SUMMARY

The malaria parasite *Plasmodium* has a complex life cycle. Red blood cells harbour most important stage of parasite and all clinical manifestations of malaria are associated with this intra-erythrocytic phase of parasite life cycle. Studies on the antigens of asexual blood stages are of paramount importance, both for antimalarial vaccine development and for the development of diagnostic tools. Malaria parasites exhibit extensive antigenic variation or diversity, and to resolve this problem, a vaccine cocktail is envisaged.

Common antigenic determinants have been reported among different species of *Plasmodium*. These antigens, shared between different *Plasmodium* species may have potential towards the development of effective vaccine. Earlier studies in the Lab demonstrated the presence of neo-antigenic determinants on *P.berghei* infected cell surface by homologous antiserum raised against infected erythrocyte membranes. So in the present study, polyclonal antibodies as well as monoclonal antibodies generated by using *P.falciparum* parasites and *P.berghei* infected erythrocyte membranes were used to identify antigens of erythrocytic stage of *P.falciparum* and those cross-reactive with *P.berghei*.

The mouse polyclonal antisera were generated against *Plasmodium falciparum* parasites (anti-Pfp antiserum) and *Plasmodium berghei* infected erythrocyte membrane rich fractions (anti-Pbm antiserum). Both the antisera reacted with their respective intracellular parasites, as revealed by immunofluorescence assay (IFA). Flow cytometry showed that both the antisera exhibited surface reactivity with their respective infected erythrocytes, albeit at low level. Low reactivity of anti-Pfp antiserum was also observed with normal human red blood cells (NRBC), while anti-Pbm antiserum was non-

reactive with mouse NRBC as seen in IFA and flow cytometry. Both the antisera were reactive with their respective parasite lysate in enzyme linked immunosorbent assay (ELISA) and exhibited the titers of 1:1600. In immunoblotting, anti-Pfp antiserum recognized multiple proteins of the molecular weight of 135 kDa, 110 kDa, 95 kDa, 80 kDa, 68 kDa, 45 kDa and 35 kDa of *P.falciparum* parasites and mainly three proteins of 60 kDa, 43 kDa and 38 kDa of human NRBC. Anti-Pbm antiserum recognized multiple proteins of > 205 kDa, 125 kDa, 115 kDa, 75 kDa, 50 kDa, 45 kDa, 35 kDa and 3 proteins of < 29 kDa of *P.berghei* infected cell membrane, while in immunoprecipitation of ^{125}I- *P.falciparum* parasites, proteins of > 205 kDa, 117 kDa, 95 kDa, 52 kDa, 35 kDa, 31 kDa and two proteins of < 29 kDa were precipitated by anti-Pfp antiserum.

Cross-reactivity pattern of anti-Pfp antiserum with *P.berghei* parasite/ infected cells and of anti-Pbm antiserum with *P.falciparum* parasites/infected cells was also studied, both the antisera were found to be reactive with other parasite lysate in ELISA. Anti-Pfp antiserum showed the binding to *P.berghei* infected cell surface as well as intracellular parasite, while the binding of anti-Pbm antiserum to *P.falciparum* was mainly confined to the intracellular parasite as seen in IFA. Identification of cross-reactive antigenic determinant(s) by immunoblotting revealed that anti-Pfp antiserum cross-reacted with 200 kDa, 155 kDa, 70 kDa, 55 kDa, 30 kDa and < 29 kDa proteins of *P.berghei* IRBC membrane, while anti-Pbm antiserum identified major proteins of > 205 kDa, 125 kDa and a doublet of 50-52 kDa of *P.falciparum* parasites. Anti-Pbm antiserum also immunoprecipitated ^{125}I-labeled proteins of 190 kDa, 105 kDa, 90 kDa, 75 kDa, 65 kDa and 35 kDa and < 29 kDa of *P.falciparum* parasites.

Monoclonal antibodies (MAbs) were generated using spleen cells of above described immunized animals. Six MAbs generated against *P.falciparum* parasites (anti-Pfp MAbs); Pf8E6, Pf9B2, Pf10G2, Pf11D5, Pf6D5 and

