Human immunization with attenuated sporozoites*

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Studies conducted between 1971 and 1975 showed that attenuated sporozoites can induce protection against human malaria. Three volunteers were protected against challenge with either a homologous or heterologous strain of Plasmodium falciparum *after being exposed to a total of 440–987 X-irradiated mosquitos on 6–8 occasions over a period of 10–38 weeks. Protection lasted for at least 8 weeks, but not 16 weeks, after the last immunization with irradiated sporozoites. Another 7 volunteers were not protected against challenge with a homologous strain after exposure to a total of fewer than 200 irradiated mosquitos, infected with either* P. falciparum *or* P. vivax, *on 2–4 occasions over a period of 4–17 weeks. These studies provide an encouraging basis for pursuing the development of a sporozoite vaccine against human malaria.*

Introduction

Studies were carried out between 1971 and 1975 to determine whether volunteers could be protected against malaria after being bitten repeatedly by mosquitos infected with attenuated sporozoites (*1*, *2*). The immunizing sporozoites were attenuated by exposing infected *Anopheles stephensi* to X-rays at a dose of at least 120 Gy (12 000 rad). The number of sporozoites inoculated into volunteers during a blood meal could not be determined but it was probably only a very small proportion of the sporozoites in the salivary glands of infected mosquitos.

After preliminary evaluation of the safety of this procedure in Navy volunteers, more extensive studies were undertaken with volunteers at the Stateville Correctional Center, Joliet, IL, USA. Of the 136 volunteers who participated in these studies, 86 were gametocyte donors, i.e., they provided the source of gametocytes to infect mosquitos. However, heavily-infected batches of mosquitos were not always forthcoming at regular intervals because variability in the tolerance of potential gametocyte donors to clinical malaria and in their response to drug suppression affected the duration and intensity of their subsequent gametocytaemia. This meant that a variable number of infected mosquitos became available at indefinite intervals of time and that, consequently, it was not possible to follow a constant immunization schedule.

Methods and results

In the actual immunization studies, 11 volunteers were bitten by 7 to 500 irradiated infected mosquitos on 2 to 8 occasions at intervals of 2 or more weeks. The remaining 41 volunteers participated as control subjects and were bitten either by irradiated non-infected mosquitos or by a few nonirradiated infected mosquitos to verify their transmission potential. About 2 weeks after their last exposure to irradiated mosquitos, both the immunized and the control volunteers were challenged with nonirradiated infected mosquitos by the interrupted bite technique. Immunized volunteers who failed to develop parasitaemia within several weeks after challenge were exposed again to mosquitos infected with a homologous or heterologous strain.

Immunization against Plasmodium vivax

Three volunteers were bitten by irradiated mosquitos infected with the Chesson strain of *P. vivax* on four occasions at intervals of 2 to 4 weeks. Each volunteer was exposed to a total of fewer than 200 irradiated infected mosquitos on these four occasions. The volunteers were challenged with the homologous strain about two weeks after the last immunization and all three of them developed patent infections. Their prepatent period and the clinical and parasitological course of their infections were similar to those observed in the control volunteers.

Immunization against P. falciparum

Five volunteers were bitten by irradiated mosquitos infected with the Ethiopian (Tamenie) strain of *P. falciparum* on 2 to 4 occasions. Each volunteer was bitten by a total of fewer than 200 mosquitos over a period of 4–17 weeks. During the course of immunization, two volunteers developed patent infections which were cured immediately with chloroquine; this indicated that 120 Gy (12 000 rad) was not always a sufficiently high irradiation dose to inactivate all sporozoites. Since one of the volunteers had to withdraw from the study for administrative reasons, only

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four men were challenged by mosquitos infected with the homologous strain. Unfortunately, none of them was protected. Their prepatent period and the course of their infections were similar to those observed in control volunteers.

Another three volunteers were bitten by irradiated mosquitos infected with the Ethiopian strain on 6 to 8 occasions. They were bitten by a total of 440 to 987 mosquitos over a period of 10 to 38 weeks.

Volunteer L.A. was exposed 6 times to a varying number of irradiated infected mosquitos (Fig. 1). The interval between exposures was precisely 2 weeks. Mosquito dissections over the 10-week period showed that he had been bitten by a total of 440 mosquitos infected with sporozoites. Two weeks after the last immunization he was bitten by 13 mosquitos infected with the homologous strain. He did not become infected whereas the control volunteer developed patent parasitaemia 12 days later. A further exposure to infected mosquitos, infected with the same strain, 16 weeks after the last immunization produced an infection during the normal prepatent period.

