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Plasmodium gallinaceum preferentially invades vesicular ATPase-expressing cells in *Aedes aegypti* midgut

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ABSTRACT **Penetration of the mosquito midgut epithelium is obligatory for the further development of *Plasmodium* parasites. Therefore, blocking the parasite from invading the midgut wall disrupts the transmission of malaria. Despite such a pivotal role in malaria transmission, the cellular and molecular interactions that occur during the invasion are not understood. Here, we demonstrate that the ookinetes of *Plasmodium gallinaceum*, which is related closely to the human malaria parasite *Plasmodium falciparum*, selectively invade a cell type in the *Aedes aegypti* midgut. These cells, unlike the majority of the cells in the midgut, do not stain with a basophilic dye (toluidine blue) and are less osmiophilic. In addition, they contain minimal endoplasmic reticulum, lack secretory granules, and have few microvilli. Instead, these cells are highly vacuolated and express large amounts of vesicular ATPase. The enzyme is associated with the apical plasma membrane, cytoplasmic vesicles, and tubular extensions of the basal membrane of the invaded cells. The high cost of insecticide use in endemic areas and the emergence of drug resistant malaria parasites call for alternative approaches such as modifying the mosquito to block the transmission of malaria. One of the targets for such modification is the parasite receptor on midgut cells. A step toward the identification of this receptor is the realization that malaria parasites invade a special cell type in the mosquito midgut.**

This is the centennial of the discovery in 1897 by Ronald Ross that mosquitoes transmit malaria (1). In the 1890s, fertilization of malaria parasites to form zygotes, the first step of *Plasmodium* development in the mosquito, also was discovered. Zygotes develop into ookinetes within the midgut and penetrate the peritrophic matrix and the midgut epithelium. In the hemolymph, parasites develop as oocysts between the midgut epithelium and basal lamina. Sporozoites develop during the hemolymph stage and penetrate the salivary glands to be injected into the human during blood feeding (2, 3). One of the most crucial aspects of the sporogonic development of malaria parasites is the crossing of the mosquito midgut epithelium by the ookinetes. The ookinetes that fail to penetrate the midgut epithelium die in the gut lumen. Blocking ookinete penetration of mosquito midgut epithelium, therefore, is a potential strategy to block malaria transmission. To exploit this invasion process, however, a better understanding of ookinete interaction with the midgut is necessary. The molecular aspects of this penetration process currently are not known.

The ultrastructure of ookinetes within the mosquito midgut epithelium has been studied (4–7). In these experiments, only a few parasites were observed because invasion and transit across the epithelial cells are asynchronous. To examine the invasion process more extensively, we have developed an *in vitro* invasion assay. This assay, along with an *in vivo* procedure, has allowed us to examine a large number of invaded cells and has provided several characteristic features of the cells that the ookinetes invade.

In this paper, we report that *P. gallinaceum* ookinetes preferentially invade a special cell type in the midgut epithelium. The invaded cells share several histochemical, ultrastructural, and immunologic features that are unique to these cells. We discuss the implications of these findings in the context of malaria transmission and mosquito physiology.

MATERIALS AND METHODS

Mosquito and Parasite. Black eye strain of *Aedes aegypti* and 8A strain of *P. gallinaceum* were used in all of the experiments. Mosquitoes were maintained in standard conditions (80% humidity/26°C) and fed on diluted Karo syrup (CPC International, Englewood Cliffs, NJ). The parasite is maintained in 4- to 5-week-old white leghorn chickens by serial passage.

Materials. M199 medium with 3× glucose and without glutamine was obtained from National Institutes of Health media unit. L-glutamine, chicken serum, penicillin, and streptomycin were purchased from Life Technologies (Gaithersburg, MD). Rabbit antiserum to the B subunit of vesicular ATPase (V-ATPase) of *Culex quinquefasciatus* was a gift from Professor Sarjeet Gill (University of California, Riverside). mAb to *P. gallinaceum* ookinete surface antigen, Pgs28, was a gift from Dr. David Kaslow (National Institutes of Health). Mosquito endocrine cell-specific antisera were kindly provided by Dr. Jan Veenstra (University of Arizona, Tucson, AZ).

