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Effect of stress conditions on the course of trophozoite-induced *Plasmodium yoelii nigeriensis* in mice recently cured of a previous infection

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Summary

The effect of stress conditions on the course of trophozoite-induced *Plasmodium yoelii nigeriensis* in mice recently treated for a previous infection was studied. It was found that when the mice were infected with up to 5943 parasites within 6 days of treating a previous infection, no patent infection was recorded. However, an inoculum of 7924 parasites induced parasitaemia, with a long period of prepatency and a short course of infection before the parasites died out. None of the mice re-infected with the parasite, and maintained at different temperatures, was positive during the period of the experiment.

Résumé

On a entrêpris un étude sûr les éffets de pression sûr la cours de *Plasmodium yoelii nigériensis* provogué par les trophozoîtes chez les mouses, récémmeut traité des infections précédente. On a decourvréze que it ny avait pas les infections mainifesté quand les mouse étaient infecté avec jusqú a 5945 parasites pendant six jours suivant la traitement précédente. Cepandant, un innoculum de 7924 parasites a provoqué la parasitemié avec une période prolonguer de pre-manifestation et une cours court, d'infections, apiès laquelle les parasîtes ont disparaitré. Aucune mouse, re-infecté avec les parasites et préserver sur différent températûres était positif pendant la période de cet experiment.

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Introduction

In an earlier study [1], it was shown that when mice that had been previously infected with *Plasmodium yoelii nigeriensis* and treated with chloroquine were re-challenged 7–14 days after treatment of the previous infection, they developed parasitaemia. However, the level of parasitaemia remained low and the parasites ran a short course before the mice finally overcame the infection.

In malarial countries like Nigeria the people are not only continuously exposed to the disease [2] but also subjected to various stress conditions. The probability that such stress conditions would affect the course of the infection in the host is very high.

The aim of the present investigation, therefore, is to study the effects of inoculum size, and temperature, on the course of a subsequent infection of *P. y. nigeriensis* in mice, following treatment of a previous infection.

Materials and methods

Effects of inoculum size on the course of P. y. nigeriensis *in mice following recovery from a previous infection*

Twenty adult male albino mice were infected with *P. y. nigeriensis*. Six days post-infection, the average parasitaemia per mouse was 225 parasitized red blood cells per 1000 erythrocytes. Chloroquine syrup (0.5 ml containing 10 mg chloroquine base) was administered orally to each mouse. These were designated the experimental mice.

Another batch of 12 uninfected mice was also given 10 mg chloroquine base, simultaneously

with the infected batch (experimental mice). These served as the first control group (chloroquine control) to monitor the effect of residual chloroquine on the subsequent infection with the parasite.

Ten days post-treatment, the blood of the experimental mice was examined and found to be free of the malaria parasites. The mice were grouped into four groups each consisting of five mice. All mice in each of the four groups were individually inoculated with one of the following doses of parasitized red blood cells: 1981, 3962, 5943, and 7924.

The chloroquine control mice (CI_1) were similarly placed into four groups. The mice in each group were infected with the number of parasitized red blood cells, corresponding with the experimental mice.

Another set of four groups of mice (neither previously infected nor treated with chloroquine) was also infected with the same number of parasitized erythrocytes as before. These groups of mice were the normal control group (CI_2).

The method of estimating the number of parasitized red blood cells in a unit volume of blood inoculated into each mouse had been described previously [1]. Stained blood films from each mouse were microscopically examined for malaria parasites. The parasitaemia was determined daily for approximately 2 weeks. The mortality rate among the mice was also recorded.

Effect of temperature on the course of infection of P. y. nigeriensis *in mice following recovery from a previous infection*

Twenty adult male mice were infected with an equal number of parasites. Eight days post-infection they were treated by administration of 10 mg chloroquine base orally. After 10 days, microscopic examination of their blood films revealed no malaria parasites.

Twenty uninfected male mice were simultaneously given 10 mg chloroquine base. This served as the chloroquine control group (CT_1). Twenty-four other uninfected male mice, to which no chloroquine was given, served as the normal control group (CT_2). All the mice in the three groups were infected with 508 parasitized red blood cells. Each of the three groups was divided into four, which were maintained at the following ambient temperatures, 9–12°C, 19–23°C, 27–30°C and 36–38°C. Six other uninfected adult mice were maintained simultaneously at each of the above temperature ranges. These served as the third group of control (CT_3) to monitor the effects of the experimental temperatures on uninfected mice.

Blood samples were taken from all the infected mice daily. Thin blood films from each were examined for parasites and, when positive, the parasitaemia was determined. General observations were also made on the mice and the daily mortality was recorded.

Results

Effects of inoculum size

The results are presented in Table 1. Three of the inocula, namely, 1981, 3962, and 5943 parasitized red blood cells, failed to give a patent infection in any of the experimental mice, throughout the period of the experiment; whereas both the chloroquine control (CI_1) and the normal control (CI_2) became positive 48 h after infection. The highest inoculum of 7924 parasitized red blood cells, however, induced parasitaemia in the experimental mice 96 h post-infection, when a parasite count of 20 parasitized red blood cells per 1000 red blood cells (2%) was obtained. After 24 h, the parasitaemia decreased to four parasitized red blood cells per 1000 red blood cells (0.4%). The mice subsequently got rid of the parasites and remained negative until the experiment was terminated 16 days post-infection.

