralization of parasites and the pathogenicity of active leishmaniasis at the molecular and cellular level in scenarios that mimic natural situations.

4. Name & address of Professor and host institute : **Shaden Kamhawi, PhD Leishmaniasis Group Leader, Associate Scientist, Vector Molecular Biology Section, Laboratory of Malaria and Vector Research, NIAID, NIH; 12735 Twinbrook Parkway, Rockville, MD 20852, Tel. 301-761-5081; Email: skamhawi@niaid.nih.gov**

5. Duration of fellowship with exact date : One Year (15.03.2023-05.03.2024)

6. Highlights of work conducted :

   i) Technique/expertise acquired :

This training program has allowed me to acquire in-depth knowledge of both fundamental and cutting-edge technologies related to vector molecular biology. These include culturing of *Leishmania* parasites; SF infections with *Leishmania* parasites by artificial blood feeding; dissecting the midgut of SFs; identification of various developmental stages of the parasite in the sand fly midgut including infectious metacyclic promastigotes; undertaking natural vector-transmission of various *Leishmania* parasites to mice models of infection by SF bites; handling infected animals including aseptic organs and tissue processing, cell recovery from different

tissues and preparation for analysing using 10-color multiplex flow cytometry, DNA/RNA isolation, qPCR, ELISA, and western blot.

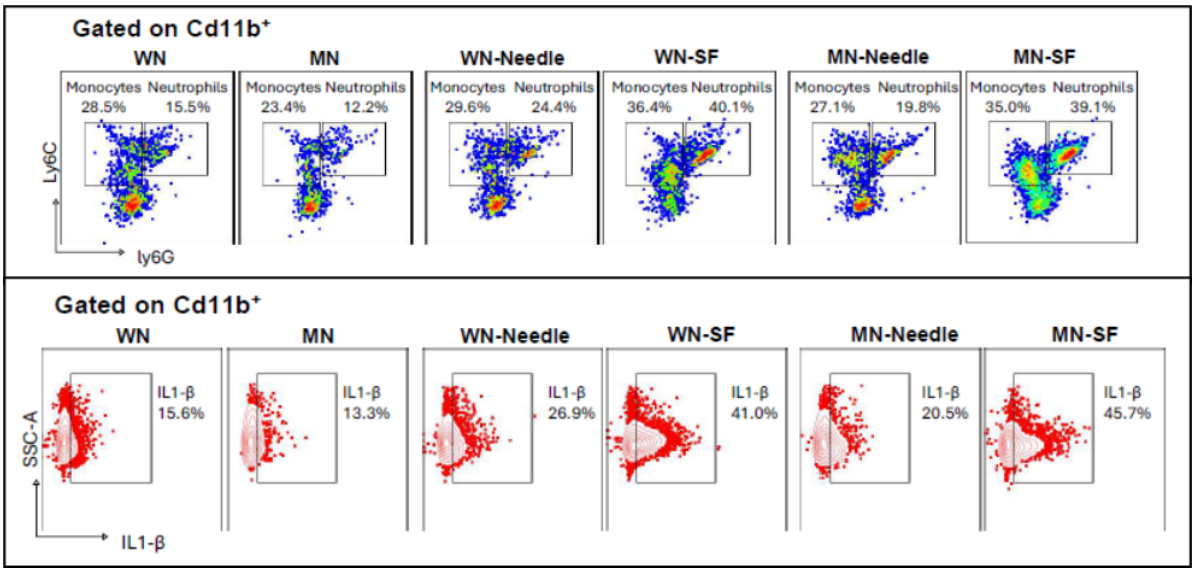
ii) Research results, including any papers, prepared/submitted for publication :

I got the opportunity to participate and contribute to two ongoing projects in Dr. Shaden Kamhawi's group (NIH/NIAID). Specifically, project 1 focused on investigating the effect of malnutrition on pathogenicity and dissemination of *L. donovani* using a mouse model of infection, and project 2 on activation of inflammasomes in the murine models of SF-transmitted experimental cutaneous leishmaniasis.