***In Vitro* Invasion of Mosquito Midgut Epithelium by Ookinetes.** Six to seven days after emergence, mosquitoes were fed on uninfected chickens. Thirty hours after the feeding, midguts were dissected and the anterior midgut, hindgut, and malpighian tubules were removed. The posterior midgut containing the blood meal was cut in half lengthwise with a scalpel. The blood and peritrophic matrices were removed to leave an isolated sheet of midgut epithelium. For ookinete invasion studies, midgut sheets were suspended in 100 μl of invasion medium (M199 medium/2 mM glutamine/10% heat-inactivated chicken serum/50 units/ml penicillin/50 μg/ml streptomycin). *P. gallinaceum* ookinetes were prepared as described (8). Approximately 10^5 ookinetes in 50 μl of invasion medium were added to the midgut epithelial sheet. The mixture was centrifuged for 1 min at 300 × *g* to bring the parasite and midgut tissue together. The tube then was tapped gently to resuspend the pellet and centrifuged again to combine them together. At this stage, the pellet was incubated at 26°C for ≈30 min, after which the epithelium was washed thoroughly to remove unbound parasites. Each parasite-

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Abbreviations: V-ATPase, vesicular ATPase: IFA, immunofluorescence assay.

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invaded midgut epithelial tissue was spread individually with a pair of dissecting needles and fixed with 2.5% glutaraldehyde and 4% sucrose in cacodylate buffer (0.1 M sodium cacodylate, pH 7.0). The fixed tissues were dehydrated in acetone and embedded in Epon or LR-White resin. Thick sections (≈1 μm) were attached to glass slides over a hot plate at 60°C. The tissues were stained with 0.1% toluidine blue for 1–2 min, after which the slides were washed with distilled water and air dried. The sections were examined with a Olympus BH2 light microscope. To distinguish midgut cells based on toluidine blue staining, a midgut cell was classified as a light cell if it was unstained or light purple and as a dark cell if it was dark purple or dark blue (Fig. 1). To count the total number of cells in an isolated section of midgut, serial sections of the entire piece were used. Twenty-five of 15,819 cells could not be classified because staining was intermediate.

Darkly stained ookinetes associated with the midgut epithelium were identified by their typical slender shape and the presence of anisotropic pigment in the cytoplasm. The pigment in female gametes acquired in the vertebrate stage remains in the cytoplasm after fertilization and transformation to ookinetes. The pigment was detected with polarized light (9). Ookinetes were found attached on the surfaces of the epithelium, invading from the lumen side and located in the midgut cell cytoplasm or close to the basement membrane. The epithelial cells that contained at least one ookinete in their cytoplasm were scored as invaded midgut cells.

Transmission Electron Microscopy. Gut sheets without exposed parasites or after invasion assay were postfixed for electron microscopy. The tissues were incubated with 0.8% $K_3Fe(CN)_6$/0.5% OsO_4 in 0.1 M sodium cacodylate for 30 min, followed by 1% tannic acid for 1 hr. The samples were stained overnight with uranyl acetate, dehydrated with a graded ethanol series, and embedded in Spurr's resin. The sections were stained with 1% uranyl acetate and Reynold's lead citrate and observed at 80 kV on a Philips CM-10 transmission electron microscope (Alpharetta, GA).

