Antigenic Similarity Between the Mosquito Vectors of Malaria and Filariasis

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ABSTRACT Rabbit antisera were raised to the malaria vector *Anopheles tessellatus*, head–thorax, abdomen, and midgut preparations. Reactivity of the antisera with *An. tessellatus* and the filariasis vector, *Culex quinquefasciatus*, were examined by enzyme-linked immunosorbent assay (ELISA) and Western blots. Although many antigens were shared between the two species of mosquitoes, tissue- and species-specific antigens also were identified.

KEY WORDS Insecta, *Anopheles tessellatus*, antigens, *Culex quinquefasciatus*

MOSQUITOES ARE VECTORS of many arboviruses and parasites that infect man. Host antibodies ingested in the blood meal can influence mosquito fecundity (Ramasamy et al. 1988a) and the transmission of arboviruses (Ramasamy et al. 1990) and malaria parasites (Ramasamy & Ramasamy 1990a). Immunization against mosquito antigens therefore may be a possible method of reducing the transmission of mosquito-borne pathogens (Ramasamy & Ramasamy 1990b). These studies implicated molecules in the mosquito midgut, particularly pathogen receptors, as targets for a transmission-blocking host immune response. The characterization of target antigens in mosquitoes is therefore a prerequisite for more detailed immunological studies. It is also important to determine the extent of cross-reactivity among homologous molecules in different mosquito species to produce immunity against a broad spectrum of mosquito transmitted diseases. In this context, we reported that vitellin of *Aedes aegypti* Linnaeus possesses epitopes that are shared among culicine mosquitoes, but that are only weakly cross-reactive with anophelines (Ramasamy et al. 1988b).

Anopheles tessellatus Theobald is a vector of the human malaria parasite, *Plasmodium vivax* Grassi (Munesinghe et al. 1986). *Culex quinquefasciatus* Say is the primary vector for *Wuchereria bancrofti* Cobbold, the predominant human filaria parasite in Sri Lanka. Hence, *An. tessellatus* and *Cx. quinquefasciatus* are suitable for studying the effects of host antibodies on mosquito physiology and disease transmission. We report here the cross-reactivity between head–thorax, abdomen, and midgut preparations of *An. tessellatus* and *Cx. quinquefasciatus*.

Materials and Methods

Mosquitoes. Colonies of *An. tessellatus* and *Cx. quinquefasciatus* were held in the laboratory at 28 ± 1°C, 80% RH and natural day–night photoperiod conditions. *An. tessellatus* larvae were fed a diet of powdered infant cereal and dry yeast, whereas *Cx. quinquefasciatus* larvae were fed powdered soya protein. Adult mosquitoes were fed on 10% glucose supplemented with multivitamins and were blood fed on guinea pigs.

Antigen Preparation and Immunization. Four- to seven-day-old sugar-fed *An. tessellatus* and *Cx. quinquefasciatus* were held at −20°C until dissected. The head and thorax, midgut, and rest of the abdomen were dissected separately in 0.01 *M* phosphate-buffered saline, pH 7.4 (PBS), and used as three antigen preparations. Pooled antigen preparations from *An. tessellatus* were homogenized in PBS and suspensions were injected, intramuscularly at multiple sites, into New Zealand white rabbits in a final volume of 1–2 ml per rabbit. The first immunization was made with Freund's complete adjuvant, mixed 1:1 with antigen in PBS. Groups of four rabbits per antigen preparation were immunized with a total of fifty mosquito equivalents per rabbit at 3–4 wk intervals. A group of three control rabbits was injected with PBS in a similar manner. After four injections of antigen, rabbits were bled through the ear vein.

Antibody Titers. Antibody titers in rabbit sera were determined by enzyme-linked immunosorbent assay (ELISA). Microtiter plate wells were coated with head/thorax, abdomen, and midgut preparations (after allowing particulate material to settle) derived from sugar fed mosquitoes (one mosquito equivalent per well) in 0.01 *M* bicarbonate–carbonate buffer at pH 9.6 overnight at 4°C. The wells were then washed in PBS and incubated with 5% wt/vol low fat milk powder in PBS (Blotto) for 2 h at 37°C to block unreacted protein binding sites in the wells. The bound antigen was then washed in PBS four times and reacted with dilutions of rabbit sera in Blotto for 1 h at 37°C. The wells were then washed with PBS four times and reacted with a 1:2,000 dilution of peroxidase conjugated sheep antirabbit IgG with H and L chain specificity (Si-

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Table 1. Titers[a] of rabbit anti-*An. tessellatus* sera

Antisera	Antigen	
	An. tessellatus	*Cx. quinquefasciatus*
Anti-*An. tessellatus* HT (four rabbits)	8.75 (8–9)	6.25 (5–8)
Anti-*An. tessellatus* AD (four rabbits)	6.25 (4–9)	4.75 (4–7)
Anti-*An. tessellatus* MG (four rabbits)	5.75 (5–7)	5 (4–6)
Control (three rabbits)	0 (0)	1 (1)

HT, head and thorax; MG, midgut; AD, abdomen excluding midgut.

