Plasmodium fieldi: Observations on the Hackeri and ABI Strains in Macaca mulatta Monkeys and Mosquitoes

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Abstract. Macaca mulatta monkeys infected with the Hackeri strain of Plasmodium fieldi had maximum parasite counts ranging from 1,300 to 301,320/µL. In 43 intact animals infected with the ABI strain, the maximum parasite counts ranged from 672 to 57,189/µL (median = 15,100/µL); in 46 splenectomized monkeys, the maximum parasite count ranged from 660 to 350,000/µL (median = 52,245/µL). Transmission through Anopheles dirus mosquitoes was obtained on 11 occasions with pre-patent periods of 9–14 days. Relapses occurred between two and eight times during a 1-year period. P. fieldi has potential for testing prophylactic and radical curative drugs.

INTRODUCTION

The human malaria parasites Plasmodium falciparum and P. vivax have been adapted to New World monkeys for drug and vaccine development.1-3 However, the number of these New World monkeys that are available for research is limited, and they are unavailable in many areas of the world. Elsewhere, macaques such as Macaca mulatta can be infected with one or more of eight different primate malaria parasites; these have many remarkable similarities to the human malaria parasites. Examination of these primate malaria parasites from macaques show characteristics that make them suitable as models for the testing of drugs and the development of vaccines that can mimic the responses that may occur in humans. During the almost 40 years since the last major publication on primate malarials,4 we have extended studies to further describe and characterize different isolates and strains of these simian parasites in Old World monkeys.

The preservation of viable malaria parasites, essentially indefinitely, by freezing and storage at ultra-low temperatures, has allowed for the collection and characterization of the many different species and isolates that infect non-human primates. Most of these have been shown to have similarities to the human infecting species of Plasmodium, and thus, could serve as models in the development of chemotherapeutic agents and vaccines and as models for basic biologic studies. A majority of the isolates were collected and described during a period of intense activity in the 1960s, when there was interest in determining whether humans could be infected with these organisms.5 Subsequent studies have been conducted with various monkey and parasite combinations to determine their potential usefulness.

Previously, we described the mosquito infection and transmission of the monkey malaria parasite Plasmodium coatneyi to M. mulatta monkeys and its transmission by Anopheles dirus mosquitoes.6 The course of parasitemia, periods when gametocytes were produced, and the laboratory-reared mosquitoes that were susceptible to infection were presented. The patterns of recrudescence and pre-patent periods after the bites of infected mosquitoes were also presented. It was proposed that P. coatneyi in M. mulatta monkeys could be a suitable model for the study of cerebral malaria, for vaccine efficacy, and for the testing of antimalarial drugs and serve as an example of the criteria needed for the selection of useful model systems.6

Presented here are some observations on the relapsing malaria parasite P. fieldi and speculation on the usefulness of this parasite in drug and vaccine development. P. fieldi was first described by Eyles and others7 after being isolated from a naturally infected Macaca nemestrina monkey in Malaysia in 1962. This line was identified as the N-3 strain of the parasite. Subsequently, the parasite was isolated on three other occasions, twice from Anopheles hackeri mosquitoes (one of which has been maintained as the Hackeri strain),8 and once from Anopheles introlatus mosquitoes (the ABI strain).8 Warren and others9 described the morphologic characteristics of the erythrocytic stages of the parasite in different species of monkeys, and Held and others10 and Coombs and others11 described the developmental stages in the liver. Anopheles maculatus, An. stephensi, and An. balabacensis (=An. dirus) were shown to be suitable laboratory vectors of P. fieldi,12 and studies indicated that it was one of the simian parasites that regularly relapses from residual liver stages.13 In 1971, the information known about this parasite was summarized by Coatney and others.4 P. fieldi was used in a study to determine the effect of splenectomy on parasitemia and mosquito infection in M. mulatta monkeys infected with the ABI strain,14 and Millet and others15 were able to culture the exoerythrocytic stages of the parasites in hepatocytes of M. mulatta in vitro. Attempts were made to infect New World monkeys with P. fieldi. Although exo-erythrocytic parasite forms were shown in liver hepatocytes, no erythrocytic stage development was seen.16 A study was also made on the susceptibility of splenectomized Macaca fascicularis monkeys from Mauritius to infection with P. fieldi.17 Frequently, P. fieldi was used as an antigen for serologic surveys because of the fact that antibodies to the human parasites P. ovale, P. malariae, and P. falciparum cross-react to erythrocytic stages of P. fieldi in the indirect fluorescent antibody (IFA) test.18-27

Previously, a summary was made of studies conducted with the N-3 strain of the P. fieldi.28 This report is a summary of studies conducted in M. mulatta monkeys with the Hackeri and ABI strains of P. fieldi. All of these strains were originally isolated from Malaysia in the 1960s; tests have been conducted.
during the ensuing years to determine their mosquito infection, transmission potential, and possible application to various biologic, chemotherapeutic, and immunologic studies.