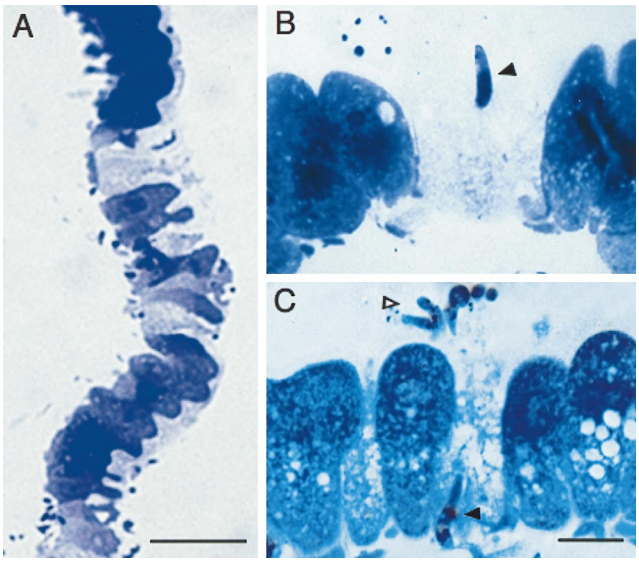

FIG. 1. Toluidine blue staining of *P. gallinaceum*-infected *A. aegypti* midgut epithelium. *In vitro*-transformed *P. gallinaceum* ookinetes were centrifuged on isolated sheets of the midgut epithelium from blood-fed *A. aegypti* (see *Materials and Methods*). Each of the irregularly shaped pieces ranged from 0.3 to 1 mm in size. Sections of these isolated midgut sheets were cut perpendicularly to the luminal plane to examine intact monolayer of epithelial cells (*A*). An isolated midgut sheet automatically curls inside out and exposes the luminal surface outward, making it suitable for *in vitro* ookinete invasion assay. (*A*) The dark and lightly stained cells in a cross section of midgut epithelium. (*B*) An ookinete invading an epithelial cell that is poorly stained by the dye (arrow). (*C*) Several ookinetes appear to be associated with the same lightly stained cell that had been invaded by another ookinete (open arrow). Scale bar: *A*, 50 μm; *B* and *C*, 10 μm.

To study the ultrastructural features of invaded midgut cells, sections were scanned for the cells that contained parasites. For each invaded cell, the surface structures (presence of microvilli, folding on apical plasma membrane, etc.), osmiophilic property of the cytoplasm, presence of rough endoplasmic reticulum, mitochondria, electron-dense secretory granules, vacuoles, and nucleus were examined.

Immunofluorescence Assay (IFA) on *in Vivo* Infected Midgut. Mosquitoes were fed on an infected chicken. Approximately 30 hr after the blood meal, the midgut was dissected and the epithelium was peeled away to remove the peritrophic matrix-encased blood meal. The gut tissue was fixed immediately in 4% paraformaldehyde, 2 mM $MgSO_4$, 1 mM EGTA, and 0.1 M Pipes buffer. The fixed tissue was dehydrated by incubating 5 min each with 20, 40, 60, and 80% methanol in PBS, pH 7.2 and finally for 30 min in 100% methanol. The tissue was rehydrated by sequentially washing with decreasing concentrations of methanol in PBS. The rehydrated and permeabilized tissue was blocked overnight with PBT-A (1% BSA/0.1% Triton X-100 in PBS). The tissue was then incubated overnight with primary antibody (rabbit anti V-ATPase antiserum or mouse anti Pgs28 mAb) diluted in PBT-B (0.1% BSA/0.1% Triton X-100 in PBS). After washing three times (1 hr each) with PBT-B containing 2% goat serum (Life Technologies), the tissue was incubated with rhodamine-conjugated goat anti-rabbit antibody and fluorescein isothiocyanate-conjugated goat anti-mouse antibody (Pierce) diluted in PBT-B containing 2% goat serum and 0.005% bisbenzimide H33258 fluorochrome (nuclear stain; Calbiochem, San Diego, CA) for 4 hr. The tissue then was washed five times (1 hr each with PBT-B) and mounted under a coverslip. The tissues were examined with a Lietz Ortholux 2 fluorescent microscope (Ernst Lietz, Ontario, Canada) by using Lietz filter block N2 (excitation filter 530 ± 25 nm and emission filter 645 ± 40 nm) for fluorescein isothiocyanate and Lietz filter block 12/3 (excitation filter 485 ± 20 nm and emission filter 530 ± 25 nm) for rhodamine.