[a] Mean of $\log_{10}$ titers determined by ELISA. The range observed is given in parentheses. Anti head/thorax, abdomen, and midgut sera were tested against head/thorax, abdomen, and midgut antigen preparations, respectively. Sera from control rabbits were tested against all three antigen preparations.

lenius, Melbourne, Australia); 2,2'-azino-bis (3-ethyl benz-thiazoline-6-sulphonic acid) (ABTS) and H_2O_2 were used as substrates (Biorad, Richmond, Calif.) to develop the color reaction.

Western Blots. Discontinuous polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate (SDS) was performed as described by Laemmli (1970). Samples of head/thorax, abdomen, and midgut preparations (at 3, 6, and 15 mosquito equivalents per lane, respectively) were dissolved in an equal volume of Laemmli sample buffer of twice the normal concentration (Laemmli 1970), placed in boiling water for 5 min, microfuged to remove insoluble material, and then electrophoresed on 10% gels. Molecular weight markers (Sigma, St. Louis, Mo.) were electrophoresed in parallel lanes. After SDS–PAGE, the proteins were transferred to nitrocellulose membrane (Towbin et al. 1979), using a Biorad Transblot SD cell. After blocking unreacted sites on the membrane with Blotto for 16 h at 4°C, the membranes were probed with the rabbit sera. A mixture of immune sera derived from four rabbits per antigen preparation was used at a final dilution of 1:100 and allowed to react for 2 h at 22°C. The membrane was washed and then incubated with a 1:2,000 dilution of peroxidase conjugated sheep antirabbit IgG (Silenius, Melbourne, Australia) for an additional 1 h at 22°C. Finally, the membrane was washed and antigen bands were visualized by reaction with 0.6 mg/ml 4-chloronaphthol and 0.015% H_2O_2.

Results

The antibody titers determined by ELISA showed that there was significant cross-reactivity between *An. tessellatus* and *Cx. quinquefasciatus* antigen preparations (Table 1). However, the titers against the immunizing antigen, which was derived from *An. tessellatus*, was generally higher for *An. tessellatus* than against *Cx. quinquefasciatus*.

Immunogenic proteins in anopheline head/thorax, abdomen, and midgut preparations were identified by probing Western blots with pooled rabbit

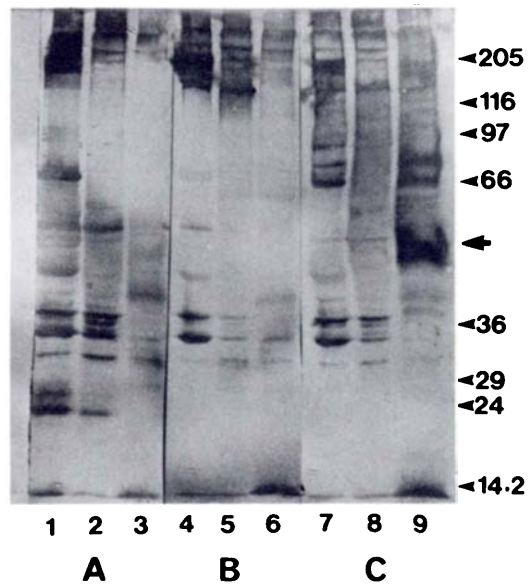

Fig. 1. Western blots of *An. tessellatus* head and thorax (HT) (1, 4, 7), abdomen (AD) (2, 5, 8), and midgut (MG) (3, 6, 9) probed with anti-HT (A), anti-AD (B), and anti-MG (C) sera, respectively. The 47–50 kdal midgut antigen is marked by an arrow. The migration positions of molecular weight standards are indicated.

anti-head/thorax, abdomen, and midgut sera (Fig. 1). Sera from rabbits injected with PBS that were used as controls showed no reaction with mosquito tissue at the same dilution as the immune sera (data not shown). A prominent reaction with a diffusely migrating, high-molecular-weight (160–190 kdal) antigen in head/thorax was observed with all three immune sera. Antigens migrating with similar mobility could be demonstrated in all three preparations. However, specific antigens also were observed. In particular, the antimidgut serum recognized a diffusely migrating 47–50 kdal antigen that was present in the midgut (arrow in Fig. 1) but not in the head/thorax or abdomen. There was extensive cross-reactivity among the preparations, with each of the immune sera recognizing multiple antigens in head/thorax, abdomen, and midgut preparations. This observation is consistent with the ELISA results reported previously for *Ae. aegypti* tissues (Ramasamy et al. 1988a).

