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

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Abstract

Para-aminobenzoic acid (pABA) is a key precursor in folic acid biosynthesis. The malaria parasite can either synthesize or scavenge it from the environment. We examined the progression of blood-stage infection by *Plasmodium berghei* in mice fed either a pABA supplemented or pABA deficient diet. Parasite multiplication rates were similar between the two groups of mice. However, mice on a pABA-supplemented diet developed severe malaria, whereas those on a pABA-deficient diet recovered, showing a 95% survival rate. CD8 + T-cells infiltrating the brain play a key role in disrupting the blood-brain barrier and triggering neurological simptoms in severe malaria. The abundance of this T-cell population varied significantly between the groups: the CD8 + to CD4 + ratio was approximately 5:1 in mice on a pABA-supplemented diet, compared to nearly 1:1 in the pABA-deficient group. Furthermore, a pABA-restricted diet increased erythrocyte membrane instability, making red blood cells more prone to echinocyte formation *in vitro*, which could lead to a more efficient clearance of infected erythrocytes by the spleen, suggesting a role for pABA distinct from its function in folate metabolism. Importantly, recovered mice developed long-term immunity that protected them from subsequent parasite exposure.

Introduction

Malaria parasites replicate asexually inside the red blood cells of a mammalian host. The rapidly replicating blood stages require large amounts of folate and its precursors, which are scavenged or de novo synthesized[12]. In contrast, mammalian hosts are auxotrophic for folate and must obtain it from their environment. Folates consist of three core chemical moieties: a pteridine ring, a para-aminobenzoic acid (pABA) bridge and a and a glutamate tail.

The *Plasmodium* genome encodes the putative aminodeoxychorismate synthase (ADCS) and aminodeoxychorismate lyase (ADCL), which are predicted to convert chorismate into para-aminobenzoate (pABA) in a two-step reaction[345]. Biochemical characterization of the *P. falciparum* enzyme confirmed that PF14_0557 encodes a functional ADCL[3]. The role of both enzymes in pABA biosynthesis during infection was subsequently demonstrated in the blood stages of the rodent parasite *P. berghei* [6]. Parasites defective in ADCS or ADCL propagate normally in mice fed a standard diet, but exhibit severe growth arrest under pABA-restricted dietary conditions, which can be fully rescued by pABA supplementation. Under standard dietary conditions, when host-derived pABA is available, salvage is preferred over de novo synthesys[6]. Conversely, the biosynthetic pathway is activated to support asexual growth when pABA is limiting, as occurs with a milk-based diet.

In this study, we examined the effect of dietary pABA restriction on the outcome of infection with wild-type *Plasmodium berghei* in a mouse model of cerebral malaria. The parasites replicated normally in mice fed a pABA-restricted diet, confirming that parasite growth does not depend on host pABA when the endogenous biosynthetic pathway is intact[6].

Mice fed a standard diet developed simptoms of severe malaria, whereas most mice fed a pABA-restricted diet cleared the infection in approximately 20 days and developed an effective immune response that protected them from subsequent exposure to the parasite.

A previous study, found that pABA prevents the destabilization of erythrocyte membrane that leads to the formation of echinocytes *in vitro*[7]. Consistent with these findings, we showed that erythrocytes from mice fed a pABA-restricted diet are more prone to forming echinocytes in an oxidant environment *in vitro*.

Overall, our results suggest that the absence of pABA in the host diet affects *Plasmodium* infection through a mechanism that is likely independent of its primary role in folate metabolism.

Results

A pABA-deficient diet affects the progression of malaria infection in mice

To investigate the role of pABA in the progression of malaria infection, we injected the lethal ANKA strain into mice fed a purified diet, either deficient in or supplemented with pABA. We chose to perform the infections in the outbred CD1 mice, which develop neurological symptoms and cerebral malaria[8]. By maintaining genetic variability, this rodent model improves robustness of the results and mirrors genetic diversity of human population better than a single inbred line.