Each tissue was scanned for V-ATPase-positive cells and the cells that contained invaded ookinetes. After identification of a parasite-invaded cell by ookinete-specific antibody, the presence of the ookinete was confirmed further by the parasite-specific anisotropic pigment.

Laser Scanning Confocal Microscopy. The midgut tissues that were prepared for IFA also were used for laser scanning confocal microscopy. The tissues were examined with a Zeiss LSM 410 confocal microscope. First, each parasite-invaded cell was detected, and then optical sections 1 μm apart were examined. Digital images of each of the optical sections were acquired in the tagged image file format and stored. To visualize the location of V-ATPase and invaded parasites in the cell, a three-dimensional image reconstruction of the cell was made with the digital images. This image allowed the studying of the relative spatial locations of the V-ATPase and the invaded parasites. For preparing the figures with selected frames, additional processing of images (which included only composing) was performed with ADOBE PHOTOSHOP software (Adobe Systems, San Jose, CA).

RESULTS

***In Vitro* Ookinete Invasion of Mosquito Midgut Epithelium.** To examine the ookinete invasion of mosquito midgut epithelium more closely, 20-hr-old *P. gallinaceum* ookinetes from *in vitro* culture were centrifuged onto isolated midgut (see *Materials and Methods*). Serial sections of the resin-embedded midgut sheets were stained with toluidine blue. Examination of these toluidine blue-stained midgut sections showed a large

number of the ookinetes attached to the lumen side of the midgut. In addition to the attached parasites, many ookinetes were found invading (Fig. 1*B*) or already within (Fig. 1*C*) the epithelial cells. The ookinetes within the midgut epithelium were identified by the shape (Fig. 1 *B* and *C*) and by the presence of anisotropic pigments in the parasite visualized with polarized light. We scanned 15,794 midgut epithelial cells in three independent experiments and found 399 (2.5%) of them were invaded by 514 ookinetes (Table 1). Of the 399 cells, 72 cells were invaded by more than one ookinete. On one occasion, we observed as many as five ookinetes within a single epithelial cell.

During this study, we found that the midgut cells showed remarkable variation in their staining patterns with the basophilic dye, toluidine blue. The cells clearly could be differentiated between darkly stained cells and lightly stained cells (see *Materials and Methods* for definitions). We noted that, relative to the majority of the midgut cells that stained darkly with toluidine blue, most of the ookinete-invaded cells were stained lightly with this basophilic dye (Table 1; Fig. 1 *B* and *C*). Of all of the invaded midgut cells, 85.5% (340 of 399) were lightly stained (Table 1). Of the cells that were invaded by more than one ookinete, 93.1% (67 of 72) were lightly stained. We hypothesized that *Plasmodium* ookinetes preferentially invade midgut cells that are less basic than the majority of the cells.

To examine whether the failure to stain with toluidine blue was caused by the ookinete invasion, we compared the frequency of lightly stained cells in uninfected and infected midgut epithelium. For this comparison, the numbers of light and dark cells were estimated in infected or uninfected midgut tissues. We found that 17.8% (2,054 of 11,541) cells in infected epithelium and 25.9% (3,733 of 14,415) cells in uninfected epithelium were stained lightly. Ookinete invasion did not increase the proportion of lightly stained cells in the ookinete-exposed epithelium, indicating that the failure to stain with toluidine blue is an inherent property of the cells and that the ookinetes preferentially invade these cells.

