The Journal of Experimental Biology 208, 3211-3218
Published by The Company of Biologists 2005
doi:10.1242/jeb.01756

A chemotactic response facilitates mosquito salivary gland infection by malaria sporozoites

Mayumi Akaki and James A. Dvorak*

Biochemical and Biophysical Parasitology Section, Laboratory of Malaria and Vector Research, National Institute of Allergy and Infectious Diseases, National Institutes of Health, 12735 Twinbrook Parkway, Bethesda, MD 20892-8132, USA

*Author for correspondence (e-mail: jdvorak@niaid.nih.gov)

Accepted 15 June 2006

Summary

Sporozoite invasion of mosquito salivary glands is critical for malaria transmission to vertebrate hosts. After release into the mosquito hemocoel, the means by which malaria sporozoites locate the salivary glands is unknown. We developed a Matrigel-based *in vitro* system to observe and analyze the motility of GFP-expressing *Plasmodium berghei* sporozoites in the presence of salivary gland products of *Anopheles stephensi* mosquitoes using temperature-controlled, low-light-level video microscopy. Sporozoites moved toward unheated salivary gland homogenate (SGH) but not to SGH that had been heated at 56°C for 30 min. We also investigated the origin of the attracted population. Attraction to SGH was restricted to hemolymph- and oocyst-derived sporozoites; salivary gland-derived sporozoites were not attracted to SGH. These data imply that sporozoites employ a chemotactic response to high molecular mass proteins or carbohydrate-binding proteins to locate salivary glands. This raises the possibility of utilizing anti-chemotactic factors for the development of mosquito transmission blocking agents.

Supplementary material available online at
http://jeb.biologists.org/cgi/content/full/208/16/3211/DC1

Key words: malaria, sporozoite, salivary gland, chemotaxis, mosquito.

Introduction

During the course of the intracellular cycle of malaria, there are critical 'gates' through which the parasite must pass in order for the cycle to be completed. One gate involves the invasion of mosquito salivary glands by sporozoites liberated from oocysts that develop on the midgut wall of the mosquito intestine. Upon release into the hemocoel, the sporozoites travel *via* the hemolymph to the salivary glands, where they invade salivary gland secretory cells and, subsequently, enter the salivary gland ducts (Wernsdorfer, 1980). However, although many aspects of the mosquito phase of the malaria life cycle are known, the mechanism used by sporozoites to locate salivary glands has not been determined.

Histologically, sporozoites have been found throughout the mosquito hemocoel (Garnham, 1966; Golenda et al., 1990; Mayer, 1920; Mülens, 1921; Wenyon, 1926). These data imply that sporozoite migration to salivary glands may be a passive process involving normal hemolymph circulation. However, it is still unclear whether sporozoites randomly delivered to salivary glands are able to detect the presence of the salivary glands during their circulation through the hemolymph. Several histological studies have shown that sporozoites are most frequently associated with the salivary glands and occasionally in spaces between but not directly on thoracic muscles (Golenda et al., 1990; Mayer, 1920; Mülens, 1921; Oelerich, 1967). These findings were confirmed and quantified using an enzyme-linked immunosorbent assay for a circumsporozoite antigen, which covers the entire surface of sporozoites (Robert et al., 1988). Therefore, the suggestion by Oelerich (1967) that once in the vicinity of the salivary glands, the location and invasion of the salivary glands may involve short-range chemotactic interactions is relevant. Chemotaxis is used by many organisms, such as bacteria and leukocytes, to detect environmental stimuli (Devreotes and Zigmond, 1988; Taylor et al., 1999). Although the involvement of chemotaxis has not been demonstrated, it is possible that the successful invasion of salivary glands by sporozoites is facilitated by a chemotactic attraction at the surface of the salivary glands to aid physical contact with a ligand–receptor binding substance.

Sporozoite motility was first observed by Vanderberg (1974). He showed that *Plasmodium berghei* sporozoites in medium exhibit a unidirectional circular gliding motion. However, the experimental setup was not designed to reveal any net forward motion. The recent development of green fluorescent protein (GFP)-expressing *P. berghei* sporozoites (Natarajan et al., 2001) has resulted in improved methods to study sporozoite motility. For example, sporozoite motility

within salivary gland ducts (Frischknecht et al., 2004) and skin (Vanderberg and Frevert, 2004) have been demonstrated. However, sporozoite motility in the hemocoel in proximity to the salivary glands has not been observed because the thorax containing the salivary glands is covered with a thick, optically opaque chitinized cuticle. Therefore, we used GFP-expressing sporozoites suspended in Matrigel to establish and maintain a three-dimensional chemical gradient (Lehmann et al., 1995), low-light-level video microscopy, precise temperature control and computer-based data acquisition for an *in vitro* assay to determine whether sporozoites display a chemotactic response to salivary gland products. The results of our study are reported here.

Materials and methods

Mosquito and parasite cultivation

Plasmodium berghei (NK65 strain) expressing GFP at the sporozoite stage (Natarajan et al., 2001) was maintained in 4–8 week-old BALB/c mice. At 72 h post-infection when blood parasitemia was 1–5%, *Anopheles stephensi* mosquitoes (NIH or Nijmegan strain) were fed on the mice and subsequently reared at 21°C and 80% relative humidity with 12 h alternating light–dark cycles.

