Experimental Infections of *Plasmodium falciparum* in *Cebus capucinus* (White Faced Capuchin) Monkeys

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Introduction

In 1934 Taliaferro and Taliaferro[10] recorded the first transfer of *Plasmodium falciparum* infection to a non-human primate, howler monkey (*Alouatta villosa*) of Panama. Later the chimpanzee (*Pan satyrus*) of Africa and the white-handed gibbon (*Hylobates lar*) of Southeast Asia also were found to be susceptible.[5,9] Only recently, however, have studies continued on the feasibility of using species of monkeys as experimental recipients of this malaria. Cadigan, et al,[2] inoculated Old World macaques, and observed the parasite's development in the Rhesus (*Macaca mulatta*), Pigtail (*M. nemestrina*), and Cynomolgus (*M. iris*). *P. falciparum* infections since have been obtained in the Panamanian marmoset (*Saguinus geoffroyi*)[8] and South American night monkey (*Aotus trivirgatus*).[4] The significance of *P. falciparum* adaptation to *Aotus* monkeys was emphasized by a subsequent report of mosquito infection from these hosts.[3] It has not been possible to infect mosquitoes with falciparum malaria induced in other simian species.

Renewed interest and the latter successes have led us to further explore the possibility of growing *P. falciparum* in other New World primates. This paper reports the susceptibility of *Cebus capucinus*, the Panamanian white faced Capuchin monkey.

Materials and Methods

Sources of primates, their maintenance, and methods of handling have been described elsewhere.[7] Infant, juvenile and adult *Cebus* monkeys were inoculated. Age determination techniques were followed as outlined by Porter, Johnson and de Sousa.[6] Infants generally weighed less than 750 grams. Juveniles were separated from adults by their lack of sexual maturity. Recipients were intact and untreated or had been splenectomized and were administered orally 5 mg/kg of an immunosuppressant, azathioprine (Imuran®), at the time of passage. Additional dosages of the drug were given as indicated.

Two blood samples for inoculation were drawn from patients naturally infected with *P. falciparum* in the Republic of Panama. Another strain, Uganda Palo Alto, was received through the kindness of Dr. Leon H. Schmidt from the National Center for Primate Biology in Davis, California. This strain of Uganda origin was shown by Geiman and Meagher[4] to infect *Aotus trivirgatus* monkeys and had been furnished to Dr. Schmidt who was maintaining it in the *Aotus*.

Parasitized blood was heparanized and inoculated into monkeys intraperitoneally. Numbers of parasites introduced into the test animals ranged from 10×10^6 to 11×10^8. Blood from recipients was examined for parasites daily from the date of passage through 30 days after the course of parasitemia. Thick blood films were taken twice-weekly for 60 days following a 30-day negative period. Prior to inoculation and after the above intervals smears were taken once weekly. Parasite concentrations were determined quantitatively by the Earle-Perez method.

None of the test animals had blood smears positive for *P. brasilianum*, the only naturally occurring malaria in Panamanian monkeys, before or after the inoculations with *P. falciparum*.

Results

Upon receipt of the Uganda Palo Alto strain of *P. falciparum*, it was inoculated into

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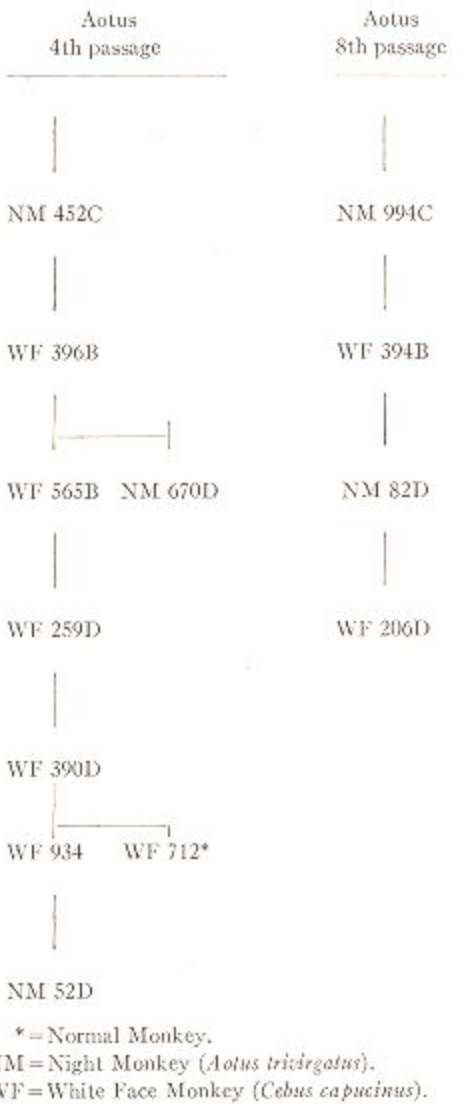

Fig. 1. Serial passages of *Plasmodium falciparum* through *Aotus trivirgatus* and *Cebus capucinus* monkeys.

