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Influence of Rodent Malaria on the Course of *Leishmania enriettii* Infection of Hamsters

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Plasmodium yoelii infection was established in hamsters, and the effect of this type of malaria on concurrent *Leishmania enriettii* infection was examined. It was found that the course of the *L. enriettii* infection was affected by *P. yoelii* and that this effect depended on the relative timing of the two infections. A chronic malarial infection with *Plasmodium berghei* was also established in hamsters, and this was found to affect the course of a concurrent *L. enriettii* infection in a similar manner to *P. yoelii*. These results are discussed in relation to current knowledge of the immunosuppressive effects of plasmodia.

Leishmania enriettii, a natural protozoan parasite of guinea pigs (15), is now well established as an experimental model of human cutaneous leishmaniasis (5). The course of *L. enriettii* infection and its eventual resolution are associated with the development of both humoral (16) and cell-mediated immune responses (6). It is not clear, however, what precise role these defense mechanisms play in eliminating the parasite. What is clear is that manipulations to suppress either humoral or cellular immunity result in an increased incidence of a metastatic diffuse form of the disease (4, 6, 7). Although human cutaneous leishmaniasis (caused by *L. tropica*) generally presents as a localized "oriental sore," a diffuse form of the infection does occur in man.

It has been suggested that this dissemination may be a result of a defect or suppression of the immunological mechanism of the host (2). This situation might result naturally if malaria occurred concurrently with the leishmanial infection. Suppression of immunological reactivity has been shown to occur during malaria both in man (9) and in rodents (11, 17). The effect of malarial infection on the course of experimental cutaneous leishmaniasis has therefore been studied.

Since mice are resistant to *L. enriettii* and guinea pigs are resistant to rodent malaria, hamsters were used because it has been found possible with this animal to establish both plasmodial and leishmanial infections in the laboratory without using protozoan species pathogenic to man.

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MATERIALS AND METHODS

Animals. Male outbred Syrian hamsters were used throughout. They were fed a pelleted diet and given water ad libitum.

Malarial infection in hamsters. (Following a recent publication [12], malarial parasites referred to previously by this laboratory as *Plasmodium berghei berghei* and *Plasmodium berghei yoelii* will now be referred to as *Plasmodium berghei* [Pb], and *Plasmodium yoelii* [Py], respectively.)

Py was maintained in the laboratory by serial passage of parasitized erythrocytes in BALB/c mice. Five days after one such passage, 10^7 parasitized BALB/c erythrocytes were taken and injected intraperitoneally into a hamster that had been splenectomized 2 weeks previously.

Five days after this, 10^7 parasitized erythrocytes were removed from this animal and passaged to a second splenectomized hamster. After another 5 days, blood was removed from this animal and, using a similar inoculum, the parasites were further passaged in normal hamsters three times. Seven days after the third passage in normal hamsters, the animal was bled out and the parasitized erythrocytes were prepared and stored as described in full elsewhere (18), in heparinized glycerine solution at −70°C.

Blood from mice infected with Pb 5 days previously was injected intraperitoneally into normal hamsters (10^7 parasitized erythrocytes per animal). Parasitized blood was passaged in these animals twice at 5-day intervals. Five days after the last passage, the parasitized blood was prepared and stored as described elsewhere (18).

***L. enriettii* infection.** Amastigotes (2×10^6) of *L. enriettii* obtained from an infected guinea pig and suspended in 0.025 ml of Earle balanced salt solution (containing 100 U of penicillin and 100 μg of streptomycin per ml) were injected intradermally into the noses of normal hamsters as described previously (2).

Quantitation. Plasmodium infections were measured by counting the proportion of parasitized

erythrocytes in Giemsa-stained thin blood smears at various times after infection. Total erythrocyte counts were performed by using a hemocytometer chamber.

The *L. enriettii* infection was quantitated by measuring the increase in lateral nose thickness with skin calipers at various times after infection.

Experimentation. All experiments were performed on groups of at least five animals. All comparative results were subjected to statistical analysis using Student's *t* test for nonpaired data.

RESULTS

Course of Py infection in hamsters. The intraperitoneal injection of 10^7 parasitized hamster erythrocytes into normal hamsters resulted in a self-limiting parasitemia that reached a peak (13%) 9 days after inoculation (Fig. 1). By day 16 no parasitized erythrocytes were detectable in Giemsa-stained thin blood smears. The mean total number of erythrocytes per cubic millimeter decreased over this period, falling to 50% of normal by day 9 (Fig. 1).