Sporozoite preparation

We isolated sporozoites at 14–19 days post-infection by two methods. To determine if sporozoite motility is similar in both medium alone and in Matrigel, we isolated sporozoites in infected salivary glands as described previously (Vanderberg, 1974) except that we used a 10 µl Hamilton syringe to disrupt the salivary glands. Liberated sporozoites were suspended in assay medium consisting of Medium 199 (Biosource, Rockville, MD, USA) containing 1.5% bovine serum albumin (Sigma-Aldrich Co., St Louis, MO, USA) and 3 mg ml^{-1} glucose, and either placed on a microslide and sealed with a coverglass or mixed with Matrigel.

We also used the centrifugation method to purify sporozoites from whole mosquito bodies (Ozaki et al., 1984). Infected mosquitoes were anesthetized with CO_2, their legs and wings removed and their neck incised. The mosquito body parts were suspended in approximately 200 µl of assay medium and layered over an approximately 100 µl volume of glass wool in a 0.5 ml microcentrifuge tube, which had an 900 µm diameter hole in its apex made with a hot 20-gauge needle. The tube was placed inside a 1.5 ml microcentrifuge tube and the assembly was centrifuged at 16 000 ***g*** for 3 min. Purified sporozoites were collected as a pellet from the bottom of the 1.5 ml microcentrifuge tube. The pellet was disbursed in assay medium and mixed with fluorescent polystyrene microsphere beads (Fluorespheres, 10 µm in diameter, normalized 1/8 brightness, Molecular Probes, Eugene, OR, USA) for some assays.

To isolate salivary gland-derived sporozoites, we removed infected salivary glands from the mosquito body, suspended them in 15 µl of assay medium, and disrupted them by passage through a 10 µl Hamilton syringe. To isolate sporozoites from hemocoels and oocysts, we used the mosquito body but excluded the salivary glands. All sporozoites harvested either from isolated salivary glands or from hemocoels and oocysts were concentrated by centrifugation as described above.

The osmolarities of all assay solution components were measured in a precalibrated vapor pressure osmometer (Model 5500, Wescor, Logan, UT, USA).

Preparation of mosquito organ homogenates

Eight pairs of salivary glands harvested from 3–10 day-old *An. stephensi* mosquitoes were suspended in 10 µl of assay medium and homogenized by sonication (30 times, 35 s total) at an output power of 8 using a Model 450 Sonifier (Branson Ultrasonics Corp., Danbury, CT, USA). As a control, some salivary gland homogenates were heated in a water bath at 56°C for 30 min. This procedure was also used to produce midgut and Malpighian tubule homogenates.

Data collection and analyses

To observe sporozoites in the hemolymph circulation, live mosquitoes were attached to a microscope slide using superglue (Axis Electronics, Inc., Damascus, MD, USA). For chemotaxis assays, we used custom-built chambers (Fig. 1A) to observe and quantify sporozoite movement. Two 9 mm×18 mm No. 1 coverglasses were adhered to a 70 mm×25 mm glass slide with a thin layer of silicon oil, leaving an approximately 5 mm wide gap between them. An

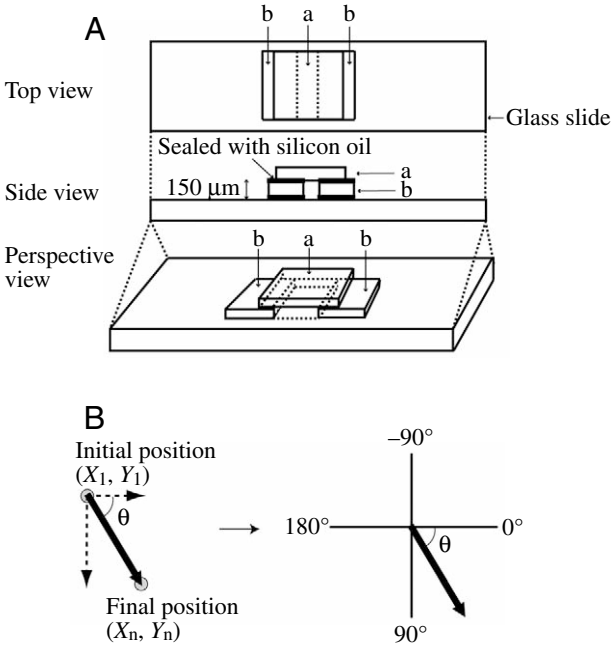

Fig. 1. (A) Schematic diagram of the custom-built chamber used for studies of sporozoite chemotaxis. a, 18 mm×18 mm coverglass; b, 9 mm×18 mm coverglass. (B) Diagram of the mathematical procedure used to analyze the direction of sporozoite movement calculated as tangency angle θ. Movement directed to the bottom of the figure is defined as 90°.

18 mm×18 mm No. 1 coverglass adhered with silicon oil bridging the two 9 mm×18 mm coverglasses was used to form a 150 μm thick observation chamber. The chamber capacity was approximately 3.5 μl. Unless otherwise noted, both sporozoites and mosquito organ homogenates in assay medium were mixed quickly at 4°C with Matrigel (Beckton Dickinson Labware, Bedford, MA, USA) individually at a final ratio of 67% assay medium to 33% Matrigel, and placed in the chamber. To prevent evaporation and exclude air bubbles from the chamber, silicon oil was added to fill the void remaining in the chamber, which was further sealed with high vacuum grease (Dow Corning, Midland, MI, USA).

The microscope slide or the chamber was placed on a custom-built thermostated stage incorporating two Peltier elements. Temperature was maintained at 20–21°C with the combination of a Model 5000 Microincubator (20/20 Technology, Inc., Wilmington, NC, USA) and a Hoefer Model RCB-300 refrigerated circulating bath (Hoefer Scientific Instruments, San Francisco, CA, USA) and monitored by a YSI Model 408 thermistor and a YSI Model 4000A Precision Thermometer (Yellow Springs Instrument Co., Inc., Yellow Springs, OH, USA).

