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ADAPTATION OF THE PANAMA II STRAIN OF *PLASMODIUM FALCIPARUM* TO PANAMANIAN OWL MONKEYS

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Abstract. The Panama II strain of *Plasmodium falciparum*, acquired at the second passage level in splenectomized Colombian owl monkeys, was adapted to owl monkeys of Panamanian origin. Patent infections were induced in 22 of 27 unaltered and 20 of 21 splenectomized recipients during 19 serial passages. The infections were significantly more virulent in splenectomized than normal Panamanian owl monkeys, however recrudescences in seven normal monkeys achieved peak parasitemias 48 times greater than in the primary attack. These results describe the first reproducible infections of indigenous falciparum malaria in Panamanian owl monkeys.

Adaptation trials at Gorgas Memorial Laboratory using 53 human isolates of Panamanian *Plasmodium falciparum* and 70 Panamanian owl monkeys yielded only transitory parasitemias in nine recipients, and subinoculation from seven was unsuccessful in continuing the infection.[1,2] Panamanian owl monkeys were shown to be capable of sustaining infection of *P. falciparum* obtained directly from a patient who became infected in Nigeria.[3] In addition, Southeast Asian and African *P. falciparum* strains, first well-adapted by passage in owl monkeys of Colombian origin, have been maintained in Panamanian owl monkeys and in some cases produce infections even more virulent than in the Colombian host.[4] We now report that the Panama II strain of *P. falciparum* can be adapted to owl monkeys from Panama after passage in three splenectomized Colombian owl monkeys.[5]

MATERIALS AND METHODS

At the time of this study, the Panamanian owl monkey was classified as *Aotus trivirgatus griseimembra*. As the taxonomy of the genus *Aotus* is being revised,[6] monkeys herein are referred to as either Panamanian or Colombian owl monkeys.

The Panama II strain of *P. falciparum* was obtained as a chilled specimen in July 1972 from W. E. Collins, Centers for Disease Control, Atlanta. The inoculum was from the second serial transfer in splenectomized Colombian owl monkeys, from a subject with a parasitemia of 320 × 10^3/mm^3.

Forty-eight Panamanian and two Colombian owl monkeys from the Gorgas Memorial Laboratory colony were used. Recipients were unaltered, splenectomized, or treated orally with a single 10.0 mg/kg dose of azathioprine (Imuran®), and parasites inoculated either intraperitoneally or intravenously; numbers of parasites in each inoculum were not standardized. Blood films from monkeys not developing patent infections were examined for 22 to 57 days after inoculation. Other procedures for monkey husbandry, blood film preparation, and interpretation have been reported previously.[7]

RESULTS

Data associated with 19 passages of the Panama II strain are presented in Table 1. As shown, the blood sample from AO-261 was inoculated into a splenectomized Colombian owl monkey and two Panamanian owl monkeys, one splenectomized and one administered azathioprine. These three recipients constituted the first passage at Gorgas Memorial Laboratory. At the second passage level, infections developed in one Colombian and two Panamanian owl monkeys inoculated from Colombian monkey 6858. Levels 3 through 19 consisted entirely of Panamanian donor and recipient monkeys. Overall, parasitemias were established in 22 of 27 (81.5%) intact and 20 of 21 (95.2%) splenectomized Panamanian owl monkeys.

Intravenous inoculation of 5 × 10^6 to 226 ×

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TABLE 1

Infection characteristics of Panamanian II strain Plasmodium falciparum *in Panamanian owl monkeys*

Passage	Monkey*	Parasites × 10³ route	Primary attack		First recrudescence			Second recrudescence			Parasites at death no./mm³‡
			Day of onset†	Peak parasitemia (no./mm³)				Days duration			
	AO-261 D										
1	6858 SC	150 ip	5	1.9 × 10⁶	130	21	611 × 10³	27			
	6901 AZ	75 ip	1	120	6	46	55 × 10³	41			
	5382 S	75 ip	—	—	—						
	6858 SCD										
2	6857 SC	5 ip	6	27 × 10³	109	44	180	15	32	110	29
	6930 S	150 ip	1	70	15	6	<10	6	15	891 × 10³	18‡
	7068 S	22 iv	1	11.7 × 10³	35						
	6902	52 ip	—	—	—						
	6908	52 ip	—	—	—						
	6901 AZD										
2	6925 AZ	0.06 ip	—	—	—						
	7057	10 ip	—	—	—						
	6930 SD										
3	6929 S	28 ip	27	<10	3‡						<10
	7075	107 iv	1	490	16						
	7041 S	226 iv	1	1.3 × 10⁶	16‡						292 × 10³
	7068 SD										
3	7071 S	10 iv	2	273 × 10³	29‡						27 × 10³
	7071 SD										
4	7177	44 iv	1	70	8	41	58 × 10³	20‡			16 × 10³
	7180 S	131 iv	1	218 × 10³	25‡						31 × 10³
	7041 SD										
4	7040 S	163 ip	3	391 × 10³	16‡						391 × 10³
	7088	163 ip	5	<10	3	29	26 × 10³	58	19	113 × 10³	21‡
	7084	195 iv	1	3 × 10³	19	22	1.2 × 10⁷	23			40
	7180 SD										
5	7195	90 iv	1	1 × 10³	13	13	1.2 × 10⁷	10‡			1.2 × 10³
	7179 S	90 iv	1	1.1 × 10⁶	20‡						165 × 10³
	7088 D										
5	7237	55 ip	4	7.7 × 10³	31‡						260
	7040 SD										
5	7089	88 ip	—	—	—						