Ultrastructural Features of the Invaded Cells. To investigate whether the ookinete-invaded cells share any unique ultrastructural features, we examined thin sections of the ookinete-infected epithelium by transmission electron microscopy. No remarkable difference was observed in the nucleus or in the abundance of the mitochondria between the ookinete-invaded and uninvaded cells. A number of features, however, were noted in invaded cells that were notably different from the majority of the uninvaded cells. The apical surface of the invaded cells lacked the dense tufts of microvilli commonly found in the columnar cells. Instead, these cells had none or very few microvilli (Fig. 2). Most remarkable was the cytoplasm of these cells, in that it was less osmiophilic (Fig. 2). The cells had sparse, rough endoplasmic reticulum and in most cases did not contain electron-dense secretory granules. Instead, these cells contained a large number of clear vacuoles, mostly near the apical end. All of the invaded cells share these common features. The unique ultrastructural features supported the hypothesis that *Plasmodium* ookinetes are biased in invading a specific type of cell in the epithelium.

Characterization of the Cells in the Midgut Preferentially Invaded by Ookinetes. From the *in vitro* invasion of midgut cells by ookinetes, it was found by light microscopy and transmission electron microscopy that the invaded cells stained lightly with toluidine blue and are less osmiophilic, respectively, than the majority of cells. To determine the type of the invaded cells, we compared them with cells described in the literature (see ref. 10). Three types of cells have been described to be present in the adult mosquito midgut. The majority are columnar cells. A typical columnar cell contains tightly packed microvilli and a large amount of smooth and rough endoplasmic reticulum. After blood ingestion, columnar cells are induced to synthesize and secrete large amounts of proteolytic enzymes and to actively participate in food digestion. Because of such metabolic activities, these cells are also likely to stain darkly with the basophilic dye, toluidine blue. The lack of dense microvilli and RER, failure to stain with toluidine blue, and absence of secretory granules indicate that the ookinete-invaded cells are not typical columnar cells.

In addition to the columnar cells, mosquito midguts also contain regenerative cells (10, 11) and different types of endocrine cells (12, 13). The regenerative cells usually are located between the columnar cells, close to the basal side of the epithelium. Because these cells are not exposed to the lumen, it is unlikely that they are invaded directly by the parasite. The endocrine cells synthesize and secrete hormones into the hemolymph. In transmission electron microscopy, some midgut endocrine cells appear electron translucent (14, 15), similar to the cells invaded by ookinetes. Endocrine cells vary in the type of hormone they secrete. Two hormones, RFamide and urotensin I, are secreted by endocrine cells dispersed in the posterior midgut of female *A. aegypti* (16), where the majority of the *Plasmodium* oocysts usually are seen in infected mosquito midgut. To determine whether endocrine cells secreting these hormones are invaded by the ookinetes, we used polyclonal antibodies specific to these hormones and a mAb to ookinete surface antigen Pgs28. Neither of these hormones colocalized with the cells invaded by the parasites (data not shown). We concluded that none of these previously described cells is a target for ookinete invasion.

We suspected that the mosquito midgut may contain other types of cells that have not yet been described. We decided to investigate the mosquito midgut epithelium for presence of the cell types described in other insects. One such cell type is an ion-transporting midgut cell of Lepidoptera. In the Lepidoptera *Manduca sexta*, midgut goblet cells are involved in ion transport across the midgut epithelium (17). We searched for

Table 1. Preferential invasion of *P. gallinaceum* ookinetes to less basic cells in *A. aegypti* midgut epithelium

	Experiment 1			Experiment 2			Experiment 3			Total		
	Cells invaded	Cells counted	% invaded	Cells invaded	Cells counted	% invaded	Cells invaded	Cells counted	% invaded	Cells invaded	Cells counted	% invaded
Light*	117	813	14.4	91	945	9.7	132	1064	12.4	340	2822	12.0
Dark†	22	4608	0.5	23	3168	0.7	14	5196	0.3	59	12972	0.4
Total	139	5421	2.6	114	4113	2.8	146	6260	2.3	399	15794	2.5
χ^2		529.9‡			214.1			565.8			1260.4	
P		<0.0001			<0.0001			<0.0001			<0.0001	

Serial sections of ookinete-exposed midgut epithelium stained with toluidine blue were examined with light microscope, and the number of cells (total and invaded) and staining pattern of each cell was recorded. Each experiment was performed independently with different batches of mosquitoes and parasites.

