AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D. C. 20523
BIBLIOGRAPHIC INPUT SHEET

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Batch 72

1. SUBJECT CLASSIFICATION
A. PRIMARY: Health — NS00-0000-0000
B. SECONDARY: Tropical diseases

2. TITLE AND SUBTITLE
Vaccination of mice against Plasmodium berghei malaria with heat inactivated parasitized blood

3. AUTHOR(S)
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4. DOCUMENT DATE: 1970
5. NUMBER OF PAGES: 14p.
6. ARC NUMBER: ARC

7. REFERENCE ORGANIZATION NAME AND ADDRESS
Ill.

8. SUPPLEMENTARY NOTES *(Sponsoring Organization, Publishers, Availability)*

9. ABSTRACT

10. CONTROL NUMBER
PN-RAB-708

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Malaria
Laboratory animals
Immunization

13. PROJECT NUMBER

14. CONTRACT NUMBER
CSD-1432 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

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CSD 1432 Res.

VACCINATION OF MICE AGAINST *PLASMODIUM BERGHEI* MALARIA WITH HEAT INACTIVATED PARASITIZED BLOOD*

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* This work supported by the United States Department of State, Agency for International Development, Contract AID/csd-1432.

ABSTRACT

Heat inactivated *Plasmodium berghei* infected blood acted as a vaccine against *P. berghei* infection in mice. The heat inactivated blood was non-infective. Intact or splenectomized vaccine treated mice, as well as *P. berghei* susceptible mice, subinoculated with whole body blood or homogenized spleens from vaccine treated animals, did not become infected. A/J, DDS and Carworth CF_1 mice were all protected against *P. berghei* challenge following vaccination. A/J and DDS mice developed good immunity following a single vaccine injection. Similar levels of immunity were obtained in CF_1 mice following at least two vaccine injections. Successfully vaccinated mice responded to *P. berghei* challenge with mild anemias and low level parasitemias. Resolution of infection occurred between the first and third weeks after challenge. Non-vaccinated mice developed progressively severe anemias and parasitemias during the same time period. The vaccine effects appear related to specific *P. berghei* antigens and could not be induced by homologous or heterologous non-infected red blood cells, *P. gallinaceum* infected blood or Freund's Complete Adjuvant.

Index Descriptors: Malaria vaccination, *P. berghei* vaccine, A/J mice, DDS mice, Carworth CF_1 mice, heat inactivated vaccine.

Systematic study of malaria vaccination is closely linked to the ready availability of effective vaccine materials and suitable laboratory animal models. The recently reported responsiveness of A/J mice to vaccination against Plasmodium berghei malaria (D'Antonio, et al., 1969b) presented a convenient model with which to investigate the vaccine character of various plasmodial preparations. Using the A/J mouse as a basic model, a comparative study of vaccination in other strains of mice became possible.

Many different blood stage plasmodial preparations have been investigated as vaccine materials in the past with varying degrees of success (Brown, 1969). These preparations have been obtained by treatment of infected blood by a variety of physical and chemical agents. Included among these have been x-irradiation, formalin fixation, various hemolytic agents, autolysis and temperature inactivation. The last, though offering one of the simplest of procedures, appears to have been the least investigated.

The following is a report on the vaccine properties of heat inactivated P. berghei. The character of protection induced in the A/J mouse model is compared to that induced in two other mouse strains.

MATERIALS AND METHODS

Mice

The highly inbred A/J[1] and DDS[2], and the outbred Carworth CF_1[3] mouse strains were used throughout.

Plasmodia

Plasmodium berghei strain NK65 (line D) was used as a source of antigen and for challenge of mice in protective trials. This line originated in our laboratories by mosquito passage through hamsters and has been continuously

1. Obtained from Jackson Laboratories, Bar Harbor, Maine.
2. Originally obtained from A. R. Schmidt, Co., Madison, Wisconsin.
3. Obtained from Carworth Farms, Inc., Portage, Michigan.

maintained in mice.

Preparation of Temperature Treated Blood

Approximately 40% infected *P. berghei* mouse blood was collected in Alsevers solution or heparin. The pooled blood was centrifuged at 1100g maximum for 10 minutes, and the buffy coat aspirated to remove the white blood cells. The packed erythrocytes were washed three times with isotonic phosphate buffered saline (PBS) (pH 7.0). Any residual buffy coat was aspirated after each wash.

The final washed packed cells were resuspended to 50% in PBS. Various lots of blood were incubated at different temperatures in a water bath for varying periods of time. The temperatures and times were 41°C for 1/2 to 4 hours, 45°C for 1/2 to 4 hours, 50°C for 10 minutes, and 56°C for 1/2 to 1 hour.

One lot of infected blood was collected and handled aseptically throughout.

Pooled non-infected control mouse blood was washed and heat treated as above.

Injection of Mice

All treated mice were injected intraperitoneally (IP) with the equivalent of 0.1 ml of packed infected blood or non-infected control blood respectively.