TABLE 1
Continued

Passage	Monkey*	Parasites ×10⁶ route	Primary attack		First recrudescence				Second recrudescence	Parasites at death no./mm³‡
			Day of onset†		Peak parasitemia (no./mm³)				Days duration	
	7084 D									
5	7092	3.5 ip	4	50	8	21	30 × 10³	28‡		3.5 × 10³
	7092 D									
6	7089	7.4 iv	1	5.8 × 10³	17‡					<10
	7179 SD									
6	7194 S	53 iv	4	1.5 × 10⁶	15‡					1.5 × 10⁶
	7194 SD									
7	7216 S	67 ip	1	1.7 × 10⁶	16‡					84 × 10³
	7216 SD									
8	7217 S	84 ip	4	914 × 10³	16‡					317 × 10³
	7217 SD									
9	7215 S	13 ip	5	1.2 × 10⁶	16‡					1.2 × 10⁶
	7215 SD									
10	7245	819 ip	1	694 × 10³	12‡					412 × 10³
	7268 S	41 ip	1	1.3 × 10⁶	10‡					568 × 10³
	7245 D									
11	7267	291 ip	1	24 × 10³	15	24	1 × 10⁶	19‡		146 × 10³
	7267 D									
12	7254 S	2.7 ip	4	1.1 × 10⁶	11‡					340 × 10³
	7297	5 iv	1	160	21‡					20
	7323	5 iv	1	10	3	30	133 × 10³	16‡		80 × 10³
	7254 SD									
13	7400 S	27 ip	1	1.9 × 10⁶	12‡					1.5 × 10⁶
	7400 SD									
14	7345 S	27 ip	2	2 × 10⁶	12‡					1.5 × 10⁶
	7428	542 ip	1	1.5 × 10⁶	10‡					1.5 × 10⁶
	7428 D									
15	7444	370 ip	1	91 × 10³	15	19	15 × 10³	9‡		15 × 10³
	7444 D									
16	7466	135 ip	1	1.2 × 10⁶	44‡					60
	7466 D									

TABLE 1
Continued

Passage	Monkey*	Parasites × 10⁶ route	Primary attack		First recrudescence	Second recrudescence	Parasites at death no./mm³‡
			Day of onset†	Peak parasitemia (no./mm³)	Days duration		
17	7606 S	(ip)§	14	787 × 10³	9‡		773 × 10³
	7462 S	1 ip	3	451 × 10³	10‡		451 × 10³
	7445	36 ip	6	121 × 10³	18‡		680
	7445 D						
18	7496	93 ip	1	84 × 10³	24‡		48 × 10³
	7606 SD						
18	7618 S	28 ip	6	756 × 10³	9‡		290 × 10³
	7612	787 ip	8	411 × 10³	17‡		411 × 10³
	7681	(ip)	28	1.5 × 10³	18¶		
	7681 S			1.6 × 10⁶	45‡		1.5 × 10⁶
	7496 D						
19	7464 S	1.7 ip	5	1.2 × 10⁶	11‡		1.2 × 10⁶
	7162 D						
19	7475	397 ip	3	96 × 10³	26¶		
	7475 S			512 × 10³	65‡		512 × 10³
	7681 D						
19	7934	0.9 ip	7	417 × 10³	44‡		54 × 10³

* S = splenectomized; C = Colombian; D = donor; AZ = azothioprine-treated.
† Primary attack = days from inoculation to establishment of patency; first and second recrudescences = days from end of patency in preceding attack to onset in reference attack.
‡ Day of death and parasitemia that day.
§ ( ) indicates inoculation of cryopreserved parasites.
¶ Splenectomized on patent day indicated.

10^6 parasites established patent infections in 11 monkeys beginning on the first day after inoculation and in two monkeys on the second and fourth days, respectively. The mean prepatent period in Panamanian monkeys inoculated intravenously was 1.3 (±0.8) days.

Parasites were detected on the day after inoculation in 11 Panamanian monkeys inoculated intraperitoneally with 27×10^6 to 819×10^6 parasites, whereas onset of the primary attack ranged from 2 to 27 days in 16 monkeys challenged with 0.9×10^6 to 787×10^6 parasites. The mean prepatent period in all Panamanian owl monkeys inoculated intraperitoneally was 3.9 (±4.9) days.