*Cells with light purple or no staining with toluidine blue (Fig. 1) were recorded as light cells.

†Cells with dark purple or dark blue staining were recorded as dark cells.

‡Chi-square test was performed on 2 × 2 contingency table (light/dark vs. invaded/uninvaded). The χ^2 values were calculated with Yates correction for continuity; degree of freedom = 1.

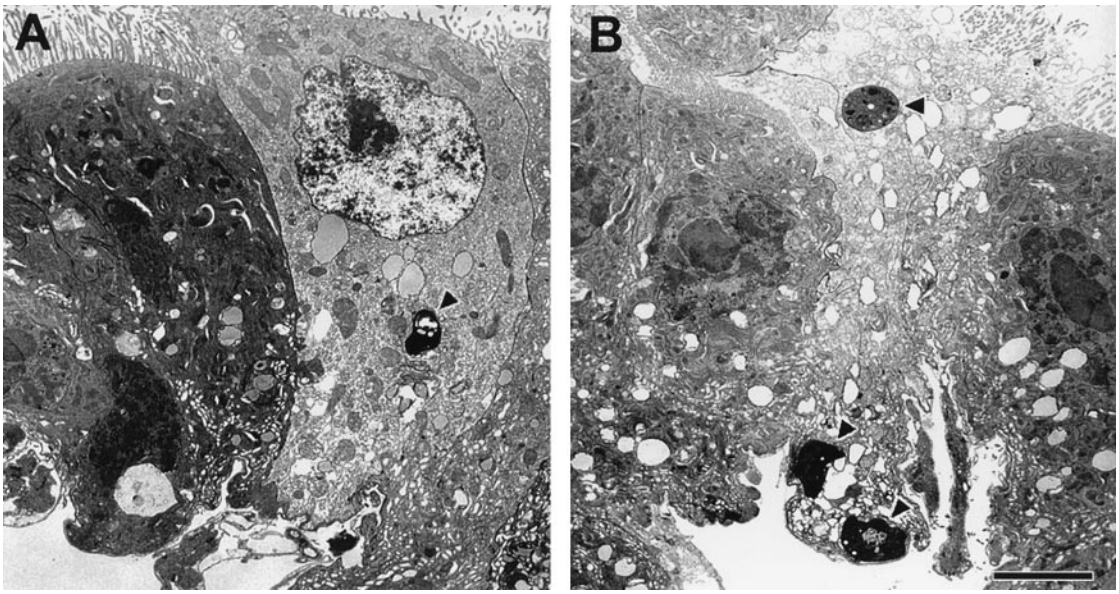

FIG. 2. Ultrastructure of two ookinete-invaded midgut cells. (*A*) An electron-translucent cell contains one parasite (arrowhead points to the parasite), and (*B*) a cell contains three parasites (arrowheads point to parasites). (Bar = 2.5 μm.)

similar cells in the mosquitoes. Blood-fed mosquitoes need to rid themselves of the high sodium load gained from ingested plasma and the potassium gained in the blood cells (18). The mechanism of ion transportation across the midgut epithelium of adult mosquitoes is not understood clearly. These cells are characterized by the presence of a typical goblet cavity filled with glycoproteins; however, typical goblet cells have not been demonstrated in mosquitoes. We considered the possibility that mosquito ion-transporting cells are structurally different from Lepidoptera cells.

