Immunosurfaceome of Plasmodium spp. infected red blood cells

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Malaria is a parasitic disease widely distributed, representing a health problem in many countries including Brazil. The two main species that can infect humans are *Plasmodium falciparum* and *Plasmodium vivax, being P. vivax* the most prevalent species in Brazil. Despite of the milestone that was the approval of malaria falciparum vaccines, there is no vaccine or immunotherapy available for malaria vivax. Thus, considering the relevance of mechanisms mediated by antibodies in malaria control, such as functional antibody dependent cytotoxicity, phagocytosis and inhibition, this work proposes the identification of *Plasmodium* spp. antigens externalized on infected-cell membrane and recognized by functional antibodies. Protein isolation was performed using a cross-linking method associated with immunoprecipitation, for this purpose two cross-linking agents were evaluated, DSP and DTSSP, against their potential of isolate membrane proteins. In *P. falciparum* samples, both presented comparable performances, additionally DTSSP was assessed and resulted in reasonable coverage of parasite protein groups that have been described on cell-infected membrane. Whilst higher enrichment of *P. vivax* proteins was reached with DSP in comparison to DTSSP. DSP-treatment resulted in 30 common proteins between individuals presenting comparable relative abundances and distributed among hypothetical proteins, glycolytic pathway proteins, ribosomal proteins, chaperones, dehydrogenases and heat shock proteins (HSPs). Trypsin shaving (TS) assays for analysis of total membrane proteins resulting in higher coverage of parasite proteins in TS fractions and much lower in IS fractions. Most of the covered peptides in shared proteins between TS and IS conditions presented amino acids predicted in linear and conformational B cell epitopes. *In silico* characterization of shared proteins in relation to the presence of transmembrane domains, signal peptide, GPI anchor, PEXEL motif, linear and conformational B cell epitopes revealed proteins with higher parameters scores such as chaperone, osmiophilic protein, putative and early transcribed membrane protein (ETRAMP). Several enzymatic groups were highlighted, and these groups need to be in-depth characterized regarding their secondary functions in Plasmodium infection as well as their immunogenic potential.

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