An examination of the reactivity of pooled rabbit anti-*An. tessellatus* head/thorax, abdomen, and midgut sera with *Cx. quinquefasciatus* antigen preparations revealed significant cross-reactivity (Fig. 2), which confirmed the cross reactions seen by ELISA. The antihead/thorax serum recognized major proteins of 215, 160–190 (diffuse band), 78, 70–74 (diffuse), 57, 55, 52, 46, 39, 36, and 25 kdal in *An. tessellatus*; and 190, 130, 70, 56, 55, 52, 46, 38, and 36 kdal in *Cx. quinquefasciatus*. Antigens of 215, 160–190, 70–74, 57, 39, and 25 kdal were unique to *An. tessellatus* head/thorax. Antibodies raised against abdominal tissues recognized several

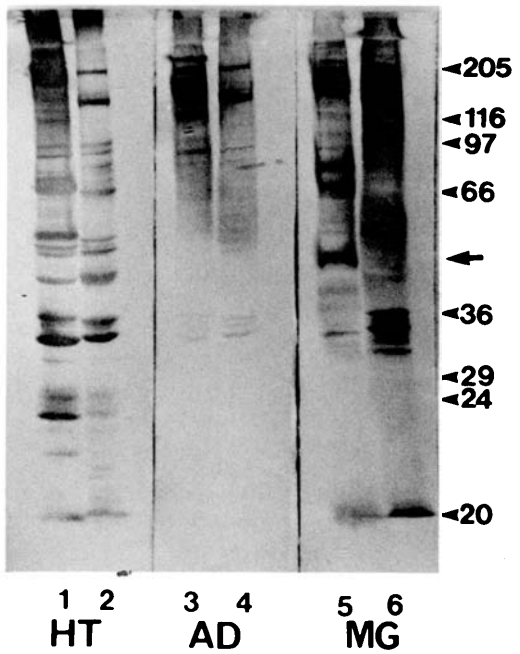

Fig. 2. Western blots on *An. tessellatus* (1, 3, 5) and *Cx. quinquefasciatus* (2, 4, 6) head and thorax (HT), abdomen (AD), and midgut (MG) tissue probed with anti-HT, anti-AD, and anti-MG sera, respectively. The 47–50 kdal antigen unique to *An. tessellatus* MG is shown by an arrow. The migration positions of molecular weight standards are indicated.

proteins that were common in both *An. tessellatus* and *Cx. quinquefasciatus* abdomen tissue. Proteins of molecular weight 215 kdal and several antigens in the range 130–180 kdal were specific for *An. tessellatus* abdomen. Similarly, specific proteins of 215, 75–80, 68–70, and 47–50 kdal were detected in *An. tessellatus* midgut serum. The 215 kdal antigen was unique to *An. tessellatus* and was found in all three preparations.

Discussion

Pooled sera from rabbits immunized with the same antigen preparation were used in Western blots to simplify analysis and to minimize variation among rabbits. The cross-reactivity observed in Western blots among *An. tessellatus* head/thorax, abdomen, and midgut preparations probably reflects the presence of common tissues (e.g., muscle) and shared (structural and "housekeeping") proteins. However, some specific antigens were identified. Because midgut antigens have been implicated as targets in artificially induced transmission blocking immunity (Ramasamy et al. 1990; Ramasamy & Ramasamy 1990a,b), it may be significant that antimidgut sera recognized a diffusely migrating 47–50 kdal midgut-specific antigen. More detailed studies on this antigen are being pursued with regard to its structure, location, and role in transmission blocking.

Shared antigens also were seen in corresponding preparations of *An. tessellatus* and *Cx. quinquefasciatus*, reflecting the presence of numerous homologous (structural and housekeeping) proteins. The absence of the 47–50, 67–70, and 74–80 kdal *An. tessellatus* midgut-specific antigens in the *Cx. quinquefasciatus* midgut is noteworthy. Although homologous *Cx. quinquefasciatus* midgut molecules may exist, the anti-*An. tessellatus* midgut serum possibly does not react with such molecules. It will be important to determine whether any of the shared antigenic molecules are useful from the point of view of immunization against multiple mosquito vectors of human disease.

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