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Fecundity of *Anopheles tessellatus* reduced by the ingestion of murine anti-mosquito antibodies

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Abstract. High titres of antibodies to antigens derived from head/thorax, midgut or abdomen of *Anopheles tessellatus* were produced in inbred mice. These antibodies, when ingested in a bloodmeal, reduced the fecundity of *An.tessellatus* by up to 29% in different experiments. It is postulated that antibodies directed against antigens shared between the head/thorax, abdomen and midgut tissues are involved in the reduction of fecundity.

Key words. *Anopheles tessellatus*, mouse anti-mosquito antibodies, fecundity.

Introduction

Antigens derived from mosquito tissues induce antibody and cell-mediated immunological responses in vertebrates, when they are introduced either naturally or artificially into vertebrate hosts (Wikel, 1982; Ramasamy & Ramasamy, 1990). The quantitation of anti-*Culex* antibody levels in man could be useful as a measure of exposure to mosquito bites in field studies (Das *et al.*, 1991). Rabbit anti-*Aedes* antibody ingested in a bloodmeal cross the midgut epithelium in *Aedes aegypti* Linnaeus (Ramasamy *et al.*, 1988a); similar observations have been made with specific rat antibodies in *Culex pipiens* L. and *Anopheles stephensi* Liston (Vaughan & Azad, 1988). However, mouse antibodies were observed not to cross the midgut of *Ae.aegypti* (Ramasamy *et al.*, 1988b).

Ingestion of rabbit anti-mosquito antibodies in a bloodmeal decreased the fecundity of *Ae.aegypti* (Sutherland & Ewen, 1974; Ramasamy *et al.*, 1988a) and *An.tessellatus* Theobald (Ramasamy *et al.*, 1992), while mouse anti-mosquito antibodies did not influence the fecundity of *Ae.aegypti* (Hatfield, 1988; Ramasamy *et al.*, 1988b) or that of *Cx quinquefasciatus* Say (Ramasamy *et al.*, 1992). The present study, however, shows that ingested mouse anti-mosquito antibodies are effective in reducing the fecundity of *An.tessellatus*.

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Materials and Methods

Mosquitoes. An.tessellatus were obtained from a laboratory colony maintained at 28 ± 1°C, 80% relative humidity and natural day:night photoperiods. Larvae were fed on powdered infant food and yeast; adults on 10% glucose supplemented with multivitamins.

Antigen preparation and immunization. 4–6-day-old *An.tessellatus* were fed on restrained BALB/C mice, and the blood-fed adult female mosquitoes stored at −20°C from 30 h after the bloodmeal. Three antigen preparations, composed of pooled head/thorax (HT), midgut (MG) containing undigested mouse blood cells and rest of abdomen (AD) were obtained by dissecting mosquitoes in 0.01 M phosphate buffered saline pH 7.4 (PBS). These pooled and homogenized preparations (approximately 5 mosquito equivalents in 0.2 ml PBS) were injected intramuscularly into 4–6-week-old BALB/C mice. The first immunization was made in Freund's complete adjuvant mixed 1:1 with antigen in PBS. Control mice were injected in parallel with adjuvant only and subsequently with PBS. A total of eight immunizations were made before the mice were used for experimental feeding of mosquitoes. Antibody titres in mouse sera were determined by an enzyme-linked immunosorbent assay (ELISA) using peroxidase conjugated goat anti-mouse IgG (Silenius, Melbourne, Australia) as described elsewhere (Ramasamy *et al.*, 1992).

Mosquito feeding and fecundity. Immunized mice were used for blood-feeding 6–10 weeks after the eighth immunization by placing a restrained mouse inside a cage of 3–4-day-old *An.tessellatus*. All non-bloodfed mosquitoes were removed immediately after feeding. Blood-fed mosquitoes were maintained on 10% glucose supplemented

with multivitamins. A surface for oviposition was not provided. The number of mature stage IV–V follicles (Clements & Boocock, 1984) present in the ovaries of mosquitoes 72 h after the bloodmeal was used as an estimate of fecundity.

Results and Discussion

Immunized mice produced antibody titres of 10^4–10^6 against immunizing antigens. Control mice, injected with Freund's adjuvant and PBS without antigen, developed titres of 10^2 by ELISA. The background reaction in control mice may be due to naturally occurring antibodies and cross-reactive antibodies produced by Freund's adjuvant.