A Leica DM IRE2 inverted light microscope (Leica Microsystems, Inc., Bannockburn, IL, USA) equipped with a Märzhäuser motorized stage (Märzhäuser, Wetzler, Germany), 20× and 100× HC PL FLUORTAR objectives and a GFP fluorescence filter module (Model XF100-2, Omega Optical, Brattleboro, VT, USA) was used to visualize the sporozoites. Microscope images were projected into a Hamamatsu Model ICCD-2400 low-light-level intensified video camera (Hamamatsu, Bridgewater, NJ, USA), captured using a DT3155 image capture card (Data Translation, Marlboro, MA, USA) and stored as AVI files. To avoid radiation-induced damage to the GFP-expressing sporozoites, a mercury lamp was not used; the preparations were illuminated with a halogen light source operating at 2.5 to 7.2 V.

Quantification of the movement of individual sporozoites was performed using the 3-D motion tracking module in Image-Pro ver. 5.0 (Media Cybernetics, Silver Spring, MD, USA). Selected time-lapse AVI files were converted to *X*, *Y*, time maximum projections with AutoDeblur ver. 9.2 (AutoQuant Imaging, Inc., Troy, NY, USA). The direction of sporozoite movement was calculated by the shift in *X*, *Y* coordinates as tangency angles; $\theta=\tan^{-1}(Y_n–Y_1/X_n–X_1)$ using the first and last video frames of each sporozoite (Fig. 1B).

Equally distributed distributions were tested for statistical significance using a two-sample *t*-test. If the samples were not equally distributed as determined by an *F*-test, we used a two-sample *t*-test with Welch's correction.

Results

Sporozoites are passively transported through the mosquito hemolymph

To quantify the real-time transport of sporozoites through the mosquito hemolymph, we recorded the process *in vivo* in the wing veins of an intact mosquito. Sporozoites were carried through the hemolymph at various speeds depending upon the diameter of the vein. For example, sporozoites flowing through a peripheral vein at the wing margin traveled at a relatively low velocity of 66–83 μm s^{-1} and often paused at the intersection between the peripheral and central veins. However, once in a larger central vein they traveled at a higher velocity of 82–98 μm s^{-1} (Fig. 2, Movie 1 in supplementary material). These data demonstrate that sporozoites are carried passively through the mosquito hemolymph.

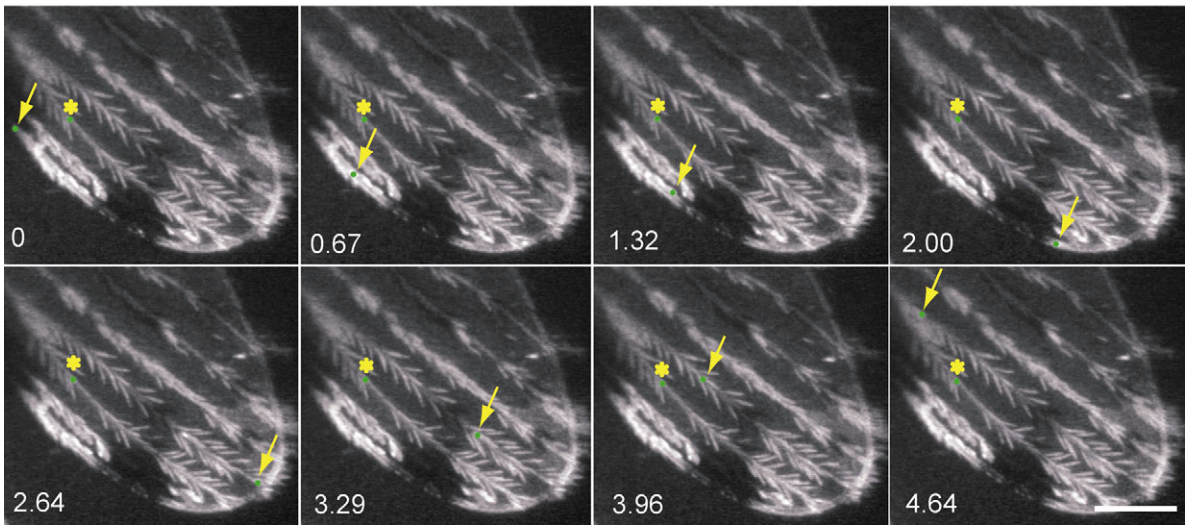

Fig. 2. Sporozoite transport through the hemolymph in mosquito wing veins. Arrows show the progress of a sporozoite (green) floating passively through a peripheral vein (upper images, 0–2 s) and subsequently through a larger central vein (bottom images, 2.64–4.64 s). Asterisks indicate a sporozoite apparently caught in a wing vein constriction. The number at the lower left in each frame is the time (s) at which the image was recorded. Scale bar, 100 μm.

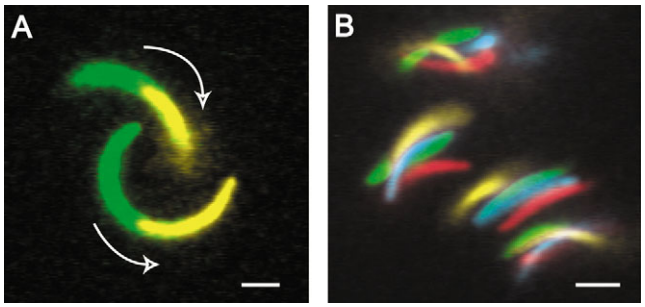

Fig. 3. Representative examples of sporozoite motility in assay medium. (A) Circular gliding motion. *X*–*Y* maximum projection of five time-lapse images acquired over a period 21 s, artificially colored as green (the first four frames) and yellow (the last frame). The white arrows indicate the direction of rotation of the two sporozoites. (B) Attached waving motion. *X*–*Y* maximum projection of four time-lapse images acquired over a 32 s period, artificially colored as red, cyan, green and yellow, respectively. Scale bars, 5 μm.