In a preliminary experiment (Experiment 1), we compared the progression of infection by injecting 10^6 parasites into groups of mice fed a standard grain-based diet (9 mice), purified diets either supplemented (8 mice) or not (9 mice) with pABA (Supplementary Table 1 and Fig. 1a).

dpi

Figure 1. Progression of *P. berghei* infection in mice on different dietary regimen

Mice fed a standard diet or a pABA -supplemented diet developed symptoms of severe disease, including cerebral malaria, between days 7 and 15, and were euthanized. In mice fed a pABA-deficient diet, parasitemia increased until days 10–15, but without causing symptoms of severe malaria. Subsequently, parasitemia gradually decreased to undetectable or barely detectable levels, and the mice recovered. No recurrence of malaria symptoms was observed when the pABA(-) diet was replaced with a standard grain-based diet.

We verified that the parasites still retained their original virulence, by the injecting blood from recovering mice into naive hosts, where circulating parasites caused severe infections (data not shown).

We also compared the parasite multiplication rate in mice fed a diet with or without pABA. Synchronous infections were performed by injecting 10^3 mature schizonts into the caudal vein of mice fed a purified pABA -deficient diet (two mice) and mice fed the same diet supplemented with pABA (2 mice). The progression of the infection, monitored until day 5 (Fig. 1b), showed no significant differences between the two groups of mice (ANCOVA test; 95% confidence interval; t = 0.537, P = 0.6).

Figure 1c shows the survival curves summarizing the results of four independent infections (Experiments 1–4 in Supplementary Table 1) conducted on two groups of mice: Group1, consisting of 37 mice fed a pABA-deficient (-) diet (Supplementary Table 2A and B), and Group 2 consisting of 22 mice fed a pABA-supplemented (+) diet (Supplementary Table 2C). Ninety-five percent of the pABA(-) mice survived, whereas all pABA(+) mice developed severe malaria (severe anemia and/or cerebral malaria) and were euthanized upon the onset of symptoms.

A pABA-deficient diet affects membrane stability of mouse erythrocytes

As shown above, the gradual decrease in parasite load in mice fed a pABA(-) diet do not depend on the genetic traits of the parasites or on their multiplication rate, which remains comparable to that observed in mice fed a pABA(+) diet.

Although mammals are auxotrophic for folate and lack a *de novo* biosynthetic pathway for pABA, it has been shown that pABA prevents the formation of spiculated erythrocytes (echinocytes) *in vitro*, and that Band3 is the primary binding site for this molecule[7]. Therefore, we investigated whether a pABA(-) dietary regimen increases erythrocyte membrane instability. To test this, we evaluated the percentage of echinocytes formed *in vitro* in the blood of mice fed a pABA(+) diet, after treatment with increasing concentration of phenylhydrazine to induce oxidative stress[9] (Fig. 2a). We then compared blood samples collected from mice fed a pABA(+) diet with those from mice fed a pABA(-) diet following phenylhidrazine treatment [9] at 200µM (Fig. 2b).

Scanning electron microscopy of the treated erythrocytes revealed a significant difference in the percentage of echinocytes (one-sided t-test, 95% confidence interval, P < 0.05), which accounted for 94.5% of erythrocytes in mice fed a pABA(-) diet, compared to 30% in those fed a pABA(+) diet. It is conceivable that, in the absence of pABA, oxidative stress caused by the *Plasmodium* parasite drastically affects the stability and deformability of the erythrocyte membrane, making the infected erythrocytes more prone to splenic clearance.

b

Figure 2. Phenylhidrazine-induced oxidative stress in mouse erythrocytes.

Mice fed a pABA(-) diet develop an effective immune response against malaria parasites

Two distinctive features characterize the course of *P. berghei* infections in mice fed a pABA(-) diet: first, parasitemia peaks between days 10 and 16 and then declines rapidly, with complete recovery of the mice. This trend is demonstrated by data from 30 infected mice grouped based on similarities in the infection progression (Supplementary Table 2A and Fig. 3a, left panel). During this period, the humoral immune response against the parasite increased progressively, as indicated by specific IgG levels measured on days 9, 11, 16 and 23 post-infection (Fig. 3a - right panel). This response was plotted as the ratio of the mean OD value of the immune sera collected at each time point to the mean of two hyperimmune mouse sera, obtained through repeated cycles of infection and treatment. The decrease in parasitemia and the concomitant increase in specific antibody production strongly suggest the involvement of the immune system in parasite clearance.