Project 1: Sand fly-initiated infections affect pathogenicity and dissemination of *L. donovani* in malnourished animals

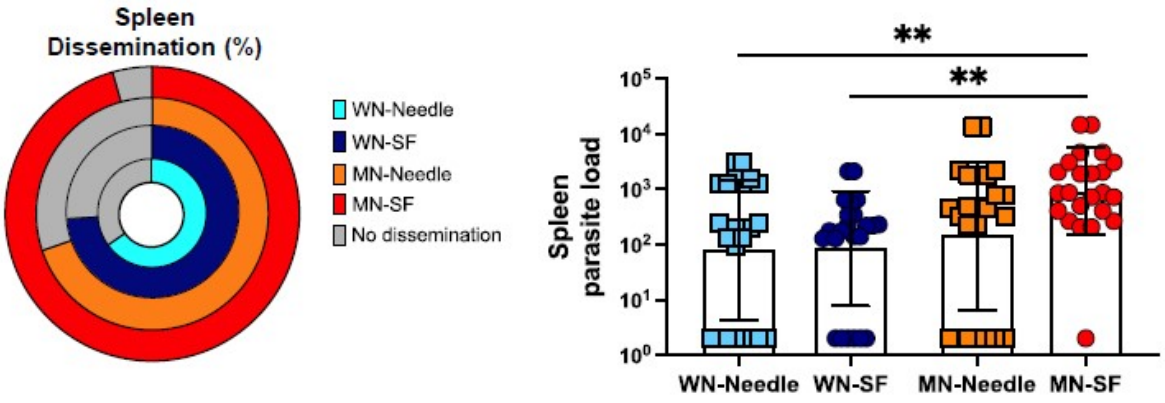
The innate inflammatory immune response at the bite site of vector-transmitted and needle-initiated *L. donovani* infections: We explored the early innate immune response in well-nourished (WN) and malnourished (MN) BALB/c mice, after a SF bite- or needle-initiated infection with *L. donovani*. The experimental WN and MN mice models were established following the diets and feeding protocols, as described previously (Ibrahim et al, 2014).

Flow cytometry analyses, gated on neutrophils and inflammatory monocytes recovered from ear cells showed the strong and persistent nature of neutrophil recruitment to bite sites at 24 hour (hr) following WN-SF or MN-SF, compared with a feeble and transient infiltration after needle-initiated infections. Monocyte recruitment was also found to be significantly higher at 24 hr in the WN-SF animals, compared to the WN-needle mice, potentially due to the SF-derived factors.

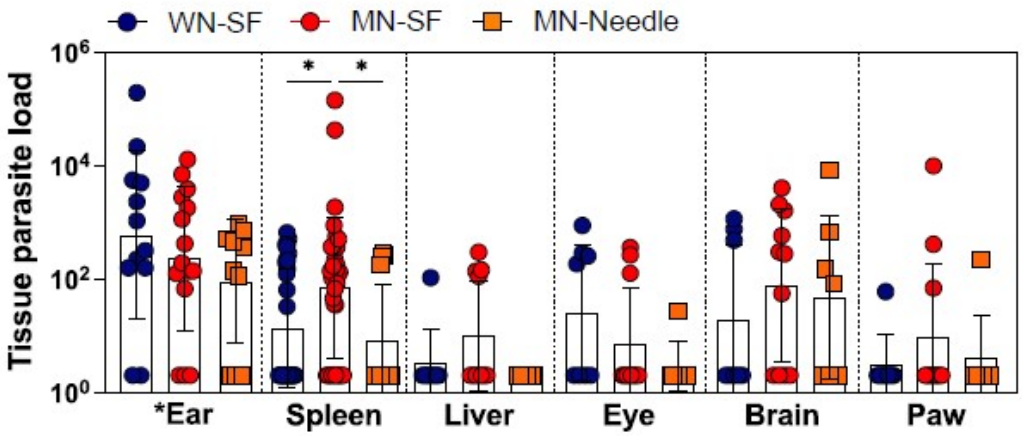


The significantly higher induction of IL1β from $CD11b^{+}$ myeloid cells at 48 hr in WN-SF or MN-SF mice, compared with needle- initiated infections, suggested that sand fly bites may activate the inflammasome complexes (unpublished data; Iniguez et al, 2024).