Sporozoites motility is modulated by vehicle

Sporozoites isolated and suspended in medium as described previously (Vanderberg, 1974) showed circular gliding motion at a velocity of 2 μm s^{-1} and attached waving motion (Fig. 3 and Movie 2 in supplementary material). In contrast to the previous report, we found that the proportions of sporozoites circling clockwise or anticlockwise were essentially identical. However, neither circular gliding nor attached waving motion resulted in any net forward motion.

To determine if forward motility could be promoted in a three-dimensional environment, we isolated sporozoites by the same method and suspended them in Matrigel in our custom-built chamber. In this environment, sporozoites exhibited a corkscrew-like forward motion with frequent random directional changes (Fig. 4A). Since sporozoites exhibited directional motility in Matrigel in addition to circular gliding motility, we mixed Matrigel with sample for all experiments to analyze sporozoite motility.

Matrigel is a fluid at 4°C and a gel at room temperature. The maintenance of sporozoites with Matrigel at 4°C results in an inhibition of sporozoite motility (Fig. 5). In order to minimize this effect, we exposed sporozoites to 4°C for less than 1 min in all experiments. After the sporozoites had been warmed to 20°C, motility increased over an approximately 20 min. However, motility again gradually decreased, probably due to a depletion of energy sources such as internal ATP and/or glucose in the assay medium (Kirk et al., 1996).

We also used the centrifugation method to isolate sporozoites and analyzed their motility. We quantified 81 salivary gland-derived sporozoites in Matrigel (Fig. 6A). 79 sporozoites (97.5%) were motile, demonstrating that sporozoite motility was well-preserved. The tangency angle (θ) of sporozoite direction was 27.2±111.0° ($\bar{\chi}$ ±S.D., *N*=74, excluding five sporozoites without vectorial movement; Table 1) demonstrating that sporozoites moved randomly. A similar result was observed with sporozoites harvested from

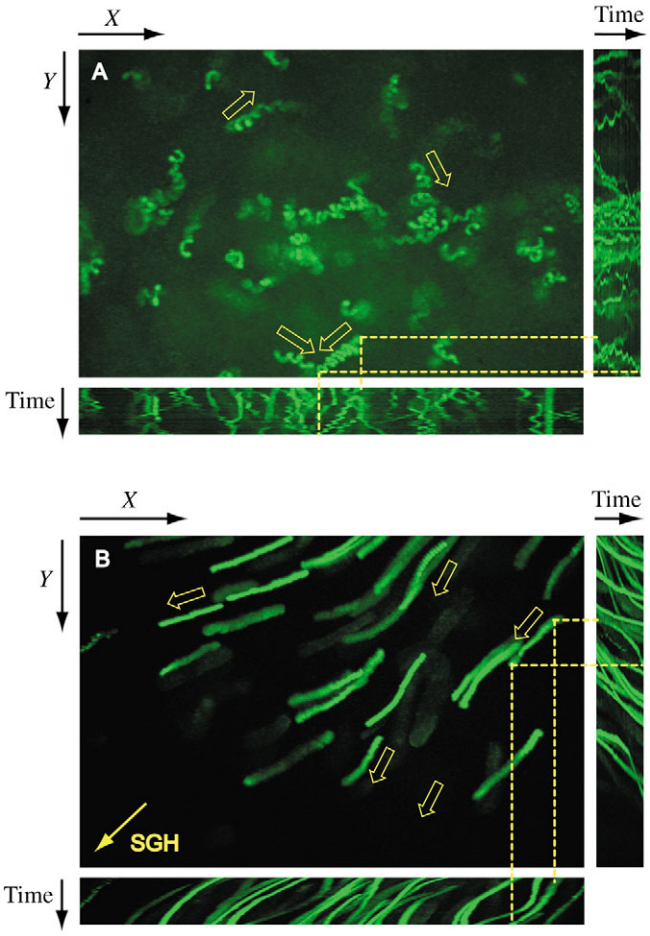

Fig. 4. Representative examples of the effects of salivary gland homogenate on sporozoite motility. (A) Random sporozoite motility in Matrigel. (B) Directed sporozoite motility in the presence of salivary gland homogenate. *X*–*Y* (upper left), *X*-time (bottom), and *Y*-time (upper right) maximum projections of 60 time-lapse images acquired over a 318 s period. The yellow outlined arrows show the directions of sporozoite movement (green). To highlight the influence of time on sporozoite direction, the yellow broken lines indicating the starting and ending points of a sporozoite moving in *X*–*Y* space were projected into both *X*- and *Y*-time space to demonstrate the influence of time on motility. The solid yellow arrow in B indicates the relative position of the salivary gland homogenate.

hemocoel and oocysts. A total of 64/74 sporozoites (90.1%) were motile (Fig. 6B) with θ=11.3±94.6° (*N*=60, excluding four sporozoites without vectorial movement; Table 1), confirming that these sporozoites also moved randomly. These data confirm that the centrifugation method preserved sporozoite motility. Therefore, sporozoites were processed by this method for all chemotaxis assays.