Second, neurological disorders associated with cerebral malaria were completely absent in infected mice fed a pABA(-) diet, despite a compromised blood-brain barrier and detectable lymphocytic infiltration (Supplementary Fig. 2). However, flow cytometric analysis of brain-infiltrating lymphocytes (Fig. 3b) revealed significant differences in T-cell composition between the experimental groups. In infected mice fed a PABA(+) diet, $CD8^+$ T cells accounted for 35% of CD45 + cells, whereas in mice fed a pABA(-) diet, this proportion dropped to 13%. This resulted in a CD8+/CD4 + ratio of approximately 5 in the pABA(+) group versus 1 in the pABA (-) group (two-tailed Welch's test, 95% confidence interval; $P = 0.02$). In contrast, splenic analysis revealed a CD8+/CD4 + ratio of about 0.25 in both groups, indicating that CD4 + T cells were four times more abundant than CD8 + T cells (two-tailed Welch's test, 95% confidence interval; $P = 0.007$). The predominance of $CD8^+$ T cells in the brain, but not in the spleen, of mice fed a pABA(+) diet may reflect a more cytotoxic or effector-oriented response, which could drive to greater local immune overactivation and lead to extensive, aggressive neuro-inflammation.

Acquired immunity against the Plasmodium parasite protects mice fed a pABA-deficient diet from reinfection

Mice fed a pABA(-) diet were maintained on a standard grain-based diet once they had recovered from the infection and were tested periodically for the presence of parasites. When present, parasites were quickly cleared from the circulation.

To verify whether recovered mice had developed long-term, parasite-specific immunity, 9 mice (Experiment 4) and 8 mice (Experiment 3) were infected with 10^6 parasites three or ten months, respectively, after the initial inoculation. Two days before reinfection, the humoral response of each mouse against malaria parasite was assessed by ELISA and compared with the OD values of two hyperimmune sera. As shown in Fig. 4, most mice maintained an IgG titre comparable to that of the

control mice. All mice were protected against reinfection after 3 months, whereas 6 out of 8 mice (75%) survived after 10 months.

Discussion

In this study, we investigated the role of pABA, a key precursor in folic acid biosynthesis, in the progression of blood-stage infection in the rodent malaria model *Plasmpdium berghei*. Previous studies[13,14,15,16] have shown that mice or rats, fed a milk-based diet deficient in pABA were not susceptible to infection with *P. berghei* or *P. yoelii*. Furthermore, monkeys fed a milk-based diet were also not susceptible to infection with *P. knowlesi* or *P. cynomolgi*[17]. In all cases, susceptibility was restored when pABA was added to the milk, suggesting a metabolic dependence on pABA.

These findings stand in stark contrast to a more recent study that, using *P. berghei* malaria model, demonstrated that the wild-type parasite is largely independent of dietary pABA[18]. In the absence of this key folate precursor, *Plasmodium* is able to synthesize pABA rather than relying on preformed folate. Growth attenuation was observed only in mice fed milk-based diet and infected with parasite mutants lacking the ADCS or ADCL enzymes, which are involved in *de novo* synthesis of pABA. The authors thus provided evidence supporting the existence of two complementary pathways: *de novo* biosynthesis and pABA salvage.

In four independent experiments, we monitored the progression of *P. berghei* infection in groups of mice fed a purified diet deficient in pABA, either supplemented or not supplemented with pABA in the drinking water. We observed no differences in the rate of multiplication among the groups, thus confirming the facultative auxotrophy of pABA[18].

However, in later stages of infection, we observed substantial differences between the experimental groups. Mice fed a grain-based diet or a purified diet supplemented with pABA, developed a severe form of malaria, accompanied by neurological symptoms, and had to be euthanized. In contrast, in mice fed a pABA-deficient diet, the infection proceeded without causing severe symptoms or cerebral malaria. Subsequently, parasitemia fell below the detectable threshold, and mice recovered with a survival rate of 95%. When the pABA-deficient diet was replaced with a grain-based diet, no recurrence of infection was observed.

