

## ABSTRACT

Malaria vaccine clinical trials in exposed populations have not always been consistent in finding robust associations between the predicted immune responses and protection against disease. The selection of most promising vaccine candidates have been based on direct antibody inhibition assays that have performed dismally. This suggests that such immune correlates employed in assessing their efficacies may not be surrogate markers of protection. Immunity to asexual blood stage malaria is complex and likely involves multiple mechanisms. IgG antibodies are thought to play a critical role and a corresponding reliable *in vitro* correlate of antibody-mediated cellular immunity has long been sought to facilitate malaria vaccine development. The aim of present work was to establish an effector cell antibody-dependent respiratory burst assay as a correlate of naturally acquired immunity that may be a useful tool to evaluate vaccine-induced immunity in malaria studies. The specific objectives of the study were: to determine if polymorphonuclear neutrophils (PMNs) and monocytes could be triggered by malaria merozoites to produce reactive oxygen species (ROS); to determine the minimum number of merozoites required for the trigger; to determine the reproducibility of the assay, to determine the applicability of the assay by testing if the blood stage malaria vaccine candidates MSP-1, MSP-2 and AMA-1 elicited antibodies that trigger effector cell respiratory burst, and to associate total antibody titres to respiratory burst activity after vaccination. This study adopted an experimental design where the characteristics of the antibody mediated effector-cell-dependent respiratory burst induction were established and the key variables determining the performance of the assay evaluated. Merozoites opsonised with either malaria hyper immune IgG from Africa or IgGs raised from vaccinations with either MSP-1, MSP-2 or AMA-1 were incubated with PMNs/monocytes and production of ROS determined by isoluminol-amplified chemiluminescence in triplicate assays. ELISA assays were performed alongside to determine if total antibody titres elicited by vaccination had any association with the magnitudes of ROS measured. Results show that monocytes (fresh or cryopreserved) and PMNs are effective at respiratory burst induction of ROS. At equivalent cell numbers, PMNs exhibited higher ROS production than autologous monocytes ( $p < 0.001$ ) and for both cell subsets, burst induction was dependent on intact merozoites. The respiratory burst activity was achieved at ratios as low as one opsonised merozoite per effector cell. For the malaria vaccine candidates, the antibody mediated ROS production increased following vaccination, with the magnitude of increase depending on the vaccine antigen. However, there was no correlation between the total serum antibody titres and the respiratory burst activity induction ( $r^2 = 0.2419$ ). These results postulate that the *ex vivo* antibody-mediated ROS assay which the study has shown to be reproducible, may be a vital tool and recommends its use in evaluating the functional relevance of anti-malaria antibodies in studies. However, given the complexity of malaria infection and the corresponding immune response, it is probable that protective immunity against the pathogen require multiple effector mechanisms and additional assays measuring other mechanisms could be needed to correlate unequivocally with protection. In conclusion, this assay offers a useful platform for elucidating malaria vaccine candidates in clinical trials and in performing malaria immuno-epidemiologic studies.