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
Transfer of adoptive immunity to Plasmodium berghei,a comparison of routes of injection

3. AUTHOR(S)
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4. DOCUMENT DATE: 1970
5. NUMBER OF PAGES: 8p.
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-706

11. PRICE OF DOCUMENT

12. DESCRIPTORS
Malaria
Injection
Immunity

13. PROJECT NUMBER

14. CONTRACT NUMBER
CSD-1432 Res.

15. TYPE OF DOCUMENT

AID 590-1 (4-74)

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TRANSFER OF ADOPTIVE IMMUNITY TO PLASMODIUM BERGHEI:

A COMPARISON OF ROUTES OF INJECTION *

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* This work was supported by the Agency for International Development, United States Department of State, Contract No. AID/csd-1432

** Submitted in partial fulfillment for the requirements for the Ph. D. in Zoology

SYNOPSIS: The ability to protect rats against *Plasmodium berghei* by the transfer of spleen cells by various routes of injection was examined. Intraperitoneal and intravenous injections of cells taken from spleens of recovered rats were successful in transferring immunity. Intramuscular injection of the same material was unsuccessful. All injections were derived from the same pool of immune spleen cells and contained equal numbers of trypan blue excluding cells.

Investigations by Phillips (5), Roberts and Tracey-Patte (7), and Stechschulte (8) have shown that immunity to the blood phase of *Plasmodium berghei* can be induced by the injection of lymphoid cells from animals recovered from this infection. Immunity has been transferred by the injection of cells taken from the lymph nodes (7,8) and spleen (5,7,8). Lymphocytes collected from the thoracic duct (7,8) and the bone marrow (7) failed to transfer this immunity. Although thymocytes alone (7) failed to transfer protection, a mixture of lymph node, thymus and bone marrow cells (5) was effective. There is conflicting evidence in regard to peritoneal macrophage transfer. While Phillips (5) was successful in protecting rats against the *P. berghei* infection with macrophage transfer, Stechschulte (8) failed to do so.

Recent experiments by Brown et al (1,6) support the idea that the immunity to certain species of *Plasmodia* is cell-mediated rather than humoral. Protection was achieved by injecting the parasites, *P. knowlesi*, mixed with Freund's complete adjuvant (FCA) in monkeys. FCA is known to induce cellular immunity to certain antigens administered with it (4).

Previous lymphoid cells transfer experiments involving *P. berghei*-rat systems have used the intravenous (IV) route for the inoculation of cells. Although Phillips (5) has been successful in transferring immunity by intraperitoneal (IP) injection, there was no precise comparison with other routes of injection.

In addition to the fact that the IV route is time consuming, inexperienced personnel may inject clumps of cells or air bubbles in the veins of experimental animals resulting in the death of these animals.

Because of difficulties in using this route of injection, animals in the same experimental group may receive varying amounts of lymphoid cells. These difficulties stimulated our interest in finding another satisfactory route of injection for the P. berghei-Lewis rat system.

MATERIALS AND METHODS

Experiment I

The NK65D clone (population) of P. berghei is kept in our laboratory by [illegible] and rat blood transfer (at 3 and 5 day intervals respectively) or in glycerinated blood stored in liquid nitrogen (-270 C). Seven week old inbred Lewis rats (105 ± 7 g) were injected IP with 1×10^6 erythrocytes (RBC's) infected with the NK65D clone and left to recover spontaneously. Three weeks after these became negative, by blood smear examination, the animals were exsanguinated and their spleens removed. The spleens were cut into small pieces and placed in cold Hank's balanced salt solution (HBS). Tissue impressions were made of all the spleens taken from the immune animals (immune spleens). These impressions were fixed in absolute methanol and stained with Giemsa's. After careful microscopical observation it was found that none of the spleens showed any free parasites or parasitized RBC's although malarial pigment was observed.

Pieces of these spleens were pushed through a 60 mesh copper screen. The cells in the suspension obtained from this procedure were washed in cold HBS 3x and then counted in a haemocytometer. Only viable cells, as determined by the trypan blue exclusion test, were counted. (RBC's were not included in the counts.)