Effect of Py infection on the course of *L. enriettii* infection. *L. enriettii* injected intradermally into the nose of a hamster results in the development of a localized, chronic, granulomatous lesion that resolves after 8 to 10 weeks (2). The level of parasitemia of the lesion has been shown to be proportional to the increase in lateral nose thickness (2). Four groups of animals were injected with 2×10^6 amastigotes of *L. enriettii* as follows: (group 1) at the same time as infection with 10^7 Py-infected erythrocytes; (group 2) 5 days after infection with 10^7 Py-infected erythrocytes; (group 3) 5 days before infection with 10^7 Py-infected erythrocytes; or (group 4) without any concurrent Py infection. Each group contained at least five animals, and the whole experiment was duplicated.

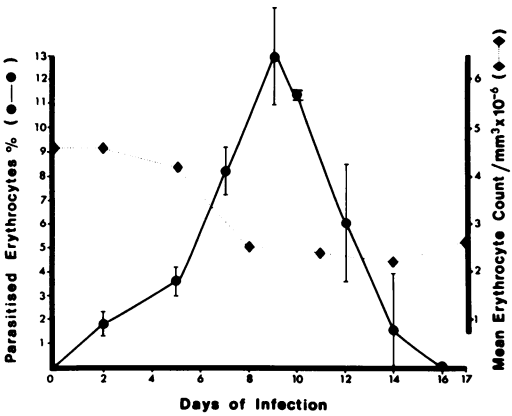

FIG. 1. *Py parasitemia in hamsters at various days after inoculation of 10^7 parasitized erythrocytes intraperitoneally. Mean ± standard deviation from groups of at least five hamsters is given at each point. The mean erythrocyte count at various days during Py infection is also given.*

The injection of *L. enriettii* into the noses of hamsters resulted in a progressive increase in nose thickness that peaked after 3 weeks and then declined, being totally resolved by week 10 (Fig. 2). The course of this infection was influenced by Py, but this influence varied depending on the relative timing of the two infections. When the two infections were given together, or when Py was given 5 days after *L. enriettii* (groups 1 and 3), there was no difference in the development of the leishmanial lesion compared with the control up to week 3. At 4, 5, and 6 weeks, however, the increase in nose thickness remained significantly higher in groups 1 and 3 ($P < 0.01$ at each time in the former; $P < 0.02$ in the latter). By week 7 the nose lesions in groups 1 and 3 had resolved to the same degree as the controls.

The development of the nose lesions in animals of group 2 only differed from the controls at week 3, when a significantly smaller increase in lateral nose thickness was detectable ($P < 0.05$).

The development of Py infection in animals concurrently infected with *L. enriettii* did not differ from that seen in animals given Py alone, whether the *L. enriettii* was given at the same time, before, or after the Py.

Development of chronic malaria in hamsters. It is known that in mice, chronic malarial infection can be established by injecting Pb some weeks after an initial acute infection with Py (20). After passage in hamsters (see Materials and Methods), the intraperitoneal injection of 10^7 Pb-parasitized erythrocytes was fatal to these animals (Fig. 3). No animal survived beyond 20 days, although death was not associated with a definite level of parasitemia. Pb infection also caused a much sharper drop than Py infection in the mean erythrocyte count, which fell to 10% of normal levels. If the injection of Pb was preceded by a Py infection, the level of mortality could be reduced.

A total of 10^7 Pb-parasitized erythrocytes were injected intraperitoneally either together with Py or 3, 4, 5, 6, 9, 15, or 19 weeks after a Py infection. As the time interval between Py and Pb increased, the animals appeared to become more resistant to the Pb (Table 1). When 6 weeks elapsed between the two infections, total protection against Pb was demonstrable. Beyond 6 weeks, however, protection was progressively lost.

When Pb was given 6 weeks after Py, animals still developed measurable parasitemia.

This was very variable, however, and in some cases quite high. In general, parasitemia lasted for 5 weeks (Fig. 4). Although parasitemia was not detectable in blood smears after this time, in some cases the blood of these animals was still infective over a year after the Pb challenge. This infection appeared to be Pb since it was lethal when subinoculated.