Aotus monkeys with resultant infections, thus confirming the findings of Geiman and Meagher.[5]

This strain was passed serially through unmodified *Aotus* monkeys. At the fifth serial passage infected blood from an *Aotus* (NM 452C) was inoculated into a *Cebus* monkey (WF 396B) and an infection resulted which persisted for 3 days (Table 2 and Figure 1). Following this there was a subpatent period of 14 days and then a return of parasitemia which persisted for 9 days at which time the monkey died. The infection was then passed serially 5 times to other *Cebus* monkeys. The last recipient, viz., WF 712D differed from the other infected *Cebus* monkeys in that it was not splenectomized or given an immunosuppressant drug.

At the ninth serial passage in the *Aotus* line, infected blood from an *Aotus* (NM 994C) was injected into a *Cebus*, WF 394B, and an infection resulted. While this parasitemia was still low, on the second day of patency, the monkey died from injuries. Eight cc of blood drawn immediately after death and containing less than 80,000 parasites, a relatively low inoculum, produced an infection in a recipient *Aotus* (NM 82D). A transfer of infected blood from this *Aotus* produced an infection in a *Cebus* (WF 206D).

A summary of the transmission attempts is shown in Table 1. The 2 attempts to infect *Cebus* monkeys from Panamanian patients with *P. falciparum* malaria failed. However, using the Uganda Palo Alto strain, established in *Aotus*, the infection was passed to *Cebus*, between the *Cebus* monkeys, and from them back to *Aotus*. When the donor was a monkey, all 11 transmission attempts were successful. Seven of the *Cebus* were splenectomized and given azathioprine but one was unmodified.

Some of the details of the infections are shown in Table 2. The prepatent periods ranged up to 30 days and the patent periods up to 72 days. In 3 monkeys the parasitemias reached high levels. After a peak parasitemia of 131,410 per cmm in WF 390D, the parasitemia spontaneously began to decline and disappeared 15 days later. In WF 206D the peak parasitemia of 126,110 per cmm occurred in the relapse. The parasitemia then declined and disappeared 9 days later.

The highest parasitemia, i.e., 662,700 per cmm, was in WF 259D. The monkey was then given 11 mg quinine in 2 doses. The parasites disappeared in a few days but reappeared after 2 negative days. The monkey died during the second day of relapse with parasites less than 10 per cmm.

In all cases, except WF 259D, the infections were self-limiting, thus not requiring intervention with drugs.

Two monkeys were administered azathi-

TABLE 1

TRANSFER OF *Plasmodium falciparum* TO AND FROM *Cebus capucinus* (WHITE FACE MONKEY) BY INTRAPERITONEAL INOCULATIONS OF INFECTED BLOOD

Source	Recipient	Surgery or Treatment	Inoc. 10^6 Range	Attempts	Successes
Man	*Cebus capucinus*	Sp. and Im.	34–39	2	0
Aotus trivirgatus	*Cebus capucinus*	Sp. and Im.	792–1,100	3	3
Cebus capucinus	*Cebus capucinus*	Sp. and Im.	10–66	4	4
		None	10	1	1
Cebus capucinus	*Aotus trivirgatus*	Sp. and Im.	10	1	1
		None	1–10	2	2

Sp. = Splenectomized.
Im. = Imuran (BD) (azathioprine): Burroughs, Wellcome and Co., Inc.

oprine one or more times subsequent to the initial dose to determine if the parasitemia would be affected. Recipient WF 565B was given 5 mg/kg on the 28th day of the prepatent period, and within two days parasites appeared. During an ensuing 72-day patent period, the drug again was administered on the 25th and 27th day (5 and 10 mg/kg), but the course of a descending parasitemia was not altered. WF 396B received 5 mg/kg on the

TABLE 2

Plasmodium falciparum INFECTIONS IN *Cebus capucinus* (WHITE FACE MONKEY) INDUCED BY INTRAPERITONEAL INOCULATIONS OF PARASITIZED BLOOD[1]

Donor		Recipient						
Monkey	Monkey	Weight Grams	Inoculum 10^6	Days Examined After Inoculation‡	Periods		Asexual Parasitemia Max.	
					Pre-patent	Patent	mm³	Pat. Day
NM452C	WF396B	550	908	39	15	3	10	—
						9*	3,250	9
WF396B	WF565B	2,450	26	158	30	72	17,610	17
WF565B	WF259D	600	66	26	11	11§	662,700†	7
WF259D	WF390D	900	10	96	19	23	31,290	10
WF390D	WF934	2,000	10	66	14	25	131,410	11
WF390	WF712	3,700	10	65	5	16	10,120	10
NM994C	WF394B	3,100	792	11	10	+	10	—
NM82D	WF206D	1,450	1,100	128	26	34	71,660	15
						18*	126,110	10

[1] All recipients except WF712 were splenectomized and administered immunosuppressant.
† Died from injuries while parasitemia patent.
* Recrudescence of infection.
NM = *Aotus trivirgatus*.
WF = *Cebus capucinus*.
† Received quinine.
‡ As of Feb. 28, 1969.
§ Died while parasitemia patent.

third day of subpatency following a three-day patent period. The parasites did reappear, although 11 days later. As shown in Table 2, the parasitemia in the relapsing monkeys reached higher parasite concentrations than were recorded initially in the primary attack.

