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Oestrous cycle perturbations and hypotrophy of clitoral glands in malaria-infected female BALB/c mice

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Abstract An experimental host-parasite association involving BALB/c female mice infected with Plasmodium vinckei petteri was used with the aim of investigating the morphological and physiological alterations induced by the parasite in the genital tract of the host. The vaginal oestrous cycle was monitored as a daily clue to the sexual physiology of the female mice, and a complete histological analysis of the genital tract was performed 36 days following parasite inoculation. The oestrous cycle showed strong transitory alterations during the first 30 days following infection. The occurrence of oestrus days increased during the first 10 days post-infection and then decreased to a subnormal value during the following 20 days. Infected mice also showed a remarkable hypotrophy of their clitoral glands 30 days after the beginning of the malarial infection. A probable cause of such perturbations is a significant hormonal imbalance triggered by the erythrocytic proliferation of the Plasmodium. The relationship between the immune response of the host and these physiological and morphological alterations, as well as the outcomes of these alterations on the sexuality of the rodent host are discussed.

Introduction

Malaria is a mosquito-borne parasitic disease affecting many vertebrate species and killing one million humans living in endemic areas each year. The erythrocytic proliferation of the Plasmodium parasite causes spectacular anatomical and physiological pathologies, well known in humans for their severity and widely studied in experimental models involving rodents (Cox 1988; Landau and Gautret 1998). Indeed, several host-parasite associations involving a genetically defined line of laboratory rodents infected by a species of Plasmodium have been established and characterized in regard to the course of the infection and to the pathologies produced.

In these murine malaria models, the erythrocytic proliferation of Plasmodium induces short-term, reversible, physiological and anatomical alterations such as fever, pyrexia, anaemia, hepatomegaly, splenomegaly, and weight loss but also long-lasting or irreversible histopathological lesions in the brain, heart and kidneys (Cox 1988; Landau and Gautret 1998; Vuong et al. 1999). In pregnant mice, the placenta is damaged and its function is impaired by the malarial parasitization (Akingbade 1992). These different pathologies are more or less specific to a host-parasite association and their relationship to the ones described in humans is not evident (Landau and Gautret 1998).

In rodents, many other parasitic infections involving protozoa, such as trypanosomiasis and toxoplasmosis (Hublart et al. 1990; Tavares et al. 1994; Stahl et al. 1995), or involving larval tapeworms (Lin et al. 1990; Morales-Montor et al. 1999), have been associated with hormonal perturbations which greatly impair the sexual functions of the host. Curiously, although Akingbade et al. (1990) demonstrated that the acute phase of a Plasmodium berghei infection induces a hormonal imbalance in female Wistar mice, no dysfunctions in the sexual physiology and no modifications of the genital tract were reported in infected females.

The present study aims at verifying the conclusions obtained by Akingbade et al. (1990) in BALB/c female mice infected by Plasmodium vinckei petteri. The physiological alterations in the sexual cycle occurring during the patent phase of the infection and the long-lasting histopathologies occurring in the genital tract of surviving female mice were investigated. The vaginal cycle
was monitored as a daily clue of the sexual physiology of the female mice.

**Materials and methods**

**Animals**

BALB/c female mice (8 weeks old) weighing 15–20 g were used. The animals were provided free of specific pathogens by the Janvier breeding company (le Genest-Saint-Ise, France). The mice were maintained in the laboratory at 24°C in individual plastic cages (250x160x136 mm) under a 12 h light/dark cycle.

**Parasite strain**

The subspecies *P. vinckei petteri* (279 BY strain) was used. Frozen blood samples, kept at –80°C, were thawed and injected into BALB/c female mice before experimentation in order to prepare fresh inocula.

**Parasitemia and inoculation**

The inoculum was prepared by counting the number of parasitized red blood cells (RBCs) and adjusting the final volume with 10% heparinized saline buffer to obtain 300,000 parasitized RBC/ml. A total of 21 mice were infected by intraperitoneal injection of 0.1 ml of this suspension containing 30,000 parasitized RBCs. Ten mice, injected with 0.1 ml of heparinized fresh blood from normal mice, were used as controls.

Follow-up of parasitemia

In order to quantitate the parasitaemias, blood smears were taken using tail-blood, fixed in methanol and stained for 45 min with 10% Giemsa diluted in phosphate buffer at pH 7.2. The parasitemia was determined by counting the total number of parasitized RBCs in 2,000 RBCs. Parasitaemias were monitored three times a week throughout the experimental period.

**Oestrous cycle**

Cyclic changes in the cell types occurring in the vaginal mucosa have been correlated to cyclic changes in the ovary and in the hormonal status of female mice (Thornton and Finn 1999). Using a very small swab, vaginal smears were taken daily in the beginning of the light phase between 09:00 and 10:00 a.m. and were stained for 15 min with 10% Giemsa diluted in phosphate buffer at pH 7.2. Occurrences of leukocytes and nucleated or cornified epithelial cells were monitored at low magnification to determine the progression of the oestrous cycle in infected and control mice.

**Histological techniques**

Surviving infected mice and control mice were killed by CO₂ inhalation 36 days after inoculation and autopsied. Clitoral glands were taken and fixed in 10% formol for 1 week. Once dehydrated, the glands were transferred to butanol before being blocked in paraffin. Transverse histological sections, 7 μm thick, were made, stained by haemalun and eosin (HE) and analysed by two independent observers. The size of the gland section, the proportion of the surface occupied by the whole acinous tissue and the relative proportion of the growing acinar cell layer were measured in a complete morphometric study. The density of mature acini containing hypertrophic acinar cells was also determined in each gland section.

Statistical analyses

Mann-Whitney U-tests were used to compare the data from controls, surviving infected and dead infected animals. Friedman’s and Wilcoxon’s tests were used to estimate the variation within the group of control mice. Spearman’s rank correlation was used to establish the relationship between parasitaemia and histological modifications. *P* values < 0.05 were considered significant. All tests were performed using STATISTICA 5.0.

**Results**

**Parasitaemia**

The development of the parasitaemia is shown in Fig. 1. In *P. v. petteri* infected mice it increased quickly and steadily to a mean value of 50% parasitized RBCs in 7 days. A third of the infected animals died within the 2 days following peak parasitaemia. The surviving mice showed an abrupt fall in parasitaemia to undetectable levels after day 14 post-infection. No reappearance of parasitized RBCs (Landau et al. 1999) occurred during the last 3 weeks of the survey.

**Oestrous cycle**

Perturbations in the progression of the vaginal oestrous cycle in infected mice occurred rapidly. These were characterized by an abnormal proportion of oestrus days within a complete cycle (Fig. 2). Whereas control mice spent a mean of 41% oestrus days throughout the 36 days of the survey (*H₃ = 3.48; P < 0.32*), surviving infected mice displayed fluctuations in the proportion of days they spent in oestrus. In fact, during the first 10 days post-infection, the surviving infected mice spent significantly more days in oestrus than controls.

![Fig. 1 Progression of the parasitemia in surviving BALB/c female mice infected with *Plasmodium vinckei petteri*. One group of 21 mice each received 30,000 infected red blood cells in 0.1 ml by intraperitoneal injection. Six of them died prior to day 10 post-infection. Data are reported as mean percentage of infected red blood cells ± SD of 15 mice for each time](image-url)