Abstract

The thesis entailed studies on strain-specific protective immunity (SSPI) induced by malaria parasites and factors affecting malarial relapses, using infections of two strains, PcCeylon and Pc746 of *Plasmodium cynomolgi* in toque monkey, an analogous model to *P. vivax* in humans. The two strains showed a certain level of genetic and immunological heterogeneity while sharing comparable courses of blood infections. This provides a solid basis to use these two strains to achieve the objectives of the thesis.

Immunization with PcCeylon or Pc746 asexual blood-stage (ABS) parasites using two successive drug-cured homologous infections (12 weeks apart) induced strain-specific protection in a subsequent mixed-strain challenge with ABS parasites of both strains. This was detected by measuring proportions of the two strains in the challenge infections using pyrosequencing (PSQ) assay based on a single nucleotide polymorphism (SNP) of *msp1* gene. Specific reduction of growth of homologous strain in homologous-infection immunized monkeys relative to those in the non-immunized monkeys in the challenge infections indicated development of SSPI to ABS parasites. Early strain-specific clearance of the parasites in the challenge infection in immunized monkeys suggests the development of strain-specific immune memory and the ability of strain-specific component of this immunity to dominate the immune responses. In the later phase of the challenge infection, there was an early clearance of detectable parasitaemia in the immunized monkeys compared to the non-immunized monkeys indicating that both strain-specific and strain-transcending components of the blood-stage immunity at work to clear the parasitaemia. In general, this work prepares for using genetic analysis to identify target antigens of SSPI in *P. cynomolgi*/Toque macaque model.

Single immunization with PcCeylon or Pc746 live sporozoite-infected mosquitoes under chloroquine (CQ) and primaquine induced parasite strain-specific immune protection in a subsequent live sporozoite challenge with both strains. This effect was detected by PSQ assay based on SNPs of *msp1* and *csp* genes as described in the previous section. In the mixed-strain challenge infection, proportion of parasites of a particular strain was significantly lower in the monkeys immunized against the homologous strain, compared to those in the monkeys immunized against the heterologous strain. This shows the induction of SSPI following live sporozoites/CQ immunization. This immunization may also induce a pan-specific effect on the parasites, as indicated by early reduction of parasitaemias in the immunized monkeys compared to the non-immunized ones during the challenge infections. It also appears that the strain-specific component of this immunity may act on the parasites during the liver phase and during early phase of the blood cycle before detectable parasitaemia in the thin blood smear whereas the pan-specific component may act during later phase in the blood cycle.

Using the two *P. cynomolgi* strains, relapse studies demonstrated that the number of relapses and the relapse intervals are dependent on the sporozoite load, the parasite strain and the co-infections of the two strains. However, peak parasitaemia of relapses is independent of all the above factors. Live sporozoite/CQ immunization has no effect on the number of relapses, relapse intervals, peak parasitaemia of relapses or on the parasite strain that emerged in relapses. Relapse parasites elicit a transient antibody response that is not boosted during successive relapse infections. Waning immunity after relapse infections itself does not seems to trigger a new relapse infection. The results also indicate that relapse parasites may be clones of the parasite populations that emerged in the primary infection and probably originated from the activation of a single hypnozoite.