Respective groups of A/J, DDS and CF_1 mice were injected one time (Table IV). Separate groups of CF_1 mice were injected two to six times. Multiple injections varied from one every other day for six times to one each week for from two to six weeks (Table IV).

At the time of initial treatment the age of the mice varied from two weeks to 14 weeks.

Weekly tail blood smears were made for 5 weeks on all mice injected with heat inactivated *P. berghei* infected blood for detection of patent parasitemias.

Test for Infectivity of Heat Inactivated Blood

The spleens were removed from 5 mice treated with heat inactivated infected blood. The spleens were homogenized and injected IP into an equal number of mice susceptible to P. berghei. Likewise, whole body blood from a number of treated mice was injected into a group of mice susceptible to P. berghei.

Weekly tail blood smears for detection of patent parasitemias were made on each of the splenectomized and P. berghei susceptible mice for 5 weeks.

Challenge of Mice

At the time of challenge all mice were injected IP with 10×10^6 P. berghei infected mouse erythrocytes.

The variously treated mice and their respective groups of non-injected control mice were challenged 5 to 17 weeks after their initial treatment.

All challenged animals were followed by weekly examination of tail blood smears for determination of parasitemias.

Weekly erythrocyte counts were made on 10 of the singly and 10 of the multiply vaccinated CF_1 mice.

RESULTS

Infectivity of the Heat Inactivated Blood

Parasitemias did not appear in any of the mice treated with heat inactivated infected blood. No patent parasitemias were observed among any of the splenectomized mice or P. berghei susceptible mice first injected with the homogenized spleens or blood originating from animals previously injected with heat inactivated infected blood.

Profile of Infection in the Variously Treated Mice

All of the various heat inactivated infected blood preparations induced protection

to challenge with virulent P. berghei (Tables I to IV). The protected mice of each mouse strain tested had lower level parasitemias and less severe anemias than the unprotected ones. Parasitemias in the protected mice rarely exceeded a few percent.

Resolution of infection occurred in a number of protected animals by the end of the first week and in all protected animals by the end of third week after challenge. Occasional protected animals experienced mild transient recrudescenses of parasitemias for up to 12 weeks following challenge.

The average red blood cells counts (RBC) for the protected, singly injected CF_1 mice was $3 \times 10^6/mm^3$ by the end of the second week post challenge. The average RBC counts for the protected multiply vaccinated CF_1 mice was $6 \times 10^6/mm^3$ by the end of the same time period. All protected mice appeared vigorous and healthy after challenge. From 70% to 90% of the multiply vaccinated Carworth and singly vaccinated A/J and DDS mice were protected (Table IV). Approximately 20% of the Carworth mice receiving a single vaccine injection were protected (Table IV).

None of the non-injected mice or mice recieving heat treated normal mouse blood were protected to challenge. All unprotected animals developed progressively severe disease characterized by high parasitemias and severe anemias. By the end of the third week post challenge, parasitemias in the unprotected A/J, DDS and CF_1 mice often exceeded 30%. The RBC counts in the unprotected CF_1 mice dropped below $2 \times 10^6/mm^3$ by the end of the same period. Unprotected animals became wasted, pale and moribund after challenge.

There were no survivors after challenge among the unprotected DDS or CF_1 mice (Table IV). Approximately 35% of the unprotected A/J mice eventually recovered (Table IV).

DISCUSSION

The heat inactivated P. berghei was clearly protective and non-infective (Tables I to IV) (D'Antonio et al, 1969a). These findings are similar to those obtained by Gingrich (1941) who studied the vaccination effects of heat inactivated Plasmodium cathemerium in canaries.

The protection pattern against P. berghei in each mouse strain was essentially the same as that reported for A/J mice following vaccination with various isolated fractions of P. berghei (D'Antonio et al., 1969b, 1970).

Although none of the unprotected CF_1 or DDS mice survived challenge, a number of the A/J mice eventually recovered (Table IV) following severe protracted disease. Recoveries of this type in the A/J mouse appears to increase with increasing age (D'Antonio, et al. 1969b). Vaccination responsiveness in the A/J mouse, however, appears to be independent of age. The A/J mice vaccinated at two weeks of age were as effectively protected as those vaccinated much later.

The vaccination appears long lasting. A/J mice have remained resistant to P. berghei challenge up to one year after initial vaccination.

The level of protection and survival attained due to vaccination appears related to the vaccine dosage and schedule of administration. As indicated in Tables II and IV, not only did a greater number of the multiply vaccinated CF_1 mice survive P. berghei challenge as compared to the singly injected ones, but the levels of anemia in the multiply vaccinated ones was significantly less. The minimum number of heat inactivated P. berghei infected cells needed to induce protection is also not known. A/J mice have been protected with as few as 24×10^6 such cells.

The vaccine protection appears related to specific plasmodial antigens. Protection against P. berghei has not resulted following the administration of Plasmodium gallinaceum, homologous (Table IV) or heterologous erythrocytes, rat plasma (D'Antonio et al. 1969b, 1970) or Freund's Complete Adjuvant. Further, the induction of protection by the aseptically prepared heat inactivated infected blood indicates the absence of non-specific effects due to outside bacterial contaminants. Vaccination may not be strain specific, however, P. berghei NYU-2 vaccine has protected against P. berghei NK65D infection.

