Anopheles gambiae salivary gland proteins as putative targets for blocking transmission of malaria parasites

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Anopheles gambiae is the primary vector of human malaria in sub-Saharan Africa. Invasion of Anopheles salivary glands by Plasmodium sporozoites is a necessary step in the transmission of malaria and is likely to be mediated by specific receptor-ligand interactions. We are interested in identifying putative an A. gambiae salivary gland receptor or receptors for sporozoite invasion as a possible target for blocking malaria transmission. By using monoclonal antibodies against female-specific A. gambiae salivary gland proteins, two molecules, one of 29 kDa and one of 100 kDa, were identified and characterized with respect to the age and blood-feeding process of mosquitoes. In an in vivo bioassay, the monoclonal antibody against the 100-kDa protein inhibited Plasmodium yoelii sporozoite invasion of salivary glands ≥75%. These results show that A. gambiae salivary gland proteins are accessible to monoclonal antibodies that inhibit sporozoite invasion of the salivary glands and suggest alternate targets for blocking the transmission of malaria by this most competent of malaria vectors.

Despite long-standing chemotherapeutic intercession and vector control programs, malaria exacts a heavy burden on human health, with 300–500 million infections and 1.5–2.7 million deaths annually. The disease is transmitted from female Anopheles mosquitoes to humans through the sporozoite stage of the Plasmodium parasite in the course of a blood meal. The penultimate event before the infectious bite is invasion of the Anopheles salivary glands by the Plasmodium sporozoites. This critical period of the sporogonic cycle presents a rarely studied point in the life cycle of the parasite, from which new strategies for blocking transmission would be derived. Invasion of the salivary glands could prevent the transmission of malaria from the Anopheles vector to humans.

Earlier studies have indicated that the sporozoite–salivary gland interaction is species specific and receptor mediated, suggesting that the glands dictate the ability of sporozoites to recognize and invade the salivary glands (1). Biochemical characterization of salivary gland components has shown that the basal lamina and the female-specific distal and median lateral lobes are heavily glycosylated (2). In addition, it has been shown that lectins that bind salivary gland-associated carbohydrates block Plasmodium gallinaceum (avian malaria parasite) sporozoite invasion of Aedes aegypti salivary glands. Concurrently, polyclonal serum against A. aegypti salivary glands inhibited P. gallinaceum sporozoite invasion as compared with preimmune serum and saline controls (3).

To date, efforts to block the invasion of mosquito salivary glands by malaria sporozoites have been carried out with A. aegypti mosquitoes and P. gallinaceum parasites. Although this system serves as an excellent model because of the relative simplicity of raising large numbers of A. aegypti mosquitoes and the ease of studying P. gallinaceum, results cannot be easily applied to the natural transmission of human malaria for two reasons. First, Aedes mosquitoes do not transmit human malaria parasites, and second, there are biological differences between many of the mammalian malaria parasites and P. gallinaceum. The goal of this study was to investigate putative receptors for mammalian malaria sporozoites on the salivary glands of A. gambiae, the primary malaria vector in Africa (5). The proposed hypothesis is that blocking the interaction between sporozoites and female-specific A. gambiae salivary gland proteins with salivary gland-specific antibodies will inhibit or prevent sporozoites from invading A. gambiae salivary glands and thus, reduce transmission. Identification of sporozoite receptors would not only increase the understanding of the biology of Plasmodium in the Anopheles vector but also suggest new molecular targets for blocking the transmission of human malaria.

Materials and Methods

Mosquitoes and P. yoelii Infection. A. gambiae (G-3 strain) and Anopheles stephensi (Dutch strain), obtained from the Laboratory of Parasitic Diseases (National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda), were reared at 27 ± 1°C and 80 ± 5% relative humidity with 12-h cycles of alternating darkness and light. Adult mosquitoes were maintained on 10% (vol/vol) Karo Dark Corn Syrup with 0.05% p-amino benzoic acid (6). Thawed P. yoelii (17XNL strain) was injected i.p. into a 6- to 8-week-old BALB/c mouse (Charles River Breeding Laboratories). When the parasitemia reached 3–5%, blood was collected and used to infect more BALB/c mice. The parasitemia was monitored daily by Giemsa-stained thin films. When the parasitemia reached 4–7% (4–5 days), the mice were anesthetized and fed to starved 4- to 5-day-old A. gambiae females.

