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Inhibition of Malaria Parasite Development in Mosquitoes by Anti-Mosquito-Midgut Antibodies

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The mosquito midgut plays a central role in the development and subsequent transmission of malaria parasites. Using a rodent malaria parasite, *Plasmodium berghei*, and the mosquito vector *Anopheles stephensi*, we investigated the effect of anti-mosquito-midgut antibodies on the development of malaria parasites in the mosquito. In agreement with previous studies, we found that mosquitoes that ingested antimidgut antibodies along with infectious parasites had significantly fewer oocysts than mosquitoes in the control group. We also found that the antimidgut antibodies inhibit the development and/or translocation of the sporozoites. Together, these observations open an avenue for research toward the development of a vector-based malaria parasite transmission-blocking vaccine.

The development of malaria vaccines has largely targeted the blood stage of the parasite, to inhibit its replication, and the sporozoite stage, to block the initial transfer of infection from mosquito to human. Further targeting of the requisite development stages of the parasite within the vector anopheline, such as the zygote, ookinete, and oocyst, may offer unique and desirable features for malaria vaccine development. In contrast to the repeated inoculation of sporozoites and the large number of emerging blood forms in the human host, the majority of the midguts of naturally infected mosquitoes contain one to three oocysts (2). Successful development of the oocyst requires 8 to 12 days of extracellular growth and division within the midgut epithlieum before sporozoites are released to migrate to the salivary glands. Although considerable development of the parasite occurs within the confines of the midgut, little is known about the role of midgut macromolecules in the process of fertilization, ookinete migration, oocyst development, and shizogony.

Previous investigations of the effect of anti-mosquito-midgut antibodies taken with a blood meal demonstrated reduced mosquito mortality and infectivity at the oocyst level (8). In the present study, using *Anopheles stephensi* as the vector and the rodent malaria parasite *Plasmodium berghei*, we undertook two separate approaches to vector-directed transmission-blocking immunity that involved the exposure of developing parasites to single or multiple blood meals containing antibody to the mosquito midgut.

Midguts from sugar-fed female mosquitoes were isolated in sterile phosphate-buffered saline and homogenized at 4°C. Mice (ICR) were immunized with complete and incomplete Freund's adjuvant at a primary dose of 50 to 100 μg of protein antigen and secondary and tertiary boosts of 50 μg each, 21 and 35 days later. Control mice were immunized and boosted with adjuvant alone on the same schedule. Midgut-immunized animals developed high-titer antibodies (>1:25,000 by enzyme-linked immunosorbent assay) that were stable for at least 3 months. Peak-titer sera from some mice were collected, pooled, and stored at −70°C for later use in membrane feeders. Additional midgut-immunized and control mice were infected with *P. berghei* (ANKA) by blood passage and served as a source of infectious blood meals for mosquitoes. Unfed mosquitoes were removed. Mosquitoes scheduled for a single blood meal were subsequently maintained on sucrose. For multiple blood meals, mosquitoes were fed on additional uninfected mice (antimidgut and control immunized) at 2- to 3-day intervals. Alternatively, multiple blood meals were taken from membrane feeders filled with the appropriate immune serum and saline-washed erythrocytes from naive mice at a final 45% hematocrit.

The results of two different multiple-feeding experiments are presented in Table 1. Significant reductions in both infection rate and number of oocysts per infected mosquito were observed in the antimidgut-fed group compared with the control group. We found that when mosquitoes were allowed to take three successive blood meals on midgut-immunized mice (group A), significantly fewer oocysts (average of 0.96 oocyst per infected mosquito) developed than in the control group (average of 19.8 oocysts per infected mosquito). In a separate trial (Table 1, group B), serum from antimidgut-immunized or control mice was passively transferred (0.3 ml per mouse, intravenously in the tail vein) into two groups of *P. berghei*-infected mice 1 h before mosquito feeding. Subsequent blood meals (immunized and control mice) were taken from membrane feeders. The number of oocysts per mosquito was quantified starting at 9 days postinfection. The results of this approach were in agreement with those of the experiments described above; the mean number of oocysts in mosquitoes that were exposed to midgut antibodies was 3.7, compared with 41.1 in control antibody-fed mosquitoes.

