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Letter to the Editor

Which Routes Do *Plasmodium* Sporozoites Use for Successful Infections of Vertebrates?

In mice, sporozoites of *Plasmodium berghei* delivered by mosquito bites are significantly more infectious than those transmitted by intravenous (i.v.) inoculation, as shown by Vaughan et al. (3). Using the chicken malaria *P. gallinaceum* in its natural host we obtained similar results. Sporozoites delivered by bites of two to five *Aedes fluviatilis* mosquitoes infected 100% of 1-week old chicks after prepatent periods (PPP) of 4 to 7 days, resulting in mortality of all birds. Sporozoites injected by syringe also cause malaria, although with lower parasitemia, longer PPP (~11 days), and lower mortality (40% to 75%). Unlike *P. berghei* and all other sporozoites that infect mammals (which develop in the hepatocytes), *P. gallinaceum* sporozoites initially invade and develop within skin macrophages at the site of injection, rather than in other tissues.

The route by which sporozoites reach the hepatocytes is still debatable although their suggested transport via the lymphoid system (3) could well be through macrophages and/or Küppfer cells. The fact that *P. berghei* sporozoites enter and leave macrophages without being destroyed and that all attempts to cultivate the mammalian plasmodium sporozoites in hepatocytes have resulted in an extremely low percentage of infections supports this hypothesis. In addition, opsonized *P. berghei* sporozoites phagocytized by macrophages or Küpffer cells are destroyed.

In the presence of stage-specific monoclonal antibodies (MAb of ≥3 μg), sporozoite invasion and/or development in macrophages is totally abrogated (2), indicating that in vitro, the primary exoerythrocytic forms of *P. gallinaceum* developing inside macrophages are susceptible to being killed by antibodies. A direct correlation occurs between the protective effect of MAb in vitro and in vivo. Thus, in vitro suspensions of sporozoites plus MAb injected i.v. did not cause infection when we used the active MAb. All control chicks receiving sporozoites with medium or specific MAb with no activity in vitro had patent malaria and high parasitemia (2).

In the mouse model, high doses of passively transferred specific MAb antisporozoites inactivated sporozoites given i.v. but not through mosquito bites (3). This result strongly suggests the presence of protective mechanisms other than the blocking of sporozoite invasion into the host cell by MAb. Furthermore, in mice challenged with mosquito bites, protection hardly occurred, despite MAb transfer. We propose that macrophage killing of the opsonized sporozoites did not occur because the parasites were taken up by skin macrophages in the presence of low immunoglobulin G levels not sufficient to opsonize the parasites.

Finally, since high homology between DNA sequences of the circumsporozoite genes has been described for *P. falciparum* and *P. gallinaceum* (1) supporting a close phylogenetic relationship between these two species, it is quite possible that other similarities between the life cycles of avian and mammalian malaria parasites do exist. However, the role of macrophages in sporozoite transport and/or in antibody-mediated destruction of *P. falciparum* sporozoites and other mammalian malaria parasites is highly relevant to vaccine development and deserves further study, since antibodies are the key to antisporozoite protection.

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Author's Reply

The question of how mosquito-transmitted sporozoites reach the liver is longstanding and unresolved. We now know that most sporozoites are not injected directly into the bloodstream, as is commonly depicted in life cycle diagrams. Since the early works of Boyd and Kitchen (1), evidence has steadily accumulated to indicate that during mosquito probing, most transmitted sporozoites are deposited as clumps within the skin and that there is a substantial delay in the movement of mosquito-transmitted sporozoites away from the site of the mosquito bite (4, 6). There are two possible routes that mosquito-transmitted sporozoites can take to move away from the bite site—entry into efferent capillaries directly or, more plausibly, via lymphatic drainage (3).

If mosquito-transmitted sporozoites enter the circulation via the lymphatics, then it is all the more remarkable that they are so efficient in reaching the liver. The lymphatic route is circuitous and seemingly fraught with danger. In order to reach the liver via the lymphatics, mosquito-transmitted sporozoites must pass through lymph nodes to reach the thoracic or right lymphatic ducts which then empty into brachiocephalic veins and into the superior vena cava. Sporozoites would then pass through the right atrium/ventricle and into the pulmonary circulation, including a passage through the alveolar capillary plexus. The left atrium/ventricle would propel the sporozoites through the aortic arch and descending aorta. If sporozoites were fortunate enough to enter the celiac trunk on their first pass through the systemic circulation, they may be sent directly into the liver via the common hepatic artery or, more likely, into the other arterial branches of the celiac trunk to the stomach, pancreas, or spleen. A bit further down the aorta, sporozoites might be sent into the superior mesenteric artery. In either case, sporozoites would have to pass through capillary beds of the lower digestive system before entering the hepatic portal system and arriving at the relative calm of the sluggish circulation within the liver sinusoids.

For mammalian plasmodia, the traditional view holds that sporozoites travel to the liver extracellularly. Drs. Krettli and Dantas offer an alternative scenario—one inspired by their work with avian plasmodia and to which I refer to informally as the "taxicab hypothesis." In this scenario, mosquito-transmitted sporozoites quickly invade macrophages (or some other leukocyte type) in the skin and are then carried inside of host leukocytes with the draining lymph, away from the bite site, through the perilous lymph nodes, and on to the liver. It has been demonstrated that sporozoites are fully capable of "actively and aggressively" moving into and out of macrophages (8). Indeed, the bioactive substances in mosquito saliva may potentiate host edema and leukocyte infiltration to the site of sporozoite deposition (2, 5). As Drs. Krettli and Dantas suggest, the taxicab hypothesis may explain why many mosquito-transmitted sporozoites are able to elude the host protective effects of passively administered anticircumsporozoite monoclonal antibodies, whereas many intravenously inoculated sporozoites (i.e., "naked" sporozoites) are not (9).

The mechanism(s) by which mosquito-transmitted sporozoites complete their journey to the liver remains unknown. But if a sporozoite vaccine is to succeed, the biology of this journey needs to be elucidated. In his classic work on sporozoite transmission (7), Vanderberg noted that ". . .until it becomes possible to label sporozoites and track them. . .there seems no way to assess the total numbers actually inoculated by mosquitoes" or, in this case, to determine how mosquito-transmitted sporozoites reach the liver. Recent success in producing a stably transformed line of *P. berghei* in which sporozoites express green fluorescent protein (Kenneth Vernick, personal communication) may prove useful in monitoring the progress of mosquito-transmitted sporozoites as they move from the skin to the liver.

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