Data from 30 infected mice fed a pABA-deficient diet were selected from the 4 experiments and grouped based on similarities in the progression of the infection. In all cases, parasitemia peaked between days 10 and 16, then declined rapidly. The concomitant increase of the humoral immune response against the parasite suggested that the immune system might be involved in parasite elimination.

We also evaluated whether the absence of dietary pABA could affect the stability of mouse erythrocytes. A previous study had shown that pABA prevents echinocytosis of healthy erythrocytes *in vitro*[7] and that Band 3 is the primary binding site for this molecule. In this study, we showed that erythrocytes from mice

fed a pABA-deficient diet are significantly more prone to forming echinocytes than those from mice fed a pABA-supplemented diet when subjected to oxidative stress *in vitro*. In *in vivo*, this could lead to more rapid clearance of infected erythrocytes by the spleen in mice fed a pABA-deficient diet.

Another noteworthy finding observed in all infected mice fed a pABA-deficient diet is the complete absence of neurological symptoms, even though the mice exhibited a breakdown of the blood-brain barrier with lymphocyte infiltrates typical of the cerebral malaria[19 20].

In particular, the two experimental groups show a significant difference in the composition of the T-cells that infiltrate the brain. In mice fed a diet supplemented with pABA, the CD8 + to CD4 + cell ratio is approximately 5, whereas in mice fed a pABA-deficient diet, it is close to 1. In contrast, in the spleen of both groups, CD4 + T cells are four times more abundant than CD8 + T cells.

It is well established that CD4 + T helper cells and CD8 + cytotoxic T cells play a key role in the immune response against the malaria parasite[21].

Once stimulated, CD4 + T cells differentiate into subsets that play different roles in the pathogenesis of cerebral malaria. Furthermore, they regulate the immune response against the malaria parasite through the secretion of pro- or anti-inflammatory cytokines[22] .

CD8 + T lynphocytes constitute the main cell population recruited to the brain vasculature and play a key role in the disruption of the blood-brain barrier[23]. These effector immune cells directly induce the death of vascular endothelial cells and infiltrate the brain parenchyma, causing severe cerebral vascular edema and fatal neurological signs[24].

The predominance of $CD8^+$ T cells in the brain, but not in the spleen, of mice fed a pABA(+) diet may reflect a more cytotoxic or effector-oriented response, which could contribute to greater local immune activation. Interestingly, it has been shown that depletion of CD8 + T cells prevents or completely reverses severe disease progression[25], consistent with the fivefold lower abundance of CD8 + T cells in mice fed a pABA-deficient diet.

We then investigated whether recovered mice, fed a grain-based diet, were protected against reinfection after a single exposure.

Naturally acquired immunity against human malaria is achieved through continuous exposure in endemic areas. Such immunity protects against morbidity and mortality, but does not prevent reinfection[26]. In the rodent model, immunity is acquired through multiple cycles of infection and cure[27].

Two groups of 9 and 8 mice were infected with a high number of *P berghei* parasites (10^6) three and ten months, respectively, after the first injection. IgG titers, measured two days before challenge, were in most cases comparable to those in sera from hyperimmune control mice obtained through several cycles of infection and cure.

Mice challenged three months after parasite injection did not develop a detectable infection, or circulating parasites were cleared within 7 days. Seventy-five percent of the mice challenged after 10 months survived (6 out of 8 mice) and circulating parasites were cleared within approximately two weeks. No signs of severe malaria were detected in either group of mice.

In conclusion, our results suggest that pABA plays a role in malaria infection that is distinct from its function in folate metabolism. A possible interaction of this molecule with erythrocyte membrane could contribute to membrane stability, which is impaired under a pABA-deficient diet. Further investigation is needed to determine whether these dietary conditions promote the elimination of infected erythrocytes by the spleen, thereby favoring the development of an effective immune response.

Methods

Animal, Diet regimen, and Plasmodium berghei infection

Animal experiments were conducted under specific pathogen-free conditions at the animal facilities of the *Istituto Superiore di Sanità* and were performed in accordance with European Union guidelines and Italian legislation (Decreto Legislativo 116/92, implementing Directive 86/609/EEC on the protection of laboratory animals). Female CD1 mice (6–8 weeks old) were purchased from Charles River Laboratories (Calco, Italy).

