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D'Antonio,L.E.; Spira,D.T.; Silverman,P.H.

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Model System for Study of Artificially Induced Resistance to Malaria

THERE is still a pressing need for a vaccination model against malarial infection[1]. The laboratory rodent has been vigorously studied for this purpose since the discovery of *Plasmodium berghei* more than 20 yr ago[2]. Of recent reports describing various methods for the induction of resistance to malaria[3–5], none have reported good success utilizing non-viable blood stage plasmodial material. The rat has been only partially responsive to such material, while the mouse has remained uniformly refractory[6].

We have recently observed the induction of a high level of resistance against *P. berghei* in A/J mice after injection of either solubilized or nonsolubilized blood stage plasmodial materials. This system seems to present an ideal model for further study of malaria vaccination.

A/J mice were used throughout, obtained either from our laboratory bred stock or from Jackson Laboratories, Bar Harbor, Maine. *P. berghei* strains NK65 (line D) or NYU–2 served as protective and infective materials. The NK65D line originated in our laboratories by mosquito passage through hamsters and was maintained in young Sprague–Dawley rats. The NYU–2 strain was continuously maintained in mice.

A modified version of the method of L. E. D'A. *et al.*[7] was used to prepare plasmodial materials. A 20 per cent suspension of pooled, washed parasitized hamster erythrocytes was passed through a French pressure cell (FPC) at 1,000 to 1,200 pounds per square inch. Isotonic saline or phosphate buffered saline (PBS) served as the processing diluent.

The FPC effluent was freed of unbroken erythrocytes by centrifuging twice in Kolmer tubes at 1,100*g* maximum for 5 min each time. The haemoglobin supernatant (preparation *A*) containing solubilized erythrocyte and plasmodial components and free parasites and parasite fragments was centrifuged for 10 min at 11,739 g maximum at 4° C. The free parasite containing sediment was washed three times and finally resuspended in 7–10 volumes of diluent (preparation *B*).

The plasmodial fragments remaining in the haemoglobin supernatant were sedimented by centrifugation at 105,650*g* maximum for 20 min at 4° C. This sediment was surface washed and resuspended as before (preparation *C*). The final haemoglobin supernatant (preparation *D*)

Table 1. WEEKLY COMPOSITE ERYTHROCYTE COUNTS AND SURVIVALS OF PROTECTED AND UNPROTECTED ANIMALS

Week after challenge	Protected: Surviving/total No.	Protected: Surviving/total Per cent	Protected: Erythrocyte counts $10^6/mm^3 \pm$ mean deviation, Surviving	Protected: Erythrocyte counts $10^6/mm^3 \pm$ mean deviation, Not surviving	Unprotected: Surviving/total No.	Unprotected: Surviving/total Per cent	Unprotected: Erythrocyte counts $10^6/mm^3 \pm$ mean deviation, Surviving	Unprotected: Erythrocyte counts $10^6/mm^3 \pm$ mean deviation, Not surviving
0	39/39	100	8·91 ± 0·46	8·17 ± 0·42	73/73	100	8·29 ± 0·65	8·37 ± 0·82
1	39/39	100	6·31 ± 0·62	5·23 ± 0·56	73/73	100	6·22 ± 0·38	5·79 ± 0·62
2	37/39	95	4·79 ± 0·84	2·41 ± 0·00	67/73	92	3·40 ± 0·60	2·88 ± 0·52
3	36/39	92	6·33 ± 0·87		58/73	78	1·70 ± 0·29	1·72 ± 0·38
4	35/39	90	6·94 ± 0·64		53/73	73	1·83 ± 0·51	1·58 ± 0·42
5	35/39	90	6·80 ± 0·81		43/73	59	2·22 ± 0·70	1·37 ± 0·36
6	35/39	90	7·59 ± 0·97		35/73	49	3·79 ± 1·54	1·51 ± 0·25
7	35/39	90	8·29 ± 0·81		33/73	45	5·18 ± 2·27	1·63 ± 0·29
8	35/39	90	7·69 ± 1·06		27/73	37	6·21 ± 1·84	1·42 ± 0·22
9	35/39	90	7·63 ± 1·03		22/73	30	6·45 ± 1·83	1·55 ± 0·44
10	35/39	90	7·73 ± 0·92		19/73	26	6·49 ± 2·23	
11	35/39	90	8·11 ± 0·63		18/73	25	5·76 ± 2·19	
12	35/39	90			18/73	25	8·15 ± 0·61	

contained solubilized erythrocyte and plasmodial components.

The respective preparations were then injected intraperitoneally (IP) into their respective groups of mice as follows: 1·00 ml. of *A*; 0·15 ml. of *B*; 0·30 ml. of *C*; 1·00 ml. of *D*. Mice in these groups were between 4 and 28 weeks old. These preparations were non-infective. Occasional infections have occurred, however, after injection of some lots of *A* or *B*.

Similar groups of control mice were either not injected or injected with one of the following: 1·0 ml. of FPC disintegrated normal hamster red blood cells prepared as *D*; 0·1 ml. of packed washed normal rat red blood cells kept at 45° C for 45 min or 0·1 ml. of a suspension of material sedimented at 225,000*g* maximum for 15 h from *P. berghei*-infected rat plasma.

Animals from each group were challenged either intravenously (IV) or intraperitoneally with 10×10^3 *P. berghei*-infected mouse cells. The NK65D challenge cells were obtained by passage into mice from infected rat donors; the NYU-2 from the continuously passed mouse line.

