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Correspondence

Assessment of Antibody Protection against Malaria Sporozoites Must Be Done by Mosquito Injection of Sporozoites

To the Editor-in-Chief:

I read with much interest the insightful article by Mueller et al[1] on genetically attenuated malaria sporozoites used for induction of pre-erythrocytic immunity in mice. On the basis of elegant experiments with genetically modified mice, the authors convincingly show that T cells and interferon-γ (IFN-γ) are important effectors in mice immunized with genetically attenuated sporozoites, which as they note have been previously shown with radiation-attenuated sporozoites.[2–5] However, Mueller et al[1] overlook an important aspect of the biology of malaria parasites when they conclude that anti-sporozoite antibodies "play a minor protective role" and that B cells are not protective in the absence of IFN-γ. They report that immunization of mice unable to express IFN-γ resulted in a strong anti-sporozoite antibody titer, but the mice were not protected against challenge with wild-type sporozoites. They cite the earlier studies done by immunization with irradiated sporozoites, which they contend also demonstrate the minor protective role played by anti-sporozoite antibodies.[2–5] Thus, they conclude "that antibodies cannot compensate for IFN-γ deficiency."

The problem with this study and the earlier studies is that challenge was by intravenous injection of sporozoites, whereas natural challenge is by mosquito injection. Recent work has consistently shown that most or all mosquito-transmitted sporozoites are not deposited in the blood circulation but in avascular portions of the skin, from where their motility enables them to invade dermal blood vessels.[6–11] Sporozoites injected directly into the circulation (as in the study by Mueller et al[1]) can invade hepatocytes within a few minutes[12]; thus, there is little time for antibodies to have an effect. Their use of intravenous challenge is perfectly appropriate for their assessment of the T-cell and IFN-γ immune mechanisms that act against infected hepatocytes. However, sporozoites injected by mosquitoes do not leave the skin rapidly[6–8,10–11]; thus, there is more time for anti-sporozoite antibodies to act by immobilizing sporozoites[13] deposited into the skin and preventing these sporozoites from entering the circulation.[8] I suggest that unless these authors are able to show that mice with high antibody levels are unprotected against a normal mosquito bite challenge, their conclusion regarding antibodies being relatively nonprotective is unsustainable.

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Authors' Reply:

In malaria, the exoerythrocytic phase is clinically asymptomatic, whereas the subsequent erythrocytic phase causes clinical symptoms and may lead to the death of the infected individual. Therefore, a vaccine that inhibits the progression of intrahepatic sporozoites to erythrocyte-infecting merozoites would completely prevent the disease. Immunization with genetically attenuated *uis3(−) Plasmodium berghei* sporozoites that are capable of infecting the liver but do not cause parasitemia completely blocks the intrahepatic maturation of wild-type sporozoites.[1] Sustained intrahepatic protection occurs irrespective of the transmission mode, ie, intravenous sporozoite injection or mosquito bite,[1] and crucially depends on T cells, especially $CD8^+$ T cells, and IFN-γ.[2] It is important to note that mice that harbor a deletion of the immunoglobulin μ-chain and, hence, are B-cell deficient enjoy sterile protection.[2] Of course, B cells might aid in protection, but antibody responses alone cannot mediate sterilizing immunity.[3] The intravenous route of infection with high doses of wild-type sporozoites is the established route to determine the level of protection, including antibody-mediated responses, induced by attenuated metabolically active liver stages.[3–5] This delivery route is preferable because it permits i) focus on the intrahepatic infection, ii) the application of exactly defined doses of wild-type sporozoites, and iii) a homogenous infection of the liver within a very short time window.

We agree with Dr. Vanderberg that antibodies might reduce or, under certain experimental conditions, prevent the spread of sporozoites from the skin to the liver.[6] As we have clearly stated, sporozoite- and CSP-specific antibodies, which are induced by the *uis3(−) P. berghei* vaccine, might additionally contribute to protection after infection under natural conditions, ie, mosquito bite.[2] However, the sporozoite inoculum transferred by individual mosquito bites varies enormously, ranging from none to 1000, despite similar loads in salivary glands.[7] Additional variables include the average length of stay in the skin and the proportion of sporozoites that enter a blood vessel and productively infect the liver.[8] For these reasons, it remains doubtful whether experimental challenge infections by mosquito bite can substitute for the intravenous route of infection in experiments studying liver-stage protective live vaccines. Experimental infections by mosquito bite are the approach of choice to study local immune responses in the skin and the draining lymph nodes and to examine the cellular events at the local site of inoculation, because they exactly reproduce the natural way of transmission.

In conclusion, we would like to stress that experimental vaccination studies should not be confused with natural transmission studies.

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