

The protozoan parasite *Plasmodium* infects mature erythrocytes during its development in the blood. This intra-erythrocytic stage of *Plasmodium* is responsible for the clinical manifestations of malaria disease and offers a potential site for parasite destruction. Various approaches which utilize the parasite-associated components for the preparation of diagnostic kits and therapeutics are circumscribed because of the intracellular habitat of the parasite. Parasite induced changes in the infected host cell membranes have been the focus of attention for the development of control remedies for malaria.

Detailed knowledge of neoantigenic determinants on parasite infected cell surface is crucial to control intracellular infection. Specific antibodies not only help in the identification and characterization of the antigen but also help in dissecting their functional role. Polyclonal antiserum was generated by homologous immunizations, in BALB/c mice, with the membrane rich fraction of erythrocytes *in vivo* infected with *Plasmodium berghei*. Viable and intact infected erythrocytes (IRBC) were taken to test the reactivity of the antiserum. Viability of IRBC and parasites was measured by Evan's Blue dye exclusion, rhodamine 123 uptake and [³H]hypoxanthine incorporation assays. While, integrity of IRBC was ascertained by non reactivity of anti-spectrin antibody.

The antiserum specifically recognized, albeit at low level, the infected cell surface as revealed by flow cytometry and immunoelectron microscopy. This binding was quantitatively proportional to the infected cell number in unfractionated and fractionated blood, as seen by ¹²⁵I-antibody binding assay. Immunoprecipitation of radioiodinated cell surface antigens revealed the presence of at least three proteins, of mol. wt. >205 kDa, 160 kDa and 100 kDa, on the infected cell surface. Among these, >205 kDa and 160 kDa antigens were parasite derived as revealed by [³⁵S]methionine labeling. Normal erythrocytes did not exhibit any reactivity with the antiserum.

Monoclonal antibodies (MAbs) were generated using spleen cells of infected cell membrane immunized animals. Two different MAbs, MAbF10 and MAbD2, were selected based on western blotting pattern obtained using infected cell membranes. Both the antibodies were of IgG1 class. Flow cytometric analysis and immunofluorescence assay (IFA) with live IRBC revealed the cell surface reactivity of these MAbs. By immunoelectron microscopy, the binding sites of MAbF10 and MAbD2 were localized on the IRBC membrane, cell cytosol and on the intracellular parasite.

Antigenic determinants of IRBC and parasites, recognized by MAbF10 and MAbD2, were identified and characterized by western blotting and immunoprecipitation of radioiodinated or [³⁵S]methionine labeled components. Immunoblot of IRBC membrane rich fraction showed the reactivity of MAbF10 and MAbD2 mainly confined to >205 kDa

and 46 kDa antigens respectively. The >205 kDa antigen was also localized on infected cell surface as revealed by immunoprecipitation of radioiodinated IRBC surface. It was parasite derived as demonstrated by immunoprecipitation of similar antigen from [³⁵S]methionine labeled IRBC extract. MAbF10 reacted with antigens of mol. wt. >205 kDa, 180 kDa and 150 kDa. While MAbD2 recognized proteins of mol. wt. 190 kDa, 160 kDa, 63 kDa and 56 kDa. MAbF10 and MAbD2 also immunoprecipitated number of proteins from [³⁵S]-labeled parasite extract. Although, none of the MAbs immunoprecipitated any antigen from NRBC yet, MAbD2 showed reactivity with 108 kDa protein of NRBC membrane in immunoblot. Similar reactivity was also observed in immunoblots of human NRBC.

The kinetic pattern of binding and uptake of ¹²⁵I-MAbs in IRBC and parasites were different under conditions that permit (37°C) or block endocytosis (4°C). At 4°C, the dose dependent binding of both the MAbs to IRBC was found to be non-saturable. Time kinetics of binding of both the MAbs revealed an attainment of plateau levels at 60 min. The overall binding of MAbD2 to IRBC was 2-3 folds higher as compared to that obtained for MAbF10. On the other hand, at 37°C, there was an approximately 2-3 folds increase in IRBC uptake and 10-12 folds increase in parasite uptake of MAbF10. This was in contrast to that observed for MAbD2, which exhibited similar patterns of binding at 4°C and 37°C. Furthermore, under endocytic condition the linear non-saturable uptake of MAbF10 was dependent on the metabolic status of the IRBC, as conditions which render the cell metabolically inactive, directly effected the uptake of MAbF10.

These MAbs were assessed for the growth inhibition of the intracellular parasite by measuring the incorporation of [³H]hypoxanthine. With MAbF10, the inhibition increased in a dose dependent manner up to a maximum value of 30% at 2.0 mg/ml and did not increase further with dose. Whereas with MAbD2 and control MAb (cMAb), the values were less than 7%.

Thus, taking rodent malaria (*P. berghei*) as a model, the important findings of this study are: i) Homologous immunization with the infected erythrocyte membranes evoked an immune response against infected cell surface antigens. This method may find wide application in other intracellular infection, particularly where animal models are available. ii) Under conditions, where the endocytic activity of the cell was minimized (at 4°C) the determinant(s) on the intracellular parasite were also accessible for binding with MAbs in the extracellular medium. iii) Although, both the MAbs were of the same class and recognized the infected cell/parasite components, only one exhibited the uptake at 37°C. This demonstrated the ^{possible} involvement of >205 kDa protein, in endocytic uptake by infected erythrocytes.