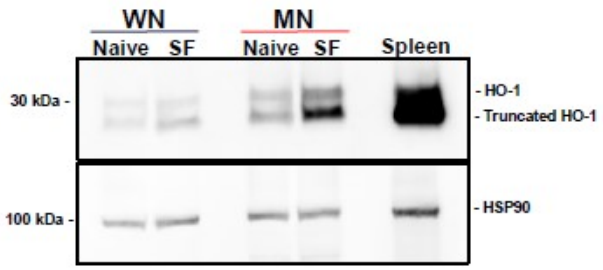
Early parasite dissemination of vector-transmitted and needle-initiated *L. donovani* infection from the site of inoculation (skin) to visceral organs (spleen) in mice: MN-SF mice showed greater *L. donovani* dissemination to the spleen (95.7% of animals) at 72 hr post-infection as compared with WN-SF (73.9%), MN-needle (60.6%) and WN-needle (65.2%) mice. Notably, the parasite burden in the spleen (Splenic parasite load) was significantly increased (**, $P \leq 0.01$) in MN-SF mice as compared to the WN-SF and WN-needle mice (unpublished data; Iniguez et al, 2024).

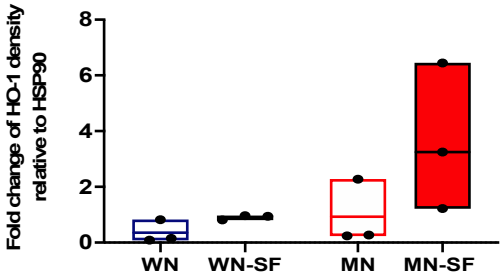


Pathogenicity of VL after infected sand fly bites in chronically infected MN-SF mice: We developed a novel model of chronic malnutrition and assessed parasite dissemination and parasite burden in various tissues by quantitative PCR targeting kDNA up to 30 weeks post-infection. MN-SF mice maintained a significantly higher parasite burden (* $P \leq 0.05$). in the spleen compared to WN-SF or MN-needle mice, suggesting that nutrient-deficiency may contribute to parasite dissemination in the malnourished hosts in infections initiated by sand fly bites (unpublished data; Iniguez et al, 2024). Interestingly, parasites disseminated to other organs including the brain, the eyes, and paws in all the groups but at a higher proportion in MN-SF mice.



Production of Heme Oxygenase-1 in the skin of MN-SF mice: Heme oxygenase-1 (HO-1), a pleiotropic cytoprotective enzyme that diminishes heme-mediated tissue damage, plays a critical role in controlling skin inflammation after infected SF bites (DeSouza-Vieira et al, 2020). To see the effect of malnutrition on its induction, we measured HO-1 levels in ear lysates by immunoblotting at 72 hr after exposure to sand fly bites. We reinforced the observation that HO-1 induction is a key response to bites of infected SFs. However, compared to the WN-SF, the HO-1 level was substantially higher in the MN-SF group. The surge in HO-1 may alter the inflammatory response in these experimental group of animals (unpublished data; Iniguez et al, 2024). Moreover, HO-1 was produced at higher levels in steady state unchallenged MN ears suggesting that a basal level of inflammation in MN animals is further exacerbated by infected sand fly bites.





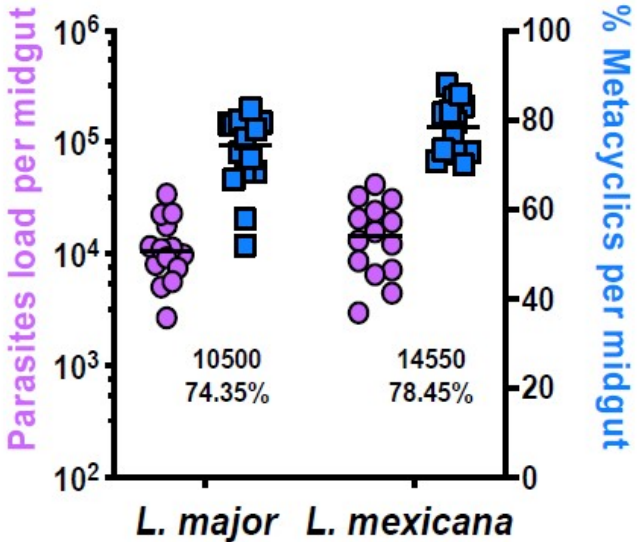
Project 2: Activation of inflammasomes in the murine models of SF-transmitted experimental murine cutaneous leishmaniasis

