

Developing Efficient Extraction Protocols to Investigate the Role of Infected Red Blood Cells Extracellular Vesicles in Cerebral Malaria

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Extracellular vesicles (EVs), which are known to be released from cell by vesiculation, have been discovered in the last decades. Remarkably EVs have been involved in many processes of cell signalling. In fact, EVs carry different cargoes including RNAs, DNAs, proteins and lipids that can be transferred from one cell to a recipient cell, in which the cargoes act as signalling molecules. This process is recognized as a new paradigm of cellular communication. There is a growing interest to investigate cell to cell communication under healthy and pathological conditions (i.e tumor growth, Brain pathologies such as Alzheimer or Cerebral malaria). Not only EVs are promising biomarkers, they might also be excellent therapeutic targets. This project is focused on Malaria, an illness caused by the parasite, *Plasmodium falciparum*, with particular emphasis on the cerebral complications resulting from the infection. Among parasites able to infect humans and cause Malaria illness, there are *P. Vivax*, *P. Malariae*, *P. Ovale* and *P. Knowlesi*, although with lesser symptoms. During the clinical stage of Cerebral Malaria, the infected red blood cells (iRBC) are sequestered in the brain capillaries. While binding to the endothelium of the blood brain barrier, iRBCs release EVs which after crossing the blood brain barrier may interact with microglial cells. While EVs released by iRBCs are known to synchronize the parasites to optimize the transmission to the mosquito, they have also strong immunoregulatory potential.

Given the central importance of these nano-sized vesicles, it is fundamental to optimize the extraction techniques in order to further analyze and use EVs in the different experimental setups. Here, we compared two extraction techniques, PEG6000 and Salting-out (SA), we concluded that SA has a higher yield of EVs extracted. In addition, it seems to provide a better extraction in the range of different sizes (smaller EVs are mined via PEG6000). The second goal of this project was to test during Cerebral Malaria, the immune response of Microglia to EVs released from the red blood cells infected by the parasite (iRBCs). In fact, it is known that these iRBCs release EVs carrying virulent proteins (PfEMP1), miRNA, non-coding Y-RNA (hY4) and other substrates of parasitic origin capable of inhibiting the pro-inflammatory reaction of immune cells. From our in vitro tests, the result of this interaction between Microglia and infected EVs has led to an increase in interleukins responsible for the stimulation of the immune response such as IL-6, IL-12 and TNF- α , whereas no inhibitory effect on the microglial cell was noticed.

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