MATERIALS AND METHODS

All monkeys were obtained commercially and were found to be free of natural malaria infection. Initially, studies were conducted at the Institute of Medical Research in Kuala Lumpur, Malaysia. Subsequent studies were conducted at the US Public Health Service laboratories in Chamblee, GA. Protocols were reviewed and approved by the Centers for Disease Control and Prevention Institutional Animal Care and Use Committee, in accordance with procedures described in the US Public Health Service Policy 1986. Animals were housed in an AAALAC (American Association for Accreditation of Laboratory Animal Care International)-approved facility. Space recommendations for laboratory animals were followed as set forth in the NIH Guide for the Care and Use of Laboratory Animals. Splenectomy, when indicated, was performed by a veterinarian using standard procedures. Animals were fed a diet of fruits, vegetables, and animal chow considered suitable and adequate for their maintenance in captivity. Daily observations of the animals’ behavior, appetite, stool, and condition were recorded. All were treated as medical conditions arose by an attending veterinarian. All observations, parasite counts, and the results of laboratory tests were recorded on a daily basis and entered into a computerized database.

Monkeys were infected by intravenous injection of parasitized erythrocytes, either freshly collected from a donor animal or with parasites that had been stored frozen. *An. freeborni* (F-1 strain originally from California), *An. dirus* (from Thailand), and *An. maculatus* (from Malaysia) were laboratory reared and maintained at the CDC/DPD insectaries. For infection, mosquitoes were allowed to feed on tranquilized monkeys as previously described. After feeding, mosquitoes were held in an incubator at 25°C until examined 1 week later for the presence of oocysts on their midguts. For transmission, infected mosquitoes were allowed to feed directly on a tranquilized animal as previously described. On other occasions, monkeys were injected with sporozoites that had been obtained from dissected salivary glands from infected mosquitoes. Beginning 1 day after injection of parasitized erythrocytes or 7 days after sporozoite inoculation, thick and thin blood films were prepared by the method of Earle and Perez, stained with Giemsa stain, and examined microscopically. At the completion of observations, animals were treated with chloroquine (150 mg daily × 2 days) or with quinine (300 mg daily × 7 days) given by oral intubation. If infection was induced by sporozoite inoculation, animals were also treated with primaquine (7.5 mg daily × 7 days).

RESULTS

Hackeri strain. There were two isolations of *P. fieldi* from *An. hackeri* mosquitoes, one was passaged through 11 *M. mulatta*, 1 *M. fascicularis*, and 2 *M. nemestrina* monkeys. This line was lost and is no longer available. The other, now known as the Hackeri strain, has been through 14 passages and has involved the infection of 1 *M. fascicularis*, 1 *M. nemestrina*, and 30 *M. mulatta* monkeys (Figure 1). Three were infected through sporozoites, including the original isolation from

![Figure 1. Genealogy of the Hackeri strain of *P. fieldi* in *M. mulatta* monkeys. Solid line, trophozoite passage; dash line, sporozoite passage.](image)

*An. hackeri* and two subsequent transmissions by *An. dirus* mosquitoes. An examination of the maximum parasite counts for the 28 animals that were not treated indicated that, in 12 intact rhesus monkeys, the counts ranged from 2,772 to 180,000/µL, with a median of 55,915/µL. In 14 splenectomized monkeys, the maximum parasite count ranged from 1,300 to 301,320/µL, with a median of 28,390/µL; many of these animals had previously been infected with heterologous species of *Plasmodium*. The counts never reached life-threatening levels. Pre-patent periods for the three sporozoite-induced infections were 17, 14, and 12 days. One sporozoite-induced infection was treated with a schizonticidal drug only and relapsed twice after being infected with sporozoites of the Hackeri strain.
ABI strain. *Macaca mulatta* were infected with the ABI strain on 102 occasions, 9 of which were through sporozoites; 2 *M. fascicularis* were infected (Figure 2). The parasite was subjected to 29 linear passages through intact and splenectomized rhesus monkeys. In 43 intact animals, the maximum parasite counts ranged from 672 to 57,189/µL, with a median maximum count of 15,100/µL. In 46 splenectomized monkeys, the maximum parasite counts ranged from 660 to 350,000/µL, with a median count of 52,245/µL. In none of the animals did parasite counts reach life-threatening levels that required drug treatment intervention. Most of the sporozoite-induced infections were treated to determine relapse patterns. Again, many of the animals had been previously infected with heterologous species of *Plasmodium*, which may be the reason for the low level maximum counts in some of the monkeys; some of these previous infections were with heterologous strains of *P. fieldi*. Infections were cured by treatment with 300 mg of chloroquine given over 3 days. Some infections were also cured with daily treatments of 300 mg of quinine for 7 consecutive days.

