CHAPTER VII:

Conclusion
7. CONCLUSION

The study was designed to assess the clone multiplicity of *P. falciparum* genotypes existing in the study area and to elucidate the humoral and cell mediated immune response of the population to identify an immune correlate of protection.

A high *P. falciparum* diversity with more alleles of each of the allelic family in the high transmission summer season was noted. Complex infections with novel genotypes in summer seen in the study tend to support the modification of *P. falciparum* genetic structure. Our data indicated that allele distribution was site dependent with some alleles common to the two sites but some were unique to a site. Multiplicity of infection could not be directly related to transmission intensity but rather host factors like ethnicity and the immune status of the individuals were implicated in complexity of infections seen in the study sites.

Anti MSP-119 antibody response of the study population was seen to protect both from risk of clinical infections and severity of the disease. A lower antibody response to MSP-119 peptides in comparison to whole merozoite extract of local *P. falciparum* strain was seen. A gradual acquisition of antibodies with age was also noted. This might be explained by the temporal variation of the *P. falciparum* structure seen in the study sites. The immune response was seen to be site dependent with a marked difference in recognition of the antigens between the two sites. A low seropositivity of Q-KNG at Guabari and a relatively higher seropositivity for WME and MSP119 peptides at KTE were observed. This was seen to be appropriately explained by the ethnicity dependent antibody response with the TT having a relatively higher immune response than the TB. A striking observation was that majority of the negative responders to Q-KNG belonged to the TB group. This observation taken together with a lower MOI in Guabari, correlation of host factors with complexity of infection suggests a strong influence of host genetics in immune response to malaria. Anti-E-TSR antibodies were also negatively associated with complicated malaria.
When cytokines response was measured in the study population with respect to different outcome of malaria infection we observed a higher TGF-β, IL-2 and IL-12α genes expression and depressed level of IL-10 with no change in mRNA level of IFN-γ in Pf infected cases suggesting induction of balanced cytokine response with activation of both Th1 and Th2 cells. In complicated malaria our data indicates suppression of T cell function in the study population with decreased levels of IL-2 and IL-12α along with increased expression of TGF-β and no change in IL-10 mRNA levels. TGF-β levels were elevated both in Pf infection as well as in complicated malaria. Further, TGF-β levels were negatively associated with age. A difference in TGF-β expression was also seen between the TB and TT with the level of cytokine significantly higher among the Tea tribes.

In addition, increased IFN-γ was directly associated with disease severity. But an increased TGF-β along with decreased IL-12α levels is not consistent with hyper activation of T cells leading to increased IFN-γ expression in complicated malaria. Therefore, we suggest that T cells are unlikely to be the source of elevated IFN-γ secretion and alternatively cells like NK cells, γδ T cells might play a role in the pathogenesis of the disease. The proposed model of the cytokine network is explained as follows.
A possible role of NK cells in pathogenesis of the disease was supported by our observation of KIR profiles in the population where the presence of the inhibitory KIR3DL1 was associated with higher risk of complicated malaria. Presence of six numbers of KIR activating genes was seen to predispose to disease severity. The increased levels of IFN-γ relating to complicated malaria might be explained by over activation of NK cells through its activating receptors signalling. Importance of a balance immune response has been
reaffirmed from our study where KIR genes profiles with four numbers of activating genes were related to protection from frequent episode in malaria.

Consistent with their history of migration, the TT and TB differed distinctly in their KIR genes frequencies. Further, a significantly higher proportion of KIR3DL1 positive individuals having complicated malaria belonged to the TT. Also, the TT was found to have a low frequency of KIR3DS1.

In conclusion, we observed a high *P. falciparum* diversity which was seen to vary with transmission seasons and between years. This could contribute to the low anti MSP1-19 antibody response of the population. Anti- MSP1-19 antibodies could confer protection from risk of infection as well as disease severity. IL-12α was seen to be an important marker of protection from complicated malaria. Risk to complicated malaria was associated with increased levels of IFN-γ, TGF-β and presence of KIR3DL1 gene. Genotypes with six activating genes were also seen to be at higher risk of complicated malaria. A strong role for genetic background was indicated in immune response to malaria. The TT and TB ethnic groups seemed to have different adaptation mechanism with KIR genes frequencies differentially associated with protection in them and the TT having a higher antibody response which conferred protection from risk of infection and severity.