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SOUTH AMERICAN MONKEYS IN THE DEVELOPMENT AND TESTING OF MALARIAL VACCINES - A REVIEW

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South American Aotus *and* Saimiri *monkeys, which are susceptible to infection with human malarias, have been used to develop models for the testing of human malaria vaccines. Studies indicate that blood-stage and sporozoite vaccines can be tested in these monkeys using appropriate strains of parasites.*

Key words: malaria – *Plasmodium falciparum* – *Plasmodium vivax* – monkey - *Aotus* – *Saimiri* – vaccines

The development of human malarias in South American monkeys has offered opportunities for testing different combinations of antigens, adjuvants, carriers, and regimens in the development of potential human vaccines. Unfortunately, because all species and strains of human malaria do not develop equally in available monkeys, we have been forced to narrow the number of parasites and animal hosts that are suitable for vaccine trials. In addition, the changing availability of certain types of monkeys has further restricted the number of strains of human malaria that can be used.

Three basic types of antimalarial vaccines are being developed: blood-stage vaccines designed to eliminate mortality and reduce morbidity by controlling asexual stages of parasite development in the host, sporozoite vaccines designed to prevent infection, and transmission-blocking vaccines designed to prevent the development of the parasite in the mosquito. We have attempted to define those model systems which best fit the needs of the vaccine development process. We have restricted our studies to the development and testing of vaccines directed against *Plasmodium falciparum* (Collins et al., 1986b, 1988a, 1991a; Ruebush et al., 1990; Coppel et al., 1987) and *P. vivax*, (Collins, 1990; Collins et al., 1989b, 1990; Charoenvit et al., 1991), although similar approaches can be made for *P. malariae* (Colline et al., 1989a, 1989c). Because *P. ovale* has not yet been adapted to growth in monkeys, no systems are available for this species. Initially, we adapted a number of strains of *P. falciparum* and *P. vivax* to South American monkeys both through blood and sporozoite inoculation (Campbell et al., 1980, 1983, 1986; Collins et al., 1973a, 1973b, 1874, 1978, 1979, 1980a, 1980b, 1982a, 1982b, 1983a, 1983b, 1985, 1986a, 1987a, 1987b, 1988b, 1988c, 1991b; Fajfar-Whetstone et al., 1987). From the list of those parasites that will grow well in these animals, we have selected the strains that allow for statistically significant trials by producing predictable parasitemia.

The current candidate vaccines are directed against the blood stages, the sporozoites, and the sporogonic stage of *P. falciparum* and the sporozoites of *P. vivax*. The models which have been developed will also serve for testing blood-stage and transmission-blocking vaccines against *P. vivax*.

MODELS FOR TESTING *P. FALCIPARUM* VACCINES

Four species of *Aotus* monkeys have been investigated as candidates for testing *P. falciparum* vaccines. *A. lemurinus griseimembra* from northern Colombia, first species made available for extensive investigation is probably the most susceptible monkey for malarial studies. Because parasites from many different geographic areas grow well in this monkey, it is the prime candidate for vaccine trials. However, although it is still available for investigators in Colombia, this monkey is no longer exported, and we are limited to the few laboratory-born animals that become available. For a short time, *A. azarae boliviensis* from Bolivia were available. Our studies, which involved

approximately 100 monkeys of this species, indicated that it had significant potential for testing blood-stage vaccines but was unsatisfactory for sporozoite vaccine trials. These animals are no longer exported, and very few laboratory-born animals are available. Through the offices of the Pan American Health Organization, arrangements were made to export two species of *Aotus* from Peru, *A. nancymai* and *A. vociferans*. We now base our vaccine development studies on these monkeys.

