A para-aminobenzoic acid-deficient diet protects mice from severe malaria and subsequent parasite exposure

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Expt1

pABA(-) diet

	4	7	10	14	16	21	dpi
m1_1(-)	0.63	0.25	0.09	0.08	0.10	0.14	
m1_2(-)	0.96	0.74	1.01	0.08	0.00	0.00	
m1_3(-)	1.21	3.38	8.11	1.83	0.21	0.01	
m1_4(-)	1.37	2.80	5.36	2.07	0.64	0.02	
m1_5(-)	1.44	4.36	6.82	33.06	31.28	0.00	
m1_6(-)	1.35	1.09	4.81	6.72	0.28	0.00	
m1_7(-)	1.87	3.87	6.89	9.60	1.79	0.01	
m1_8(-)	1.88	3.50	5.41	44.93	30.82	0.06	
m1_9(-)	0.79	1.97	3.49	4.04	0.64	0.01	

standard diet

	4	7	10	14	16	dpi
m1_1(s)	0.01	7.60				
m1_2(s)	0.65	3.70	5.80			
m1_3(s)	0.00	2.00	40.00			
m1_4(s)	0.01	0.14	24.88			
m1_5(s)	0.50	2.50	12.00	51.00		
m1_6(s)	0.80	3.50	4.30	28.60		
m1_7(s)	1.00	2.30	14.40	31.30		
m1_8(s)	4.77	5.50	17.08	43.76		
m1_9(s)	0.00	1.29	8.49	23.00	32.00	

pABA(+) diet

	4	7	10	14
m1_1(+)	0.01	5.00		
m1_2(+)	0.50	6.20		
m1_3(+)	1.38	4.97	19.81	
m1_4(+)	1.31	3.61	18.32	
m1_5(+)	2.55	4.26	28.80	
m1_6(+)	0.80	1.50	20.60	23.00
m1_7(+)	1.10	3.20	31.40	35.00
m1_8(+)	2.45	5.60	10.88	38.98

Expt2

pABA(-) diet

	4	7	9	14	16	21	36	42	dpi
m2_1(-)	3.37	4	15.28	23.53	29.66	0.04	0	0	
m2_2(-)	5.31	5.4	24.59	29.32	43.99	0.75	0	0	
m2_3(-)	7.76	5.77	21.75	32.79	37.21	5.99	25.87	0	
m2_4(-)	3.34	3	8.08	31.2	12.83	1.21	0.01	0	
m2_5(-)	3.4	3.68	13.04	57.76	32.16	0.03	0.02	0	
m2_6(-)	4.22	1.5	3.49	22.61	1.91	0.01	0.16	0	
m2_7(-)	4.6	1.12	1.68	4.41	0.99	18.45	22.92	0	
m2_8(-)	5.07	2.26	1.05	0.11	0.06	0.12	0.01	0	
m2_9(-)	8.29	5.68	8.12	0.97	0.34	0.07	0	0	

Expt3

first inoculum

	4	7	9	11	14	16	21	25	dpi
m3_1(-)	0.02	0.01	0.02	0.11	5.05	1.32	0.01	0	
m3_2(-)	0.02	0.01	0.01	0.02	3.39	0.34	0	0	
m3_3(-)	0.04	0.01	0.04	0.13	3.87	1.56	0	0	
m3_4(-)	0.18	0.74	1.29	2.43	20.56	1.61	0	0	
m3_5(-)	0.19	1.44	3.99	4.7	12.64	6.86	0	0	
m3_6(-)	0.02	0.03	0	0.02	4.08	4.65	0.08	0	
m3_7(-)	0.02	0.01	0	0	0.93	4.77	0.57	0.19	
m3_8(-)	0.08	0.34	1.42	3.49	14.42	20.65	0	0	
m3_9(-)	0.2	1.79	3.31	6.86 (×)					
m3_10(-)	0.22	2.35	4.05	3.83 (×)					