Sporozoite movement is modulated by the presence of salivary gland homogenate

To determine whether salivary gland homogenate (SGH) influenced the direction of sporozoite movement, we quantified motility in the presence of either fresh or heated

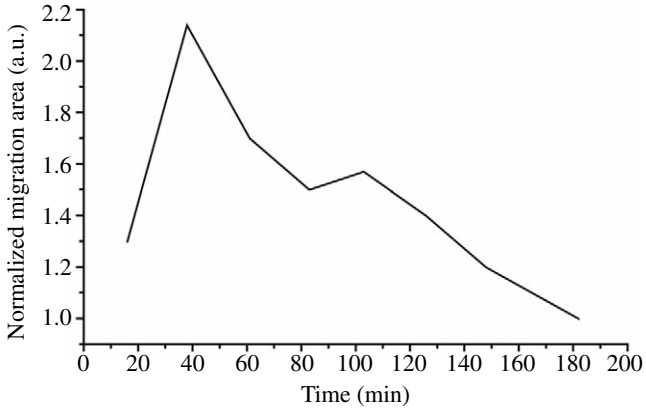

Fig. 5. Effects of sample preparation temperature on sporozoite motility. Sporozoites in Matrigel were exposed at 4°C for ≈40 min and transferred to the chamber at 20°C at time 0. The graph is a summary of 240 time-lapse images acquired over a 21 min period. Migration area of sporozoites in each image was normalized to sporozoite number.

SGH. Sporozoites collected from whole bodies of infected mosquitoes were suspended in Matrigel together with 10 µm diameter fluorescent beads as fiducial points and either unheated or heated SGH. In a typical experiment, a total of 21 out of 22 sporozoites (95.5%) moved toward unheated SGH (Fig. 7A, Movie 3 in supplementary material) with θ=77.5±15.7° (*N*=21, Table 1). In contrast, sporozoites in the presence of heated SGH moved randomly (Fig. 7B) with θ=–58.1±127.1° (*N*=20, Table 1). Sporozoite movement in the presence of SGH was relatively uniform with time, compared to sporozoites in the absence of SGH (Fig. 4A,B). The difference in θ between sporozoites in the presence of SGH *vs* heated SGH was significant ($P<0.001$, two-sample *t*-test with Welch's correction), demonstrating that the majority of the sporozoites isolated from whole mosquito bodies were attracted to unheated SGH. Mosquito organ extracts of midguts and Malpighian tubules did not attract sporozoites (data not shown). The fluorescent beads remained stationary in all cases, demonstrating that no passive fluid streaming occurred. To further confirm the lack of passive fluid movement, the osmolarities of all solutions were determined (Table 2). There was no significant difference in osmolarity between SGH and sporozoites suspended in Matrigel ($P>0.4$, two-sample *t*-test), demonstrating that solution osmolarity differences were not responsible for the directional motility of sporozoites.

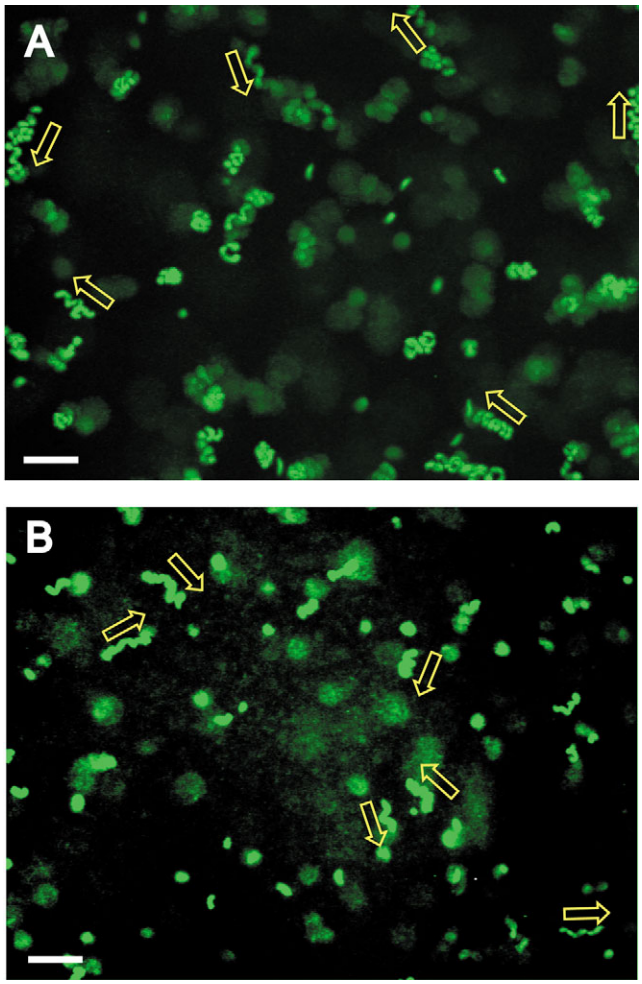

Fig. 6. Sporozoite motility in the absence of salivary gland homogenate. (A) Salivary gland-derived sporozoites. (B) Oocyst- and hemolymph-derived sporozoites. The images represent *X–Y* maximum projections of 50 time-lapse images acquired over a 265 s period. The yellow outlined arrows show the direction of sporozotie movement (green). Scale bars, 50 µm.