In the single-blood-meal-fed mosquitoes, the number of oocysts per infected mosquito was also reduced in the immune-fed group, although only modestly (Fig. 1A). At 12

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TABLE 1. Transmission-blocking immunity in mosquitoes receiving multiple blood meals

Group[a]	No. of mosquitoes/group		% Infected mosquitoes		Mean no. of oocysts/mosquito (range)		% Transmission-blocking immunity[b]
	Control	Anti-MG	Control	Anti-MG	Control	Anti-MG	
A	32	28	75	25	19.8 (0–116)	0.96 (0–19)	95.2
B	10	10	70	60	41.1 (0–113)	3.7 (0–13)	90.9

[a] A, all blood meals were on live animals (control or midgut [MG] immunized); B, infectious blood meal was on MG-immunized and control-immunized animals; subsequent blood meals were by membrane feeders.

[b] Determined by the method of Ponnudurai et al. (7) as [(mean oocyst no. in control − mean oocyst no. in anti-MG)/(Mean oocyst no. in control)] × 100.

days postinfection and later, paired midgut and salivary gland preparations were analyzed. The number of sporozoites in the salivary gland was counted in crushed preparations of salivary glands (Fig. 1B). Whereas a 42.9% (6 of 14) salivary gland infection rate was observed in the control group between 17 and 21 days postinfection, no sporozoites were observed in the salivary glands of the immune-fed group (0 of 19) as late as 29 days postinfection. Curiously,

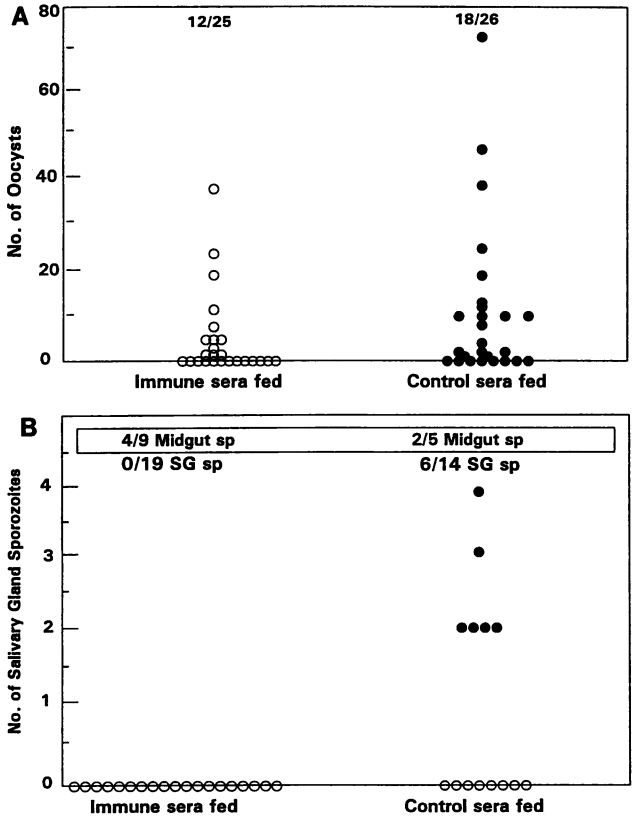

FIG. 1. Effect of a single blood meal on *P. berghei* oocyst production (A) and sporozoites in salivary glands (B). (A) Mosquitoes received infectious blood meals from *P. berghei*-infected mice with comparable circulating gametocytemia. The mice had passively received either antimidgut immune serum or control mouse serum. ○, antimidgut blood meals; ●, control blood meals. Each symbol represents an individual mosquito. (B) Salivary gland (SG) sporozoite (sp) infections in mosquitoes that received antimidgut or control blood meals were determined between 17 and 29 days postinfection. ○, uninfected salivary gland; ●, salivary gland in which sporozoites were found. The number of sporozoites is represented on the ordinate scale as follows: 1, 1 to 10; 2, >10; 3, >100; and 4, >1,000.

free sporozoites (within the midgut lumen and outside the midgut epithelium), late-stage oocysts, and degenerate oocysts were observed in the midguts of approximately 50% of the antimidgut-fed mosquitoes at 29 days postinfection. It was not determined whether the free sporozoites observed in the midgut preparations were released from oocysts by the mechanical pressure of dissection or had been released prior to dissection. Nonetheless, it was not until 30 days postinfection that any salivary gland sporozoites were observed in the immune-fed group (sporozoites in the gland in 2 of 12 individuals). Midgut sporozoites or oocysts were rarely observed after 20 days postinfection in control mosquitoes. Similar results were obtained when a cohort of mosquitoes that had received multiple blood meals (Table 1, group A) were monitored for the presence of salivary gland infections; the reduced oocyst infection rates in the antimidgut-fed group became more pronounced at the salivary gland level, with only 7.7% of the dissected mosquitoes containing sporozoites in the salivary gland. Among the normal blood-fed control mosquitoes, 75% had the salivary gland infection.