Animals receiving preparations *A*, *B* and *C* with their respective controls were challenged as early as 6 weeks and those receiving preparation *D*, as well as their controls, 14 weeks post-inoculation.

Composite results obtained after challenge of the various groups are given in Tables 2 and 3. Animals injected with preparations *A*, *B*, *C* or *D* experienced mild transient anaemias and parasitaemias. Their disease began to resolve by the end of the second week after challenge. Thirty-five out of thirty-nine totally recovered. The course of infection was similar in all protected groups independent of route of infection, challenge strain or its immediate origin, plasmodial preparation given, origin or age of the mouse at the time of protection or challenge.

Table 2. WEEKLY COMPOSITE PERCENTAGE PARASITIZED ERYTHROCYTES AND RANGES OF PROTECTED AND UNPROTECTED ANIMALS

Weeks after challenge	Protected				Unprotected			
	Surviving		Not surviving		Surviving		Not surviving	
	%	Range	%	Range	%	Range	%	Range
0	0	0	0	0	0	0	0	0
1	1·7	0–5·6	3·7	0·0–8·6	2·7	0·5–18·6	2·3	<0·4–0·8
2	2·4	0–27·0	12·7	8·7–17·2	18·6	3·0–37·2	18·9	5·5–44·0
3	<0·1	0–0·0	20·4	20·4	41·2	22·6–56·3	37·1	17·4–56·3
4	0·2	0–3·1			38·5	4·4–68·6	37·0	11·0–67·5
5	0·2	0–2·6			32·1	0·0–60·9	52·7	25·6–77·6
6	<0·1	0–0·7			11·8	0·0–44·8	52·3	30·1–70·5
7	<0·1	0–0·4			13·8	0·0–78·5	52·5	23·6–74·6
8	0	0			4·3	0·0–35·2	54·6	19·5–69·5
9	<0·1	0–0·0			4·8	0·0–61·9	61·9	46·2–79·5
10	0	0			14·2	0·0–58·9		
11	0	0			3·2	0·0–12·7		
12	0	0			0	0		

Table 5. AGE, PARASITE STRAIN AND PREVIOUS TREATMENT INFLUENCE ON SURVIVAL OF UNPROTECTED AND PROTECTED ANIMALS

	Non-protected survivors/total	Protected survivors/total
(1) Age at time of challenge		
(*a*) Less than 5 months	0/8	10/11
(*b*) 5 months or more	18/65	25/28
(2) Challenge strain of *P. berghei*		
(*a*) NYU-2	11/42	11/13
(*b*) NK65D	7/31	24/26
(3) Previous treatment		
(*a*) Solubilized normal hamster red blood cells	4/17	
(*b*) Normal rat red blood cells	2/8	
(*c*) Infected rat plasma components	3/8	
(*d*) None	9/40	
(*e*) Preparation *A*, French press effluent of infected red blood cells		10/11
(*f*) Preparation *B*, washed free parasites		11/12
(*g*) Preparation *C*, sedimented plasmodial fragments		4/5
(*h*) Preparation *D*, solubilized infected red blood cells		10/11

All control animals experienced profiles of severe persistent anaemias and parasitaemias. All mice younger than 5 months succumbed within 28 days. Those 5 months or older succumbed at any time from the second to the eleventh week. Eighteen of seventy-three control animals had recovered by the end of the eleventh week. Recovery began slowly from the fourth week. The course of infection in animals in the various control groups was similar to and independent of that with the previous treatment. In contrast to the healthy appearance of the surviving protected animals, all non-protected animals looked wasted at the peak of their disease. One group of unprotected mice at that time averaged 14·5 g; a similar protected group averaged 21·4 g.

The A/J mouse seems to be particularly responsive to these protective procedures, although preliminary experiments indicate that Carworth mice will also respond with higher and possibly multiple doses. The protection seems to be induced by the presence of plasmodial components. The absence of protection in animals treated with the preparations not containing plasmodia seems to rule out non-specific effects caused by foreign materials or specific host contaminants. In addition, preparations such as *B*, known to have little if any host contaminants[7], still induced protection. Also preparation *D* obtained homologously from infected mouse erythrocytes had protective properties. The method of preparation and handling of the non-viable protective plasmodial materials reported here seems crucial[6]. Finally, there was no evidence of other disease in our mice which might account for the results obtained.

The special responsiveness of the A/J mouse might be related to its unusual and unexpected reaction to *P.*

berghei infection. It seems to have a degree of age resistance after 5 months. This is reflected in prolonged median survivals as well as in the spontaneous recovery of at least 25 per cent after severe protracted disease. The older mice have also experienced up to 80 per cent recoveries following severe infection induced by FPC free parasite preparations still contaminated with viable infective cells. This contrasts with the behaviour of younger mice. In more than 100 similarly studied, 100 per cent died within 4 weeks.

This protective system with its special behavioural characteristics and striking responsiveness to induction of resistance seems to offer an excellent model for further understanding the mechanisms of induced protection, as well as opening the way for isolation and characterization of malarial vaccine materials.

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LAWRENCE E. D'ANTONIO
DAN T. SPIRA*
PAUL H. SILVERMAN

Department of Zoology,
University of Illinois,
Urbana, Illinois 61801.

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* Permanent address: Hebrew University, Hadassah Medical School, Jerusalem.

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