In this study, we investigated the early immune responses of C57/BL6 mice to *L. mexicana*- and *L. major*- infected sand fly bites by:

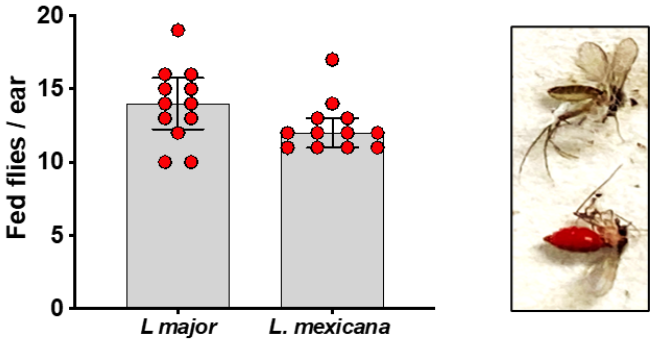
- Assessing vector competency of *L. mexicana* in *Lu. longipalpis* infected-sand flies
- Studying the recruitment of innate immune cells & the induction of IL1β after infected sand fly bites
- Exploring the activation of inflammasome mediators after transmission of *L. mexicana* and *L. major* by *Lu. longipalpis* sand flies

Briefly, *Lu. longipalpis* sand flies were infected with either *L. major* or *L. mexicana* parasites by artificial blood feeding, and the parasites were transmitted to C57/bl6 mice by the bites of infected SFs (1-2 hr in dark). 20 flies per ear were placed for 1-2 hr. After 6 hr, the mice were sacrificed and the cells were collected for flow cytometry, and whole cell lysates were collected for western blot analyses (DeSouza-Vieira et al, 2020). The early immune response to *L. mexicana*-infected *Lu, longipalpis* ($LmxSF_i$) to *L. major*-infected ($LmjSF_i$) bites in mice, were compared.

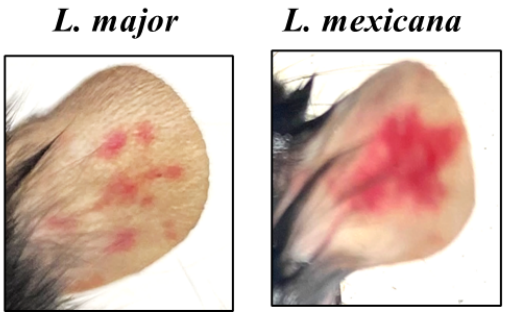
Comparable mature infections in *L. mexicana* and *L. major- infected Lu. longipalpis* sand flies: The total number of parasites per midgut (10,500 and 14,500 respectively) and the percent metacyclic promastigotes (73.5% and 78.5%, respectively) from *Lu. longipalpis* midguts infected with *L. major* or *L. mexicana* were comparable. This demonstrates that *Lu. longipalpis* can develop mature and transmissible infections with both *L. major* and *L. mexicana.*