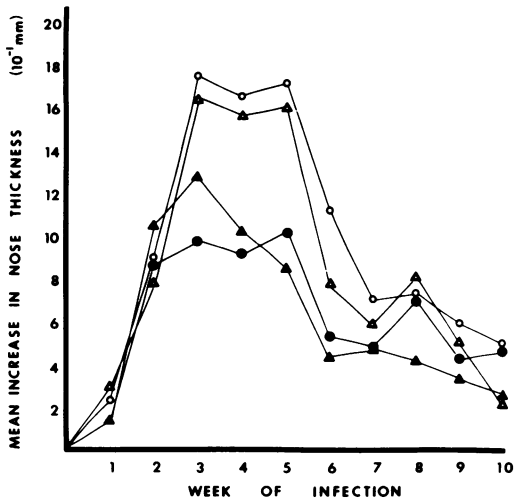

FIG. 2. *Effect of concurrent Py infection on the course of L. enriettii infection as detected by increase in lateral nose thickness after intradermal inoculation of 2 × 10^6 amastigotes on day 0. Symbols: (▲) L. enriettii alone (control) (group 4); (○) L. enriettii and Py together (group 1); (●) L. enriettii 5 days after Py (group 2); (△) L. enriettii 5 days before Py (group 3). Each point is the mean reading from groups of at least 10 animals. Standard deviations have been omitted to retain clarity.*

Effect of chronic malaria on *L. enriettii* infection. Since a concomitant Py infection was shown to affect the course of *L. enriettii* infection (see above), it was felt of interest to see whether the more chronic malaria produced by injecting Pb 6 weeks after Py would have a more dramatic effect on *L. enriettii*. Five groups of five animals were used and infected as shown in Table 2. Although this experimental regime meant that the time interval between Py and Pb in groups 2 and 4 was 5 and 7 weeks, respectively, we felt it important that all animals be infected with the same population of *L. enriettii*. In all animals, the nose thickness was measured each week after infection with *L. enriettii*.

The increase in nose thickness in control group 5 was maximal 2 to 3 weeks after infection (Fig. 5). It then declined and had resolved by week 8. The development of the nose lesions in animals of group 4 was not significantly different from that in control group 5. Of the three groups that received the Pb infection, group 2 (Pb 7 days before *L. enriettii*) showed a delayed development of the nose lesion. Two weeks after infection, the increase in nose thickness was less than in the control group, although not significantly so ($0.05 > P < 0.1$). By week 5, lesions were significantly greater

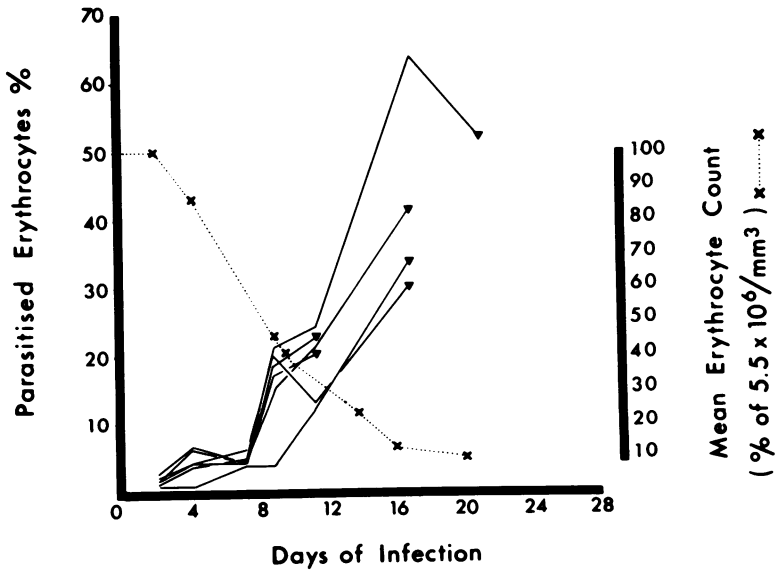

FIG. 3. *Pb parasitemia in individual hamsters at various days after inoculation of 10^7 parasitized erythrocytes intradermally. (▲) Death of individual animals. The mean erythrocyte count at various days during the Pb infection is also given.*

TABLE 1. *Susceptibility of hamsters to Pb after recovery from Py infection*[a]

Infective agent[b] (day 0)		Challenge with Pb at week:	% Mortality
Py	Pb		
+	−		0.0
−	+		100.0
+	+		100.0
+	−	3	83.3
+	−	4	83.3
+	−	5	33.3
+	−	6	0.0
+	−	9	33.3
+	−	12	33.3
+	−	15	50.0
+	−	19	66.7

[a] Six animals were used per group.
[b] Infective doses of parasitized erythrocytes was 10^7 in each case.

