MALARIA VACCINE STUDIES ON *ANOPHELES* MIDGUT GLYCOPROTEINS AND A *PLASMODIUM FALCIPARUM* MEROZOITE SURFACE PROTEIN

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ABSTRACT

Vaccination is an alternative approach to control malaria because of the development of insecticide resistance in mosquito vectors and drug resistance in parasites. In this context, two different approaches viz, transmission blocking, using mosquito midgut (MG) glycoproteins, and eliciting immunity to asexual stage parasite antigens, using *Plasmodium falciparum* merozoite surface antigen 2 (PfMSA2) were investigated.

MG glycoproteins have been shown to act as receptors for recognition of ookinetes. Effect of the antibodies raised against Concanavalin A binding proteins (ConABP) of MG of *Anopheles tessellatus* on mosquito physiology and infectivity were investigated. Although a reduction (14%) in fecundity and an increase (11%) in percentage mortality were noticed in mosquitoes by *in vitro* feeding of anti-ConABP serum the effects were not significant. Antigenic relationships between proteins of MG and peritrophic matrix (PM) of *An. tessellatus* larvae and adult and MG glycoproteins of other vector mosquitoes were studied using anti-ConABP and anti-WGA binding protein (WGABP) antisera. While adult and larval MG glycoproteins of *An. tessellatus* are antigenically almost similar, glycoproteins in the PMs of the two developmental stages differed extensively. Antigenic cross-reactions are also observed between adult MG glycoproteins of different *Anopheles* species and, to a lesser extent, between *An. tessellatus* and *Culex* and *Aedes* mosquitoes.

Screening an *An. gambiae* cDNA expression library with anti-WGABP serum identified many myosin clones and yielded a unique clone (clone E) for a protein with a transient receptor potential (TRP). Homologous search analysis showed that clone E

encodes a homologue of TRP gamma cation channel protein of *Drosophila*. These findings illustrate the potential of MG glycoproteins as targets in transmission blocking as well as drawbacks in producing specific antisera.

Pf MSA2 has been identified as potential candidate molecule for developing an erythrocytic stage vaccine against malaria. PfMSA2 was inducibly expressed and displayed in lactic acid bacteria, *Lactococcus lactis* and *Lactobacillus* spp, in two different forms (1) covalently attached to the surface of live *L. lactis* cells through PrtP anchor system (MSA2cP) (2) non-covalently attached to *L. lactis* cell wall ghosts and to live *Lb. salivarious* and *Lb. reuteri* through AcmA anchor system (MSA2cA). Immunogenicity of MSA2 was investigated in three inbred strains of mice (Balb/c, C57 and C3H) and an outbred strain, ICR, by combined oronasal immunisation.

Oronasal immunisation of both immunogens (MSA2cP and MSA2cA) elicited high serum IgG antibodies (highest Abs observed at 405nm was 1.99 with MSA2cA and 0.69 with MSA2cP) against MSA2. Balb/c and C3H mice responded better to MSA2cP and MSA2cA on *L. lactis* respectively. The IgG isotypes to both immunogens reflected Th1 and Th2 influences. IgM response was significantly ($p<0.001$) low. IgA antibodies were observed in ICR, Balb/c and C3H strains and antisera of these strains reacted with native MSA2 on the surface of *P. falciparum* merozoites. However the responses of mice to MSA2cA on *Lactobacillus* were weak (highest Abs observed at 405nm was 0.34). Antigen specific spleenic IFN-γ secreting T cells were demonstrable by ELISPOT assay in Balb/c, C3H and C57 strains immunised with MSA2cA and in C57 mice immunised with MSA2cP. Oronasal immunisation with PfMSA2 was able to generate systemic antibodies and cellular

immunity in mice and in general MSA2cA was better than MSA2cP in eliciting immune responses in mice. Also the immune responses were dependent on the strain of mice and the way of antigen presentation.

Immune responses to the two immunogens were weaker and T-independent in old (24-30 wks) Balb/c mice. IFN-γ secreting cells were not detected in old mice. Histological studies revealed that there was a tendency for enlargement of mesenteric lymph nodes but not the spleen, increase in number of primary but not secondary follicles in mesenteric lymph nodes and spleen and presence of enlarged lymphoid aggregates in lamina propria in animals orally immunised with live *L. lactis* or *L. lactis* cell walls. However, immunisation with MSA2cP and expression inducer strain NZ9700 *L. lactis* together improved the immune responses in both oral and subcutaneous immunisation.

Mice immunised with live *L. lactis* cells had significantly ($p<0.05$) higher levels of anti-lactococcal antibodies than mice immunised with cell walls. Levels of antibodies tended to plateau between $5x10^8$ and $5x10^9$ live *L. lactis* per dose in ICR mice. Anti-lactococcal antibodies were noticeably lower against bacteria expressing MSA2 suggesting that presence of the heterologous antigen may divert the immune response from *L. lactis* antigens. These findings demonstrate the potential use of MSA2 as a vaccine candidate and use of *L. lactis* as vaccine delivery system in mucosal immunisation. Also it demonstrates the need to monitor anti-lactococcal responses to avoid possible hypersensitivity reactions in the recipient, to monitor local inflammatory responses in the gut mucosa and its implications in the induction of immune responses in old people.