The mean peak parasitemia in 19 intact Panamanian monkeys was 240 (±426) × $10^3/mm^3$. Of these monkeys, 10 died on mean patent day 23.8 (±11.5) during the primary attack and the mean peak parasitemia in eight of these animals was 316 (±406) × $10^3/mm^3$; the highest parasitemia in the two other monkeys (7428 and 7612), viz. 1.5×10^6 and 411 × $10^3/mm^3$, respectively, occurred on the day prior to death. Monkeys 7681 and 7475 were splenectomized during the primary attack and neither animal survived the higher parasitemias that ensued.

The mean peak parasitemia in the 10 intact monkeys surviving the primary attack was 12 (±27) × $10^3/mm^3$, and recrudescences occurred in nine of them. A mean peak parasitemia of 186 (±334) × $10^3/mm^3$ could be defined in seven of these survivors during the first recrudescence. In the primary attack the mean peak parasitemia for the same monkeys was 3.9 (±8.3) × $10^3/mm^3$, but the difference between parasitemias was not significant ($P > 0.1$). The infection in monkey 6901 was self-limited after the first recrudescence and monkey 7088 died during the second recrudescence after a peak parasitemia of 113 × $10^3/mm^3$.

Of the 20 splenectomized Panamanian monkeys, parasitemia peaked during the primary attack in 13 at a mean of 967 (±660) × $10^3/mm^3$. In the six that died without a definable peak, the mean parasitemia was 920 (±411) × $10^3/mm^3$. The infection of one monkey (7068) was self-limited in the primary attack; one (6930) died of malaria during second recrudescence; and one (6929) died of a nonmalarial infection during that period. Seventeen monkeys died during primary malaria attack on mean prepatent day 14.9 (±5.4).

There was indication of increased virulence of this strain, as measured by peak parasitemias of the primary attack, during passage in intact Panamanian owl monkeys. For passages 1 through 5, designated group A (8 monkeys), the mean peak parasitemia was 1.6 (±2.5) × $10^3/mm^3$; in passages 6 through 15, designated group B (6 monkeys), the mean peak parasitemia was 136 (±252) × $10^3/mm^3$; for passages 16 through 19, designated group C (4 monkeys), the mean peak parasitemia was 456 (±449) × $10^3/mm^3$. Mean peak parasitemias between groups A and B were not significant ($P > 0.1$), nor were they significant between groups B and C ($P > 0.1$); however, mean peak parasitemias between groups A and C were significant ($P < 0.05$).

DISCUSSION

The Panama II strain was adapted to Colombian and Panamanian owl monkeys through the following process: On 9 March 1972, falciparum-infected blood from a Panamanian patient was inoculated into an intact Colombian owl monkey (AO-356); a 43-day prepatent period followed before splenectomy on day 15 of patency.[5] On 23 May 1972, infected blood from this monkey was transferred into another splenectomized Colombian owl monkey (AO-261), whose blood was later (25 July) passed into a third splenectomized Colombian and two Panamanian owl monkeys (Table 1).

Parasites from the splenectomized Colombian owl monkey (6858) were then inoculated into five monkeys, one of which (6930), a Panamanian splenectomized recipient, was the donor for monkeys comprising passage level three. After three passages in splenectomized Colombian owl monkeys, it was possible to adapt these parasites to both intact and splenectomized owl monkeys of Panamanian origin. Ideally, infected blood from the patient should have been inoculated concurrently into Panamanian owl monkeys, but previous adaptation trials using some 70 Panamanian owl monkeys inoculated with 53 isolates of indigenous *P. falciparum* yielded only transient parasitemias in nine recipients.[1] Based on this experience it appeared unlikely that the Panama II strain from a human would have been infective for indigenous owl monkeys.

Infections were significantly more virulent in splenectomized than in intact Panamanian mon-

keys ($P < 0.001$), as indicated by death rate and peak parasitemias.

Initial studies[8] in Colombian owl monkeys referred to a peak recrudescent parasitemia in one intact animal that was 2.5 times greater than the primary peak parasitemia (280×10^3 vs. 720×10^3 per mm^3). A similar course of infection was noted during the present study in intact Panamanian owl monkeys, as peak parasitemias during the first recrudescence averaged 2,900 times greater (42–13,300) than in the primary attack among six of seven monkeys. Usually, parasitemias of well-adapted *P. falciparum* in owl monkeys are significantly lower during recrudescence.[4,9] Increased virulence during recrudescence may be a reflection of parasite adaptation to a new host or an expression of antigenic diversity, as demonstrated recently in cases of acquired immunity.[10,11]

The alteration of the Panama II strain parasites that appears to have resulted from passage in Colombian owl monkeys, and rendered them infective to a highly probable nonsusceptible Panamanian owl monkey, has not been defined. If modification of parasite receptor sites to achieve erythrocyte penetration is demonstrated, then a reversal of such modification, possibly blocking parasite penetration, could have significance in the development of a merozoite vaccine.

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