The testing of antisporozoite vaccines requires a host that we can infect with sporozoites at a highly predictable rate. Our initial studies indicated that splenectomized *A.L. griseimembra* monkeys could be infected. Thirty-four out of 49 attempts to transmit the Santa Lucia strain of *P. falciparum* by mosquito bite were successful, with prepatent periods ranging from 15 to 46 days (mean of 24.9 days) (Fig. 4). When the animals received the bites of 10 or more infected mosquitoes, 25 (78%) of 32 animals became infected. If trial groups of 10 to 15 animals are challenged by the bites of 10 or more infected mosquitoes, we should be able to detect the effectiveness of antisporozoite vaccines. Since these animals are available in numbers only in Colombia, most trials would need to be conducted there. Almost all of the successful transmissions have been with the strain of *P. falciparum* from El Salvador (Santa Lucia strain). Unfortunately, this parasite has not developed well in culture, and gametocytes for infection of mosquitoes have all come from infected animals.

The susceptibility of *A. nancymai* to *P. falciparum* is limited to a few of the commonly studied strains, such a Uganda Palo Alto (FUP) and Vietnam Oak Knoll (FVO). We have now completed five immunization trials involving 170 of these monkeys and have protocols for testing additional candidate vaccines (Collins et al., 1991a; Ruebush et al., 1990). Initially, we used the Uganda Palo Alto strain. An examination of the daily parasite counts of 44 animals infected with the FUP strain (Fig. 1) indicated that the maximum parasite count occurred on day 13, and parasitemia was undetectable 4 weeks after inoculation. In a retrospective analysis, we determined that variations in maximum parasitemia made it difficult to determine significant differences between control and immunized groups of animals. Maximum parasitemia in control monkeys ranged from 1,610 to over 1,000,000 parasites per mm^3, and 11 of 28 monkeys had maximum parasitemia of < 100,000 per mm^3 (Fig. 2).

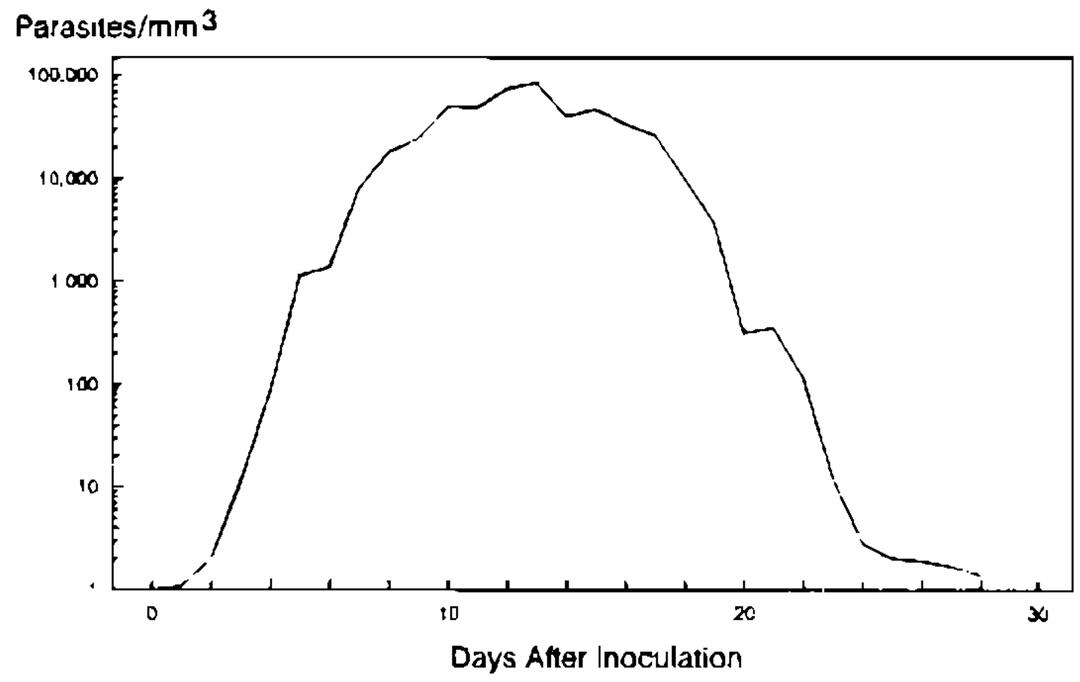

Fig. 1: mean daily parasite counts for 44 *Aotus nancymai* monkeys infected with the Uganda Palo Alto (FUP) strain of *Plasmodium falciparum.*