Volunteer D.S. was exposed 8 times to a total of 954 irradiated mosquitos infected with sporozoites (Fig. 2). Although the first four and the last two exposures were at intervals of 2 weeks, there was an interval of about 19 weeks between the fourth and sixth exposure. Immunization covered a period of about 30 weeks. The volunteer was then challenged at 2 and 8 weeks after the last immunization by mosquitos infected with the homologous strain. He did not become infected although, on both occasions, control volunteers developed patent infections within 2 weeks after challenge. However, he was not protected during subsequent challenge at 17 and 25 weeks after the last immunization. Mosquitos used for challenge during week 17 had been infected with the heterologous Vietnam (Marks) strain of *P. falciparum*.

Volunteer W.D. was exposed 7 times to a total of 987 irradiated infected mosquitos (Fig. 3). Intervals between exposures were very irregular during the 38-week period of immunization. About 8 weeks after the last immunization, he was exposed to mosquitos infected with the Vietnam (Marks) strain. He was protected against this heterologous challenge whereas the two control volunteers developed patent parasitaemia at 11 to 13 days after challenge. However, subsequent challenge with the homologous strain at 18 weeks after immunization failed to protect him against malaria.

Fig. 1. **Immunization and challenge schedule of volunteer L.A.** Open arrows represent times of exposure to irradiated mosquitos. Solid arrows represent times of exposure to nonirradiated infected mosquitos. Week 0 is the time of last exposure to irradiated mosquitos.

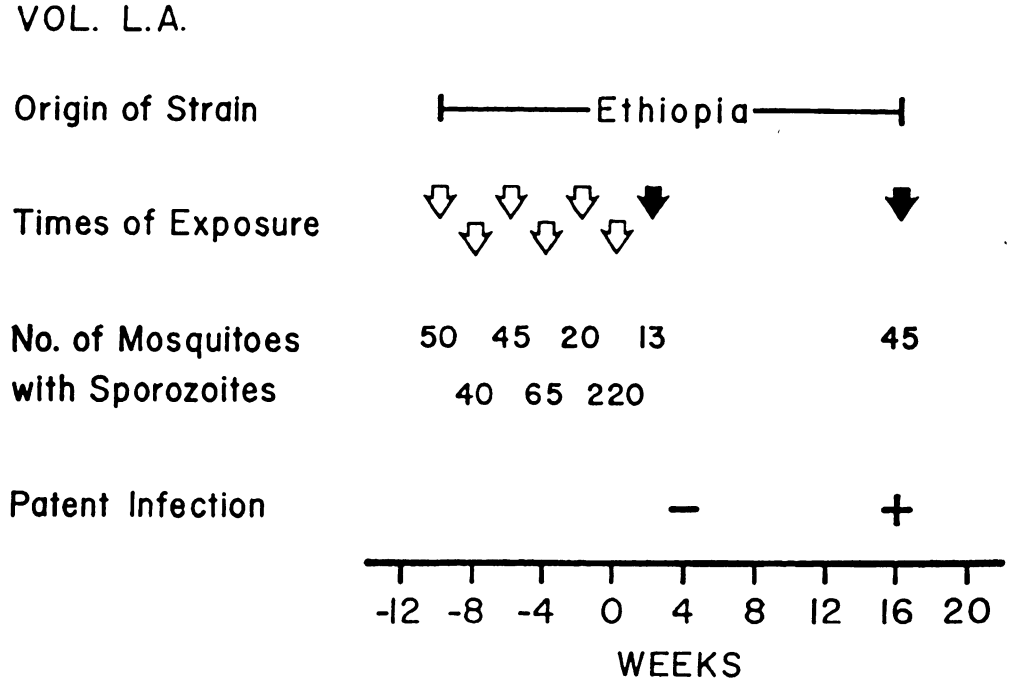


Fig. 2. **Immunization and challenge schedule of volunteer D.S.** Refer to Fig. 1 for explanation.