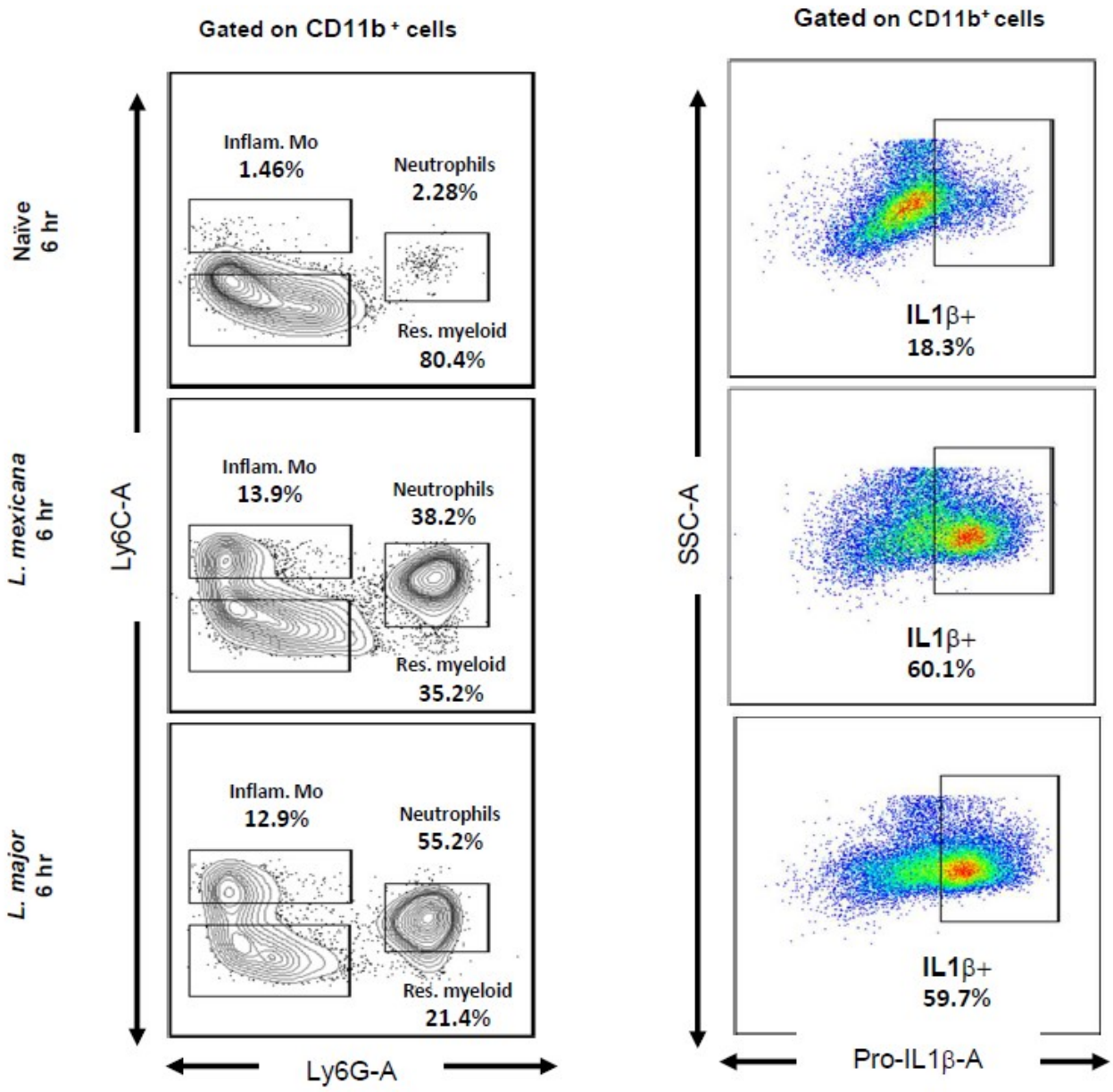
Feeding score after parasite transmission to mouse ears: The numbers of fed flies per ear were also comparable between *L. major*- and *L. mexicana*- infected SFs; but notably, the inflammation and severity of the skin lesion, characterized by acute redness and bleeding, was found to be much more prominent after *L. mexicana* infected SF bites.