SDS/PAGE Analysis of Salivary Gland Proteins. Dissected salivary glands (two pairs per lane) extracted in SDS/PAGE sample buffer containing 10% (vol/vol) β-mercaptoethanol and heated at 95°C for 10 min were analyzed on a 5–20% gradient SDS/PAGE gel (7). The gel was silver stained (Rapid Ag Stain, ICN) according to the manufacturer’s protocol and photographed with the Kodak ID (New Haven, CT) system. To determine the effect of a blood meal on salivary gland protein expression, female A. gambiae mosquitoes were deprived of sugar water for 12 h and then fed human erythrocytes in an equal volume of heat-inactivated normal human serum (NHS) through a membrane feeder at 39°C for ~1 h. Intact pairs of salivary glands were dissected from each mosquito immediately after they fed on the blood meal and at the indicated intervals after feeding and were then analyzed by SDS/PAGE.

Abbreviation: NHS, normal human serum.

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Salivary Gland Protein Expression Analysis. *A. gambiae* mosquitoes deprived of sugar water for 12 h were fed 1 mCi (1 Ci = 37 GBq) $^{35}$S-methionine (Trans 35S-Label, ICN), dried in a SpeedVac (Savant), and reconstituted in 300 µl of 10% (vol/vol) sugar water with 50 µl of red food coloring for 1 h through a membrane feeder heated to 39°C. Glands were dissected from radiolabeled mosquitoes at the indicated time points and were processed for SDS/PAGE and autoradiography. To analyze the protein content of the saliva, radiolabeled *A. gambiae* (4–7 days after emergence) were allowed to probe for 3 h through a membrane feeding apparatus containing distilled water. The contents of the feeder (water plus the saliva of mosquitoes that probed) were collected and dried in a SpeedVac. Intact salivary gland pairs were also dissected from mosquitoes before and after saliva collection to compare protein profiles to those of saliva.

Monoclonal Antibody Preparation and Immunoprecipitation Analysis. BALB/c mice were immunized i.p. with 50 *A. gambiae* female salivary glands (sonicated and freeze-thaw extracted) emulsified with Freund’s complete adjuvant (Sigma). Mice were boosted twice with 50 salivary glands emulsified in Freund’s incomplete adjuvant (Sigma) at 20-day intervals, and spleen cells were fused with P3-X-Ag8.653 myeloma cells (ATCC CRL 1580). For immunoprecipitation, salivary glands from $^{35}$S-methionine-fed *A. gambiae* females were extracted in 1% Triton X-100 in the presence of a protease inhibitor mixture as described (8). These procedures resulted in the selection of two clones, 2A3 (IgG2a) and C26 (IgG1) for further investigation.

Immunoelectron Microscopy and Indirect Immunofluorescence Assay. Female *A. gambiae* salivary glands were fixed for 10 min in 1% paraformaldehyde/0.2% glutaraldehyde in 0.1 M PBS, pH 7.4, and washed in 0.1 M PBS. Fixed glands were dehydrated in ethanol and embedded in LR Gold Resin (London Resin, Basingstoke, U.K.) at −20°C (9). Thin sections were cut with a diamond knife and placed on 300-mesh nickel grids. Sections were etched with saturated aqueous sodium metaperiodate and incubated with 1:100 dilutions (vol/vol) of salivary-gland-specific monoclonal antibodies 2A3 and C26 and a negative control, 6B6. Gland sections were then incubated with 15-nm goat anti-mouse IgG Immunogold (Janssen) diluted 1:20 (vol/vol) in PBS/Tween-20. Glands were stained with uranyl acetate and Reynold’s lead citrate and were coated with carbon. Grids were examined with a JEOL 100 CX electron microscope.

To localize salivary gland proteins by indirect immunofluorescence assay, intact pairs of salivary glands were incubated in a drop of 0.1% Evans blue in PBS on a glass slide at room temperature for 10 min. The glands were carefully washed with PBS and used in an indirect immunofluorescent assay as described (10) by using a 1:10 dilution (vol/vol) of mouse ascites (2A3, C26, and 6B6 as a negative control) as the primary antibody and a 1:50 dilution (vol/vol) of fluorescein-conjugated sheep anti-mouse Ig (Amersham Pharmacia) as the secondary antibody.