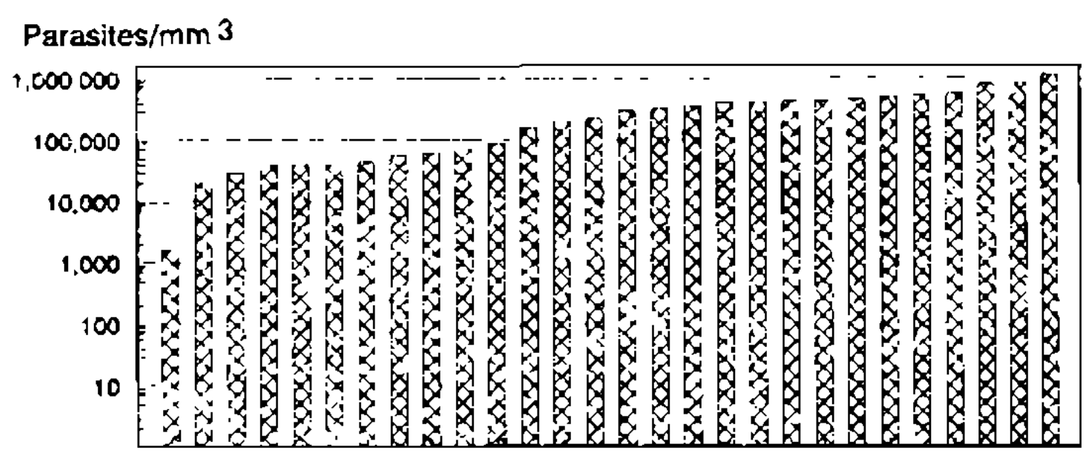

Fig. 2: maximum parasite counts in 28 *Aotus nancymai* infected with the Uganda Palo Alto strain of *Plasmodium falciparum.*

With the FVO strain of *P. falciparum* that we have used in recent studies (Ruebush et al., 1990), maximum parasite counts in 23 control animals from two different trials were consistently higher than those obtained with the FUP strain (Fig. 3). Maximum parasite counts ranged upwards from 296,000 and all animals required treatment to control their infections.

Aotus nancymai also has been successfully used by Dr Patarroyo and his coworkers in Colombia (Patarroyo et al., 1987; Rodriguez et al., 1990). Challenge to date has been restricted to blood-stage parasites because this monkey is highly resistant to infection via sporozoite inoculation.

Aotus vociferans from Peru was used in our initial vaccine trials with recombinant vaccines directed against the ring-infected erythrocyte surface antigen of *P. falciparum* (Collins et al., 1986, 1988a; Coppel et al., 1987). It is highly susceptible to infection with the Indochina I/