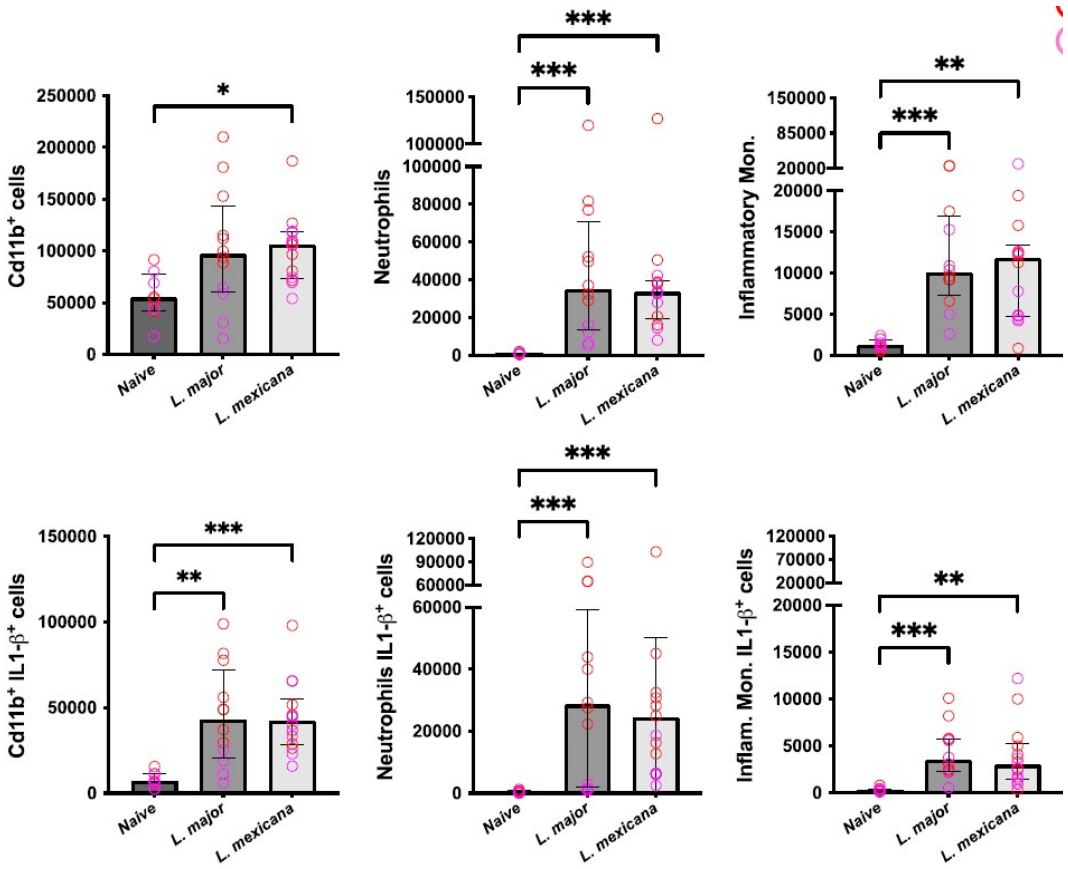
6 h post-infected bites



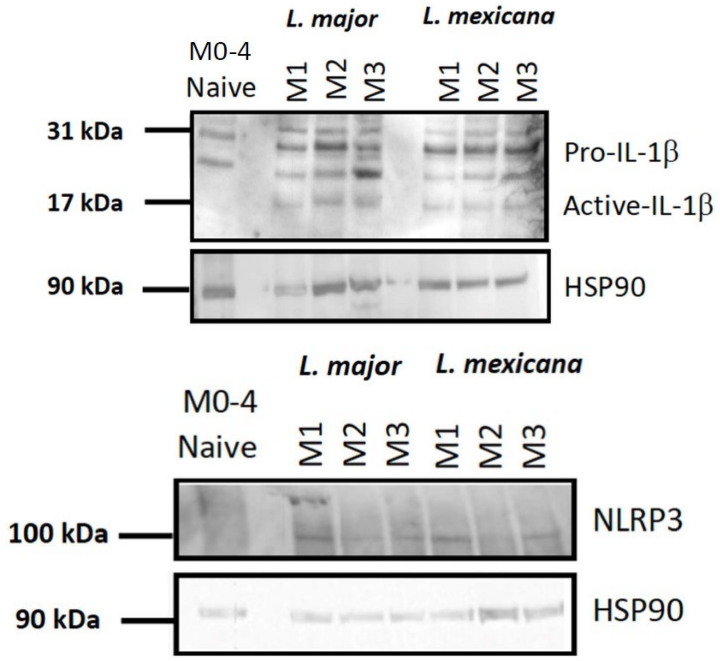
Regulation of inflammation at the bite site by the recruitment of neutrophils and inflammatory monocytes and the production of IL1-β: 6 hours after *L. mexicana*- ($LmxSF_i$) and *L. major*- ($LmjSF_i$) infected SF bites, we observed an acute inflammatory response with a 1.94-fold and 1.77-fold higher recruitment of $Cd11b^+$ cells, respectively, compared to steady state ear skin. Sand flies infected with either $LmxSF_i$ or $LmjSF_i$ produced a comparable influx of both neutrophils (median [M] of 33400, and 34750), and inflammatory monocytes (iMOs; M of 11750 and 10000), respectively, compared to steady state for neutrophils (M= 925) and iMOs (M=1250).