pABA(-) diet
ELISA OD_{450}

2 days before second inoculum

1:1000	1:5000	1:25000
2.72	1.44	0.42
1.24	0.41	0.12
3.09	1.40	0.38
3.05	1.36	0.42
1.58	0.47	0.11
1.23	0.34	0.09
0.28	0.07	0.03
3.40	1.78	0.46

c1	2.30	0.99	0.30
c2	2.03	0.69	0.17

second inoculum after 10 months

3	7	9	14	17	dpi
0.56 (×)					
0.57 (×)					
0.39	7.95	8.85	0.2	0.14	
0.02	1.07	0.06	0	0	
0.16	10.24	6.99	0	0	
0.55	9.34	0.82	0	0	
0.26	2.95	0.87	1.11	0.03	
0.09	0.1	0.01	0	0	

pABA(+) diet

	4	7	9	10	dpi
m3_1(+)	2.82				
m3_3(+)	1.27	14.51			
m3_4(+)	2.47	5.29	18.86		
m3_5(+)	4.88	8.04	37.22		
m3_6(+)	2.36	6.8	20.19	36.93	

Expt4

pABA(-) diet
ELISA OD_{450}

first inoculum

	4	7	10	14	21	28
m4_1(-)	0.06	0.06	0.20	0.06	0.01	0.01
m4_2(-)	0.20	2.45	10.34	4.00	0.00	0.02
m4_3(-)	0.20	1.91	10.17	0.60	0.10	0.00
m4_4(-)	0.12	0.70	4.06	8.06	0.05	0.00
m4_5(-)	0.15	3.32	6.61	37.35	0.03	0.01
m4_6(-)	0.08	0.09	0.16	0.26	0.00	0.00
m4_7(-)	0.44	3.57	13.70	44.05	0.01	0.22
m4_8(-)	0.01	0.01	0.01	0.06	0.02	0.00
m4_9(-)	0.03	0.01	0.01	0.20	2.54	0.00

2 days before second inoculum

1:1000	1:5000	1:25000
1.75	0.69	0.18
2.04	0.80	0.20
1.80	0.55	0.14
3.01	2.12	1.09
1.70	0.54	0.13
1.73	0.63	0.18
2.26	1.13	0.39
3.38	2.98	1.51
0.27	0.09	0.04

c1	3.21	2	0.57
c2	2.97	1.5	0.46

second inoculum after 3 months

3	5	7	10	dpi
0	0.48	0.01	0	
0.06	0.19	0.03	0	
0.29	2.73	0.08	0	
0.01	0	0	0	
0.11	0	0.01	0	
0.22	0.01	0	0	
0	0	0	0	
0	0	0	0	
0.75	3.47	0.03	0.01	

pABA(+) diet

	4	7	10	14	dpi
m4_1(+)	0.10	8.50			
m4_2(+)	3.07	6.14			
m4_3(+)	0.60	2.40	12.70		
m4_4(+)	0.40	2.90	3.80		
m4_5(+)	1.79	3.95	11.37		
m4_6(+)	3.16	4.75	14.56		
m4_7(+)	1.10	3.10	10.00		
m4_8(+)	1.80	3.70	18.40		
m4_9(+)	1.36	5.97	3.87	7.13	

Table S1. parasitemia values in mice fed a pABA-deficient diet pABA(-) and pABA supplemented diet pABA(+)
Experiments are numbered (Expt1-4); the labels in each experiment indicate the mouse(m), the experiment (1-4) and the mouse number (1-10)
Parasitemia values for each mouse are reported at different days post-infection (dpi).
For each pABA(−) mouse, the maximum parasitemia value during the first infection is highlighted in a different color according to the day on which the peak occurs. (day 10: yellow; day 14: light blue; day 16: green; day 21: brown). For pABA(+) mice, gray-shaded cells indicate the day of death.
Mice in Experiments 3 and 4 received two parasite inoculations with the second inoculation administered 10 months (Expt 3) and 3 months (Expt 4) after the first.
For Expt3 and 4, ELISA results (optical density at 450 nm, OD450) obtained at three different serum dilutions are reported.
Serum samples were collected two days before the second parasite inoculation. ELISA results from two hyperimmune mice (c1 and c2) are also included for comparison.

