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Depressed Malarial Immunity in Pregnant Mice

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A proportion of mice solidly immune to the rodent malaria parasite *Plasmodium berghei* exhibited a pregnancy-associated depressed immunity with a transient or even lethal recrudescence.

Attenuated immunity and analysis of the immunodepressive effect of pregnancy-associated substances have been the subject of many investigations. In human malaria a pregnancy-associated depression of immunity was inferred from an observed increase in parasite rate and parasite density in pregnant women in a holoendemic area and from an increased severity of malaria during pregnancy (e.g., cerebral malaria) in areas with unstable malaria (13, 17, 21). Some authors reported that primigravidae are at an especially high risk (2, 3, 17, 20; H. Kortmann, M.D. thesis, Royal Tropical Institute, Amsterdam, 1972), whereas a decline in the prevalence and density of malaria parasites in pregnant as well as in nonpregnant women with increasing age has been described (2, 4). Prematurity and possibly abortion were also found to be associated with malaria during pregnancy in humans (3, 4, 17, 18, 21, 28). Comparable phenomena were observed in mice immunized against the rodent malaria parasite *Plasmodium berghei* during pregnancy. Moreover, with recrudescence as a marker for depressed immunity, this model can be used to analyze the relevance to the immune status of pregnancy-associated changes in immune responsiveness and levels of immunologically active proteins and hormones during pregnancy.

Approximately 6-week-old mice of several strains (Swiss outbred, C3H/StZ, and B10LP inbred) were immunized against *P. berghei* (strain K173) by an intraperitoneal injection of 10^7 parasitized erythrocytes, followed by sulfadiazine treatment (30 mg/liter of drinking water: estimated daily consumption, 3 ml per mouse) from days 2 to 33 after infection. Two days later mice were challenged with 10^5 parasitized erythrocytes given intraperitoneally to assess immunity (9). The acquired immunity was of the premunition type with low, subclinical numbers of persisting parasites. Spontaneous recrudescences were rare (7). A considerable proportion of such immune mice, however, suffered a recrudescence during pregnancy (Table 1). Patent infection (parasites are readily observed in a thin blood smear) was found in approximately the same proportion of mice in all strains tested.

A parasitemia of more than 5% infected erythrocytes, as determined from thin blood films, was taken to indicate depressed immunity. Patency was not observed before the last, i.e., third, week of pregnancy. As a prepatent period (period between infection and patency) of several days is common for *P. berghei* infection in mice, presumably the actual breakdown of malarial immunity occurred before the third week of pregnancy in at least a considerable proportion of the mice. In human malaria the situation is even less clear as some authors reported a prevalence of malaria early in pregnancy (2, 26), and others reported it late in pregnancy (17, 21).

The results of Table 1 also show that, depending on the strain, a varying proportion of mice recovered from a pregnancy-associated recrudescence. Recovery was never observed before parturition. These results seem to indicate that at least in a proportion of the mice the immunodepressed state is abrogated after parturition, in accordance with changes in many other immunological phenomena observed during pregnancy. Although the mechanism of recovery of mice suffering a recrudescence during pregnancy is unknown, a possible mechanism was discussed in relation to a time-dependent waning of immunity (8, 9a).

A proportion of mice did not suffer a recrudescence during pregnancy, possibly due to the clearance of parasites before the immunodepressive period. The clearance of parasites in an increasing proportion of mice in relation to increasing periods since the last challenge has been described (7). For evaluating the status of nonrecrudescing mice, blood was subinoculated immediately after parturition. Of 28 Swiss mice without a recrudescence during pregnancy, 19 were found to harbor parasites after parturition. Such mice may either have developed better immunity or a less severe immunodepression during pregnancy.

The *P. berghei* mouse model also revealed malaria-associated prematurity and abortion.

The results of Table 2 indicate that 10% of the mice with a recrudescence delivered prematurely (before day 20). Abortion was also observed.

Though the percentage of premature deliveries was low (10%), 20% of the recrudescing mice died from malaria before parturition and were not included in the count.