Significant production of IL-1β was also observed for both $LmxSF_i$ and $LmjSF_i$, albeit at a lower magnitude in iMOs (M= 2600,3450) compared to neutrophils (M = 24280, 28400), respectively. Production of inflammasome-derived IL-1β after *Leishmania*-infected sand fly bites is associated with the recruitment of neutrophils to bite sites.



Activation of the nlrp3 inflammasome in mice after infected sand fly bites: Western blots of ear cell lysates from both *Leishmania spp*. showed expression of NLRP3 and cleavage of pro-IL-



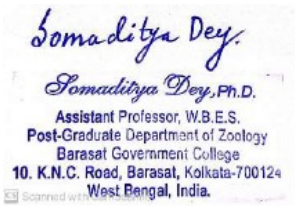
1β into its active form (a 3-4 folds increase, and 10-50 folds increase was observed in the levels of pro- and cleaved IL1-β respectively), indicative of pore formation and canonical inflammasome activation. Like previous studies (Dey et al, 2018), we showed that *L. mexicana* produces IL-1 β at the site of the bite, 6 hrs post-transmission, indicating that it is a signature host immune response to infected sand fly-bites.

Papers prepared/submitted for publication:

1. **NLRP1-dependent activation of Gasdermin D in neutrophils controls cutaneous leishmaniasis.** Michiel Goris[1,2], Katiuska Passelli[1,2], Sanam Peyvandi[1,2], Oaklyne Billion[1,2], Borja Prat-Luri[1,2], Benjamin Demarco[1], Chantal Desponds[1,2], Manon Termote[1,2], Eva Iniguez[3], **Somaditya Dey**[3,4], Miriam Díaz-Varela[1,2], Bernard Malissen[5], Shaden Kamhawi[3], Benjamin P. Hurrell[1,6], Petr Broz[1] and Fabienne Tacchini-Cottier[1,2*]. ***(Under Review; Plos Pathogens PPATHOGENS-D-24-00132)***
2. **Malnutrition exacerbates pathogenesis of sand fly transmitted *Leishmania donovani*.** Eva Iniguez[1], Johannes Doehl[1#], Pedro Amado Cecilio[2#], Tiago Donatelli Serafim[1], Caroline Percopo[1], Yvonne Rangel-Gonzalez[1], **Somaditya Dey**[1,3], Elvia J. Osorio[4], Patrick Huffcutt[1], Sofia Rotmain[4], Claudio Meneses[1], Mara Short[1], Peter C. Melby[4], Shaden Kamhawi[1*]**. (Manuscript is in the final stages of preparation for submission**).

iii) Proposed utilization of the experience in India :

- This training may help us to set up an insectary to rear Indian sand flies, most importantly, the VL vector *Phlebotomus argentipes,* to be collected from VL endemic regions of West Bengal, India, and establish the methodologies for experimental infections with local strains of *L. donovani*.
- The establishment of an SF- bite initiated experimental murine VL model, and utilising this system to explore the pathology would be the first to be established in India and could be a promising future direction to test vaccines and assess efficacy of drugs towards control of this lethal neglected tropical disease.
- Additionally, this training has provided me the expertise to initiate field studies to elucidate the transmission dynamics of VL in West Bengal that is poorly understood to date.



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