SUMMARY OF THE THESIS

Antibody mediated selective delivery of drugs has been studied widely in concerous cells. However, application of this approach has not received much attention towards parasitic diseases. In the present study, attempts were made towards the antibody mediated drug delivery to malaria infected erythrocytes.

Erythrocytic stage of malarial parasite, being clinically important stage, is the prime target for various therapeutic agents. Antigenic properties of erythrocyte change prominently as the parasite matures. Polyclonal antiserum was raised against later stages of *P. berghei* infected red blood cells (trophozoite/schizonts). Antiserum recognized NRBC as well as IRBC as revealed by IFA and ELISA. However, repeated absorption of the antiserum with NRBC resulted in the IRBC specific antiserum with non significant reactivity towards NRBC. NRBC absorbed antiserum did not show any reactivity with reticulocytes.

Absorbed antiserum recognized specifically surface component(s) on IRBC as established by hemagglutination assay, radiolabeled protein A binding assay and immunogold electronmicroscopy. Intracellular parasite labeling observed in immunoelectron microscopy indicated the possible parasite origin of these surface determinant(s).

Radioimmunoprecipitation of specific antigenic determinant(s) on IRBC surface demonstrated the presence of two major protein bands of Mol. wt. > 200 kDa and 108 kDa and two minor protein bands of Mol. wt. 45 kDa and 40 kDa. Among these, 108 kDa protein band looks a novel protein which has not yet been reported in *P. berghei* infected erythrocyte membrane.

Selective delivery of drugs to *P. berghei* infected erythrocytes was attemped using two approaches: (i) *in vitro* targeting of hemolytic non steroidal anti inflammatory drug conjugated to $F(ab')_2$ portion of specific antibody in order to test selective hemolysis of IRBC and (ii) *in vivo* efficacy of IRBC specific $F(ab')_2$ bearing chloroquine (Chq) laden liposomes in malaria infected animals.

Many non steroidal anti infalmmatory drugs such as flufenamic acid, FA; indomethacin, IM; ibuprofen, IB and fluribprofen, FB were tested for their hemolytic activity. Among these, FA and IM exhibited higher hemolytic potency at lower concentrations. Conjugation of these two drugs was tried with $F(ab')_2$ using water soluble carbodiimide method. No immunoconjugate could be obtained with FA while IM yielded an immunocnjugate with a $F(ab')_2$: IM molar ratio of 1:10-12. However, this conjugation resulted a loss of around 20% binding of $F(ab')_2$ in conjugate to IRBC as revealed by ELISA. Accordingly, furhter attemps were not made to obtain higher drug-$F(ab')_2$ molar ratio.

The efficacy of $F(ab')_2$-IM conjugate towards selective hemolysis of IRBC was tested *in vitro* for time and dose dependent parameters. IRBC hemolysis caused by specific $F(ab')_2$-IM conjugate was time as well as dose dependent.

While non specific F(ab')2-IM conjugate, corresponding concentrations of drug alone or along with free form of specific F(ab')2 didn't induce significant lysis.

Efficacy of IRBC specific F(ab')2 carrying chloroquine (chq) laden liposomes (targeted) was checked in mice infected with *P. berghei.* Tissue distribution studies of immunoliposomes in normal and malaria infected (2% parasitaemia) mice showed higher recognition of RBC in infected animals compared to that of normal animals. This indicated the selective recognition of IRBC in infected animals.

A single intravenous dose of targeted liposomized chq was administered on day four post infection (1-2% parasitaemia) for testing its efficacy in controlling parasitaemia *in vivo.* Both, percent inhibition of parasitaemia as well as survival of animals significantly increased through chq delivery by targeted liposomes as compared to those treated with free or nontargeted liposomized chq. Even single administration of low dose (2.5 mg/kg) of chq in targeted liposomes suppressed parasitaemia for a longer period of time. Thus, use of IRBC specific polyclonal antibodies significantly improved the efficacy of liposomized chq delivery in malaria infected animals.