Infectivity studies were made with *An. freeborni* mosquitoes because this mosquito is highly receptive to infection, feeds well on monkeys, and is relatively easy to culture. However, as with some other species of Old World primate malaria parasites such as *P. coatneyi*, *P. simiovale*, and *P. fragile*, *An. freeborni* will support oocyst development but not the concentration of sporozoites in the salivary glands. Transmission of these species with this mosquito can be accomplished by the injection of sporozoites harvested from crushed mature oocysts. It is used only as an indicator of gametocyte infectivity because oocyst development is normal. Thirteen intact monkeys were infected, and *An. freeborni* mosquitoes were allowed to feed on them daily for the first 30 days of patent parasitemia to determine periods of and intensity of infection. The results (Figure 3) indicated a peak in the asexual parasite count between the 6th and 12th days of patent parasitemia. However, the oocyst numbers per infected gut for the mosquitoes that fed on the 13 animals were maintained and even tended to increase during the latter half of the 30-day feeding period. An examination of the data indicated that, throughout the 30-day feeding period, 58.9% of the lots fed were infected and 2,339 of the 10,721 individual mosquitoes (21.8%) examined had at least one oocyst on the midgut. There was a total of 23 days on which a fed mosquito group had > 10 oocysts per positive gut. This occurred with eight different animals; six of

Figure 2. Genealogy of the ABI strain of *P. fieldi* in *M. mulatta* monkeys. Solid line, trophozoite passage; dash line, sporozoite passage.
DISCUSSION

Both the Hackeri and the ABI strains of *P. falciparum* that were studied were derived from mosquito isolations. Although this parasite was isolated > 40 years ago, it has received little attention except for biologic and serologic studies. The parasite as characterized in rhesus monkeys 1) produces relatively moderate-density parasite counts that are not life threatening, 2) readily produces infective gametocytes, 3) produces multiple relapses after sporozoite inoculation, 4) fails to complete sporogonic development in *An. freeborni*, but readily develops in *An. dirus* and *An. maculatus* mosquitoes, and 5) cross-reacts serologically in an IFA test with other species of *Plasmodium*.

Of the eight malaria parasites that naturally infect Old World macaques, *P. knowlesi* is the one that has been used most extensively for drug and vaccine development, extending back to the 1930 and 1940s with the efforts of Coggshall, Freund, and others to develop effective vaccines against malaria.33-37 This is primarily because of its high level of virulence, which results in the death of > 95% of rhesus monkeys after infection. Because of this, many workers considered this parasite to be a suitable model, because the endpoint in vaccine or drug trials (death) was so clear cut. However, the 24-hour asexual development cycle is unique and is unrelated to any of the human-infecting parasites. It does not sequester like *P. falciparum* and it does not relapse like *P. vivax*. Because of this, *P. knowlesi* is probably a poor selection as a model for vaccine or drug developmental studies that could be related to human malaria.

Another parasite, *P. cynomolgi*, has also received considerable attention. This parasite in *M. mulatta* has the characteristics of an excellent model in that it mimics the human malaria parasite *P. vivax*. This parasite basically mirrors *P. vivax* both in its tertian developmental cycle and in the presence of a frequent relapse pattern. It has been used in different drug studies and in vaccine trials as well as in basic biologic investigations.33-37 *Plasmodium cynomolgi* can be transmitted experimentally by many different species of mosquitoes, from both the *Anoph eles* and *Cellia* subgenera. In contrast to *P. knowlesi*, this is an example of an excellent choice of a model for studies that relate to a human malaria parasite, such as *P. vivax*.