Table 1. *Effects of sporozoite origin and salivary gland homogenate (SGH) preparation on sporozoite motility*

Sporozoite origin	θ	*N*	Movement	SGH	Fig. in text
Whole mosquito bodies	77.5±15.7[a]	21	Directed	Unheated	7A
	–58.1±127.1[a]	20	Random	Heated	7B
Oocysts and hemocoel	71.8±34.0[b]	15	Directed	Unheated	8B
	11.3±94.6	60	Random	–	6B
Salivary glands	–32.5±97.4[b]	12	Random	Unheated	8A
	27.2±111.0	74	Random	–	6A

[a,b]The differences are significant by two-sample *t*-test with Welch's correction ([a]$P<0.001$, [b]$P<0.005$).

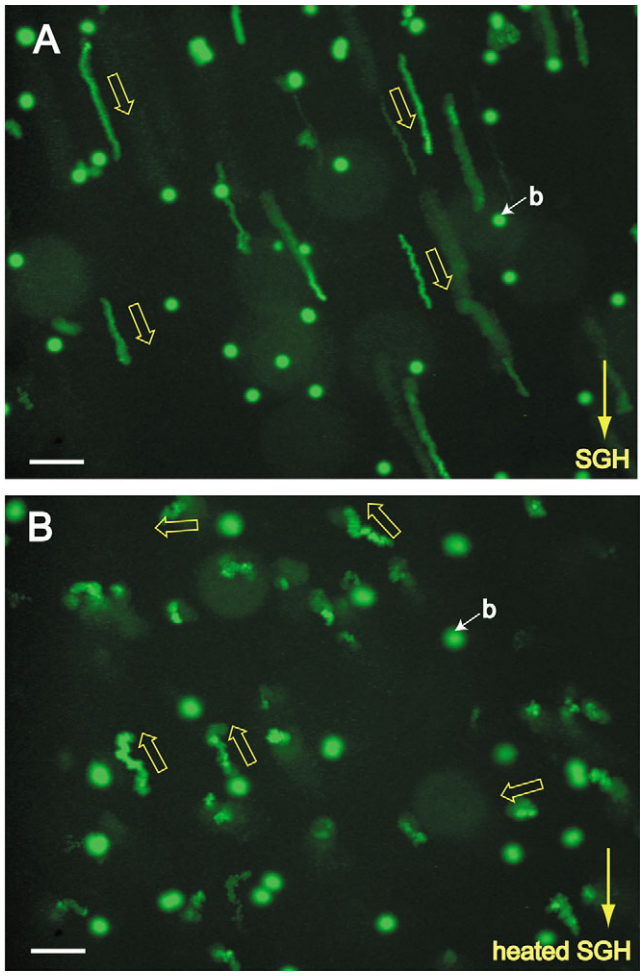

Fig. 7. The effects of salivary gland treatment on sporozoite motility. Sporozoite motility in the presence of (A) unheated or (B) heated salivary gland homogenate. *X*–*Y* maximum projections of 106 time-lapse images acquired over a 561 s period. The yellow outlined arrows show the direction of sporozoite movement (green). The yellow solid arrows points to the relative position of the unheated or heated salivary gland homogenate. The white arrows point to fluorescent beads (b). Scale bars, 50 μm.

Table 2. *The osmolarities of assay solution components*

Assay solution components	Osmolarity (mmol kg^{-1})	
	Experiment 1	Experiment 2
Assay medium	312.0±3.6	320.5±3.7
67% assay medium + 33% Matrigel	288.5±10.7	310.5±4.9
Matrigel	230.0±5.4	262.8±9.3
Salivary gland homogenate[a]	297.0±7.9[b]	311.0±6.1[c]
Sporozoites + fluorescent beads[a]	292.8±6.6[b]	313.5±11.4[c]

Each osmolarity value was measured four times and is presented as the mean ± S.D.

[a]Suspended with 67% assay medium +33% Matrigel.

[b,c]No significant difference on the basis of a two sample *t*-test ([b]P=0.44, [c]P=0.71).

Mosquito isolation site modulates sporozoite movement

Sporozoites in whole mosquito bodies on days 14–19 post-infection were present in both salivary glands and hemolymph plus oocysts (data not shown). To determine whether the attraction of sporozoites to SGH is dependent upon the site from which the sporozoites were isolated, we compared the motility of salivary gland-derived sporozoites to sporozoites derived from hemocoels and oocysts. Salivary gland-derived sporozoites moved randomly in the presence of SGH (Fig. 8A) with θ=–32.5±97.4° (N=12, Table 1). In contrast, 15 hemocoel- and oocyst-derived sporozoites out of 19 (78.9%) were attracted to salivary gland homogenate (Fig. 8B) with θ=71.8±34.0° (N=15, Table 1). The difference in θ between salivary gland-derived *vs* hemocoel- and oocyst-derived sporozoites was significant (P<0.005, two-sample *t*-test with Welch's correction).

Discussion

In this report, we describe and utilize an *in vitro* system for real-time studies of the interaction between sporozoites and mosquito salivary glands. Although sporozoites float freely in the hemolymph and travel throughout the mosquito body, ultimately they reach the salivary glands. Our results show that sporozoites are attracted to salivary gland homogenate *in vitro*. These data imply that sporozoites may detect and utilize chemotactic gradients in the vicinity of the salivary glands; however, the composition of the chemotactic gradient remains to be determined. The maintenance of a chemotactic gradient may involve a reduced hemolymph velocity in the vicinity of the salivary glands. Details of mosquito hemolymph dynamics are unknown; however, the complex anatomical structures in the vicinity of the salivary glands should result in a reduction in hemolymph velocity, which would facilitate the attraction of sporozoites to the surface of salivary glands. Salivary glands are enclosed by a basal lamina, consisting of fibrilliar components, such as laminin and collagen IV, which are also major components of Matrigel. These components may aid sporozoite approach towards salivary glands by providing a suitable vehicle to facilitate sporozoite movement.