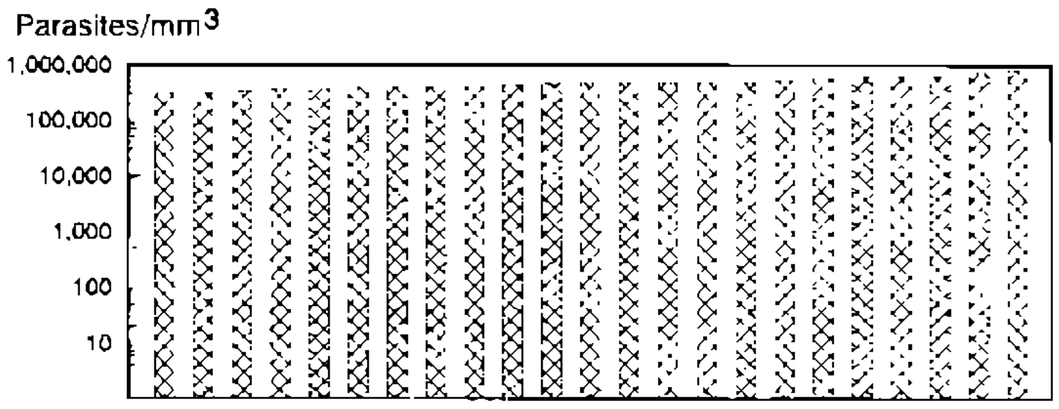

Fig. 3: maximum parasite counts in 23 *Aotus nancymai* infected with the Vietnam Oak Knoll strain of *Plasmodium falciparum*.

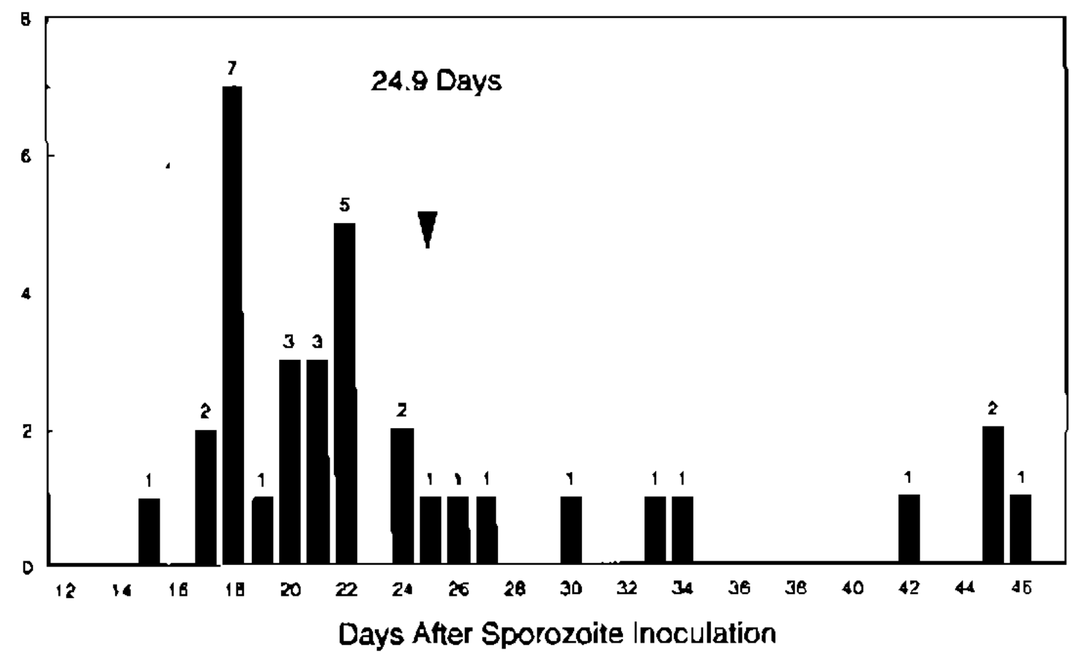

Fig. 4: prepatent periods in 34 splenectomized *Aotus lemurinus griseimembra* monkeys inoculated with the Santa Lucia strain of *Plasmodium falciparum* via the bites of infected mosquitoes.

CDC strain of this parasite. Only recently has this species been made available for additional studies. All indications are that it is highly susceptible to infection with many strains of *P. falciparum*. More importantly, our recent studies indicate that this species can be infected via sporozoite inoculation; more than 50% of splenectomized monkeys have been infected following the intravenous inoculation of sporozoites harvested from dissected salivary glands. Eventually, we hope to have a model system that can be challenged with *P. falciparum* sporozoites to test both antisporozoite and blood-stage vaccines. Presently, only *A.l. griseimembra* and *A. vociferans* appear capable of fulfilling this requirement.

Additional vaccine trials have been conducted in the Republic of Panama using Panamanian *A.l. lemurinus* monkeys which may prove to be useful hosts. Because these animals cannot be exported, the establishment of additional centers for immunization trials in countries where susceptible animals can be readily obtained is a welcome development.