A

pABA(-) diet

mice	day 4	day 7	day 9	day 10	day 11	day 14	day 16	day 21	day 25	day 28
m1_2	0.96	0.74	0.87	1.01	0.54	0.08	0.00	0.00	nd	nd
m4_1	0.06	0.06	0.13	0.2	0.13	0.1	0.04	0.01	0.01	0.01
m4_2	0.2	2.45	6.40	10.3	7.17	4.0	2.00	0	0.01	0.02
m4_3	0.2	1.91	6.04	10.2	5.39	0.6	0.35	0.1	0.05	0.00
m1_3	1.21	3.38	5.74	8.11	4.97	1.83	0.21	0.01	nd	nd
m1_4	1.37	2.80	4.08	5.36	3.71	2.07	0.64	0.02	nd	nd
m1_5	1.44	4.36	5.59	6.82	19.94	33.06	31.28	0.00	nd	nd
m1_6	1.35	1.09	2.95	4.81	5.76	6.72	0.28	0.00	nd	nd
m1_7	1.87	3.87	5.38	6.89	8.24	9.60	1.79	0.01	nd	nd
m1_8	1.88	3.50	4.46	5.41	25.17	44.93	30.82	0.06	nd	nd
m1_9	0.79	1.97	2.73	3.49	3.76	4.04	0.64	0.01	nd	nd
m4_4	0.12	0.7	2.38	4.1	6.06	8.1	4.06	0.05	0.03	0.00
m4_5	0.15	3.32	4.97	6.6	21.98	37.4	18.69	0.03	0.02	0.01
m4_6	0.08	0.09	0.13	0.2	0.21	0.3	0.13	0	0.00	0.00
m4_7	0.44	3.57	8.64	13.7	28.88	44.1	22.03	0.01	0.12	0.22
m4_8	0.01	0.01	0.01	0.01	0.04	0.06	0.04	0.02	0.01	0.00
m3_1	0.02	0.01	0.02	0.07	0.11	5.05	1.32	0.01	0	nd
m3_2	0.02	0.01	0.01	0.02	0.02	3.39	0.34	0	0	nd
m3_3	0.04	0.01	0.04	0.09	0.13	3.87	1.56	0	0	nd
m3_4	0.18	0.74	1.29	1.86	2.43	20.56	1.61	0	0	nd
m3_5	0.19	1.44	3.99	4.35	4.7	12.64	6.86	0	0	nd
m2_4	3.34	3	8.08	8.08	19.64	31.2	12.83	1.21	nd	nd
m2_5	3.4	3.68	13.04	13.04	35.40	57.76	32.16	0.03	nd	nd
m2_6	4.22	1.5	3.49	3.49	13.05	22.61	1.91	0.01	nd	nd
m3_6	0.02	0.03	0	0.01	0.02	4.08	4.65	0.08	0	nd
m3_7	0.02	0.01	0	0.00	0	0.93	4.77	0.57	0.19	nd
m3_8	0.08	0.34	1.42	2.46	3.49	14.42	20.65	0	0	nd
m2_1	3.37	4	15.28	15.28	19.41	23.53	29.66	0.04	nd	nd
m2_2	5.31	5.4	24.59	24.59	26.96	29.32	43.99	0.75	nd	nd
m2_3	7.76	5.77	21.75	21.75	27.27	32.79	37.21	5.99	nd	nd

