ABSTRACT

"Never give up on your dream, fight your hardest for it"

-The Pursuit of Happyness

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Malaria, which affects millions of people, is caused by a unicellular parasitic protozoa, Plasmodium. There are five distinct species of *Plasmodium*, *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae* and *P. knowlesi*, which infect humans. Among them *P. vivax* is the most widely and frequently occurring malaria. Monocytes, represent a connecting link between inflammation and the innate defence against microorganisms to adaptive immune responses. Monocytes convert into macrophages, and are involved in the removal of the parasite by phagocytosis. This is mediated by complement components (opsonisation) or by parasite specific IgGs. During malaria infection monocytes and macrophages are involved in interaction with the *Plasmodium* infected erythrocytes (IEs) and helps in its removal from the spleen thereby playing a major role in protection from the disease. Also in several instances of vaccine studies, monocytes are not effective at controlling the parasite. So the aim of this study was to determine if recombinant malarial antigens had any effect on the monocyte functionality and thus the objectives of the study were as follows:

1. Cloning, expression and purification of *P. vivax* antigens AMA-1, MSP7 and WARP.

2. To study the immune reactivity of patients to the recombinant antigens and the in vitro effect of the recombinant antigens on monocyte function.

3. To study the chemokine receptor expression in monocytes in individuals infected with *P. vivax*.

P. vivax antigen AMA1, MSP7 and WARP were used in this study. Primers were designed and procured commercially for these three antigens. PCR products of 1338 bp (AMA-1), 1263 bp (MSP-7) and 888 bp (WARP) were obtained and the amplicons were then cloned and expressed. Thereafter, these recombinant proteins were purified using nickel affinity chromatography and precipitated by methanol chloroform method.

The recombinant antigens were blotted onto nitrocellulose membrane followed by treating the blot with serum from infected individuals. It was observed that 12 sera reacted with AMA-1 (85.71%), 10 sera reacted with MSP-7 (71.42 %) and 14 sera reacted with WARP (100%). To study the phagocytosis rate, monocytes from controls were isolated and treated with the recombinant antigens. Post incubation, the monocytes were incubated with fluorescent beads and were examined under a fluorescent microscope. It was observed that the rate of phagocytosis of both, single and multiple beads in monocytes treated with rPvAMA-1 was reduced from 41.92% to 32.67% and from 24.29% to 17.29% respectively. Similarly, for rPvMSP7 the rate of phagocytosis was reduced to 30.17% and 14.03% for single and multiple beads respectively and for rPvWARP to 30.66% and 15.55%. NBT reduction assay was performed and it was observed that blue colour formation was more in the case of AMA-1 and MSP7 compared to that of the untreated and WARP treated monocytes in NBT assay.

The chemokine profile in vivax patient and the effect of the purified recombinant antigen on control monocytes (healthy) was also studied. Monocytes were isolated from 10 patients and 3 healthy individuals. RNA was isolated followed by cDNA preparation. The cDNA was then used to study the chemokine receptor mRNA levels. It was observed that gene expression of CCR2 and CX3CR1 were upregulated while CCR7 and CXCR4 were downregulated in all the patients infected with *P. vivax*.

GAPDH was used as an internal control. Exposure of recombinant AMA-1 and WARP led to a reduction of CCR2, CX3CR1 and CXCR4, while no difference in the fold expression for CCR7. But upon exposure of MSP7 the monocyte showed a reduction in all the chemokine receptor levels. For the fold expression of mRNA for various chemokine receptors of monocyte derived from activated PBMCs, CX3CR1 was either unchanged (AMA-1) or increased (MSP7 and WARP). The fold expression of other receptors were reduced.