*Plasmodium coatneyi* and *P. fragile* are also tertian parasites with similarities to the human parasite *P. falciparum*. Neither of these parasites has been shown to relapse from residual liver-stage parasites. They can be highly virulent to rhesus monkeys, and the parasites sequester from the peripheral blood, similar to *P. falciparum* in humans. Both parasites have been used in vaccine and immunologic studies to a limited extent.36-43 *P. cynomolgi*, *P. coatneyi*, and *P. fragile* mimic the two major human malaria parasites (*P. vivax* and *P. falciparum*) and are readily transmitted by laboratory vectors. These three parasites are thus the leading candidates as models for drug and vaccine studies when using Old World primates such as rhesus monkeys as the hosts.

*Plasmodium inui* is a complex of quartan (72-hour asexual developmental time) parasites of Old World monkeys that has been studied biologically as a model for the human parasite *P. malariae*.44 This parasite has not received much interest for vaccine or drug development, nor does it offer any advantages over the previous three parasites in that respect. *P. gonderi*, *P. simiovale*, and *P. fieldi* have received only biologic study since their original isolation, and neither has been selected for use as models for vaccine or drug development, although they have some potential for such use.

The Hackeri, ABI, and N-3 strains of *P. falciparum* have been maintained for decades, yet still have not been selected for use in the development of drugs or vaccines. As indicated, they produce moderate-density parasite counts in rhesus monkeys but never to a level to be life threatening. They are transmitted experimentally through the bites of *An. dirus*, *An. maculatus*, and *An. freeborni* mosquitoes fed on 13 *M. mulatta* monkeys daily infected with the ABI strain of *P. fieldi*.

**Figure 3.** Mean numbers of oocysts per positive gut in *An. freeborni* mosquitoes fed on 13 *M. mulatta* monkeys daily infected with the ABI strain of *P. fieldi*.
and An. stephensi mosquitoes to rhesus monkeys, and infections will relapse in these animals. Pre-patent periods after sporozoite injection are predictable and range from 9 to 14 days. P. fieldi, like the human parasites, P. vivax and P. ovale, and the monkey malaria parasites, P. cynomolgi and P. simiovale, relapse from resting exoerythrocytic stages (hypnozoites) in the liver. This study indicated that, during a 1-year observation period, the number of relapses varied from two to eight. A previous report indicated as many as 14 have occurred in a year. Thus, this parasite could find use in the testing of causal prophylactic drugs as well as radical curative drugs directed against the liver-stage parasites. In studies to determine the gametocytoidal activity of a drug, treatment of an animal infected with P. fieldi followed by the feeding of An. freeborni or An. dirus mosquitoes and subsequent midgut examination for the presence or absence of oocysts could make this combination useful in drug development; however, P. cynomolgi could be used for the same studies.

At this time, P. cynomolgi and P. simiovale, along with P. fieldi in rhesus monkeys, are the non-human primate models that could be used to test these types of drugs. This study indicates that An. freeborni mosquitoes are readily infected by feeding on intact and splenectomized M. mulatta monkeys over extended periods of time after infection with the ABI strain of P. fieldi. The same would be expected by feeding An. dirus or An. maculatus, both of which are capable of supporting salivary gland infection with P. fieldi. It has already been shown that P. fieldi cross-reacts with P. ovale, P. malariae, and P. falciparum in the indirect fluorescent antibody test and has been used in serologic surveys when the homologous antigen is unavailable. It has never been shown specifically what is the shared antigen in P. fieldi that cross-reacts with these human parasites in the IFA test. Possibly, research in this area would be fruitful. At this time, no homologous antigen is unavailable. 18–27 It has never been shown that this parasite could be used for the testing of radical curative and causal prophylactic drugs in Old World monkeys, whereas P. cynomolgi infects both and continues to be the parasite of choice for most studies.

It is not anticipated that P. fieldi will have a significant role in the development of vaccines or drugs. The most suitable Old World primate and parasite models are P. cynomolgi, P. coatneyi, and P. fragile for these studies. Nonetheless, for the molecular, evolutionary, and basic biologist, P. fieldi is sufficiently different from the other species to warrant study.

Received January 12, 2009. Accepted for publication January 16, 2009.

Acknowledgments: The authors thank the staff of the Animal Resources Branch, National Center for Preparedness, Detection and Control of Infectious Diseases for the care of the animals. Financial support: This study was supported in part by an Interagency Agreement 536-3100-AA6-P-00-0006-07 between the US Agency for International Development and the Centers for Disease Control and Prevention. Aliquots of the Hackeri and the ABI strains of P. fieldi have been deposited with the American Type Culture Collection. Disclaimer: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention.

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