Proposal for Masters Project 2022-2023
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Mechanisms of γδ T cell activation during the hepatic stage of *Plasmodium* infection

The co-evolution of *Plasmodium spp*., the etiological agents of malaria, and humans over thousands of years has established an intricate equilibrium between the immunity of the host and parasitic virulence factors, guaranteeing maximal parasite transmission with limited host damage. A major factor contributing for this "quid pro quo" is the development of naturally acquired immunity (NAI).

The nature of NAI is, most certainly, multifactorial. Although cumulative exposure to natural *Plasmodium* infections results in decreased levels of circulating parasites, the major readout of acquired immunity to malaria is protection from severe disease and death. While the establishment of such protective responses has been extensively explored, no single molecular signature, key cellular determinant, or immune mechanism has been causally associated with clinical protection from severe malarial disease[1].

We have developed a rodent model of infection that recapitulates the main features of NAI, i.e., decreased parasite burden and protection from severe pathology, echoing what is observed in clinically-asymptomatic humans living in high- to holoendemic malaria regions[2]. This experimental infection system allows for the uncoupling of the two distinct development stages of *Plasmodium* within the mammalian host, while controlling for the initial blood-stage (BS) parasite inoculum. It also bypasses the mosquito stage of *Plasmodium* development, which has been suggested to alter parasite virulence[3]. In accordance, C57BL/6 mice pre-exposure to a single bolus of non-productive, irradiated hepatotropic parasites (sporozoites; spz), followed 2 days later, by a BS infection initiated by the transfusion of PbANKA-infected red blood cells (iRBCs) are fully protected from severe malarial disease. This is in sharp contrast to control mice receiving the same inoculum of iRBCs 2 days after a hepatic mock infection (equivalent amount of uninfected mosquitoes' salivary gland material) that succumbed all from experimental cerebral malaria (ECM). This rodent neurological syndrome largely recapitulates the main features of severe neuropathology following *Plasmodium* infection in humans, and is typically associated with significant intravascular accumulation of mononuclear cells, intracerebral haemorrhage, enhanced blood-brain barrier permeability and oedema[4,5].

Until now, we were able to establish that the cellular basis for the protection against ECM afforded by exposure to spz prior to a BS infection relies on the activation of a subset of T lymphocytes harbouring the gamma (γ) and delta (δ) chains of the T cell receptor, i.e., γδ T cells. In fact, while wild-type (WT) C57BL/6 mice pre-exposed to irradiated spz, followed 2 days later, by a BS infection are fully protected from ECM establishment, C57BL/6 mice with genetic depletion of γδ

T cells (TCR$\delta^{-/-}$ mice) succumb to ECM in the same experimental setting. Production of IL-17 by $\gamma\delta$ T cells associates with increased extra-medullary erythropoiesis and concomitant reticulocytosis throughout infection, thus impacting on parasite virulence and the concomitant establishment of ECM.

We now aim at identifying (and characterizing) the signals generated by spz during the hepatic stage of infection leading to the activation of hepatic (and potentially, of non-hepatic) $\gamma\delta$ T cells. We will do so by using a library of genetically-modified parasite strains to functionally identify parasite-derived processes (and molecules) relevant for protection from ECM upon spz exposure followed by a BS infection. For this, C57BL/6 mice exposed to spz from each parasite line will receive a BS infection and infection progression and ECM establishment will be assessed. In parallel, analysis of hepatic $\gamma\delta$ T cell activation (CD44 expression) and functional specification (cytokine production) will be assessed by flow cytometry at relevant time points.

We then intend to disclose the molecular signature(s) of $\gamma\delta$ T cell activation following exposure to *Plasmodium* spz. For this, hepatic $\gamma\delta$ T cells (and other liver-resident and infiltrating leukocytes) will be isolated from the liver of C57BL/6 mice receiving spz, or from non-infected mice, at defined time points, at a single cell level. Single cells will be sorted into separate wells of a 384-well plate, with 5 plates per cell population per mouse. We will apply the MARS-seq protocol[6] to obtain the single cell transcriptomes of thousands of single cells of each type and the data obtained will be analyzed using the Seurat software[7]. Cells will be clustered in an unbiased manner and differential gene expression will be performed between the transcriptomes of the different cell types isolated from spz-exposed, or non-exposed, hosts. Time allowing, we will proceed by functionally validating candidate molecules and/or molecular pathways disclosed by our unbiased approach.

Trainee Goals:

During the development of the experimental work in the laboratory, the student is expected to:

- take part in all common activities in the laboratory, engaging and interacting with other team members and understand, present and scientifically discuss the data obtained in periodical presentations during laboratory meetings;
- acquire the basic knowledge on the techniques required for malarial infection establishment and follow-up in the rodent model, including isolation of both parasitized RBC and hepatocyte-infectious parasites (spz) from mosquitoes salivary glands; isolation of and determination of parasite load both during the liver and blood stages of infection;
- learn basic techniques of animal experimentation including handling and restraining; maintenance of mouse colonies necessary for the development of the project by genotyping of mice offspring and layout of husbandry schemes; administration of substances by intraperitoneal and/or intravenous routes and in vivo or post-mortem collection of biological samples;

- learn laboratory techniques including: isolation of tissue immune and non-immune cell subsets; flow-cytometric acquisition and analysis of isolated cells; fluorescence-activated single cell sorting; basic analysis of transcriptomic large data sets; cell culture techniques and; gene expression quantification by real time quantitative PCR.

Bibliography

1. Langhorne, J., Ndungu, F. M., Sponaas, A. M. & Marsh, K. Immunity to malaria: More questions than answers. *Nature Immunology* **9,** 725–732 (2008).
2. Lindblade, K. A., Steinhardt, L., Samuels, A., Kachur, S. P. & Slutsker, L. The silent threat: Asymptomatic parasitemia and malaria transmission. *Expert Review of Anti-Infective Therapy* **11,** 623–639 (2013).
3. Spence, P. J. *et al.* Vector transmission regulates immune control of Plasmodium virulence. *Nature* **498,** 228–231 (2013).
4. Langhorne, J. *et al.* The relevance of non-human primate and rodent malaria models for humans. *Malaria Journal* **10,** (2011).
5. Dorovini-Zis, K. *et al.* The neuropathology of fatal cerebral malaria in Malawian children. *Am. J. Pathol.* **178,** 2146–2158 (2011).
6. Brette, F. *et al.* Massively Parallel Single-Cell RNA-seq for marker-free decomposition of tissue into cell types. *Science (80-.).* (2014). doi:10.1126/science.1242747
7. Butler, A., Hoffman, P., Smibert, P., Papalexi, E. & Satija, R. Integrating single-cell transcriptomic data across different conditions, technologies, and species. *Nat. Biotechnol.* **36,** 411–420 (2018).