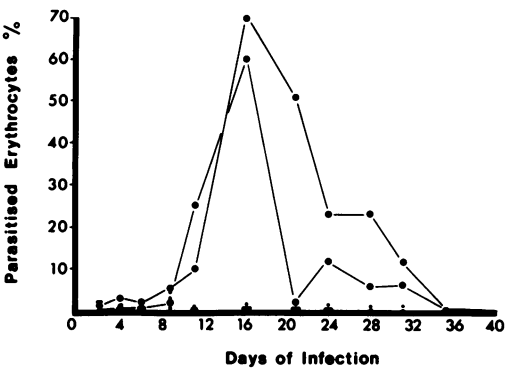

FIG. 4. *Course of parasitemia in six individual animals given Pb (10^7) 6 weeks after Py (10^7). Percentage of parasitized erythrocytes calculated by counting on Giemsa-stained thin blood films.*

than in the controls ($P < 0.01$), which were resolving by this time. The resolution of group 2 lesions was rapid after this time and was complete by week 8.

When the Pb was given at the same time as the *L. enriettii* (group 3), a rapidly developing and more severe nose lesion resulted which peaked at week 2 ($P < 0.01$ compared with control) and was still more severe than that of the controls at weeks 4 and 5 ($P < 0.01$ at each time). This still resolved by week 8.

Group 4 animals (Pb 7 days after *L. enriettii*) showed an early development of lesions that was similar to that in the controls. However, in this group four of the five animals died between weeks 4 and 5 as the leishmanial infection appeared to affect the course of the Pb infection (see below).

Effect of *L. enriettii* infection on malaria. All animals that received Pb developed some parasitemia, but this was very variable from animal to animal. No direct relationship between the level of parasitemia and size of nose lesions was observed. In the group of animals that received Pb 7 days after *L. enriettii* group 4, however, a more rapid development and higher level of Pb parasitemia was observed, and four out of five of these animals died 3 to 4 weeks after Pb inoculation. A comparison of Pb parasitemia in this group with that of animals that received *L. enriettii* at the same time as the Pb (group 3) is shown in Table 3.

TABLE 2. *Regime for infection of hamsters*

Group	Infecting organism at week:			
	0	5	6	7
1	Py		*L. enriettii*	
2	Py	Pb	*L. enriettii*	
3	Py		*L. enriettii*	
4	Py		*L. enriettii*	Pb
5			*L. enriettii*	

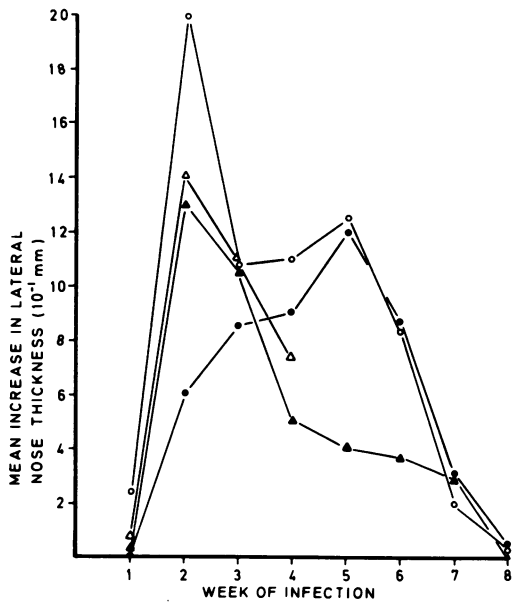

FIG. 5. *Effect of concurrent Pb infection on the course of L. enriettii infection as detected by increase in lateral nose thickness after intradermal inoculation of 2 × 10^6 amastigotes on day 0. (All animals receiving Pb had received Py 6 weeks previously.) Symbols: (▲) L. enriettii alone (control) (group 5); (○) L. enriettii + Pb infections together (group 3); (●) L. enriettii 7 days after Pb (group 2); (△) L. enriettii 7 days before Pb (group 4). Group 1 received L. enriettii 6 weeks after Py but did not receive a Pb infection. These animals developed lesions identical to those of group 5 and are omitted. Each point is the mean reading from groups of five animals. Standard deviations have been omitted to retain clarity.*

TABLE 3. *Percentage of parasitized erythrocytes at various weeks after Pb infection in hamsters infected at the same time or 7 days after infection with L. enriettii*

Regime	Animal no.	% Parasitized erythrocytes					
		1[a]	2	3	4	5	6
Pb + *L. enriettii*	1	<0.01[b]	<0.01	<0.01	<0.01	<0.01	<0.01
	2	1	10	30	26	5	<0.01
	3	0.25	18	30	17	<0.01	<0.01
	4	1.5	30	20	9	<0.01	<0.01
	5	1	1	<0.01	D[c]		
Pb 7 days after *L. enriettii*	1	1	60	50	D		
	2	<0.01	20	55	D		
	3	0.5	25	32	D		
	4	0.1	20	18	D		
	5	<0.01	5	<0.01	<0.01	<0.01	<0.01

[a] Weeks after Pb infection.
[b] 0.01% is the limit of detectable parasitemia using thin blood films.
[c] D, Death of individual animals.