We demonstrate that sporozoites do not exhibit directed movement toward heated salivary gland homogenate. These data imply that chemoattractant factors may be denatured or inactivated by heating (56°C for 30 min), suggesting that they may be high molecular mass protein(s) or carbohydrate-binding protein(s). High molecular mass protein(s) are usually denatured (Shin et al., 2002) and carbohydrate-binding proteins lose sugar-binding activity (Gaikwad et al., 2002) when heated under the conditions we used. High molecular mass glycoproteins could be secreted from the basal lamina into the extracellular space (Mariassy et al., 1989). Also, it has been shown that *P. gallinaceum* sporozoites recognize lectins when they invade the salivary glands of *Aedes aegypti* mosquitoes (Barreau et al., 1995). Consequently, these sporozoites may chemotactically detect secreted carbohydrate-

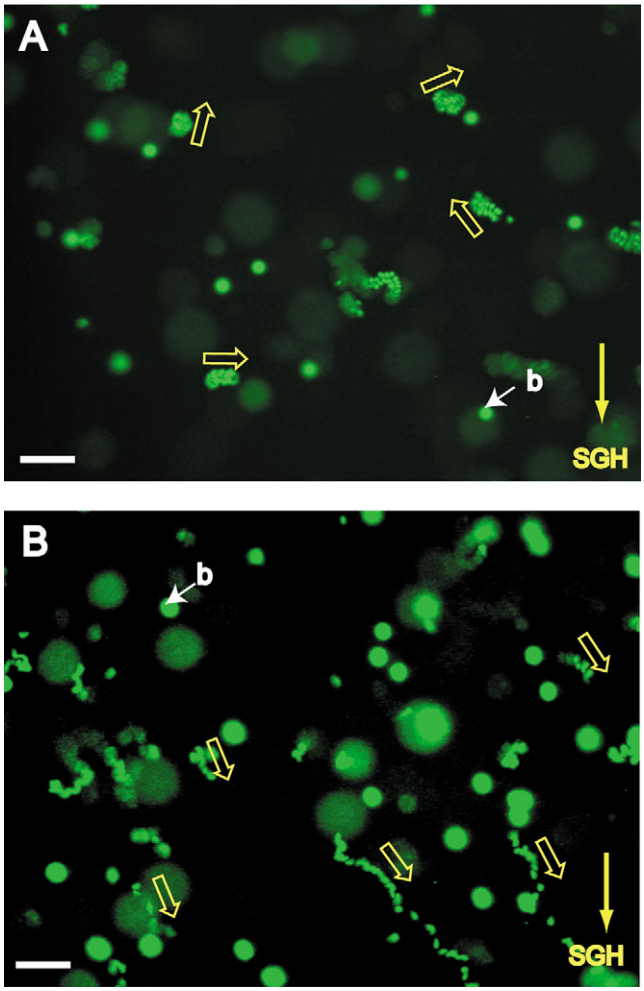

Fig. 8. The effects of the site of origin of sporozoites on motility in the presence of salivary gland homogenate. (A) Salivary gland-derived sporozoites. (B) Oocyst- and hemolymph-derived sporozoites. The images represent *X–Y* maximum projections of 140 time-lapse images acquired over a 742 s period. The yellow outlined arrows show the direction of sporozotie movement (green). The yellow solid arrows indicate the relative position of the salivary gland homogenate. The white arrows indicate fluorescent beads (b). Scale bars, 50 μm.

binding proteins and a similar mechanism may exist for *P. berghei* sporozoites.

We also demonstrate that there is a marked difference in chemoattraction between salivary gland-derived sporozoites and oocyst- and hemolymph-derived sporozoites. Salivary gland-derived sporozoites were not attracted to salivary gland homogenates. In contrast, the majority (80%) of oocyst- and hemolymph-derived sporozoites are attracted to salivary gland homogenate. These data can be explained by the fact that once sporozoites enter salivary glands they no longer need to locate the salivary glands and may lose their chemotactic ability quickly after invasion, possibly through the saturation of chemoattractant receptors. The remaining ≈20% did not show directed motility. This is probably the consequence of the heterogeneous maturation state of the oocyst- and hemolymph-derived sporozoite population. It has been shown that sporozoite mobility, infectivity of the vertebrate host, and the ability to invade salivary glands change during their maturation (Touray et al., 1992; Vanderberg, 1974; Vanderberg, 1975) in a time-dependent manner (Al-Olayan et al., 2002). The subset of the population that did not display directed motility in the presence of salivary gland homogenate is probably immature with respect to the development of a chemotactic response.

In conclusion, we demonstrate that sporozoites locate mosquito salivary glands by chemotaxis, suggesting the possibility that chemical component(s) can be identified and synthesized to block or suppress mosquito salivary gland invasion as a malaria transmission blocking strategy.

The authors thank Mr Andre Laughinghouse and Mr Kevin L. Lee, Laboratory of Malaria and Vector Research (LMVR), National Institute of Allergy and Infectious Diseases (NIAID) for providing mosquitoes. We are grateful to Robert W. Gwadz, Jose M. Ribeiro, Fuyuki Tokumasu, LMVR, NIAID, and Jerome P. Vanderberg, Department of Medical and Molecular Parasitology, New York University School of Medicine, for discussions and suggestions during the course of this work.

References

Al-Olayan, E. M., Beetsma, A. L., Butcher, G. A., Sinden, R. E. and Hurd, H. (2002). Complete development of mosquito phases of the malaria parasite *in vitro*. *Science* **295**, 677-679.