The course of infection following challenge of mice vaccinated in the manner described here may in part be determined by the predominantly reticulocyte infecting character of P. berghei NK65D. In contrast to the rapid progress of normocyte infecting P. berghei strains, the progress of the NK65D strain is much slower and parallels the rate of formation of reticulocytes. This slower, though still lethal progress, may give the vaccinated animal an opportunity to mobilize its primed but still meager defenses. No such opportunity is offered by the more fulminant P. berghei strains and, unless vaccination is sufficiently solid, the animal may be overwhelmed early in the disease.

Many of the paramaters related to optimum vaccination against the blood stage of malaria remain to be determined. The basic immunobiology and immunochemistry of malaria vaccination are still in their earliest stages. The mouse models and vaccine preparations described here promise to contribute much to these facets of malaria vaccine immunology and technology.

ACKNOWLEDGEMENTS

I thank Miss Donna Dagnillo, Mrs. Rosa Chang Fu, and Mr. David Rubin for their active participation and contributions to this work.

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TABLE I

Average Percent Parasitized Erythrocytes with Overall Ranges for All Mice Successfully Vaccinated Against and then Challenged with *P. berghei*

Weeks After Challenge	Mouse Strain A/J %	DDS %	Carworth %	Overall Range For A/J, DDS & Carworth
0	0	0	0	0
1	2	8	5	0-14
2	2	0	7	0-26
3	<1	0	<1	0-1
4	<1	<1	<1	0-3
5	<1	<1	2	0-4
6	1	-	<1	0-2
7	-	-	<1	0-<1
8	-	-	<1	0
9	<1	-	0	0-<1
Total No. of Mice	67	10	23	100

TABLE II

Average Percent Parasitized Erythrocytes and Ranges in All Non-vaccinated Mice Challenged with *P. berghei*

	Mouse Strain							
	A/J				DDS		Carworth	
Weeks After	Non-Survivors		Survivors					
Challenge	%	Range	%	Range	%	Range	%	Range
0	0	0	0	0	0	0	0	0
1	2	<1 - 5	<1	0 - 6	8	<1 - 9	4	<1 - 9
2	19	1 - 35	14	3 - 29	22	16 - 29	32	2 - 70
3	35	0 - 55	32	15 - 42	33	32 - 34	* 18	-
4	52	5 - 61	22	0 - 69	71	68 - 74	* 5	-
5	48	20 - 72	14	0 - 56		-	* 12	-
6		-	5	0 - 36		-	* 19	-
7		-		-		-		-
8		-		-		-		-
9	59	55 - 66	<1	0 - 3		-		-
Total Number of Mice	85		34		12		47	

* One animal

TABLE III

Average Erythrocyte Counts in Successfully Vaccinated and Non-Vaccinated Carworth Mice Following Challenge with *P. berghei*

Weeks After Challenge	Treatment: One Injection of Heat Inactivated Infected Blood RBC x $10^6/mm^3 \pm$ M.D.†	Three to Four Injections of Heat Inactivated Infected Blood RBC x $10^6/mm^3 \pm$ M. D.†	None or One to Three Injections of Non-Infected Incubated Blood RBC x $10^6/mm^3 \pm$ M. D.†
0	8.57 ± 1.16	9.06 ± 0.51	8.72 ± 0.79
1	3.07 ± 0	6.40 ± 0.86	4.68 ± 1.21
2	3.19 ± 1.31	7.04 ± 1.52	1.97 ± 0.68
3	5.63 ± 1.45	8.30 ± 0.92	* 1.36
4	6.57 ± 0.36	7.37 ± 0.83	* 1.72
5	6.85 ± 2.14	6.85 ± 1.13	* 0.94
6	6.81 ± 0.47	6.97 ± 1.29	* 0.75
7	6.98 ± 2.40	6.96 ± 1.20	
8	8.89 ± 1.64	9.70 ± 0.78	
9	8.12 ± 0.84	9.45 ± 0.85	
No. of Mice Protected			
Total	2/10	8/10	0/27

*One Animal

† Mean Deviation

TABLE IV

Survival of Mice Treated as Indicated and then Challenged with *P. berghei*

	No. Surviving/Total
I. Carworth CF_1 Mice	
Treatment	
A. One injection of heat inactivated infected blood.	2/10
B. Two to 6 injections of heat inactivated infected blood.	21/25
C. No treatment or 3 injections of heat treated non-infected blood.	0/27
II. DDS Mice	
Treatment	
A. One injection of heat treated infected blood.	10/12
B. No treatment.	0/10
III. A/J Mice	
Treatment	
A. One injection of heat inactivated infected blood.	67/73
B. No treatment or one injection of heat treated non-infected blood.	28*/85

* Eventually recovered following severe protracted parasitemias (see text and Table II).