SUMMARY/ABSTRACT

In the present study, MAb B9A10, generated against *P. falciparum* infected cell membrane by homologous immunization (Kumar, 2011) specifically recognized parasite associated components. Further, the maximal expression of these components was found at trophozoite stage of the parasite. Infected cell surface reactivity of B9A10 indicated the involvement of these components in invasion or parasite development. Attempts to identify 112 kDa component reactive to B9A10 were not successful. However, the mimotope recognized by this antibody showed interesting results. The antisera, generated against various B9A10 mimotopes, exhibited parent antibody type immunoreactivity with *P. falciparum* parasite and infected erythrocytes. Interestingly, one of the mimotope A10p4 antisera, like B9A10, displayed significant effect on parasite growth, suggesting its close structural as well as functional similarity with antibody epitope. Different attributes of this mimotope i.e., alignment profile, reactivity pattern of generated antisera in immunoblotting and its pattern of fluorescence in confocal microscopy with parasite and infected cell surface, significant *in vitro* parasite growth inhibitory efficacy, suggested that 112 kDa antigen recognized by MAb B9A10 may be *P. falciparum* rhoptry neck protein 6 (PfRON6). Further experimentation is needed to validate this observation. Nevertheless, A10p4 peptide mimotope can be a good candidate for vaccine development in malaria.

Further objectives of the study were: i) characterization of phage display derived peptides specifically recognizing parasite infected cells, ii) development of rapid screening assay for cytotoxicity analysis of peptide and drugs and, iii) exploiting combined use of CPPs and AMPs for increasing antimalarial efficacy. Among the panel of screened phages (Shah, 2009), five phages, I3p4, I3p7, I3p9, I3p10 and I3p23 showed *P. falciparum* infected cell specific reactivity. Besides, I3p4 and I3p10 phages also displayed cross reactivity with *P. berghei* infected erythrocytes in vitro. Also, I3a7 phage exhibited in vivo reactivity with *P. berghei* infected cells. However, in *in vitro P. falciparum* growth inhibition test, synthetic peptides corresponding to I3a4 and I3a7 phages did not show any effect on parasite growth. It remains to be seen whether modifications in these IRBC specific peptides lead to their antimalarial effect.

CPPs, Tat, Penetratin and two recently designed CPPs, P3 and P8 showed localization in the nucleus of *P. falciparum* IRBCs and exhibited binding to parasite DNA in solution. Our results

emphasize the need of validation of antimalarial activity, observed at higher concentrations of cationic CPPs in SG dye based assay, by gold standard [^{3}H]hypoxanthine incorporation assay. In case of cationic CPPs, it was noticed that removal of excess peptide, before addition of lysis buffer containing SG dye, significantly reduced the false readout of antimalarial activity of cationic CPP. Like CPPs, DNA intercalating agents also inhibited *in vivo* SG binding to DNA but being antimalarial in nature this effect was observed by taking early period of parasite growth. Unlike CPPs, no effect on false readouts in short assay was observed even after removal of excess intercalators. Further, based on this observation, rapid assay for *in vivo* high throughput screening of DNA intercalating agents was established. Number of DNA intercalating agents or drugs when checked in this assay displayed different extent of *in vivo* DNA intercalation. Alternatively, for majority of DNA intercalating agents, this assay can be used for screening DNA targeting antimalarial agents. Thus, this study describes an assay which can be used for rapid and high throughput screening of antimalarial agents and simultaneously can be exploited for analysis of toxicity in mammalian cell lines.

Cytotoxicity analysis data demonstrated that: i) there exists a disparity in readouts of hemolytic activity exhibited by various hemolytic agents in hemoglobin release assay and turbidity method at higher hematocrit indicating erroneous results of hemolysis in later method, ii) the appearance of anomalous results in turbidity assay is due to the interference caused by the ghost or membrane fractions in suspension, iii) a comparative analysis of the two readouts at 2% hematocrit provides a rapid and easy tool to classify the hemolytic agent based on their membrane acting properties.

Peptide based studies revealed that CPP, TP10 inhibited *in vitro* growth of *P. falciparum*, within 1 h of incubation, at all stages of the parasite. Our results further showed that hemolysis was also responsible for antimalarial activity of this peptide. Interestingly, KLA after conjugation with TP10 or P8 i.e., KLA-TP10 or KLA-P8, exhibited strong antimalarial activity while no such effect could be seen when these peptides were checked alone. Further, KLA-P8 conjugate, at non hemolytic concentration, exhibited parasite growth inhibitory property. Thus, this study provide a novel approach for delivering intracellular target specific peptides after conjugation with potent CPPs.