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SUMMARY OF THE THESIS

Plasmodium, the protozoan parasite, responsible for deadly malarial disease invades and infects erythrocyte during asexual stage of its life cycle, causing extensive modification in the host cell. Structural and functional modifications of the host cell are not only essential for parasite survival but also responsible for clinical manifestation of the disease. A number of parasite proteins such as MSP-1, AMA-1 etc. are implicated to be intricately involved during invasion process. Also, the parasite derived proteins exported to the erythrocyte membrane are thought to aid in nutrient uptake, ionic balances and cytoadherence for successful survival of parasite within the null host cell. Infected cell surface determinants along with parasite proteins involved in invasion process have been the focus of extensive research since past few decades in understanding the mechanisms of pathogenesis as well as for developing immunodiagnostics and therapeutic interventions.

Earlier studies in laboratory, using monoclonal antibody, mAbF10 generated by immunizing *P.berghei* infected erythrocyte membrane rich preparation, identified different forms of Merozoite Surface Protein-1(MSP-1) i.e. 230 kDa ($MSP\text{-}1_{230}$), 195 kDa ($MSP\text{-}1_{195}$) and a 156 kDa doublet ($MSP\text{-}1_{156}$) on parasite as well as infected erythrocyte (Bhattacharjee, 2003). Host and parasite cell membrane/surface association of MSP-1 was confirmed by a series of experiments involving cell surface biotinylation and electron microscopy. Further, kinetic studies revealed that unlike isotypic control, mAbF10 exhibited a temperature-dependent uptake in parasite and IRBC *in vitro* and selectively delivered drug to the infected erythrocytes *in vivo*. In the current study, panel of antibodies against *P.berghei* infected erythrocytes and *P. falciparum* parasite generated in earlier lab studies (Choudhury, 1996; Indu, 1998) were used to: i) dissect the conserved epitope(s) on MSP-1, recognized by growth inhibitory antibody and possibly involved in macromolecular uptake in parasite and infected erythrocytes and, ii) identify and characterize a target molecule present on *P. falciparum* parasite/infected cell surface involved in invasion/growth of the parasite.

Initially, the studies were focused on secretion/release of MSP-1 by the parasite or infected cells and its possible association with infected cell membrane. The current study for the first time reports the presence of higher forms of MSP-1 i.e. 230 kDa ($MSP\text{-}1_{230}$), 195 kDa ($MSP\text{-}1_{195}$) and a 156 kDa doublet ($MSP\text{-}1_{156}$) along with 95 kDa ($MSP\text{-}1_{95}$) in

the plasma of *P.berghei* infected mice with 10-15% level of infection. The stated forms of MSP-1 were observed in western blotting of plasma probed with anti-MSP-1 mAbF10. The secretory nature of MSP-1 was also supported by the bioinformatics analysis of amino acid composition of secretory and non-secretory proteins of *Plasmodium* origin. Further, it was confirmed that the specific association of MSP-1 with infected cell membrane, observed in earlier lab study, was due to intracellular parasite and not because of non-specific attachment of MSP-1 present in infected animal sera as observed in NRBC binding assay. Moreover, the surface expression of MSP-1 was further reiterated by the observation of non-reactivity of anti spectrin antibody with the fraction containing MSP-1 fragments, affinity purified from IRBC extract.

Monoclonal antibody, mAbC3, was selected from panel of antibodies raised against *P. berghei.* Selection was made based on its reactivity with MSP-1 components ($MSP\text{-}1_{230}$, $MSP\text{-}1_{195}$, and $MSP\text{-}1_{156}$) purified using mAbF10 affinity column. Identity of mAbC3 reactive components was confirmed as MSP-1 by 100% identical N-terminal sequence (EVYNDLIQ) of 156 kDa molecule purified using mAbC3-sepharose affinity column. Further, binding pattern of mAbC3 with IRBC as observed in immunoflourescence assay was quite similar to that of MSP-1 reactive mAbF10 reported earlier (Bhattacharjee, 2003). However, unlike mAbF10, mAbC3 exhibited inhibition towards *in vitro* growth of *P. falciparum.*