The inconsistent response to the azathioprine does not permit any conclusion as to its effect upon the parasitemias.

All stages of the asexual parasites circulated in the peripheral blood. Segmenters usually became apparent during the first week of patency, but were seen as early as the second day. Gametocytes were produced in three monkeys (WF 206D, WF 390D, WF 712), however most of these forms appeared to be immature. In WF 206D and WF 712 sexual stages were noted continuously during primary patent periods from the ninth and 11th patent days. Respective counts ranged from 10 to 750 and 10 to 50 per mm.[3] In contrast, gametocytes appeared in WF 390D on the 22nd day of patency, and were seen intermittently through nine days following termination of the asexual patent period in concentrations not exceeding 20 per mm.[3] Data regarding unsuccessful attempts to infect mosquitoes from these hosts are reported by Baerg and Young.[1]

Discussion

After it was found that a human malaria, i.e., *P. vivax*, would grow well in New World monkeys,[11] we have been testing the susceptibility of other monkeys to different species of malaria.

Our results with *P. falciparum* of Panama obtained from naturally infected Panamanians have given only partial success. Infections were obtained in *Saguinus geoffroyi* which persisted as long as 15 days.[8] Subinoculations to other *Saguinus* failed. Similar attempts to infect *Aotus trivirgatus* and *Cebus geoffroyi* from human sources of *P. falciparum* have failed.

However, using falciparum blood from a human who had acquired the infection in Uganda, Geiman and Meagher[4] established this malaria species in *Aotus*. After obtaining an inoculum, we have maintained this strain since in *Aotus* monkeys.

When we used the Uganda strain growing in the *Aotus*, infections were readily obtained in the *Cebus*, successfully subinoculated into other *Cebus*, and passed between them and the *Aotus* monkeys.

The poor success with the Panama strains of *P. falciparum* contrasts sharply with the almost uniform success with the foreign (Uganda) strain. It is not known whether the ability of the African strain to grow in the *Cebus* is due to virulence, its prior adaptation to *Aotus*, or to some other factors.

The recipients were not of uniform weight and age as their use was dependent on availability at the laboratory. Even so, the magnitude and duration of the parasitemias in monkeys of comparable size were variable and were independent of the concentrations of parasites inoculated. In most of the *Cebus*, the prepatent periods were relatively long. The maximum parasitemias occurred usually in the second week of the primary or relapse patent period or within 3 days thereafter. The maximum parasitemias were in the ranges seen in human patients.

Gametocytes appeared in 3 of the infected animals.

No consistency in host response was evident through serial transfer. Rectal temperatures could not be correlated with the presence of or the height of parasitemias as readings fluctuated within the same limits before, during, and after parasitemias.

Three animals died. One was from a fractured skull caused by a misjudged jump. The second was the only death associated with the significant parasitemia (2230 per cmm). As others have survived parasitemias 50 times greater, this fatality may not have been due to malaria. The third (WF 259D) had experienced the highest parasitemia seen in these monkeys, and although it appeared to respond to treatment, its death a few days later could have been due to the malaria.

In contrast, most of the *Aotus* monkeys died during the parasitemia period unless treated.

It seems apparent that the *Cebus* monkey tolerates well the *P. falciparum* infections. This together with the character of the induced infections and the hardiness of this monkey in captivity indicates that it should be very useful for the study of certain *P. falciparum* strains of human malaria.

Summary

Cebus capucinus (white faced Capuchin) monkeys were shown to be susceptible to *Plasmodium falciparum* malaria of human origin. The malaria strain originated in Uganda and had been adapted to *Aotus trivirgatus* monkeys. The *Cebus* monkeys were from Panama.

The malaria was passed serially through *Cebus* and *Aotus* monkeys by intraperitoneal injections of parasitized blood. The eight *Cebus* tried became infected. Significant parasitemias were produced, one of which persisted for 72 days. All stages of the asexual parasite appeared in the peripheral blood. Gametocytes were present in some of the infections.

The infections were tolerated well by the *Cebus*.

Two attempts to infect *Cebus* monkeys with *P. falciparum* blood from Panamanian patients failed.

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