DISCUSSION

The course of *L. enriettii* infection has been shown to be affected by procedures designed to alter the immune response of the host. These include the use of antilymphocyte serum (4), the use of adjuvants and substances toxic to macrophages (6), and the use of the immunosuppressive drug cyclophosphamide (1). When those manipulations have resulted in suppression of either humoral or cellular immunity, the incidence of metastasis of the leishmanial infection has appeared to increase. In man, cutaneous leishmaniasis due to *L. tropica* also develops in some cases into diffuse cutaneous leishmaniasis. If the experimental results are related to the natural occurrence of diffuse cutaneous leishmaniasis, an indigenous agent that could cause immunosuppression might be involved in the development of this form of the disease. Such an agent could be malaria, which has been shown to suppress the immune response to many antigens both in mice and man (9, 11, 17). The present paper describes experiments where concomitant malaria and leishmanial infections were studied in hamsters.

The normal course of *L. enriettii* infection in hamsters has been fully described elsewhere (2). Py infection of the hamsters was achieved by initially passaging mouse Py in splenectomized hamsters, since splenectomy has been shown to increase the severity of malaria in a variety of hosts (8). Once adapted, Py infection of hamsters was similar to the infection in mice, as described by others (18).

When animals were infected with *L. enriettii* at the same time or 5 days before Py, the nose lesions reached a larger size and remained higher than the controls for 3 weeks. There appeared to be no difference in the time of resolution, however. If Py was injected 5 days before *L. enriettii*, a slight reduction in the peak size of the nose lesion was seen. It should be noted that no metastatic lesions developed in any of these animals.

By injecting the lethal-strain Pb 4 to 14 weeks after Py, it is possible in mice to create a model of chronic malaria (21). This has now been achieved in hamsters, using an interval of 6 weeks between infection with Py and Pb. Experiments were therefore performed giving *L. enriettii* infection before, after, or at the same time as Pb in hamsters that received Py 6 weeks previously. It was hoped that in this situation immunosuppression (if it occurred at all) might be increased or at least occur for a longer period. In this event, the effect of Pb on the *L. enriettii* was not too dissimilar from that of the Py.

Animals receiving the Pb 7 days before *L. enriettii* were slower to develop the nose lesion, which reached a maximum after 5 weeks, whereas animals receiving both infections together developed a significantly greater nose lesion than controls infected with *L. enriettii* alone. It seems clear, therefore, that the development and resolution of *L. enriettii* infection can be affected by malaria, but the effect is dependent on the relative timing of the two infections. This type of observation has been made in other systems.

The immune response to sheep erythrocytes, for example, is most strongly suppressed by injecting plasmodia 7 to 10 days before antigen, whereas lymphomagenesis due to Moloney virus is most strongly enhanced by plasmodia given between 5 days before and 5 days after the virus (20). Greenwood et al. (11) showed that Py had no effect on skin graft rejection

when injected 5 or 10 days before grafting, whereas Wedderburn (20) found some lengthening in rejection time in animals given Py on the day of grafting, and others (19) found a considerably longer rejection time if the malarial disease could be prolonged for the whole period of rejection.

The mechanisms whereby malaria causes immunosuppression are still unresolved. Workers have reported reductions in numbers of B and T lymphocytes during malaria (13), whereas others have described that functionally, T cells at least appear unaffected (11). Defects in macrophage function have been suggested as resulting from malaria, both in man and animals (14). In general, antibody production to some antigens certainly is suppressed (10, 11, 20). No attempt has been made in the present communication to resolve this question, but one point that does become evident is that there is no marked difference between the influence of an acute Py infection on *L. enriettii* as compared with the more chronic Pb infection.

In both situations it seems likely that the effect of malarial infection on *L. enriettii* is only transient and is probably associated with the primary immune response, whether it be on antibody production, macrophage function, or T lymphocyte sensitization. None of the experimental situations described resulted in the development of metastatic lesions in hamsters, probably because these animals are naturally more resistant to *L. enriettii* than are guinea pigs (2). Although malarial infection has been shown quite clearly to affect the course of *L. enriettii* infection, it must be remembered that no direct evidence of Py or Pb being immunosuppressive in hamsters has as yet been presented.

ACKNOWLEDGMENTS

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