Although most of our effort has been directed towards the use of *Aotus* monkeys, *Saimiri* or squirrel monkeys have proven to be useful hosts for the testing of blood-stage vaccines. Extensive studies with these monkeys have been conducted in Guyana. In addition workers at the University of Illinois used *Saimiri* monkeys and the Geneve strain of *P. falciparum* for blood-stage vaccine trials (James et al., 1985), and Australian investigators have immunized *Saimiri* monkeys of Guyanan origin in their vaccine trials with the Indochina I/CDC strain of *P. falciparum*. However, we have had no success in infecting *Saimiri* by sporozoite inoculation.

MODELS FOR TESTING *PLASMODIUM VIVAX* VACCINES

Plasmodium vivax is sometimes neglected in the quest for antimalarial vaccines because of the more lethal characteristics of *P. falciparum* and the emergence of drug resistance in *P. falciparum* which has rightfully alarmed malaria control people. In addition, the successful adaptation of *P. falciparum* to *in vitro* culture by Trager & Jensen (1976) made parasites of this species readily available to many investigators. Unfortunately, adapting *P. vivax* to *in vitro* culture has proven very difficult. Recent reports of chloroquine-resistant strains of *P. vivax* (Reichmann et al., 1989) should stimulate added efforts to develop vaccines against this parasite.

The *P. vivax*-monkey model has been somewhat easier to develop than the *P. falciparum* model. Infections with a high degree of predictability can be readily established in both intact and splenectomized monkeys via sporozoite inoculation. In addition, the availability of strains with distinctly different repeat regions of the circumsporozoite protein offers opportunities to identify those regions of homology that may be of importance in protection. Our initial studies with *Aotus* monkeys indicated a rate of *P. vivax* sporozoite transmission lower than that of *P. falciparum* (Collins, 1990). However, studies with splenectomized *Saimiri sciureus boliviensis* monkeys have resulted in a highly predictable rate of transmission. Following the intravenous inoculation of 10,000 sporozoites of the Salvador I strain of *P. vivax* into splenectoized *S. sciureus boliviensis*, 33 of 34 monkeys developed infections after prepatent periods of 14 to 38 days (mean of 19.9 days) (Fig.5). An ex-

amination of the maximum parasite counts in these 33 monkeys (Fig. 6) indicated that 26 of the monkeys supported the development of high density asexual parasitemia (³ 10,000/mm³), suggesting that blood-stage vaccines could be readily tested in this host. In our trial design, all monkeys immunized with antisporozoite or anti-blood-stage vaccines or combinations of both would be challenged with *P. vivax* via sporozoite inoculation. Transmission-blocking vaccines could also be tested for their effect on mosquito infection in the same system since *P. vivax* infections in splenectomized *Saimiri* are infective to mosquitoes.

