Instituto de Medicina Molecular João Lobo Antunes

Master Project Proposal

TITLE: ROLE OF RETICULOCYTES IN THE ESTABLISHMENT OF SEVERE MALARIA

SYNOPSIS: Despite major advances in the development and implementation of novel intervention strategies in the last decades, malaria still continues to impose an immense public health and economic burden in vast areas of the globe. In 2020 alone, 241 million cases of malaria were reported globally, resulting in 627 000 deaths[1]. Such striking numbers highlight the importance of continuing developing disease intervention strategies and broadening our current knowledge on the biology of *Plasmodium*, the causative agent of malaria, and the complex interaction it establishes with its host.

Plasmodium has a complex life cycle[2] initiated upon injection, by an infected *Anopheles spp*. mosquito, of sporozoites (spz) into the skin of the mammalian host. After entering the circulatory system, spz reach the liver and infect hepatocytes, in the range of dozens to hundreds, where they undergo major transformations and initiate a period of remarkable schizogynous division. The liver stage (LS) of infection culminates with the release of thousands of new parasites into the bloodstream where they infect red blood cells (RBC), initiating the blood stage (BS) of infection. The latter is associated with the establishment of disease and all its clinical complications.

Previously, we created a rodent model of infection where the two developmental stages of *Plasmodium* infection are uncoupled. With this model, we established that the obligatory LS of infection, until now considered not to contribute to the clinical outcome of infection, is critical for protection from experimental cerebral malaria (ECM)[3]. 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]. LS-elicited protection from ECM relies on the production of IL-17 by γδ T cells, which associates with increased extra-medullary erythropoiesis and concomitant reticulocytosis throughout infection. Ultimately, increased reticulocyte availability curbs the establishment of Plasmodium-associated neuropathology[3].

We now aim at identifying (and characterizing) the mechanisms by which reticulocyte availability impact the establishment of severe malarial disease. For that, we will start by establishing the kinetics of reticulocyte infection during the BS of infection upon transfer of reticulocyte-enriched blood. We then intend to characterize the expression of virulence factors in parasites infecting reticulocytes *versus* fully matured red blood cells. At selected time points of infection, infected reticulocytes and infected RBCs will be sorted at the single cell level 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 parasite within reticulocytes *versus* fully matured RBCs. Time allowing, we will proceed by functionally interrogate the importance of molecules and/or molecular pathways differentially regulated in parasites infecting reticulocytes for the development of ECM.

SUPERVISOR: Ângelo Ferreira Chora (angelochora@medicina.ulisboa.pt), Maria Mota lab

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