ELISA Determination of in Vivo Binding of Monoclonal Antibodies to Salivary Gland Proteins. *A. gambiae* females (5–6 days after emergence) were fed 200 µl of a 1:3 dilution (vol/vol) of monoclonal antibodies 2A3, C26, and 6B6 (mouse ascites) in washed human erythrocytes and heat-inactivated NHS through a membrane feeder. Unfed mosquitoes were removed and used as the control. Previous studies have shown that fed antibodies reach maximum hemolymph titers 3 h after feeding (11). One pair of whole, intact salivary glands was dissected from each of 15 mosquitoes 3 h post feeding and extracted by 10 freeze-thaw cycles followed by centrifugation (Brinkmann) at 10,000 rpm for 2 min. The pellet was resuspended in 400 µl of carbonate buffer (4 mM Na$_2$CO$_3$/NaHCO$_3$, pH 9.6), vortexed thoroughly, and used to coat ELISA plate wells in triplicate for each serial dilution. After blocking with 5% (vol/vol) milk in PBS/Tween-20, 100 µl of a 1:1000 dilution (vol/vol) of horseradish peroxidase-conjugated goat anti-mouse antibody (Bio-Rad) was added (37°C for 1 h) and developed with 100 µl of 2,2’-azinodi(3-ethylbenzthiazoline-6-sulfonate) (Kirkegaard & Perry Laboratories). The plate was read with an ELISA reader (Molecular Devices) at 405 nm.

In Vivo Sporozoite Invasion-Blocking Assay. *A. gambiae* females were allowed to feed on an anesthetized BALB/c mouse infected with *P. yoelli* 17XNL for 30 min. Fed mosquitoes were kept at 24°C and 68% relative humidity with alternating light and dark cycles. At 5 days after feeding, mosquitoes were given a second, uninfected blood meal of washed human erythrocytes in an equal volume of heat-inactivated NHS to decrease mosquito mortality (12). A small sample of mosquito midguts was dissected 4–5 days later to determine oocyst infection rates. On day 10, after feeding on infected blood meal, mosquitoes were divided randomly among four cages and were fed monoclonal antibodies through a membrane feeder apparatus in a double-blinded fashion. Salivary glands from individual mosquitoes were removed 4–5 days after antibody feeding and were homogenized in microtissue grinders (Kontes) with 35 µl of 1% BSA in PBS on ice. A 10-µl aliquot was placed on a hemocytometer, and the mean number of sporozoites per infected salivary gland was determined with a phase-contrast microscope. The significance of the sporozoite counts was analyzed by using STATVIEW 5.0 and the two sample t test.

Results

Monoclonal Antibody Preparation and Immunoprecipitation. The two monoclonal antibodies (2A3 and C26) with the highest reactivity for female *A. gambiae* salivary gland proteins, as determined by ELISA, recognized proteins of 100 kDa and 29 kDa, respectively, in the female *A. gambiae* salivary gland (Fig. 1B). We limited the scope of our study to the 29-kDa and 100-kDa proteins of *A. gambiae*, primarily because the epitopes may be mosquito species specific, and moreover, *A. gambiae* is a major malaria transmission vector. The expression of these two proteins in relation to the life cycle of female *A. gambiae* mosquitoes and any role they have in sporozoite recognition of salivary glands became the focus of our investigation.

SDS/PAGE Analysis of Salivary Gland Proteins. Salivary gland extracts were analyzed by using SDS/PAGE to investigate the species and sex specificity, the age-dependent expression, and the effect of a blood meal on salivary gland protein expression. The protein expression profiles of 4-day-old *A. gambiae* and *A. stephensi* female and male salivary glands show that males and females express some salivary gland proteins of similar molecular masses. There are, however, a number of proteins of approximately 25 kDa, 29 kDa, 42 kDa, and 67 kDa (Fig. 1A) that are present in female salivary glands but conspicuously absent in male salivary glands when equal numbers of glands are compared. *A. stephensi* female and male salivary glands were run as a comparison to show that the differences in salivary gland protein profiles are consistent between sexes, regardless of *Anopheles* species. When the salivary gland protein profiles of *A. gambiae* females are compared with those of *A. stephensi* females, it can be seen that some proteins are present across species lines, notably those of apparent molecular masses of 21 kDa, 25 kDa, and 32 kDa. However, many proteins of noncorresponding molecular masses are also detected in the two species compared (Fig. 1A).

Analysis of salivary glands from female *A. gambiae* of different ages, maintained on sugar water, also revealed that the 29-kDa
protein is not expressed until 3 days after emergence, and thereafter it is expressed in abundant amounts (not shown).

