ABSTRACT

The present investigations have been carried out on Indian malaria vector *Anopheles culicifacies*, which is responsible for about 65-70% of malaria in India. During this study an attempt has been made to develop the indigenous techniques for mosquito transgenesis and to identify an effector/antiparasitic molecule for transmission blocking and for anti-mosquito immunity.

For this, the ultra structure of *An. culicifacies* eggs has been observed with the help of scanning electron microscopy so as to prepare the eggs for microinjection/transgenesis. *An. culicifacies* egg were boat shaped, black in color, moderate float length (14-15 ridges) and 3 oval shaped lobe tubercles at both anterior and posterior ends. Micropylar orifice was surrounded by collar with incomplete hexagonal rays. Chorionic cells were present on anterior-lateral surface with distinct boundaries. The frill was moderate in height. The ultra structural analysis should enable to prepare the eggs for microinjection. In addition, the construction of pBac [3xP3-EGFP-Afm] plasmid was standarized for microinjection.

The temporal pattern of soluble proteins in the midgut of *An. culicifacies* has been studied to reveal the polypeptides involved in glucose feeding. The age and sex-specific polypeptides in the midgut have also been investigated. The soluble midgut proteins pattern of different species of *Anopheles* and in sibling species complex of *An. culicifacies* (Type A, B and C) has also been investigated. N-terminal sequencing of low molecular weight polypeptides was also analyzed.

N-terminal sequencing of low molecular weight proteins and sequence analysis shows that 13 kDa polypeptide was ~80% homologous to *An. gambiae* cecropin like polypeptide. Hence, 13 kDa polypeptide was used to raise antisera. The antibody titer was measured by ELISA. The putative immunogenic polypeptides were identified by Western blotting. The binding of antibodies with different tissues was studied by *in vivo* ELISA. The malaria parasite *P. vivax* development was blocked by ingesting anti-mosquito cecropin like antibodies along with blood meal. The effect of anti-mosquito antibodies was also studied on other *Anopheles* species.

High antibody titer was observed against the cecropin like protein during present investigations. Four immunogenic polypeptides (85, 38, 29 & 13 kDa) were identified by the antisera raised against cecropin like protein. However, 13 & 38 kDa polypeptides were specific to mosquito midgut. Cross reactivity with other tissues as well as with other mosquito species was extensive which could be attributed to the sharing of common epitopes. The maximum fecundity reduction was 40.8% after feeding of antimosquito antibodies. Engorgement remains unaffected in all the cases. The increase in mortality was found to be insignificant. The effect on the reproductive capacity seems to be species-specific. The transmission of malaria parasite *P. vivax* was reduced by 87.6% when parasites were digested with anti-sera raised against glucose fed anti-mosquito antibodies.

In addition, molecular analysis of *Cecropin A* was carried out by amplifying it from genomic DNA by PCR. The amplified fragment was sequenced and cloned. *Cecropin A* gene of *An. culicifacies* was ~916bp in length with a 343bp 5’ UTR and a 228 bp 3’ UTR. Homology search alignment shows *An. culicifacies* cecropin A gene is 98% homology with *An. gambiae* cecropin A, 95% with *An. merus, An. bwambae* and *An. melas*, 97% with *An. arabiensis* and *An. quadriannulatus*.

Identification of target molecules from different tissues can be exploited for the development of immunological tools as well as for genetic engineering of mosquitoes to alter the ability of the mosquito to transmit malaria. Cecropin could be used as an effector/antiparasitic molecule along with sex, tissue and stage-specific promoters for transgenic formation.