Mice were randomly allocated into three experimental groups: the first group received a standard grain-based rodent diet; the second group received the purified diet AIN-93M by Mucedola supplemented with pABA in the drinking water (25 mg/ml)), whereas, the third group received the same purified diet without PABA supplementation. We verified that the chosen PABA concentration was similar to that of the grain-based diet, which causes a lethal infection in CD1 mice. The feeding regimen was maintained throughout the course of infection. Animal health was monitored daily by routine visual checks, mice exhibiting signs of severe disease were humanely euthanized to minimize suffering.

Mice were inoculated intraperitoneally with blood infected by *Plasmodium berghei* ANKA line that constitutively expresses the green fluorescent protein (GFP) under the control of the *P. berghei* HSP70 promoter. The transgenic line was obtained by transfection with the plasmid pBAT-SIL6, kind gift of Dr. Taco Kooij[10] .

Synchronous infections were performed by intravenous injection of schizonts matured in colture and purified from uninfected erythrocytes by Nycodenz cushions[11].

Infected erythrocytes were counted by flow cytometry using a FACSCalibur cytometer (Becton Dickinson, CA, USA) on blood samples collected from the tail vein (10 µl per mouse) and diluted in PBS containing EDTA. Erythrocytes were gated based on size using FSC-H and SSC-H parameters to exclude debris, and a total of events were acquired per sample. Parasitemia was calculated as the percentage of infected erythrocytes on total erythrocyte population.

Immunoglobulin detection in mouse sera

Antibodies against *P. berghei* parasite were detected in mouse sera by ELISA at different infection time points. Cell extracts in carbonate buffer (pH 9.4) from infected erythrocytes or uninfected controls were adsorbed onto 96-well Nunc MaxiSorp plates (NUNC) (0.25 µg/well) and incubated overnight at 4°C. Plates were washed in PBS containing 0.1% Tween-20 (PBS-T) and blocked for 2 hours at 37°C in PBS containing 3% non-fat dry milk (NFDM). Serial dilutions of mouse sera in 1% NFDM-PBS were added and incubated at room temperature for 2 hours. Specific antigen-antibody complexes were detected using horseradish peroxidase-conjugated anti-mouse IgG and TMB (VECTOR) as the substrate. After 30 minutes, the enzymatic reaction was stopped by adding 50 µl of 1 M sulfuric acid per well, and optical density measured at

450 nm.

Isolation and flow cytometry analysis of brain-infiltrating lymphocytes in mice infected by Plasmodium berghei ANKA.

Infected mice at day 8 post-infection as well as infected controls were sacrificed and intracardially perfused with PBS. Brains were collected, mechanically dissociated, and digested with 7 mg/ml Type III Collagenase (Worthington Biochemicals) and 4 mg/ml DNase I (Sigma). The homogenate was then passed through a 70 µm cell strainer and the resulting cell suspension washed in RPMI complete medium. Mononuclear cells were enriched by centrifugation over a 30% Percoll cushion, followed by red blood cell lysis. Cells were washed, resuspended in RPMI, and viable brain-sequestered lymphocytes were counted by Trypan blue exclusion. Brain-infiltrating lymphocytes were washed and stained with fluorescently conjugated monoclonal antibodies (all from Biolegend) targeting CD45 (clone 30-F11), CD3 (clone 145-2C11), CD4 (clone H129.19), and CD8 (clone 53 – 6.7) for 20 min on ice. Samples were run on a Gallios flow cytometer and analyzed with Kaluza Analysis Software (Beckman Coulter). Appropriate unstained and single-stained controls were included for gating and compensation.