Experimental evidence of acquired resistance to hematophagous arthropods was first reported by Trager in 1939 (11). In these studies, dog ticks, *Dermacentor variabilis*, fed less on guinea pigs and rabbits that had been sensitized by exposure to feeding larval ticks. Since then, a variety of mammalian hosts have been immunized with homogenates of arthropods, extracts of them, or purified proteins (1, 14). The concept of using host antivector immune responses has also been successfully exploited for other tick-borne infectious diseases. These studies have led to experimental cattle vaccines based on tick gut antigens (3, 6). In other studies, it has been shown that antibodies against midgut antigens of *Aedes aegypti*, when ingested with an infected blood meal, reduce the susceptibility of mosquitoes to infection with arboviruses (9). In the present study, we have chosen (as have several other investigators) to target the mosquito midgut as a source of protein antigens that may be critical to successful sporogony of the malaria parasite.

Mosquito midgut homogenates were chosen as a source of antigens for immunization of mice in anticipation that antibody interaction with midgut antigens in the infected mosquito might interfere with normal parasite development. Although the mechanism by which antibodies against mosquito midgut components affects parasite infectivity is unclear, the observed reduction in oocyst numbers in mosquitoes that received antimidgut antibodies may reflect a disturbance of normal vector-parasite interactions and/or changes in the gut physiology rather than a direct effect on the developing parasites. Although the findings reported here are consistent with those in an earlier report (8), our study differs in many respects. In addition to investigating the effects of single antimidgut blood meals, multiple blood

feeds were tested. Even when allowed to engorge completely, many mosquitoes will feed as often as every 3 to 4 days if given the opportunity. Thus, although levels of hemolymph antibody drop quickly within 18 h after a blood meal (12, 13), the level may be maintained by multiple blood meals. It is also important to point out here, as has been mentioned before, that naturally infected mosquitoes in regions of Africa where the malaria rate is high contain an average of one to three oocysts (2). Therefore, under field conditions, less drastic reductions in oocyst numbers than those observed here may effectively abolish most mosquito infections.

The observation that salivary gland sporozoites are absent in mosquitoes that carry oocysts suggests a delayed or incomplete development of oocysts in response to antimidgut blood meals. These are particularly interesting results, because just over one-third of the natural population of two members of the *Anopheles gambiae* complex survive for more than 1 week and only 9% survive for more than 2 weeks (4). Therefore, a delay in the time needed for translocation of sporozoites from midgut tissue to the salivary glands together with the short natural lifespan of the mosquitoes would increase the chance of eliminating sporozoite-containing mosquitoes from the existing population.

Other recent studies suggest a mosquito species-specific mortality response to antimidgut blood meals (10). Interestingly, similar antimidgut blood meals among *P. berghei*-infected *Anopheles farauti* resulted in decreased mortality (8). Evidence suggesting penetration of midgut epithelial cells by *P. berghei* ookinetes (5), resulting in midgut damage and increased mosquito mortality, has been reported (8). In this case, a reduced infection rate among antimidgut-fed mosquitoes was proposed to negate the mortality effect normally seen in *P. berghei*-infected mosquitoes (8). In our experience, mortality rates among both antimidgut-fed and control-fed populations were low, and no distinguishable differences were found. A transmission-blocking scheme based on the interruption of vector-dependent parasite development, in which the vector experiences minimal adverse effects, may be desirable. Under such conditions, there would be little or no selection of random vector variants in which vector-dependent parasite development was restored in the face of transmission blockage.

In conclusion, this study shows that rodent antibodies against mosquito midgut antigens, when ingested by mosquitoes along with the infected blood, affect the development of oocysts in the midgut and the release and/or translocation of sporozoites into the salivary gland. The inhibitory effect of antimidgut serum on parasite development is further manifested by multiple immune blood meals. Clearly, additional work is needed to identify which of the midgut antigens are involved in interacting with the parasites. Once these proteins have been identified, midgut antigen-based antimosquito vaccines can be developed for use independently or as a part of a multivalent immune intervention strategy.

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