

Title : Attempted Immunologic Protection Against Plasmodium berghei in Newborn Mice by Immune Rat Spleen Cells

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Objectives P. berghei produces a fatal infection in adult and newborn mice, whereas adult rats usually survive. The mechanism of host defense in the rat is thought to be acquired immunity. The experiments described here were designed to determine whether immunity against malaria can be passively transferred from the rat to the mouse by the transplantation of spleen cells.

Description All animals used were random-bred albinos maintained in the animal colonies of SEATO Medical Research Laboratory. Because of the danger of runt disease an attempt was made to set up cross tolerance between groups of rats and mice. A litter of rats less than 18 hours old was given, by the intracardiac route, 0.8 million spleen cells from newborn mice. The Spleens were removed from the mice, ground in Hank's solution, and diluted so that 0.05 ml. contained 8×10^5 cells, predominantly lymphocytes. When the rats that had received the mouse spleen had grown they were exposed to P. berghei. When their peripheral smears had become negative their spleens were removed, ground and diluted in the same way as the mouse spleens, and under microscopic control, injected into the hearts of newborn mice (less than 18 hrs. old) using a fine glass needle. Two days later all of the surviving mice were given 1 million parasites (P. berghei) intraperitoneally. Control mice were not given the spleen suspensions but were given the same amount of parasites on the same day of life. The mice were followed with frequent peripheral blood smears and were autopsied upon death. All animals which survived five days after malaria parasites had been given and subsequently died were considered to have died of malaria. Portions of liver, spleen and serum were frozen from those animals which had received rat spleen. These frozen tissues were then cut on the cryostat, treated with fluorescein-conjugated chicken anti-rat serum and examined by fluorescent microscopy in an attempt to localize surviving rat cells. The serum was reacted on an Ouchterlony plate against the same antiserum unconjugated. Controls of normal rat and mouse serum were used.

Results The results are summarized in Table 1. The fluorescent-antibody studies show cells, appearing to be lymphocytes and plasma cells, coated with anti-rat serum. These have been few in number and scattered. So far those tissues which have been "blocked" by unconjugated anti-rat serum prior to staining with conjugated serum, have shown no such coated cells. Normal mouse tissue has also been negative while rat tissue is strongly positive. These studies are not yet finished.

Anti-rat serum which reacted on the Ouchterlony plate has shown no precipitate bands with the sera of these mice but does show such bands with the sera of rats.

None of the mice survived infection with P. berghei, but the shape of the survival curve (Fig. 1) suggests that there was some element of protection given to the mice by the rat spleen. A statistical analysis of these data is not yet complete. Fluorescent microscopy, so far, suggests that some of the rat cells survived in the mice but much more work remains to be done on this point.

Summary Data presented suggest that spleen cells transfused from immune mice offered some protection to newborn rats infected with a lethal dose of P. berghei. Fluorescent microscopy studies indicated that some rat cells survived in the mice.

Table 1. Survival of Mice Given Immune Rat Spleen Prior to Infection with P. Berghei

Group	No. of mice surviving for 5 days after malaria parasites given	No. surviving malaria	Percentage of survivors	Animals which died			
				No.	Range days survived	Average days survived	Median day
Controls	31	0	0	31	13-30	13	10
Mice with Immune Rat Spleen	56	0	0	56	8-38	14	12

Fig. 1. SURVIVAL OF NEWBORN MICE WITH P. BERGHEI