Plasma membrane V-ATPase plays a crucial role in transporting ions in insect goblet cells (19, 20). V-ATPase in these cells generates a proton gradient across the midgut lumen and cell cytoplasm, which is used subsequently for ion transport across the epithelium. We used *Culex quinquefasciatus* V-ATPase B-subunit antiserum (21) as a marker for ion-transporting cells in *A. aegypti*. The V-ATPase antiserum strongly labeled a subset of the mosquito midgut cells; ≈29% (373 of 1,268) of the midgut cells were V-ATPase positive.

We examined the invasion of V-ATPase-expressing cells by ookinete-specific mouse mAbs (anti-Pgs28) and rabbit anti-V-ATPase antiserum. Initial attempts of using *in vitro*-invaded midgut epithelium for these experiments failed because of high background caused by the ookinetes that attached to the surface of the epithelium. The large amount of attached ookinetes, compared with few invaded ones, made it difficult to differentiate invaded ookinetes from the uninvaded. We decided to use natural infection by using mosquitoes fed on infected blood. For these experiments, 6- to 8-day-old adult *A. aegypti* were fed on a *P. gallinaceum*-infected chicken. About 30 hr after the blood meal, midguts were dissected and epithelial sheets were prepared. The isolated epithelium was processed for IFA. All of the cells ($n = 122$) that were invaded by ookinetes were also positive for V-ATPase. Altogether, 32.7% of the V-ATPase-positive midgut cells were invaded by ookinetes.

In *M. sexta* goblet cells, V-ATPase is present on the apical plasma membrane and in intracellular locations (22). To determine the spatial distribution of the V-ATPase and the invaded ookinetes, we examined the samples prepared for IFA with laser scanning confocal microscopy. Serial optical sections of the invaded and uninvaded cells were examined for the site of V-ATPase expression. Reconstructions of the images provided a three-dimensional view of the cells. Parasites were observed only in cells that expressed V-ATPase (Fig. 3 *A* and *B*). In both invaded and uninvaded V-ATPase-positive cells, the enzyme was associated with three distinct locations. In-

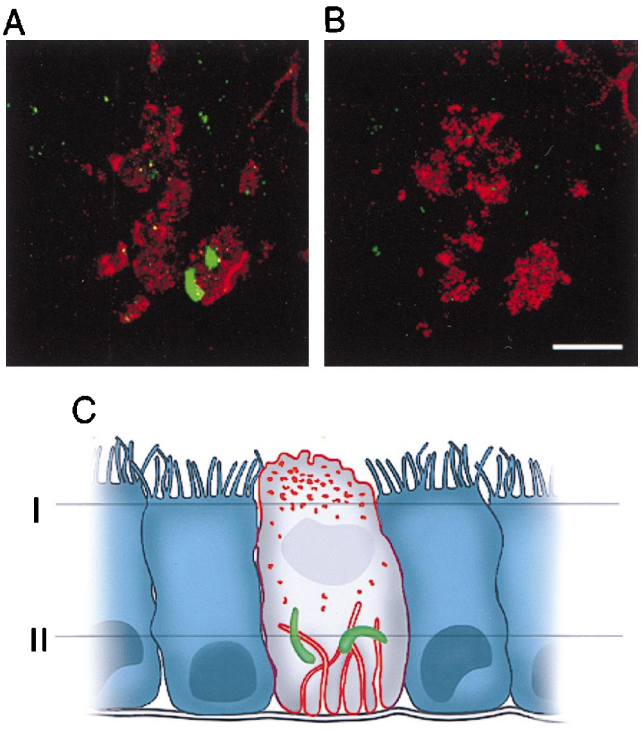

FIG. 3. Confocal microoscopic images of V-ATPase-positive cells and location of invading parasites in mosquito midgut cell. (*A*) A cell in a cluster of V-ATPase-positive midgut cells is invaded by two parasites (green). The distance of the focal plane of the image relative to the apical surface of the cell is 27 μm. The sections of the tubular structures (red) are seen in both parasite-invaded and uninvaded V-ATPase cells. (*B*) The same cells as in *A* at 12 μm below the apical cell surface. At this distance, numerous vesicles (red) were present in both invaded and uninvaded V-ATPase-positive cells. (Bar = 20 μm.) (*C*) A schematic drawing describing the location of mosquito V-ATPase and invaded parasites in the cells shown in *A* and *B*. Horizontal lines I and II show the planes of the images in *B* and *A*, respectively.