The blood-feeding process initiates malaria infection in both the vertebrate and mosquito hosts and is essential to female oviposition. It is therefore reasonable to assume that this process may induce protein expression changes in the mosquito to facilitate the feeding process and aid in digestion of the blood meal and the accompanying physiological changes. When compared with equal numbers of sugar-fed female *A. gambiae* salivary glands, salivary glands dissected immediately after a blood meal showed a marked increase in a number of high and low molecular mass proteins. At 1 h after ingestion of a blood meal, the salivary gland protein profile, as revealed by silver staining, is once again similar to that of sugar-fed mosquitoes. The most notable difference seen was the expression of the 100-kDa protein in response to a blood meal in mosquitoes 4 days after emergence that otherwise do not express the 100-kDa protein. The expression of the 29-kDa salivary gland protein changes little in response to a blood meal and was comparable to that of sugar-fed mosquitoes (Fig. 1C).

**Immunoelectron and Immunofluorescence Localization.** To localize the binding sites of the two monoclonal antibodies specific to *A. gambiae* female salivary glands, immunoelectron microscopy was used. 2A3 and C26 monoclonal antibodies were found to bind exclusively to the median and lateral lobes of female *A. gambiae* salivary glands. The monoclonal antibody C26, directed against the 29-kDa protein, showed extensive decoration of salivary glands (Fig. 2B). In contrast, the monoclonal antibody 2A3, which is specific for a 100-kDa protein, showed sparse localization dispersed throughout the female salivary gland (Fig. 2A). These results suggest that the 29-kDa protein is more abundant in female *A. gambiae* salivary glands than the 100-kDa protein.

We also used an indirect immunofluorescence assay to localize these proteins further in the context of the whole gland. Both C26 and 2A3 bind in diffuse patterns to the median and lateral lobes of paraformaldehyde-fixed female salivary glands, indicating that these two proteins have similarly diffuse dispersion throughout the salivary gland (Fig. 2 C and D).

**Biosynthetic Expression of Salivary Gland Proteins.** To follow the expression of salivary gland proteins, *A. gambiae* male and female mosquitoes were fed $[^{35}S]$methionine (1 h) to determine...
the time of maximum incorporation of $^{35}$S methionine into the proteins of the salivary glands. Equal numbers of salivary glands were dissected from mosquitoes at different time points and were analyzed by SDS/PAGE and autoradiography. The incorporation of $^{35}$S methionine into salivary glands was detected immediately after feeding, with maximum expression 6 h after ingestion of $^{35}$S methionine in sugar water (Fig. 3A). Male A. gambiae incorporated $^{35}$S methionine into their salivary gland proteins at barely detectable levels on prolonged exposure of gels. Based on these results, the incorporation of $^{35}$S methionine into salivary gland proteins was used to elucidate further the age-dependent expression profiles as well as the stability of expressed proteins. In all of the subsequent studies, salivary glands were dissected 6 h after $^{35}$S methionine feeds.

To investigate salivary gland protein expression profiles during the adult life of the mosquito, A. gambiae females of different age groups were fed on $^{35}$S methionine under identical conditions. Salivary gland proteins are expressed in increasing amounts until the 7th day after emergence, with maximum expression on day 7. Whereas the expression of the 29-kDa protein was detectable in all of the age groups, the expression of the 100-kDa protein was not seen until mosquitoes were 6 days old, and the protein was expressed at maximum levels on the 7th day after emergence (Fig. 3B).

Sporozoite infection of salivary glands can occur at various times throughout the adult mosquito life. Thus, a putative salivary gland sporozoite receptor would have to be expressed throughout the life of the mosquito after the first infected blood meal. To investigate the stability of the 100-kDa and 29-kDa salivary gland proteins, 7-day-old A. gambiae females were fed $^{35}$S methionine. Beginning 6 h after feeding, and at 48-h intervals thereafter, equal numbers of salivary glands were dissected until day 23 after emergence and were then analyzed by SDS/PAGE and autoradiography. The labeled proteins remained generally stable throughout the life of the mosquito. Notably, the radiolabeled 100-kDa and 29-kDa salivary gland proteins could be seen from day 7 through day 23 after emergence (not shown).

Finally, a putative sporozoite receptor on the A. gambiae salivary gland would be expected to be a component of the gland, and not secreted in the saliva. Analysis of radiolabeled whole salivary glands and radiolabeled saliva from 4- to 7-day-old A. gambiae females revealed a number of protein bands in the saliva; however the 29-kDa and 100-kDa proteins were not among the secreted proteins (Fig. 4).