Barreau, C., Touray, M., Pimenta, P. F., Miller, L. H. and Vernick, K. D. (1995). *Plasmodium gallinaceum*: sporozoite invasion of *Aedes aegypti* salivary glands is inhibited by anti-gland antibodies and by lectins. *Exp. Parasitol.* **81**, 332-343.

Devreotes, P. N. and Zigmond, S. H. (1988). Chemotaxis in eukaryotic cells: a focus on leukocytes and *Dictyostelium*. *Annu. Rev. Cell Biol.* **4**, 649-686.

Frischknecht, F., Baldacci, P., Martin, B., Zimmer, C., Thiberge, S., Olivo-Marin, J. C., Shorte, S. L. and Menard, R. (2004). Imaging movement of malaria parasites during transmission by *Anopheles* mosquitoes. *Cell Microbiol.* **6**, 687-694.

Gaikwad, S. M., Gurjar, M. M. and Khan, M. I. (2002). *Artocarpus hirsuta* lectin. Differential modes of chemical and thermal denaturation. *Eur. J. Biochem.* **269**, 1413-1417.

Garnham, P. C. C. (1966). *Malaria Parasites and Other Hemosporidia*. London: Blackwell.

Golenda, C. F., Starkweather, W. H. and Wirtz, R. A. (1990). The distribution of circumsporozoite protein (CS) in *Anopheles stephensi* mosquitoes infected with *Plasmodium falciparum* malaria. *J. Histochem. Cytochem.* **38**, 475-481.

Kirk, K., Horner, H. A. and Kirk, J. (1996). Glucose uptake in *Plasmodium falciparum*-infected erythrocytes is an equilibrative not an active process. *Mol. Biochem. Parasitol.* **82**, 195-205.

Lehmann, T., Cupp, S. M. and Cupp, W. E. (1995). Chemical guidance of *Onchocerca lienalis* microfilariae to the thorax of *Simulium vittatum*. *Parasitology* **110**, 329-337.

Mariassy, A. T., McCray, M. N., Lauredo, I. T., Abraham, W. M. and Wanner, A. (1989). Lectin-detectable effects of localized pneumonia on airway mucous cell populations: role of cyclooxygenase metabolites. *Exp. Lung Res.* **15**, 113-137.

Mayer, M. (1920). Über die Wanderung der Malariasichelkeime in den Stechmücken und die Möglichkeit der Uberwinterung in diesen. *Medizinische Klinik* **16**, 1290-1291.

Mülens, P. (1921). Das Verhalten der Malaria Sporozoiten in der Anophelesmücke. *Archiv. für Schiffs- und Tropen-Hygiene* **25**, 58-61.

Natarajan, R., Thathy, V., Mota, M. M., Hafalla, J. C., Menard, R. and

Vernick, K. D. (2001). Fluorescent *Plasmodium berghei* sporozoites and pre-erythrocytic stages: a new tool to study mosquito and mammalian host interactions with malaria parasites. *Cell Microbiol.* **3**, 371-379.

Oelerich, S. (1967). Vergleichende Untersuchungen über das Auftreten von Malaria-sporozoiten in den Speicheldrüsen und in den übgrigen Organen der Mucke. *Zeitschr. Tropen. Med. Parasitol.* **18**, 285.

Ozaki, L. S., Gwadz, R. W. and Godson, G. N. (1984). Simple centrifugation method for rapid separation of sporozoites from mosquitoes. *J. Parasitol.* **70**, 831-833.

Robert, V., Verhave, J. P., Ponnudurai, T., Louwe, L., Scholtens, P. and Carnevale, P. (1988). Study of the distribution of circumsporozoite antigen in *Anopheles gambiae* infected with *Plasmodium falciparum*, using the enzyme-linked immunosorbent assay. *Trans. R. Soc. Trop. Med. Hyg.* **82**, 389-391.

Shin, I., Wachtel, E., Roth, E., Bon, C., Silman, I. and Weiner, L. (2002). Thermal denaturation of *Bungarus fasciatus* acetylcholinesterase: Is aggregation a driving force in protein unfolding? *Protein Sci.* **11**, 2022-2032.

Taylor, B. L., Zhulin, I. B. and Johnson, M. S. (1999). Aerotaxis and other energy-sensing behavior in bacteria. *Annu. Rev. Microbiol.* **53**, 103-128.

Touray, M. G., Warburg, A., Laughinghouse, A., Krettli, A. U. and Miller, L. H. (1992). Developmentally regulated infectivity of malaria sporozoites for mosquito salivary glands and the vertebrate host. *J. Exp. Med.* **175**, 1607-1612.

Vanderberg, J. P. (1974). Studies on the motility of *Plasmodium* sporozoites. *J. Protozool.* **21**, 527-537.

Vanderberg, J. P. (1975). Development of infectivity by the *Plasmodium berghei* sporozoite. *J. Parasitol.* **61**, 43-50.

Vanderberg, J. P. and Frevert, U. (2004). Intravital microscopy demonstrating antibody-mediated immobilisation of *Plasmodium berghei* sporozoites injected into skin by mosquitoes. *Int. J. Parasitol.* **34**, 991-996.

Wenyon, C. M. (1926). Protozoology. In *A Manual for Medical Men, Vetenarians and Zoologists.* London. Balliére, Tindall and Cox.

Wernsdorfer, W. (1980). The Importance of Malaria in the World. In *Malaria*. Vol. 1 (ed. J. Kreier), pp. 1-93. New York: Academic Press.