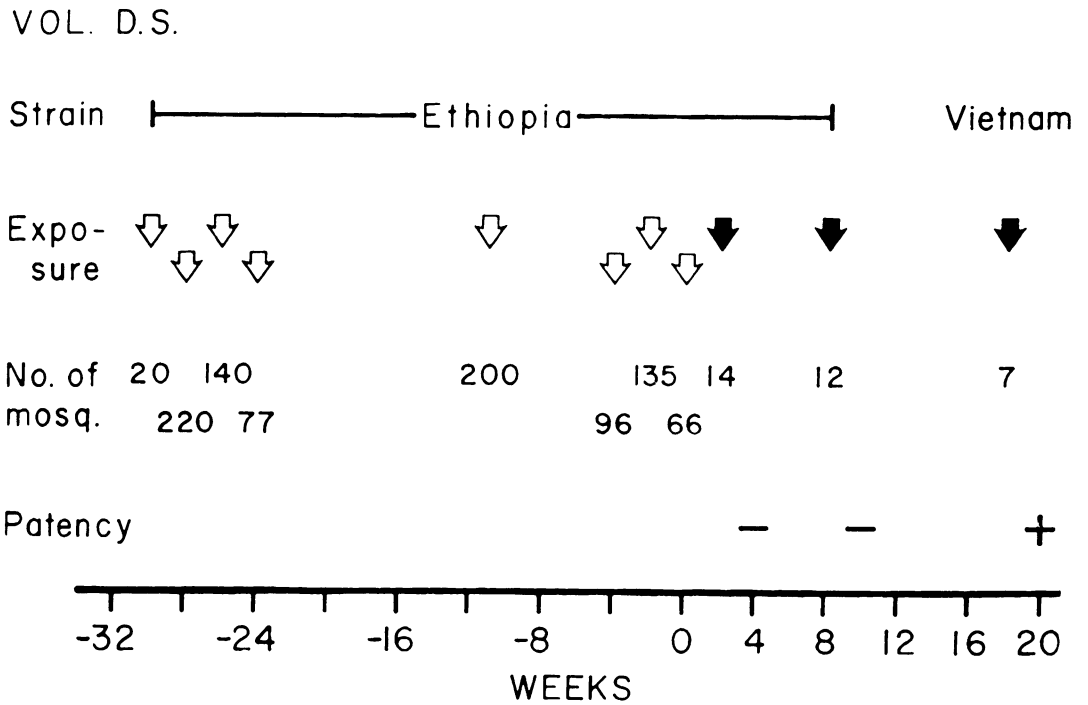
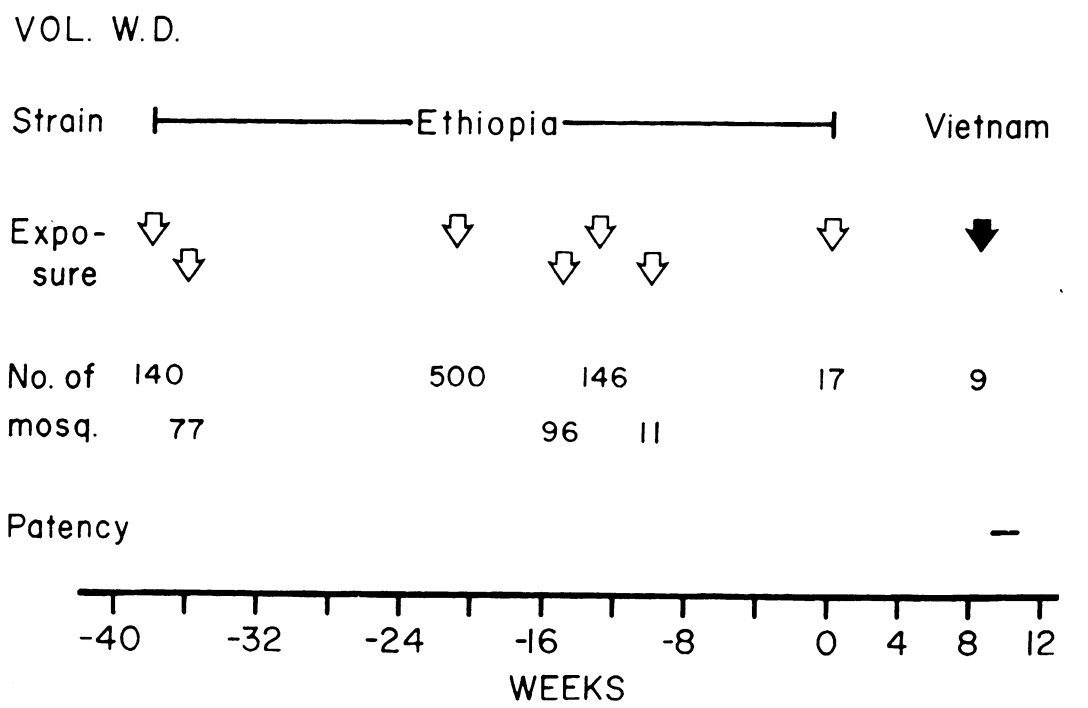


Fig. 3. **Immunization and challenge schedule of volunteer W.D.** Refer to Fig. 1 for explanation.



The results obtained in these studies show that attenuated sporozoites can induce protection against falciparum malaria. Protection lasted for at least 8 weeks following 6–8 exposures to 480–987 irradiated infected mosquitos. However, protection was no longer evident at 16–18 weeks after immunization. The fact that protection could be induced to a strain of *P. falciparum* geographically remote from that used during immunization should strengthen our resolve to develop a sporozoite vaccine against human malaria.

References

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