

SPOROZOITE-INDUCED *Plasmodium cynomolgi* INFECTIONS  
IN CAPTIVE BORN *Macaca fascicularis*

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OBJECTIVES :

1. To study sporozoite induced *Plasmodium cynomolgi* infections in young malaria naive cynomolgus monkeys.
2. To determine effects of high sporozoite inoculae and splenectomy on the *P. cynomolgi* parasitemias in captive born cynomolgus monkeys.
3. To determine if captive born cynomolgus are capable of substituting for rhesus monkeys in the radical curative antimalarial compound testing model.

**BACKGROUND AND METHODS :** Rhesus monkeys have become quite expensive and since malaria naive captive born cynomolgus were available from the AFRIMS breeding colony, the possibility of their substitution in the radical curative anti-malarial drug testing model was proposed. Methods were identical to the ongoing drug testing project and rhesus were thus available as infection comparison controls. Eighteen *Macaca fascicularis* (cynomolgus) were divided into three groups and given a high or low dose of sporozoites intravenously. One group of four received  $0.8 - 1.6 \times 10^6$  and a second group of eight received  $0.3 - 1.0 \times 10^6$  sporozoites. The third group of six was splenectomized and then received  $3.0 - 4.0 \times 10^6$  sporozoites. Parasitemias were followed for a minimum of 120 days. White and red cell counts were obtained before infection and during the first five weeks post infection.

**RESULTS :** Parasitemias became patent on an average of 7.9 days. Group mean parasitemia data for the low dose, high dose and high dose-splenectomized are shown in Table 1. Data from six rhesus mosquito feeders infected concurrently in the ongoing drug screening program have been included for comparison. Although low and high dose infection of intact cynomolgus monkeys resulted in a persistent parasitemia, the maximum parasitemia was less than in splenectomized cynomolgus or intact rhesus and the percent of positive blood films was lower. Mean parasitemia in splenectomized cynomolgus was much higher than intact cynomolgus throughout the infection. Total red cells (rbc), leukocytes (wbc) and the number of lymphocytes at preinfection, 2 weeks and 3 weeks following sporozoite inoculation are listed in Table 2. At 3 weeks post infection mean rbc had decreased by  $1.12 \times 10^6$  in the intact groups and  $2.66 \times 10^6$  in the splenectomized group. There was a 24% increase in lymphocytes from preinfection to 2 weeks post infection in the splenectomized group. In this group, mean wbc count from post-inoculation day 12 through 22 was 30,464.

Mosquitoes fed readily on cynomolgus monkeys, but gametocyte production was poor in the intact monkeys. Only five of 12 intact monkeys produced gametocytes. Sporozoite production resulted in only 1 out of 6 feedings on intact monkeys. All splenectomized monkeys produced abundant male and female gametocytes, and all lots of mosquitoes that fed on the splenectomized monkeys developed sporozoites or oocysts (Table 3). A harvest of sporozoites from mosquitoes which fed on one splenectomized monkey (AF-18) was used as inoculum for inducing infection in another (AF-9). The resulting infection was indistinguishable from those induced with sporozoites harvested from mosquitoes which fed on rhesus.

Relapse potential was tested in two splenectomized monkeys. A curative dose (10 mg/kg) of the blood schizonticide chloroquine was given to AF-9 following 113 days of continuous parasitemia. Following clearance, a relapse occurred on a post-treatment day 27. AF-13 was given chloroquine and became negative on post-patent day 34. A relapse occurred on the 4th day following the last treatment. Sub-curative doses (0.3 & 0.1 mg/kg) of the tissue schizonticide primaquine along with 10 mg/kg chloroquine were then given to AF-13 and AF-9 respectively. Following clearance of the parasitemia, these monkeys relapsed 28 and 26 days post-treatment. To terminate the infection in all monkeys, chloroquine at 10.0 mg/kg and primaquine at 1.3 mg/kg were administered. All cynomolgus responded to these doses without subsequent relapse.

The intact captive born cynomolgus does not appear to be capable of substituting for the rhesus in the radical curative model even when given a high inoculum of sporozoites. Splenectomized cynomolgus were capable of sustaining a monkey-mosquito-monkey cycle. From these limited numbers, the captive born, splenectomized cynomolgus given  $3 - 4 \times 10^6$  sporozoites seems promising as a potential replacement, however, the disadvantages of a splenectomized monkey must be considered.