As to the prevalence of recrudescences in primigravidae, the situation in the *P. berghei* mouse model is complicated. Though the incidence of recrudescences was much lower in multigravidae, a high proportion of the mice that recrudescenced during their first pregnancy died from the infection and could not be studied as multigravidae. In a different approach to the problem, the possibility of a change in the immune status of the mouse from the first to subsequent pregnancies was investigated. A group of 24 immune Swiss mice received curative treatment with chloroquine (0.8 mg per mouse intraperitoneally for 5 days before mating, supplemented with 100 mg of chloroquine per liter of drinking water for the duration of the first pregnancy). After parturition the mice were reinfected and allowed to mate again. During the second pregnancy 46% (11 mice) exhibited a recrudescence, and this percentage was the same as that observed in primigravidae (Table 1). A fundamental difference in the immunological capacity of the host between the first and subsequent pregnancies is, therefore, not indicated by these results.

In older mice a decreased proportion of recrudescences was observed (Fig. 1). Although this result is comparable with the situation in humans (2, 4), the enhanced clearance of parasites in older mice (9a) may have been influential.

Our results show that the *P. berghei* mouse model is an excellent experimental model for the analysis of the mechanism of depressed malarial immunity during pregnancy, exhibiting several similarities with the human model. Moreover, it is also suitable for the study of the relevance of changes of immunological responses in general or of immunologically active substances to immunity. The potential immunodepressive activity of substances like prolactin (15), α-macroglobulins (29, 30, 31), α-fetoproteins (6, 24, 25), soluble fetal antigens in the maternal circulation (1), progesterone (27), and corticoids (14, 19), which are present during pregnancy, has been established, but is not directly related to the actual immunological status of the host. With recrudescence as a marker for depressed immunity a direct correlation is possible. The same holds true for pregnancy-associated changes in lymphocyte reactivity (5, 11, 12, 22, 23) or antibody responses (10, 16, 24). As to changes in antibody responses during a malaria-associated pregnancy in humans, the situation is not clear. The inhibition of an antiparasitic immunoglobulin G response has been suggested (2), but others did not observe changes in anti-parasitic antibody titers during pregnancy (13; Kortmann, M.D. thesis).

TABLE 1. *Recrudescence and recovery in pregnant mice*[a]

Mouse strain	No. of pregnant mice	No. of mice with a recrudescence during pregnancy (%)	No. of mice recovering from a recrudescence (%)
C3H/StZ	63	31 (49)	6 (19)
B10LP	94	38 (40)	23 (61)
Swiss	233	107 (46)	32 (30)

[a] Age of mice at mating varied from 11 to 22 weeks (i.e., immediately to 11 weeks after immunization). Approximately 25% of Swiss mice were older at mating (up to 46 weeks).

TABLE 2. *Recrudescence and prematurity in Swiss mice*

Mice with	% with parturition on day						% Dead before parturition
	17	18	19	20	21	22	
No recrudescence ($n = 48$)				50	33	17	0
Recrudescence ($n = 46$)	2	4	4	33	37		20

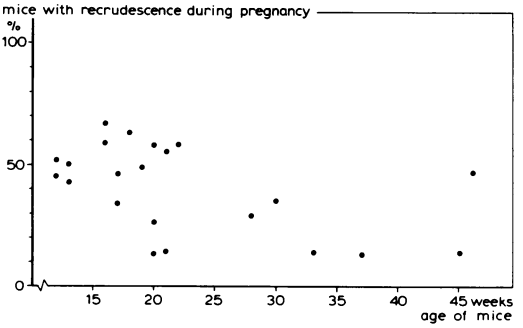

FIG. 1. *Effect of age on the recrudescence of malaria in primigravidae. The figure shows the results of 22 groups, each containing from 12 to 56 pregnant Swiss mice. Mice were immunized at the age of 6 weeks (9). Before the mice were allowed to mate, immunity was (re)confirmed by the resistance to challenge (10^5 P.E.) The time indicated in the figure reflects the age at mating.*

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