

8. Title: P. falciparum in small rodents.

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OBJECTIVE

It has been reported that P. falciparum can be detected in the blood of the mouse as late as five days after intravenous injection of parasitized human blood.¹ If this observation could be repeated uniformly, the system would constitute an interesting and potentially very useful device for the study of the interaction of the parasite with antibody, drugs, and other materials which modify plasmodial growth and development. The objectives of the present study are to explore various regimens designed to retard the destruction of human erythrocytes in the mouse circulation and to determine the effect of such treatment on the persistence of P. falciparum in the circulation of the mouse.

DESCRIPTION

Attempts to induce immunological tolerance to human red cells in mice have been made by (a) injection of packed cells during the first 24 hours after birth and at weekly intervals thereafter for 12 weeks and by (b) a combined antigen-cyclophosphamide treatment² of adult mice.

Survival times of human erythrocytes in mice were estimated by the rate of disappearance of intravenously injected Cr-51 labelled cells.

P. falciparum infected blood was obtained from a patient admitted to Sri Raja Cross Hospital.

The persistence of P. falciparum in the mouse circulation was monitored by examination of thick and thin blood films. Provision for the detection of repenetration of erythrocytes by P. falciparum in the mouse was made by the admixture of type matched human fetal erythrocytes with the infectious inoculum and elution of hemoglobin A from the thin blood films by established techniques before staining with Giemsa's stain. The initially uninfected fetal cells can be distinguished microscopically from the adult human and mouse cells after this procedure.

Hemagglutinins were determined by standard techniques.

PROGRESS

Intravenous erythrocyte challenge of mice given human erythrocytes during the neonatal period resulted in almost immediate death of the animals. Hemagglutination titers on the sera of mice from this same group were as high as 1/65,000 whereas litter mates were negative (except for one mouse with a positive reaction at $\frac{1}{2}$). Therefore attempts to induce tolerance by this method were abandoned.

Simultaneous injection of human erythrocytes and the nitrogen mustard derivative cyclophosphamide according to the method of Aisenberg² gave more encouraging results. In a representative experiment the apparent half survival time of human erythrocytes in treated animals ranged from 300 to 900 minutes whereas the range in control animals was from 44 to 92 minutes. Although a great deal more work is required on the survival of normal human red cells in mice, we took advantage of the final phase of the

TABLE 1

Survival of P. falciparum in normal and human erythrocyte—
Cyclophosphamide (E_H - CY) treated mice (Thick Films).

Time after injection, hours	Ratios of the number of positive animals to the number studied	
	Normal mice	E_H - CY treated mice
23	10/10	9/9
40	4/10	9/9
47	2/10	5/9
64	0/10	1/9
70	0/10	0/9

malaria season and performed one experiment involving P. falciparum parasitized cells. The washed cells, in admixture with type matched fetal erythrocytes, were injected intravenously into normal and erythrocyte-cyclophosphamide treated animals and thick and thin films were prepared at various times thereafter. The results are shown in Table 1. It is evident that the treatment improved survival of the parasitized cells to only a limited extent. In addition, no fetal cells were found to be parasitized, thus making it difficult to determine whether or not repenetration of erythrocytes took place during the observation period. However, fetal cells were observed throughout the 70 hour observation period. Hemagglutination tests on sera collected one month after P. falciparum challenge demonstrated that immunological tolerance had been achieved; of the six tested animals in the control group, five were positive (two at 1/128) whereas all of the five tested cyclophosphamide treated animals were negative. It is encouraging that P. falciparum persists for so long even in normal animals. Renewed attempts to extend this period of survival through variations in the drug-antigen regimen and via other super imposed treatments are planned.

SUMMARY

A slight prolongation of survival of P. falciparum in the mouse was observed in animals made immunologically tolerant to human erythrocytes by cyclophosphamide treatment.

REFERENCES

- (1) Weinman, D., et al, 1966, Plasmodium falciparum in Mus musculus, Trans. Roy. Soc. Trop. Med. Hyg. 60, 562.
- (2) Aisenberg, A.C., 1967, Studies on Cyclophosphamide induced tolerance to sheep erythrocytes, J. Expt. Med. 125, 833-845.