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THE EFFECT OF IMMUNE SERUM ON THE INFECTIVITY OF SONICALLY DAMAGED *PLASMODIUM BERGHEI* INFECTED ERYTHROCYTES[1]

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ABSTRACT. The reduction in the infectivity of those *Plasmodium berghei* containing erythrocytes which remained unlysed following exposure to sonic energy and treatment with immune serum was shown to be far greater than that caused by the sum of the separate actions of immune serum and sonic energy applied separately. This indicates that, after exposure to sonic energy, plasmodia in surviving, parasitized erythrocytes are more susceptible to neutralization by immune serum than are plasmodia in unsonicated, parasitized erythrocytes. The membranes of parasitized erythrocytes that survive sonication thus appear to be permeable so that intracellular parasites are accessible to antibodies in immune serum.

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INTRODUCTION

Plasmodia freed by continuous flow sonication and differential centrifugation (Prior and Kreier 1972a, b) have been useful for studies of immunization (Saul and Kreier 1977, Grothaus and Kreier 1980) and for studies of host-parasite interactions *in vitro* (Green and Kreier 1978, Brooks and Kreier 1982), but their use as infective inocula for *in vivo* studies (Hamburger and Kreier 1975, 1976a, b, Kreier et al. 1976) has been questioned. McAlister and Gordon (1977) reported that free plasmodia prepared by continuous flow sonication and differential centrifugation were not capable of initiating infection, probably due to their immature state or, if mature, their short duration of infectivity after release from the erythrocyte. McAlister and Gordon (1977) suggested that the infectivity of free plasmodial preparations prepared by continuous flow sonication was due to small numbers of contraminating infected erythrocytes.

Infectivity of the free plasmodial preparations is susceptibile to neutralization by antibody to a degree that infectivity of unsonicated infected erythrocytes is not (Hamburger and Kreier 1975, 1976a, b). The present study was designed to determine if infected erythrocytes that had been exposed to sonic energy would be affected differently by antibody than would unsonicated infected erythrocytes.

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METHODS AND MATERIALS

PARASITES. The *Plasmodium berghei* strain was obtained from the Walter Reed Army Institute of Research.

HARVESTING OF INFECTED RED BLOOD CELLS. Blood was collected from phenylhydrazine-treated mature rats after parasitemia had reached 20% as described by Hamburger and Kreier (1975). A portion of this blood was subjected to a modified continuous flow sonication procedure (Prior and Kreier 1972a, b). The modification was that no attempt was made to separate free parasites from intact red blood cells by differential centrifugation; instead, the sonication liquid, containing free parasites, erythrocyte fragments, infected red blood cells (IRBC) and uninfected red blood cells (URBC), was collected and adjusted to appropriate concentrations with Alserver's solution and utilized directly as infective inocula in passive protection tests.

ANIMALS. Mature Sprague-Dawley rats (Charles River Breeding Laboratories) were used as the source for serum and parasites. Adult (25 g) male Swiss mice (Charles River Breeding Laboratories) were used as the test animals for the protection tests.

SERUM. Normal rat serum was harvested from Sprague-Dawley rats and stored at −20°C. Immune serum was raised in rats as follows. Rats were infected by intravenous injection of 1×10^8 IRBC and then allowed to recover. The rats were then injected with a similar dose of IRBC at biweekly intervals for a total of four injections. Two weeks after the last injection, the rats were bled and the immune serum obtained was stored at −20°C.

PROTECTION TEST PROCEDURE. Suspensions containing approximately 20% blood cells from infected rats were prepared. The exact concentrations were determined by the microhematocrit technique and by haemocytometer counts. After sonication, concentrations of unlysed erythrocytes were again determined and concentrations adjusted appropriately. One volume (2.5 ml) of a suspension containing 1×10^5 unsonicated IRBCs or an amount of a suspension of sonicated IRBCs containing an equal number of unlysed IRBCs was mixed with an equal volume of either normal or immune serum, and incubated at room temperature for 15 min. The mixtures were centrifuged in the cold at 10,000 × g for 10 min.; the supernatent fluids were discarded and the pellets resuspended to the original volumes (5 ml) in Alserver's solution. Then 0.5 ml of the resulting suspensions, containing approximately 1×10^4 unlysed IRBC, was injected into the tail veins of each of five Swiss mice. Blood smears were taken daily from the Swiss mice, stained with Geimsa, and parasitemias were determined by observing 10,000 erythrocytes.