Scanning Electron Microscopy analysis of phenylhidrazine treated erythrocytes

Erythrocytes from mice fed a pABA(-) or a pABA(+) diet, were treated *in vitro* with phenylhidrazine at different concentrations (50-100-200-400 µM) in PBS and then fixed with 2,5% glutaraldehyde in Na-cacodilate buffer 0,1M while let to adhere on poly-lysine treated round glass coverslips for 3h at RT. Samples were processed as previously described[12] and analysed by a SEM-FEG Quanta INSPECT F (FEI –ThermoFisher Scientific). Cell morphology (echinocytes vs. discoid erythrocytes) was assessed in five microscopic fields per sample, with an analyzed horizontal field width (HFW) of 85 µm, and the percentage of each morphology, in erythrocytes from mice fed a diet supplemented or not with pABA, was calculated.

Declarations

Competing interests

The authors declare no competing interests.

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Author contributions

F.G., T.P. and M.P. designed experiments; S.M. performed *P. berghei* infections; L.B. and F.I. performed electron microscopy analysis; G.S. and S.A. performed flow cytometry analysis of brain and spleen lymphocytes; E.P. performed statistical analysis; E.P. and M.P. wrote the manuscript. All authors read and approved the final version of the manuscript.

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Data availability

All data regarding parasite infections and ELISA assays are available in the supplementary materials.

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Figures

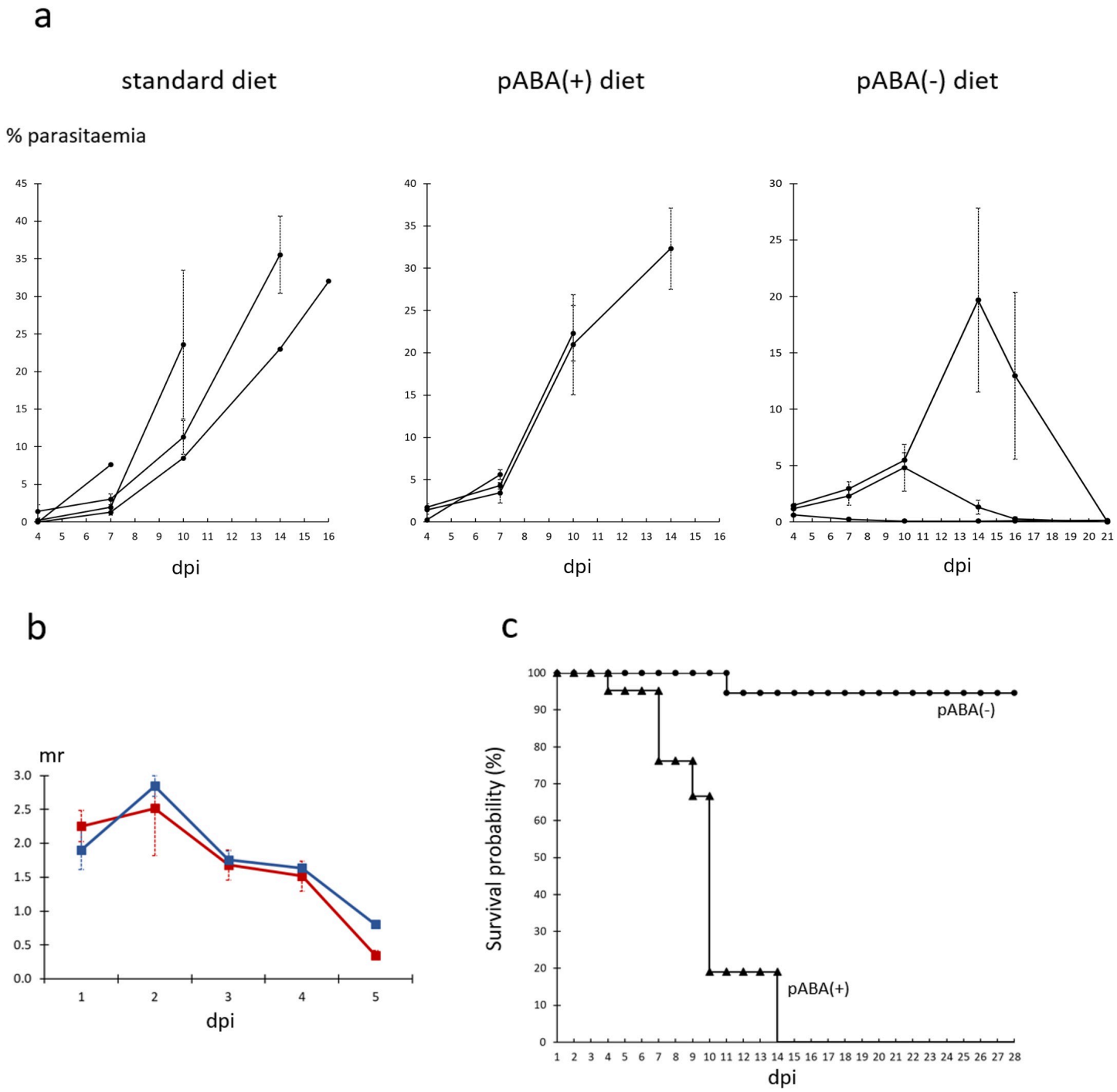
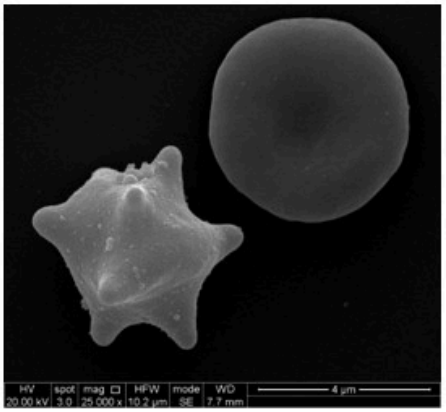
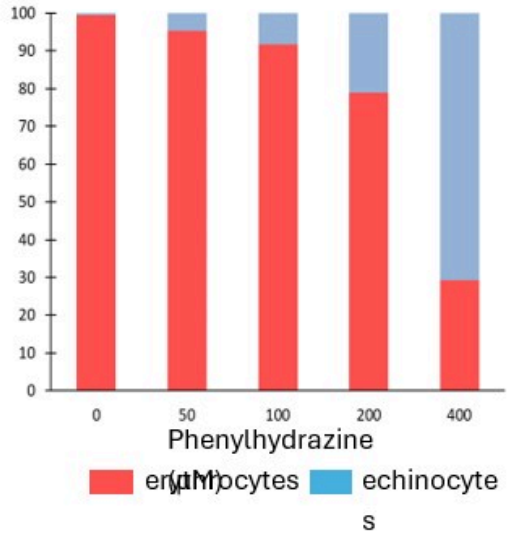


