Introduction
INTRODUCTION AND AIM OF STUDY

INTRODUCTION

Malaria remains a scourge to the mankind, with recent figures of 350-500 million clinical disease episodes with mortality figure of around 2.0 million and death every 30 seconds (Hay et al., 2009). The main contributing factors are emergence of drug resistant strains of the parasite, the appearance of mosquitoes that are resistant to insecticide, environmental changes and lack of potential vaccine (Pierce and Miller, 2009). Clinical manifestation of the disease is associated with asexual stage of the parasite in the host erythrocyte. Therefore, this stage has been the focus of extensive research since past few decades for developing immunodiagnosics and therapeutic interventions.

Malaria infected red blood cells (IRBCs) undergo drastic membrane changes, including the appearance of knobs (Nagao et al., 2000), changes in lipid composition (Maguire and Sherman, 1990), insertion of parasite-derived proteins (Smith et al., 2001) and modifications of host erythrocyte proteins (Winograd et al., 2005). About 400 *P.falciparum* proteins i.e. 8% of the proteome are exported into the host erythrocyte where they are involved in antigenic and structural alterations of the erythrocyte (Smith and Craig, 2005). These exported proteins are unique having no homology to other proteins. However, many among these molecules suffer from allelic heterogeneity between strains, antigenic variation within a single strain and high sequence polymorphism of critical target epitopes (Blythe et al., 2004; Craig and Scherf, 2001; Florens et al., 2004).

One of the great challenges in malaria research is the identification of potent target molecule among the plethora of parasite antigens and those associated with infected cell. Ever since the release of *Plasmodium* genome, the latest proteomic approach combined with bioinformatics tools have been adding vast amount of valuable data for malaria research (Wu et al., 2003; Johnson et al., 2004; Corradin et al., 2007). Inspite of this, out of 5300 proteins expressed during the life cycle of plasmodium, the functionality of majority of these proteins remain unknown (Charpian and Przyborski, 2008; Maier et al., 2009). Thus, in order to counter the malaria parasite survival strategies and to combat the disease, it is important to: i) identify structurally/functionally important, but non polymorphic, antigens/epitopes present on parasite and infected cells and, ii) to develop unique control strategies, based on their detailed functional characterization.
Phage display system has been exploited for the identification of targets in many diseases (Beckmann et al., 2005; Fairlie et al., 2008; Greenwood et al., 1991; Mukai et al., 2006; Zhang et al., 2001). Besides, phage display derived mimotope sequences have been used for both diagnosis and disease prevention (Ghosh et al., 2002; McIntosh et al., 2007). While in malaria this system has been invaluable in investigating host pathogen interactions (Fairlie et al., 2008; Ghosh et al., 2001).

Studies from our laboratory, using monoclonal antibodies, have demonstrated the presence of MSP-1 and RhopH3 not only on P.berghei parasite but also on infected cell surface (Bhattacharjee, 2003; Indu, 1998; Tiwari, 2008). MAbs F10 and C3 were directed against high molecular weight forms of MSP-1 (MSP\textsubscript{230}, MSP\textsubscript{195}, MSP\textsubscript{156}), while MAbD2 reacted with infected cell surface associated RhopH3. Among these antibodies, MAbC3 cross reacted with P.falciparum parasite and inhibited its in vitro growth (Tiwari, 2008). These observations emphasized the need for identifying their epitopes/mimotopes for their possible exploitation as vaccine candidates.

Taken together these observations and recent reports describing the utility of phage display system, the goal of this study was to identify and characterize structural/functional domains of parasite membrane/infected cell associated molecules using phage display. In order to achieve this, the following objectives were laid down:

1) Dissection of structurally/functionally important epitope(s) of MSP-1 and RhopH3 reactive antibodies.
2) Screening and selection of phage peptides reactive with parasite/infected cell followed by their characterization.
3) Identification and characterization of peptides binding to functional domain(s) of the target protein i.e. V-H\textsuperscript{+} ATPase.
4) Checking if any of these peptides arrests/blocks the growth of intracellular parasite.