Normal spleens were also obtained from inbred Lewis rats of the same age as the immune spleen donors. The procedure for the isolation

of the normal cells was the same as the one used to isolate the cells from the immune spleens.

Thirteen groups of 6-7 week old rats (83 ± 15 grams) were used as recipients of 10×10^6 spleen cells (immune or normal) in a total volume of .5 cc. All rats were challenged four days later with 1×10^6 P. berghei infected RBC's The experimental design is shown in Table I.

Experiment II

The animals used in this experiment were similar to the ones used in the previous experiment. The experimental design is presented in Table II.

Blood films were made from all the rats every other day. These smears were fixed and stained, and the percent of cells parasitized was recorded.

RESULTS

Experiment I

The individual peak parasitemias ranged from less than 1 to 64%. Any animal presenting a peak parasitemia of 5% or less was considered immune to the blood phase of P. berghei. Rats with a larger percentage of parasitized RBC's were considered non-immune. The experimental design and results are expressed in Table I.

The average peak parasitemia of all the control groups was 33% with a range from 22 to 43%. The difference between control groups was not statistically significant (all statistics were done by analysis of variance and Duncan's multiple range test at the 1% level of significance). All the animals injected IV showed an average peak parasitemia of only 1.5%. The groups of rats injected intramuscularly (IM) with immune spleen cells showed an average peak parasitemia of 21%. The difference

between the IP injected group and the control groups was not significant. The 6 immune rats that received the IP injections of immune spleen cells showed a significantly lower average peak parasitemia (1.1%) than any of the controls. The rats injected IP with immune spleen cells that did not show immunity to the challenging dose of P. berghei, had a peak parasitemia no different than the control rats (30%).

Experiment II

In order to demonstrate that a low grade parasitemia does not protect the animal against a higher challenging dose, Experiment II was carried out. Table II gives the average peak parasitemia of the groups used. None of the groups showed any degree of protection against the challenging dose. Some delay of the prepatent period was detected as expected. This delay has been demonstrated by many researchers on different strains and species of Plasmodia (2,3,9,10,11), and is inversely proportional to the size of the innoculum.

DISCUSSION

Immunity to the blood phase of P. berghei was transferred by both IP and IV injections of spleen cells. Since the IP route of injection is an easier, more consistent way of introducing material into animals, it can be used in subsequent experiments of this type. At least 6 animals should be used in each group in order to have a good statistical comparison.

The failure to transfer immunity by way of the IM route could be due to a time factor. Perhaps the immune cells had no time to migrate to the target organ(s) in the four days allowed. It is possible that if a larger interval is allowed between the time of injection of the immune cells and the day of challenge, a better result could be obtained with the IM route.

TABLE I

EXPERIMENTAL DESIGN AND RESULTS FOR EXPERIMENT I

			MATERIAL INJECTED			
Group	Route of Injection	Route of Challenge	Immune Spleen Cells	Normal Spleen Cells	Hank's BSS	None
1	--	IP	--	--	--	0/6
2	IV	IP	6/6*	0/6	0/6	--
3	IP	IP	5/6	0/6	0/6	--
4	IP	IV	4/6	0/6	0/6	--
5	IM	IP	0/6	0/6	0/6	--

* Number of immune animals per number of animals in the group.

TABLE II

EXPERIMENTAL DESIGN AND RESULTS FOR EXPERIMENT II

Group	No. Animals	Inoculum Injected On Day 0	Inoculum Injected On Day 5	Average Peak Parasitemia
A	6	$1 \times 10^{6*}$	$1 \times 10^{6*}$	51.3
	6	1×10^{6}	None	51.8
B	6	1×10^{4}	1×10^{6}	48.5
	6	1×10^{4}	None	41.2
C	6	1×10^{2}	1×10^{6}	41.8
	6	1×10^{2}	None	41.0
D	6	None	1×10^{6}	40.0

* Number of parasitized RBC's injected IP

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