PLASMODIUM VIVAX: PATHOGENESIS AND INFECTIVITY

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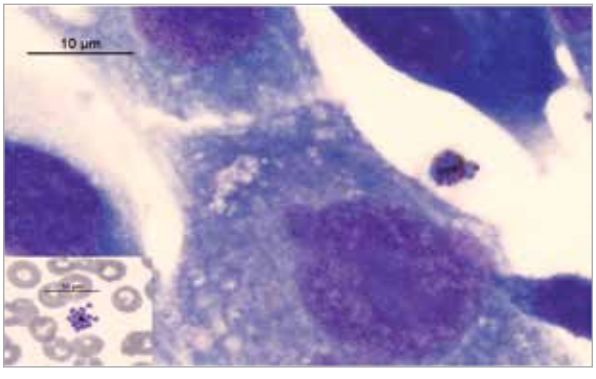


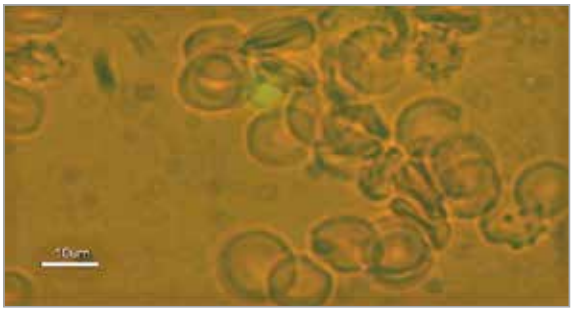
Figure 1. Plasmodium vivax cytoadhesion. Giemsa-stained light photomicrography revealing P. vivax schizont cytoadhered to human lung endothelial cell. Insert shows a schizont of P. vivax after maturation in a thin film blood smear Giemsa-stained

Malaria parasite infects more than 200 million individuals and lead to death of 600 thousand people annually. Fatalities are normally associate to *Plasmodium falciparum* infections as *P. falciparum*-infected erythrocytes (Pf-iEs) can sequester into the microvasculature of vital organs, playing a key role in the pathogenesis of life-threatening malaria complications, such as cerebral malaria (CM) and malaria in pregnancy (MiP). Moreover, despite the appropriate antimalarial treatment and the development of several adjunctive therapies, a significant proportion of individuals still succumb to CM and survivors develop neurological sequelae. Sequestration is marked by the cytoadhesion of Pf-iEs to host receptors on the surface of endothelial cells and on non-infected erythrocytes (rosettes). Although *P. falciparum* is the most lethal parasite and is responsible for the majority of malaria cases, *Plasmodium vivax* infects 22 million cases per year, with strong social impact in South-East Asia and the Americas. Moreover, it is estimated that 2.6 billion people are at risk of *P. vivax* infection worldwide. In Brazil, where transmission is almost exclusively restricted to the Amazon region (99.8%), 85% of malaria infections are caused by *P. vivax* accounting for 50-60% of all malaria cases reported in the Americas. Recently, it has been reported that in Brazil and Asia-Pacific region *P. vivax* infections may also lead to severe clinical complications. These observations challenge the concept that *P. vivax* is a "benign" species of parasite and open new avenues to study *P. vivax* pathogenesis and its mechanisms of infection. Thus, as long-term *in vitro* culture of *P. vivax* is still not feasible, the study of the biology of this parasite species remains restricted to endemic areas. Thus, as a result of a close collaboration with referral hospitals and research institutions in malaria endemic areas in the Amazon, the team was able to establish functional *ex vivo* assays allowing us to understand the mechanisms related to *P. vivax* cytoadhesion (including rosette formation) and to identify potential parasitic ligand(s) involved in this process. Moreover, it is also planned to determine the impact of *P. vivax* infections in pregnant women, the consequences to the placental tissue and the innate immune response involved in the adhesion to the placental. Finally, it is intended to verify the potential vaccine antigens of *P. vivax*.

SUMMARY OF RESULTS TO DATE AND PERSPECTIVES

Based on *P. vivax*-infected patient's clinical complications, it is hypothesized that these poor outcomes could be related to adhesion of *P. vivax*-infected erythrocytes (Pv-iEs) to the endothelium. Thus, Pv-iEs harvested from Brazilian patients were used in *ex vivo* cytoadhesion assays to brain and lung endothelial cell and to placenta cryosections. It is observed that Pv-iEs were able to adhere under static and flow conditions to both cell-lines and to placenta via ICAM-1 (mainly) and chondroitin sulfate A (CSA) cell receptors. In addition, the parasite surface ligand named VIR was implicated in this binding. Recently, it is confirmed cytoadhesion of *P. vivax* via ICAM-1 by performing adhesion assays to lung endothelial cells with Colombian isolates. Rosette formation has been investigated using Brazilian and Asia-Pacific isolates. It is revealed that the majority of isolates rosettes, however without an association between blood type and severity. Moreover, these rosettes were, mostly, formed by late stage-forms of Pv-iEs to normocytes via glycophorin C, rather than reticulocytes (*P. vivax* target cells). In addition, Pv-iEs when bound to non-infected erythrocytes were less likely to be phagocyted. Assuming Pv-iEs display adhesiveness, therefore disappearing from the peripheral blood (sequestration), it is performed *ex vivo* adhesion assays with Pv-iEs, before and after maturation. It is observed a higher binding potential of schizonts compared to other asexual stages (young forms). These results were correlated with observations in vivax malaria patients in which schizonts were almost absent in the peripheral blood in a patient with negative peripheral parasitemia, Pv-iEs VIR-positive were observed bound to lung endothelium. Next, although MiP has been largely studied in falciparum infections, little is know regarding the consequences to the placental tissue and to the innate immune response in pregnant women bearing *P. vivax.* A recent study revealed more lesions in the placenta of *P. vivax*-exposed women in comparison to healthy volunteers. Moreover, by means of an experimental murine model representing MiP, it is demonstrated that MyD88 plays a major role in the severity of pregnant-infected mice. Collectively, the findings provide, so far, evidence that Pv-iEs cytoadhere and sequester, however it is not clear if this adhesiveness is direct related with *P. vivax* severity. Therefore, these observations prompt a paradigm shift in *P. vivax* biology and open new avenues to investigate the role of sequestration in *P. vivax*, especially its involvement as a mechanism of immune evasion and its infectivity to the reticulocytes.

Figure 2. Rosetting formation in Plasmodium vivax. Fluorescence photomicrography of a P. vivax mature-stage form stained with acridine orange surrounded by non-infected red blood cells (rosette)



MAIN PUBLICATIONS

Carvalho BO, Lopes SC, Nogueira PA, Orlandi PP, Bargieri DY, Blanco YC, Mamoni R, Leite JA, Rodrigues MM, Soares IS, Oliveira TR, Wunderlich G, Lacerda MV, Del Portillo HA, Araújo MO, Russell B, Suwanarusk R, Snounou G, Rénia L, Costa FT. 2010. On cytoadhesion of *Plasmodium vivax*-infected erythrocytes. *The Journal of Infectious Diseases*. **202**: 638-647.

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