RESULTS

The infected erythrocytes, either sonicated or unsonicated, incubated with normal or immune serum, washed by centrifugation, and resuspended in Alsever's solution to the appropriate concentration to be used were injected into the mice. All the mice which received either sonicated or unsonicated IRBCs incubated in normal serum became infected and died. All the mice which received unsonicated erythrocytes incubated in immune serum also became infected and died. However, none of the mice which received sonicated infected erythrocytes which had been incubated in immune serum became infected. The times to death and the forms of the parasitemia curves in the mice of the first three groups were roughly similar (fig. 1).

DISCUSSION

Hamburger and Kreier (1975, 1976a, b) studied neutralization by antibody of preparations of sonically freed plasmodia. They suggested that their results indicated action of antibody on free parasites and not parasitized erythrocytes. McAlister and Gordon (1977), however, showed that the infectivity of the preparations of free plasmodia prepared by sonication was probably due to contaminating, unlysed IRBCs. This observation cast doubt on the validity of the conclusions drawn from studies of action of antibody on preparations of sonically freed plasmodia.

The data of Hamburger and Kreier (1975, 1976a, b), however, clearly indicated that the infectivity of the free parasite preparations was susceptible to neutralization by antibody while infectivity of intact infected erythrocytes was not. As incubation in immune serum in this study had less effect on unsonicated infected erythrocytes than on infected erythrocytes exposed to sonic energy it is probable that the damage sustained by the erythrocytes during passage through the sonicator, while not lethal to the contained parasite, made their membranes permeable to antibody and thus permitted antibody action on the plasmodia. This study thus explains why the infectivity of the free parasite preparations used by Hamburger and Kreier (1975, 1976a, b) was susceptible to neutralization by antibody despite

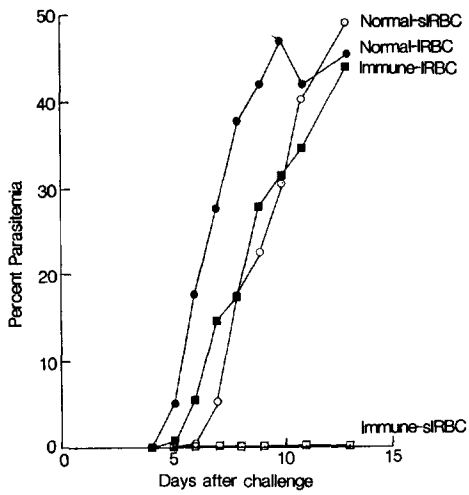

FIGURE 1. Percentage parasitemias in Swiss mice injected with 1×10^4 *Plasmodium berghei* infected erythrocytes. The infected erythrocytes had either been passed through a continuous flow sonication chamber (S-IRBC) or held for an equal time without exposure to sonic energy. Subsequently the sonicated or unsonicated infected erythrocytes were either incubated in normal serum (Normal-sIRBC; Normal-IRBC) or in serum from a rat immune to *P. berghei* (Immune-sIRBC; Immune-IRBC).

Infectivity of the sonicated and unsonicated infected erythrocytes incubated in normal serum was similar as was infectivity of unsonicated infected erythrocytes incubated in immune serum. Infectivity of sonicated infected erythrocytes on the other hand was abolished by incubation in immune serum. This indicates that the sonic treatment modifies the erythrocyte membrane in such a manner that the contained parasite becomes vulnerable to the action of the immune serum.

the fact that it was due to contaminating infected erythrocytes rather than the free parasites.

The concept that antibodies many act on plasmodia by passage thru a leaky erythrocyte membrane late in the development of the meront was advanced by Green et al. (1981). It is probable that sonic energy damages the erythrocyte membrane and allows access of antibody to the parasite earlier in its development than would normally occur, and this may be a useful model of an antibody permeable membrane.

The results of the present study, therefore, support the assumption that antiplasmodial antibody may act directly on plasmodia, in this case through damaged and permeable host cell membranes.

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