Mosquitoes fed on mice immunized with antigen derived from either head/thorax, midgut or abdomen produced significantly fewer eggs in experiments 1 and 2 (Table 1). In experiment 3, mosquitoes were fed on three mice immunized with midgut antigen, or on two control mice. No significant difference was observed between the numbers of eggs produced by mosquitoes fed on either of the control mice (C_{71} and C_{74}). The number of eggs produced by mosquitoes fed on one of the three mice (C_{22} but not C_{23} nor C_{25}) immunized with midgut antigen was significantly lower than the numbers of eggs produced by mosquitoes fed on either control mice (C_{11} and C_{74}).

When the numbers of eggs produced by mosquitoes feeding on mice immunized with the same antigen were combined over experiments 1–3, mosquitoes feeding on control mice produced 62.0 ± 1.1 eggs/female compared to 50.0 ± 1.6, 52.6 ± 1.2 and 58.8 ± 1.6 eggs/female produced by mosquitoes fed on mice immunized with antigens derived from head/thorax, midgut or abdomen tissues respectively. These reductions of 19% and 15% produced in the presence of anti-mosquito antibodies to head/thorax and midgut antigens were statistically significant ($P < 0.05$).

The reduction in fecundity observed in the presence of antibodies to head/thorax, midgut and abdomen tissue preparations suggests that antibodies to a common antigen shared between all three preparations may be involved in the process. Antigens shared between head/thorax, midgut and abdomen tissues have been demonstrated in *An.tessellatus* (Ramasamy *et al.*, 1991). Rabbit antibodies directed against shared antigens decreased the fecundity of *An.tessellatus* (Ramasamy *et al.*, 1992). The nature and cellular or extracellular location of such shared antigens are not known.

Ingested specific antibodies were detected in the haemolymph of *Culex pipiens* up to 3 h after the bloodmeal, while in several anopheline species such antibodies persisted in the haemolymph for up to 24 h after a bloodmeal (Vaughan & Azad, 1988). Mouse anti-mosquito antibodies to *Cx quinquefasciatus* antigens had no detectable effect on the fecundity of *Cx quinquefasciatus* (Ramasamy *et al.*, 1992). The ability of rabbit and mouse anti-mosquito antibodies to reduce the fecundity of *An.tessellatus* may therefore be related to contrasts in processing ingested anti-mosquito antibodies in the midgut and the haemocoel in the different mosquito genera. Vitellogenesis or other processes concerned with egg development could also be differentially susceptible to antibodies in culicine and anopheline mosquitoes. Determining the mode of action of ingested antibodies should resolve this question.

Table 1. Fecundity of *An.tessellatus* fed on BALB/C mice immunized with *An.tessellatus* antigens. HT = head and thorax; MG = midgut; AD = abdomen remnants. Significance of differences between total number of eggs/female determined by '*t*' tests. In each experiment, the numbers of eggs laid by mosquitoes fed on immunized mice were compared with numbers produced by those fed on control mice.

Mouse no. (antigen)	No. engorged females	Egg development (%)	Eggs/female (mean ± SE)	*P*
Experiment 1				
C_{75} (Control)	41	97.5	68.00 ± 2.62	
C_{11} (HT)	34	97.1	54.97 ± 2.22	<0.01
C_{21} (MG)	36	94.4	55.77 ± 1.55	<0.01
C_{34} (AD)	40	97.4	61.74 ± 2.31	<0.05
Experiment 2				
C_{72} (Control)	40	100	62.15 ± 2.00	
C_{12} (HT)	38	81.6	45.27 ± 1.91	<0.001
C_{22} (MG)	39	66.7	52.31 ± 4.46	<0.05
C_{32} (AD)	34	100	55.47 ± 1.83	<0.05
Experiment 3				
C_{71} (Control)	31	100	59.00 ± 1.63	
C_{74} (Control)	32	96.9	54.55 ± 1.91	
C_{22} (MG)	33	72.7	41.92 ± 2.08	<0.001
C_{23} (MG)	30	96.7	52.47 ± 3.00	NS
C_{25} (MG)	32	96.9	58.75 ± 2.03	NS

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