tense staining of V-ATPase was distributed homogeneously on the apical plasma membrane of the cells. In the cytoplasm, numerous vesicles were labeled with V-ATPase antiserum (Fig. 3*B*). The majority of these vesicles were within 20 μm of the apical surface. Close to the base of the cells, the vesicles were less abundant. Instead, in this region, tubular structures extending inward to the cell cytoplasm labeled heavily with V-ATPase (Figs. 3*A*).

DISCUSSION

Immediately after ingestion by a susceptible mosquito, *Plasmodium* parasites emerge from red blood cells and fertilize to form zygotes. The zygotes transform into ookinetes that cross the midgut epithelium and develop as oocysts between the basement membrane of the epithelial cells and the basal lamina. In this paper, this description of the sporogonic development of *Plasmodium* has been extended with the finding that the parasites invade a specific cell type in the mosquito midgut that differs from the majority of the microvilli-covered columnar cells. We have demonstrated by their different histochemical, ultrastructural, and immunologic features that these cells are unique. We propose to name these cells "Ross cells" to honor one of the most significant discoveries in medical history by Sir Ronald Ross 100 years ago: that mosquitoes transmit malaria.

Invasion of specific cells by pathogens is common in the gut of vertebrates (see ref. 23). *Salmonella typhi* in humans and *S. typhimurium* in the mouse both bind to intestinal M cells and are transported rapidly into the Peyer's patch mucosa. *Shigella flexnerii*, a Gram-negative bacterium that causes dysentery, and *Yersinia paratuberculosis*, which is related closely to plague bacillus *Y. pestis*, also is delivered across the epithelium via M cells. Peyer's patch explant studies from mouse and rabbit showed that HIV-1, a human retrovirus that causes AIDS, binds selectively to M cells and is transported into the epithelial pocket. Because lymphoid follicles and follicle-associated epithelia containing M cells are abundant in mucosal surfaces, including the rectal mucosa, it has been suggested that human M cells can serve as an entry port for HIV. Although Ross cells share some common features with M cells (such as lacking microvilli and serving as a doorway for a pathogen), it is not yet clear whether they are related.

The high level of V-ATPase in Ross cells suggested that they are related to Lepidoptera ion-transporting goblet cells. In these cells, V-ATPase was present on the apical surface and on the lining of the goblet cavity. V-ATPase also is present on the surface of insect malpighian tubules and vertebrate kidney epithelial cells (24). These V-ATPase-containing cells also are involved in the regulation of ion concentration, suggesting that V-ATPase plays an important role in ion transport through these cells. Blood-fed female mosquitoes need to remove the high sodium and potassium load gained from ingested blood. Although mosquitoes are extremely efficient in removing these ions, it is not clear how these ions are transported from the midgut lumen into the hemolymph where malpighian tubules absorb them. If the cells that are invaded by ookinetes are ion-transporting cells, it would be interesting to know why ookinetes have evolved to invade these cells.

The discovery of the invasion of this cells in *A. aegypti* by the bird *Plasmodium, P. gallinaceum*, has opened a new area of malaria transmission-blocking research. Further studies are needed to determine whether Ross cells in *Anopheles* mosquitoes are also invaded by human malaria parasite *P. falciparum* and by other primate and rodent *Plasmodia*. The identification of the cells in *Anopheles* mosquitoes may lead to new strategies to block the transmission of malaria. If invaded by *P. falciparum*, the receptors on these cells are candidates for a malaria transmission-blocking vaccine.

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