Pf14F9 exhibited binding with the intracellular parasites as seen in IFA and ELISA and did not react with IRBC surface determinant(s), as revealed by flow cytometry. Four MAbs raised against *P.berghei* IRBC membrane components (anti-Pbm MAbs); Pb1B6, Pb5G8, Pb3C5 and Pb42C3, exhibited binding with intracellular parasites, as revealed by IFA and ELISA. Among these, three MAbs; Pb1B6, Pb3C5 and Pb42C3 exhibited binding with *P.berghei* infected cell surface as revealed by flow cytometry. MAb Pf10G2 belonged to IgM class, rest all MAbs were of IgG_1 class. Antigenic determinant(s) of IRBC and parasites, recognized by these ten MAbs were identified and characterized by immunoblotting. Proteins of *P.falciparum* antigens recognized in immunoblotting by Pf8E6, Pf9B2, Pf10G2 and Pf6D5 were : 50 kDa and < 29 kDa; doublet of 95-97 kDa; 52 kDa; doublet of 78-80 kDa, respectively, while, Pf11D5 and Pf14F9 were non reactive. *P.berghei* infected cell membrane components recognized in immunoblotting by Pb1B6, Pb5G8, Pb3C5 and Pb42C3 were : 160 kDa, 99 kDa; 175 kDa, 125 kDa, 90 kDa, 84 kDa and 80 kDa; 175 kDa, 125 kDa, 90 kDa, 84 kDa and 80 kDa; 78 kDa, respectively.

Cross reactive antigens of *P.berghei* infected cell membrane, recognized by anti-Pfp MAbs : Pf8E6, Pf9B2, Pf10G2, Pf6D5 and Pf14F9 were : 130 kDa, 96 kDa and < 29 kDa; doublet of 87-90 kDa; < 29 kDa; 125 kDa, 70 kDa and < 29 kDa; < 29 kDa proteins, respectively in immunoblotting. Anti-Pbm MAb: Pb1B6 recognized 125 kDa protein of *P.falciparum* in immunoblotting. Thus, 125 kDa antigen was present both in *P.falciparum* parasites and *P.berghei* infected erythrocyte membranes.

To assess the intracellular parasite growth inhibitory potential of monoclonal antibodies, a microassay was first developed using *in vitro* incorporation of [^{3}H] hypoxanthine by *P.berghei* infected erythrocytes. Anti-Pfp MAbs : Pf8E6, Pf10G2 and Pf6D5 showed 4 %, 9.2 % and 11 % inhibition

of [^{3}H] hypoxanthine incorporation, respectively, while anti-Pbm MAbs; Pb1B6, PB5G8, Pb3C5 and Pb42C3 showed 25.2 %, 20%, 9.5% and 9.1% inhibition of [^{3}H] hypoxanthine uptake, respectively. The inhibition of intracellular parasite growth, as observed with Pf6D5 and Pb1B6 was dose dependent. The reactivity of various anti-Pfp monoclonal antibodies was further analysed with 16 Indian isolates of *P.falciparum* by enzyme linked immunosorbent assay (ELISA). MAb Pf8E6 found to be reactive with all the isolates except one. Pf9B2 was reactive with half of the isolates studies and exhibited moderate reactivity. Pf10G2 and Pf11D5 were poorly reactive with almost all the isolates. Pf6D5 and Pf14F9 exhibited reactivity with more than half of the isolates thus showing differences towards antigenic recognition.

The cross reactivity of anti-Pbm antiserum with 16 Indian isolates of *P.falciparum* was also analyzed. MAb Pb1B6 exhibited moderate reactivity with 8 isolates; Pb5G8 and Pb3C5 showed cross reactivity with 1-2 isolates, whereas, Pb42C3 was almost non reactive with all the isolates studied.

Studies were further extended to see the differential antibody response towards *P.falciparum* and *P.berghei* parasites using antisera from 14 malaria patients. Five patient sera no. 10, 11, 12, 13 and 14, which exhibited reactivity with parasite lysate of two *P.falciparum* isolates and also cross-reacted with *P.berghei* parasite in ELISA, were further used to identify antigens of *P.falciparum* by immunoprecipitation. Autoradiogram revealed that patient sera no. 10, 11, 12, and 14 immunoprecipitated > 205 kDa; 205 kDa; > 205 kDa, 78 kDa, 55 kDa, 50 kDa, two proteins of < 29 kDa; 50 kDa antigens, respectively of *P.falciparum* parasites.

Thus, important findings of the present investigation are : (i) Polyclonal antisera and monoclonal antibodies generated using *P.falciparum* parasites or *P.berghei* infected erythrocyte membranes recognized number of erythrocytic stage antigens of *P.falciparum* and *P.berghei*, respectively. (ii) Some of these

MAbs were found to be partial inhibitory to intracellular parasite growth. (iii) One MAb was reactive towards all Indian isolates of *P.falciparum* tested. (iv) Number of cross- reactive antigens among *P.falciparum* and *P.berghei* were identified by these antibodies. Among these, 125 kDa protein appears to be an important novel antigen, since antibodies directed towards this antigen partially inhibited the growth of intracellular parasite. (v) These antigens may be of potential importance towards the development of antimalarial vaccine.