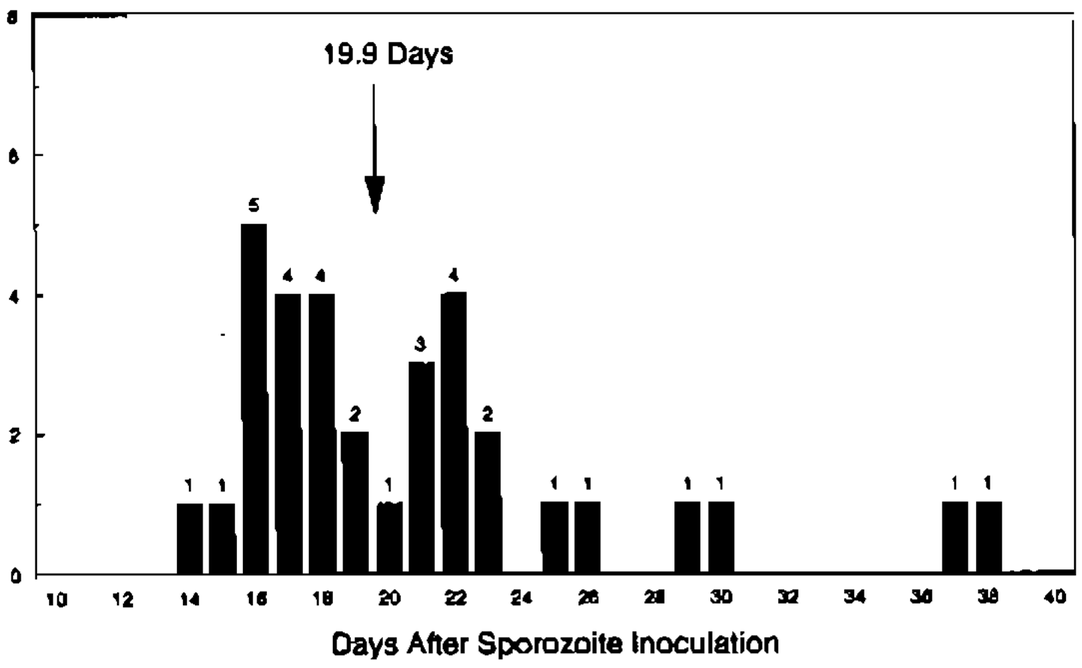

Fig. 5: prepatent periods in 33 splenectomized *Saimiri sciureus boliviensis* monkeys inoculated with 10,000 sporozoites of the Salvador I strain of *Plasmodium vivax*.

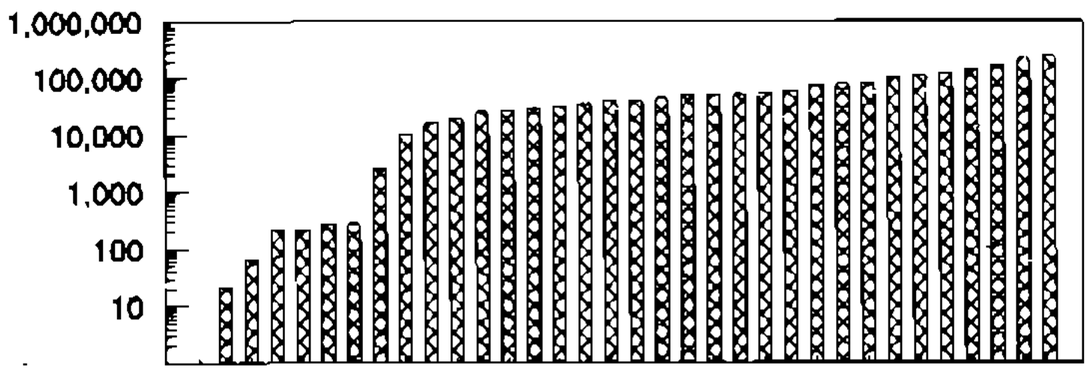

Fig. 6: maximum parasite counts in 33 splenectomized *Saimiri sciureus boliviensis* monkeys infected with the Salvador I strain of *Plasmodium vivax*.

Saimiri monkeys from Peru are also highly susceptible to infection with sporozoites. However, because feral animals are often infected with *Plasmodium brasilianum*, extensive screening is necessary to select animals suitable for inclusion in vaccine trials.

South American *Aotus* and *Saimiri* monkeys, which are susceptible to infection with human malarias, have been used to develop models for the testing of human malaria vaccines. Studies to date indicate that blood-stage and sporozoite vaccines can be tested using appropriate strains of parasites. The major problems appear to be associated with the availability of the most useful species of monkey. Therefore, extensive trials may have to be conducted in Colombia, Bolivia and Peru where the monkeys are readily available. These animal models may be vital for the selection of appropriate antigens, carriers, and adjuvants in the development of human vaccines and for basic immunologic studies of the mechanisms involved in protection. The importance of these studies is such that efforts to establish centers for the testing of vaccines where animals are available should be strongly considered.

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