It is of interest to note that the course of infection of the parasite in both the chloroquine control mice and the normal control mice was approximately the same when both groups were inoculated with the same number of parasites (Table 1). No correlation was found between inoculum size and parasitaemia.

Effect of temperature

None of the experimental mice maintained at the following ambient temperatures, 9–12°C, 19–23°C, and 27–30°C, was found to have *P. y. nigeriensis* during the 12 days of the experi-

Table 1. Average daily parasitaemia induced by different numbers of inoculum in both the experimental and control mice

Days after infection	Inoculum size — parasitized red blood cells											
	1981			3962			5943			7924		
	EI*	CI_1†	CI_2‡	EI*	CI_1†	CI_2‡	EI*	CI_1†	CI_2‡	EI*	CI_1†	CI_2‡
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	2	3	0	4	6	0	0	6	0	10	12
3	0	7	9	0	13	12	0	20	35	0	17	28
4	0	7	8	0	26	15	0	22	40	20	63	76
5	0	33	41	0	33	50	0	46	75	4	73	88
6	0	32	46	0	32	47	0	39	68	0	44	132
7	0	100	87	0	103	90	0	88	120	0	142	168
8	0	218	190	0	211	230	0	150	244	0	258	270
9	0	385	375	0	325	480	0	220	478	0	270	282
8§	—	—	—	—	—	—	—	—	—	—	—	—
9§	—	—	—	—	—	—	—	—	—	—	—	—
10§	—	—	—	—	—	—	—	—	—	—	—	—
11§	—	—	—	—	—	—	—	—	—	—	—	—
12	0	240	302	0	255	375	0	360	492	0	90	100
13	0	206	346	0	154	374	0	100	290	0	80	190
14	0	298	382	0	287	280	0	48	¶	0	60	400
15	0	161	365	0	—§	—§	—§	—§	—§	—§	—§	¶
16	0	325	¶	0	360	450	—§	370	—§	—§	391	—§

*EI: Experimental mice.
†CI_1: Chloroquine control.
‡CI_2: Normal control.
§ Mice were not bled for microscopic examination.
¶ All mice were dead.

ment. No death was recorded for the mice. The chloroquine and the normal control groups, however, showed patent parasitaemia 48 h after infection, and the parasitaemia increased, ultimately culminating in the death of the mice (Table 2). Both the experimental animals and the two groups of control mice maintained at an ambient temperature of between 36°C and 38°C did not show parasite positivity throughout the period of the experiment, and no mortality was recorded.

Discussion

The results of this study showed that an inoculum size of up to 5943 *P. y. nigeriensis*, administered within 10 days after effective treatment of a previous infection, in mice failed to give a patent infection. When the inoculum was increased to 7924 parasites, patency was established 96 h after infection. Thereafter, decreased parasitaemia was recorded before the mice finally got rid of the parasite.

It is obvious that the first exposure of the mice to infection with the parasite induced the mice to develop some protective activity against further infection. Such protective activity may be humoral, as reported by Hamburger and Kreier [3] who used free-stage parasites to demonstrate protective humoral activity in serum of recovered rats. McGregor and Cohen [4] also reported that serum from immune patients or even animals contains antibodies that, by passive transfer, will reduce parasitaemia and bring about clinical cure of acute infection. Conversely, the protective activity could be cellular because it is well known that host phagocytic cells do phagocytose malaria para-

Table 2. Average parasitaemia in mice exposed to various temperatures

Days after infection	Temperature											
	9–12°C			19–23°C			27–30°C			36–38°C		
	ET*	CT_1†	CT_2‡	ET*	CT_1†	CT_2‡	ET*	CT_1†	CT_2‡	ET*	CT_1†	CT_2‡
1	0	0	0	0	0	0	0	0	0	0	0	0
2	0	14	20	0	0	22	0	19	16	0	0	0
3	0	39	80	0	64	68	0	106	104	0	0	0
4	0	206	102	0	290	341	0	291	321	0	0	0
5	0	199	287	0	401	432	0	484	515	0	0	0
6	0	149	342	0	334	374	0	270	353	0	0	0
7	0	135	263	0	103	205	0	§	212	0	0	0
8	0	147	205	0	99	174	0	§	175	0	0	0
9	0	120	183	0	323	335	0	§	387	0	0	0
10	0	§	297	0	224	259	0	§	§	0	0	0
11¶	—	—	—	—	—	—	—	—	—	—	—	—
12	0	§	172	0	125	145	0	§	112	0	0	0

*ET: Experimental mice.
†CT_1: Chloroquine control.
‡CT_2: Normal control.
§ All mice were dead.
¶ No reading was taken.
Note: Each figure is the average parasitaemia of the surviving mice.

sites either in erythrocytes or free in the circulation. It is, however, probable that both humoral and cellular protective activities are in action in the present study.

This study also shows that an imunological state developed in the host depending on the inoculum size, with a large inoculum overcoming the immune state of the host and resulting in the establishment of the parasite. The course of infection of the parasite in such 'partially immune' hosts, however, is short, resulting in the spontaneous recovery of the mice. It appears that the challenge infection acted as a booster dose for the host's reaction against the parasite.

It is perhaps not surprising that none of the infected mice maintained at different temperature ranges was positive. In other words, stress condition induced by temperature had no effect on the development of the parasite. This is probably due to the host's physiological activities, i.e. mice are warm blooded and are able to keep their internal temperature constant, regardless of the external temperature. As such, any variation in the external temperature of the mice would not be transmitted to their internal environment, which is the micro-habitat of the malaria parasites.

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