In Vivo Accessibility of Monoclonal Antibodies to Salivary Glands and Inhibition of Sporozoite Invasion. An ELISA-based assay was used to determine whether the identified 100-kDa and 29-kDa female A. gambiae salivary gland proteins may have a role in the invasion of salivary glands by Plasmodium sporozoites. A. gambiae were blood fed on mice infected with P. yoelii 17XNL. At 9 days after the infectious blood meal, midguts were dissected to determine infection rates, and the numbers of oocysts were counted. There was no significant difference in the percentage of oocyst infection (87.5%, 75%, and 75%) between each of the three experiments. The mosquitoes were then fed monoclonal antibodies to the aforementioned salivary gland proteins on the 10th day after infection. One whole, intact pair of salivary glands was dissected and homogenized from each mosquito on days 4 and 5 after monoclonal antibody feeding, and the number of oocysts per salivary gland pair was counted. The negative controls were fed 6B6, a nonsalivary gland-specific monoclonal antibody, and the controls were fed heat-inactivated NHS. Mosquitoes fed 2A3 had 73% fewer sporozoites ($P = 0.0604$) than controls fed NHS (Fig. 5A). Mosquitoes fed C26 and the negative control antibody (6B6) showed no significant differences compared with controls. Salivary gland infection rates were not significantly different between any of the treatment groups (67–71%, 50–65.2%, 34.5–41.67%) in the three experiments.
necessary for sporozoite recognition, survival, and further trans-
expression was detected on the 3rd day after emergence, with maxi-
mum expression on day 7. The expression of the 29-kDa protein
was not significantly affected by blood feeding, nor is it a
component of the saliva. In sharp contrast to the 29-kDa protein,
the 100-kDa salivary gland protein was not detectable by silver
staining until after blood feeding. Similarly to the 29-kDa
protein, the 100-kDa protein was expressed at maximum levels
on the 7th day after emergence, remained stable throughout the
life of the mosquito, and is not a component of the saliva.

These findings prompted us to speculate and test a hypothesis
that these two proteins are putative candidates for sporozoite
recognition. The sporozoites recognize the female-specific lobes
of the salivary glands (14, 15). Both the 29-kDa and 100-kDa
salivary gland proteins are expressed on these lobes and fit the
logical parameters we considered for a putative salivary gland
receptor for sporozoites.

With this information in mind, we developed an in vivo
sporozoite invasion-blocking assay to ascertain the role of the
29-kDa and 100-kDa proteins in salivary gland infection. Before
we could implement this assay, we determined, by ELISA,
that monoclonal antibodies fed to mosquitoes in a blood meal are
indeed capable of binding salivary glands. In the sporozoite
blocking assay, infected mosquitoes were fed the monoclonal
antibodies on the 10th day after infection. This time period
 corresponds with the release of P. yoelii sporozoites into the
hemocoe(12), and a large majority of the free sporozoites would
be expected to still be circulating in the hemolymph. The results
show that the mosquitoes fed 2A3 displayed a 73% reduction in
the average number of sporozoites per infected salivary gland.
Mosquitoes fed the C26 monoclonal antibody showed little
difference in the average number of sporozoites per infected salivary
gland when compared with controls. Because the salivary
gland infection rates between treatment and control groups
were similar, it is highly unlikely that antibody 2A3 inhibits the
ability of sporozoites to enter salivary glands. Perhaps a more
reasonable explanation is that the antibodies reduce the avail-
able number of target sites that participate either in the binding
or entry of sporozoites.

Studies investigating the possibility of blocking sporozoite
invasion of mosquito salivary glands used a fixed number of
sporozoites injected intrathoracically along with anti-salivary
gland antibodies (3). Although assays performed in this man-
ner may serve to evaluate more accurately the role of salivary
gland proteins in sporozoite invasion, we feel that our assay
better emulates the natural course of malaria transmission,
but with it comes the inherent variability of any biological system.
This variability can be attributed to a number of possible
factors. The number of oocysts on the midgut of an infected
mosquito is highly variable, as it was in our experiments; thus,
the number of sporozoites released is consequently highly
variable as well. In addition, the release of sporozoites from
the oocyst is an ongoing process (16), and it is possible that
sporozoites were released and invaded the salivary glands after
the monoclonal antibodies had lost their potency and the
ability to bind their targets or before the monoclonal antibod-
ies were fed to the mosquitoes. It is also possible that not all
of the salivary gland targets were bound by presumed insuf-
cient concentrations of monoclonal antibody fed in a blood
meal. The number of 29-kDa and 100-kDa antigens present on
the salivary glands has not been quantitated; thus, it is not
possible to determine whether saturation occurs with the
concentration of monoclonal antibody present in the hemo-
coel. Nonetheless, the monoclonal antibody 2A3 consistently
resulted in fewer sporozoites in the salivary glands (average
reduction of 73% in three experiments), which approaches the
level of statistical significance. Taking into account all of the
biological considerations above and the fact that a single
epitope-specific antibody was an effective inhibitor, further
studies with a monospecific polyclonal antibody must be