Table 1. Mean parasitemia data by group.

| Group<br>(Number)        | Sporozoite<br>Dose    | Day of<br>Patency | Maximum<br>p/cmm | Mean Parasitemia<br>From Day 41 to<br>120 p/cmm |
|--------------------------|-----------------------|-------------------|------------------|---|
| M fas (4)                | $0.8-1.7 \times 10^6$ | 8.3               | 13,257           | 16  |
| M fas (8)                | $3-10 \times 10^6$    | 8.3               | 32,026           | 44  |
| M fas-S <sup>1</sup> (6) | $3-4 \times 10^6$     | 7.7               | 102,159          | 571   |
| M mul (6)                | $0.8-2.0 \times 10^6$ | 7.4               | 543,417          | ND <sup>3</sup>                                 |

M fas = *Macaca fascicularis*; M mul = *Macaca mulatta*.

1 - S = splenectomized prior to infection

2 p/cmm = number of parasites per cubic millimeter of blood

3 ND = not done.

Table 2. Mean rbc, wbc and lymphocyte counts at preinfection, 14 and 21 days post-infection.

| Group (Number)           | Time                   | RBC x 10 <sup>6</sup> | Total WBC | Lymphocytes |
|--------------------------|------------------------|-----------------------|-----------|-------------|
| M fas (4) L <sup>1</sup> | Preinfection           | 7.52                  | 11,550    | 6,953       |
| M fas (8) H <sup>2</sup> | "                      | 7.23                  | 13,963    | 6,414       |
| M fas-S (6) H            | "                      | 7.21                  | 11,850    | 7,607       |
| M mul (6) L              | "                      | ND                    | ND        | ND          |
| M fas (4) L              | 14 Days Post-infection | 6.3                   | 12,150    | 9,455       |
| M fas (8) H              | "                      | 6.5                   | 14,538    | 9,021       |
| M fas-S (6) H            | "                      | 5.32                  | 32,250    | 26,483      |
| M mul (6) L              | "                      | 5.6                   | 11,383    | ND          |
| M fas (4) L              | 21 Days Post-          | 6.23                  | 9,800     | 5,680       |
| M fas (8) H              | "                      | 6.29                  | 14,175    | 10,159      |
| M fas-S (6) H            | "                      | 4.55                  | 28,500    | 22,412      |
| M mul (6) L              | "                      | 4.86                  | 12,217    | ND          |

<sup>1</sup> L = 0.8 - 2 x 10<sup>6</sup> Sporozoite Inoculum

<sup>2</sup> H = 3 - 10 x 10<sup>6</sup> Sporozoite Inoculum

Abbreviations as Table 1.

Table 3. Gametocyte, oocyst and sporozoite production in splenectomized *Macaca fascicularis*.

| Monkey #  | AF-18    | AF-9             | AF-16  | AF-13    | AF-11   | AF-14 |
|---|----------|------------------|--------|----------|---------|-------|
| # Days Mosquitoes Fed                                     | 5        | 2                | 2      | 2        | 2       | 1     |
| Gametocytemia Range <sub>3</sub> (x 10 <sup>3</sup> /cmm) | 0.5-16.8 | 0.7-5.6          | 4.8-71 | 3.2-23.5 | 0.4-2.2 | 3.0   |
| Male to Female Gametocyte Ratio                           | 1:13.1   | 1:4.1            | 1:9.0  | 1:6.7    | 1:6.6   | 1:2.7 |
| Oocyst Positive Mosquitoes/Total                          | 22/25    | 5/8              | 5/5    | 5/5      | 4/5     | 4/5   |
| Mean Oocyst #   | 59       | 1.7*             | 91     | 91       | 95      | 100   |
| Sporozoite Positive Mosquitoes/Total                      | 25/25    | ACF <sup>1</sup> | 10/10  | 8/8      | 9/10    | 5/5   |
| Sporozoite Density  | 4+       | ACF              | 4+     | 4+       | 4+      | 4+    |

1(\*) - Air conditioner failure in insectary.

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