B

m2_7	4.6	1.12	1.68	nd	nd	4.41	0.99	18.45	nd	nd
m4_9	0.03	0.01	0.01	0.01	0.11	0.20	1.37	2.54	1.27	0.00
m1_1	0.63	0.25	0.17	0.09	0.09	0.08	0.10	0.14	nd	nd
m2_8	5.07	2.26	1.05	0.58	0.11	0.11	0.06	0.12	nd	nd
m2_9	8.29	5.68	8.12	4.55	0.97	0.97	0.34	0.07	nd	nd
m3_9	0.2	1.79	3.31	5.08	6.86×					
m3_10	0.22	2.35	4.05	3.94	3.83×					

C

pABA(+) diet

mice	day 4	day 7	day 9	day 10	day 11	day 14
m3_1	2.82					
m1_1	0.01	5.00				
m1_2	0.50	6.20				
m3_3	1.27	14.51				
m4_1	0.10	8.50				
m4_2	3.07	6.14				
m3_4	2.47	5.29	18.86			
m3_5	4.88	8.04	37.22			
m3_6	2.36	6.80	20.19	36.93		
m1_3	1.38	4.97	12.39	19.81		
m1_4	1.31	3.61	10.97	18.32		
m1_5	2.55	4.26	16.53	28.80		
m4_3	0.60	2.40	7.55	12.70		
m4_4	0.40	2.90	3.35	3.80		
m4_5	1.79	3.95	7.66	11.37		
m4_6	3.16	4.75	9.66	14.56		
m4_7	1.10	3.10	6.55	10.00		
m4_8	1.80	3.70	11.05	18.40		
m1_6	0.80	1.50	11.05	20.60	21.80	23.00
m1_7	1.10	3.20	17.30	31.40	33.20	35.00
m1_8	2.45	5.60	8.24	10.88	24.93	38.98
m4_9	1.36	5.97	4.92	3.87	5.50	7.13

TableS2. Aggregated parasitemia values of mice fed a pABA-deficent diet and pABA-supplemented diet
Table A. Data from Expt 1-4 were combined into a single table using a common days post-infection (dpi) scale.
Missing values were estimated by linear interpolation between adjacent measurements.
pABA(+) mice were grouped according to the day of peak parasitemia, as indicated by the colored cells
(day 10: yellow; day 14: light blue; day 16: green).
Table B. Data from mice that could not be assigned to any of the defined groups.
Table C. pABA(-) mice from Expt1-4 were grouped according the day of death

	Expt2 day 9	Expt1 day 11	Expt1 day 16	Expt2 day 16	Expt3 day 23
ELISA OD_{450} (Ev)	0.06	0.31	0.53	0.90	2.39
	0.18	0.47	1.00	0.99	1.97
	0.26	0.17	0.82	0.96	2.65
	0.30	0.12	0.69	1.12	2.10
	0.00	0.20	0.52	0.61	1.85
	0.24	0.35	0.67	0.79	2.49
	0.06	0.29	0.50	0.81	2.18
	0.03	0.13	0.68	0.55	2.20
	0.05	0.21	0.78	0.82	
c1	1.03	1.02	1.02	1.03	3.19
c2	1.16	1.13	1.13	1.16	2.66
mean value (mc)	1.10	1.08	1.08	1.10	2.93
Ev/mc	0.05	0.28	0.48	0.82	0.82
	0.16	0.43	0.91	0.90	0.68
	0.24	0.15	0.75	0.87	0.91
	0.27	0.11	0.63	1.02	0.72
	0.00	0.18	0.47	0.55	0.64
	0.22	0.32	0.61	0.72	0.86
	0.05	0.26	0.45	0.74	0.75
	0.03	0.12	0.62	0.50	0.76
	0.05	0.19	0.71	0.75	
mean values	0.12	0.23	0.69		0.77
standard deviations	0.03	0.03	0.05		0.03

Table S3. ELISA values (Ev) of mice sera at day 9, 11, 16, 23 post infection (1:1000 dilution)
ELISA data from serum samples of mice from Expt1-3 are reported,
together with those from two hyperimmune mice used as controls (c1 and c2).
The mean ELISA values (mc) of the control mice were used to normalize individual ELISA values
The mean and standard deviation of the normalized values were also calculated.

(Ev/mc).