



## 2011 Research Grant Program Winning Abstract

### Role of RBC Derived Microparticles in Human Malaria

By Matthias Marti

---

Malaria is an infectious disease with tremendous public health impact around the world (Snow 2005). Most cases of severe malaria and most deaths are caused by the blood stage forms of *Plasmodium falciparum*. Severe and life-threatening malaria represent a combination of syndromes that arise from a few key processes. These include the sequestration of infected red blood cells (iRBC) in target organs, parasite dependent and independent activation of a pro-inflammatory response, and the recruitment and infiltration of inflammatory cells. Local and systemic disease syndromes are the consequence of atypical inflammatory cascades that are maintained by infiltrating cells through positive feedback cycles (Schofield 2005). What determines whether an infection will be clinically silent, symptomatic, or even severe? In most cases, homeostasis corrects the cascade effect, and responses are downregulated appropriately. However, in severe disease the cascade effect is not controlled, with fatal consequences. Appropriate regulation of immune responses is therefore key to healthy outcome, and identifying the factors that trigger and regulate these responses will be instrumental in the development of novel intervention strategies. What are the parasite-dependent pathways that trigger pro-inflammatory responses during malaria infection? The current model suggests that the interaction of parasite factors (“toxins”) with cells of the innate system such as macrophages and dendritic cells is the initial step in induction of the pro-inflammatory response. It is assumed that these factors are released into the blood stream when the parasitized red blood cell bursts and invasive daughter parasites emerge. Recent studies in the mouse malaria model suggest that microparticles (MPs) derived from infected red blood cells (iRBC) are a second source of circulating parasite factors (Couper 2010). Moreover, studies on the plasma from malaria-infected patients have demonstrated a link between MPs derived from platelets and endothelial cells, and disease (Combes 2004, Faille 2009).

Microparticles are small vesicles (0.1–1  $\mu\text{m}$  in size; smaller vesicles are termed exosomes) that are released as messengers from cells undergoing damage, activation, or neoplastic transformation (Cocucci 2009, Doevre 2009). These lipid particles contain both protein and RNA and are secreted at higher rates in cancer and inflammation (Mitchell 2009). They act as information messengers delivering antigens, lipids, and RNA from parent cells to elicit effects on downstream targets, and their composition reflects their cell of origin. Recent evidence suggests that iRBC-derived MPs significantly contribute to the stimulation of macrophages in the experimental rodent malaria model (Couper 2010). We hypothesize that iRBC-derived microparticles play an equally important role in activating the host immune response during human infection with *P. falciparum*. We have recently established protocols for the cytometric detection and biochemical purification of RBC-derived MPs. We have also performed mass spectrometry experiments with purified MPs and identified a series of RBC surface proteins as well as secreted parasite antigens as major components of these structures (manuscript in preparation).



Based on these promising preliminary studies, we propose a detailed functional characterization of MPs derived from iRBCs cultured under in vitro conditions and in patient serum. Our specific aims are: i) Quantity and dynamics of MPs derived from iRBCs by cytometric and microscopic analysis of MP production during parasite development. We will use a panel of antibodies against parasite and host protein markers identified in the pilot proteomics experiment to characterize and quantify MPs released from iRBCs during the in vitro cell cycle. ii) Role of MPs in immunomodulation and receptor activation. We will characterize the MP-dependent activation of macrophages (MF), dendritic cells (DC), lymphocytic, and endothelial cells in in vitro stimulation assays. iii) MPs as disease markers. We will analyze MPs from three malaria patient cohorts (cerebral malaria, uncomplicated malaria, and healthy controls) and use a panel of antibodies and beads to identify the cellular origins, composition, and quantity of the MPs. Importantly, our department has a large collection of patient sera from multiple malaria endemic sites.

We have a unique special order BD FACSAria™ III system equipped with special optics and photomultipliers, in addition to photodiodes for forward scatter (FSC) measurements. The instrument has greater sensitivity compared with conventional cytometers and allows evaluation of MPs based on size and fluorescence starting from 200 nm. Specific BD reagents that we propose to use for this project include multiple fluorochrome-conjugated antibodies to characterize maturation and activation of MF, DC, T cells, and endothelial cells including CD14, CD45, CD3, CD25, and CD19, cell culture reagents including TNF, IL-4, TGF- $\beta$ , and GM-CSF, viability dyes, cell enrichment kits, and cell fixation and permeabilization kits.

Identification of parasite molecules in iRBC-derived particles is of great relevance. First, the potential capability of such molecules to activate the innate immune system during infection makes them promising candidates for vaccine adjuvants. Second, their presence in the plasma of malaria patients may be exploited for diagnostic purposes in prediction of disease outcome and transmission, and to monitor treatment. The proposed experiments will lay the foundation for further studies on the role of MPs for the development of vaccine and biomarkers in malaria.

---

The BD Biosciences Research Grant Program aims to reward and enable important research by providing vital funding for scientists pursuing innovative experiments to advance the scientific understanding of disease.

Visit [bdbiosciences.com/grant](http://bdbiosciences.com/grant) to learn more and apply online.