Discussion
In this study, we characterized female-specific salivary gland
proteins of A. gambiae mosquitoes and attempted to determine
whether these proteins play a role in facilitating sporozoite
invasion of the salivary glands. When whole female and male A. gambiae salivary gland protein expression profiles are compared,
there are a few proteins common between the two sexes.
However, there are many proteins that are not shared between
the two sexes. Previous studies have shown that Plasmodium
sporozoites preferentially migrate to mosquito salivary glands
and that salivary gland factors may be responsible for sporozoite
recognition and subsequent invasion (1, 13). Therefore, the
different protein content of female and male A. gambiae salivary
glands visible in Fig. 1A may represent one or more proteins
necessary for sporozoite recognition, survival, and further trans-
mission to a host.

Monoclonal antibodies made against female A. gambiae sali-
vary glands identified two proteins, one with a molecular mass
of 29 kDa and one with a molecular mass of 100 kDa. Because
these proteins are expressed in a sex-specific fashion, they were
characterized with respect to the life of the adult mosquito to
determine whether the expression of these proteins corresponds
with the biological and physiological needs of the A. gambiae
vector and for Plasmodium sporozoite invasion. The 29-kDa
protein was found to be abundant as revealed by intense labeling
with [35S]methionine and immunoelectron microscopy. Expression
was detected on the 3rd day after emergence, with maxi-

![Fig. 5. In vivo binding of fed monoclonal antibodies to salivary glands (A)
and in vivo blocking of sporozoite invasion of salivary glands (B). Female A.
gambiae were fed monoclonal antibodies in a blood meal, and salivary
gland-bound antibodies were detected by ELISA (A). The graph shows the
average absorbance of three replicates with two salivary gland equivalents
per well. B shows the combined results of three in vivo sporozoite blocking
assays. P values as compared with the NHS control are: 2A3, 0.0604 (n = 51);
C26, 0.8829 (n = 50); 6B6, 0.6865 (n = 42); NHS, (n = 71). The third experiment
was performed with ammonium sulfate-precipitated mouse ascites to elimi-
nate any inhibitory ascites factors.](image-url)
undertaken for in vivo blocking assays. Finally, we believe that sporozoite recognition and invasion may involve more than one salivary gland component. Thus, although our assay is more applicable to natural malaria transmission (and perhaps possible vaccine applications) than other methods, further work and refinements are necessary.

Ultrastructural studies have shown that sporozoites invading salivary glands may encounter one receptor at the basal lamina of the salivary glands and another at the plasma membrane of the secretory cells (15). Other studies have indicated that the salivary gland components are highly glycosylated (2) and that specific lectins can inhibit sporozoite invasion of the salivary glands (3). In this study, we did not attempt to determine whether the 29-kDa or 100-kDa A. gambiae salivary gland proteins were part of the basal lamina or the secretory cells. We only confirmed that they are part of the salivary gland structure and not a component of the saliva. If it is assumed that the basal lamina is impermeable to antibodies, the binding of the two monoclonal antibodies to the salivary glands suggests that the 29-kDa and 100-kDa proteins are most likely components of the basal lamina. Further studies are needed to determine the precise ultrastructural location of these proteins and any posttranslational modifications.

As previously stated, A. gambiae is responsible for the devastating effects of malaria in sub-Saharan Africa, a region that bears the greatest burden of the world’s malaria problem. As vector control becomes more difficult because of insecticide resistance and the fact that limited funds need to be continuously redistributed to address the diversifying health needs of regions with a high malaria endemcity, new control strategies are required. Effectively blocking malaria transmission will rein in the prevalence of the disease, and established measures may make it possible finally to control malaria. Although the results of this study are preliminary, they do demonstrate that A. gambiae salivary gland proteins can be additional putative targets for new transmission blocking strategies and warrant a detailed molecular investigation.

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