Following observations, pertaining to the comparable internalization of surface MSP-1 reactive both mAbs F10 and C3, strongly indicated the involvement of MSP-1 in specific antibody uptake by *P.berghei* parasite and infected erythrocytes:

i) Time dependent increase in uptake, within parasite, of only radiolabelled mAbs F10 and C3 but not of BSA.

ii) Competitive inhibition of radiolabelled mAbF10 uptake in parasite by excess of unlabeled MSP-1 reactive antibodies only, and not by other isotypic control antibody.

iii) Distribution and localization of labelled antibody fragments within cytosolic fraction and membrane fraction of parasite incubated at 37^0C and 4^0C respectively.

The conserved nature of MSP-1 in rodent and human malarial parasite species was evident in *in silico* analysis of MSP-1 sequence from the two diverse species which was further corroborated by the binding of anti-*P.berghei* MSP-1antibodies mAbs F10 and C3 in immunoblotting with *P. falciparum* parasite extract. Parasite reactivity and infected cell surface localization of mAbs F10 and C3 binding site in immunoflourescence assay, confirmed that mAbs F10 and C3 recognized conserved epitopes of MSP-1 in rodent and human malarial parasite species. The mAbC3, showing effective and greater internalization within IRBC as compared to mAbF10, substantially inhibited (46.5%) the *in vitro* growth of *P. falciparum.*

The mimotopes reactive to mAbC3, obtained after screening random peptide phage library, aligned in the amino acid region 681-698 of MSP-1 sequence of *P.berghei* Further, clustal alignment of this region with MSP-1 protein from *P. berghei, P. yoelii, P.chabaudi,* and *P. falciparum* showed that the region is well conserved among all the species.

Among the panels of mAbs, mAbB4 was selected based on its cross reactivity with rodent and human parasite in ELISA, recognition of infected cell surface in immunoflourescence assay and *in vitro P. falciparum* growth inhibitory activity. Affinity matrix, prepared using mAbB4-Sepharose, isolated components of 82 kDa and 70 kDa. mAbB4 was reactive to both 82- kDa as well as 70 kDa molecules indicating that 70 kDa might be a proteolytic product of 82 kDa. However, N-terminal sequence of these purified components could not be determined, in spite of repeated attempts, probably due to the N-terminal blockade.

The screening of random peptide phage library, while mapping of mAbB4 reactive epitope, yielded many peptides with consensus sequence --WH---W[ST]W[WP][LI]SXSP-. The mimotope B4p4 having sequence "WHWSWQRNYPSY" upon blast search retrieved a hypothetical protein, PFL1050w with 83% identity corresponding to molecular weight of 82 kDa, thus indicating that PFL1050w might be the antigen reactive to mAbB4. Following evidences further strengthened the above interpretation:

ii) Anti-B4p4 peptide phage antisera showed good binding with infected cells in immunoflourescence assay, with binding sites localized on the parasite and the membrane of infected RBC (IRBC).

Further, the hypothetical protein (PFL1050w) was highly conserved among various rodent malarial parasite species. Besides, the epitope recognized by inhibitory mAbB4 was also conserved in rodent and human malarial species.

Overall, the important findings of the current study are:

i) Parasite and infected cell membrane associated MSP-1 might be involved in specific antibody uptake in parasite as well as infected erythrocytes.

ii) Amino acid region between 681-698 of MSP-1 contains epitope which is recognized by *P. falciparum in vitro* growth inhibitory antibody and conserved among rodent and human malaria parasite.

iii) A novel hypothetical protein (PFL1050w) of *P. falciparum* was recognized by growth inhibitory antibody. Epitopic region of PFL1050w, recognized by this growth inhibitory antibody, was also identified which was found conserved among different malarial parasite species.

The biochemical, immunological and functional characterization of above identified regions on MSP-1 and hypothetical protein PFL1050w on malaria parasite and infected cell surface, and involved in parasite invasion/growth, might be of paramount importance in designing drug/vaccine against malaria.