Plasmodium berghei Malaria: effect of acute phase serum on immunity generated in rats by infection and by vaccination

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Abstract. Acute phase serum (APS) given at the time of challenge with Plasmodium berghei inhibited the generation of immunity to the infecting plasmodia. Administered with a single dose of vaccine, it inhibited induction of immunity by the vaccine. Three weekly doses, the last given two weeks before infection, induced immunity. Administration of vaccine simultaneously with infection neither aggravated nor ameliorated the infection. These results indicate that the effect of administration of APS on immunity generated by immunization or infection is dose- and time-dependent. The depression of immunity induced by this serum is thus temporary, the host finally overcoming the depression and responding to the plasmodial antigen in the serum. The interaction of vaccine and infection observed indicates that the introduction of vaccine is not detrimental to the individual incubating infection; rather, the vaccine is rendered useless, the reducing the aggregate benefit of the immunization to the group.

Materials and methods

Parasites

The organism used was a Plasmodium berghei strain always lethal for mice, but from which rats often recover. The plasmodia were cryopreserved in liquid nitrogen (−193°C) by standard techniques (Trager and Jensen 1980). The frozen plasmodia were retrieved from storage when needed, thawed, and infection was initiated by i.p. injection of Swiss mice.

Animals

Outbred Sprague-Dawley (SD) rats were the source of the infected red blood cells (IRBC) used in the preparation of the formalin-fixed vaccine and for challenge. SD rats were also the source of immune serum (IS) and acute phase serum (APS). SD rats weighing 130 g were the test animals in the vaccine trials. Five rats were in each experimental group.

Vaccines

The technique for preparation of the formalin-fixed IRBC vaccine was adapted from Murphy and Lefford (1979). A suspension containing 10⁶ IRBC/ml was gently agitated at 4°C for 24 h in 0.1% formalin. The vaccine, distributed in aliquots, was stored at −20°C until used. Administration of the vaccine was by i.v. injection of 10⁶ IRBC at weekly intervals for a total of 1, 3, or 5 injections. Rats in the control group received i.v. 10⁶ normal red blood cells (NRBC) in 0.1% formalin.

The free-parasite vaccine consisted of sonically released, free P. berghei parasites (Prior and Kreier 1972a, b). One dose
of the vaccine contained $10^9$ free parasites in 0.85% saline with 0.13 mg of saponin (Kodak) as an adjuvant; it was injected i.m. into the rear hindquarters. Rats in the control group were injected i.m. with NRBC membranes in 0.85% saline with the same saponin adjuvant. These vaccines were prepared by methods that have previously been described (Saul and Kreier 1977; Grothaus and Kreier 1980).

**Sera**

*Acute phase serum.* Acute phase serum (APS) was collected from SD rats just after the parasitemias began to fall, normally 15–17 days after the first detectable parasitemia. The APS was injected i.p. in 1.0-ml amounts into the rats of the various vaccine groups on the day before and the two days after the injection of the vaccine. Other rats were injected i.p. with 1.0 ml APS on the day before and the days after challenge. In subsequent discussion, a dose of APS means three injections according to the aforementioned schedule. In the experiment in which the interaction of acute serum and immune serum was studied, a volume of APS was mixed with an equal volume of immune serum and 2.0 ml were injected following the same schedule.

*Immune serum.* Immune serum (IS) was produced in the SD rats by four cycles of infection and recovery: the rats were inoculated i.p. with $10^9$ IRBC at biweekly intervals. One week after the last injection, the rats were exsanguinated by cardiac puncture and the serum was frozen for future use. Immune serum was injected i.p. in 1.0-ml amounts by the same schedule as APS. Again, these three injections are subsequently referred to as a single dose of serum.

**Challenge**

All animals were challenged by the i.v. injection of $10^4$ IRBC. Unless indicated otherwise, challenge occurred two weeks after immunization was completed.

**Determination of parasitemias.** Parasitemias were determined by daily examination of thin blood films taken from the tail. About 10000 erythrocytes, or a fraction of 10000 erythrocytes with increasing parasitemia, were examined to determine each data point.

**Data presentation and statistical analysis**

For ease of presentation, parasitemia values from each group were averaged for each day and then compiled into an average value for days 1–15. This allowed presentation of multiple parasitemia curves in a single histogram. The parasitemia values for the trial depicted in Fig. 2 were averaged for days 1–20, since the parasitemia developed slightly later in the rats used in this experiment.

Group parasitemias were analyzed using one-way analysis of variance. When a significant difference was indicated, the values were tested individually by the Student-Newman-Kuhl test. A $P$ value less than 0.05 was taken to indicate a significant difference.

**Results**

**Standardization of the immunization procedures**

The average percent of parasitemia during the first 15 days after infection in rats which were immunized by i.m. injection of $5 \times 10^8$ small, free *P. berghei* in saponin was approximately half that in rats which had received a control vaccine of erythrocyte membranes (Fig. 1). Three weekly i.v. injections of $10^8$ formalin-fixed, infected erythrocytes were required to induce a degree of immunity roughly similar to that induced by one injection of free-parasite vaccine. The degree of immunity induced by the IRBC vaccine was proportional to

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Fig. 1. Parasitemias following challenge in rats which received one injection of NRBC membranes in saponin (EM 1 ×) or one injection of free parasites in saponin (FP 1 ×) two weeks before challenge. Parasitemia in the immunized rats was about half that in the hosts in the control group.

Fig. 2. Parasitemias following challenge in rats which received five weekly injections of formalin-fixed i.v. NRBC (5 ×), or one (1 ×), three (3 ×), or five (5 ×) weekly injections of formalin-fixed IRBC two weeks before challenge. The degree of reduction in parasitemia in the rats which received three injections of IRBC was roughly equal to that in the rats which received one injection of free parasites (cf. Fig. 1).