Figure 1

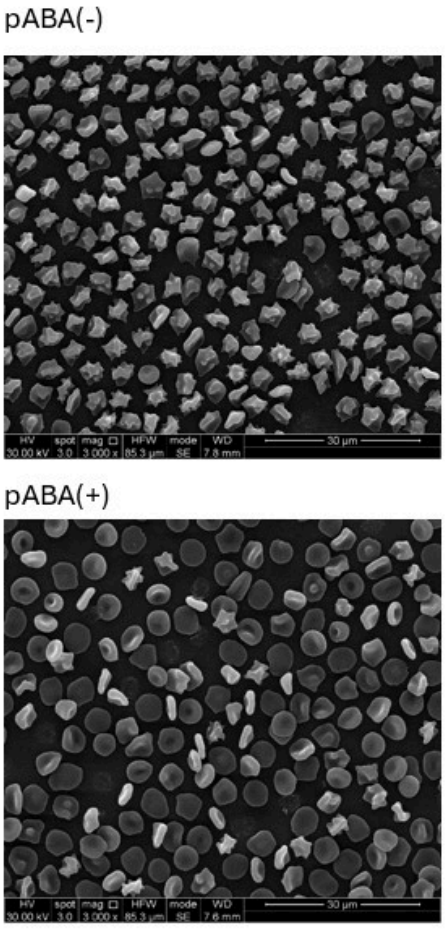
Progression of *P.berghei* infection in mice on different dietary regimen. a) 10^6 parasites were injected into groups of mice fed a standard grain-based diet (9 mice), or purified diets either supplemented (8 mice) or not supplemented (9 mice) with pABA (experiment 1); days post-infection (dpi). For the standard and pABA-supplemented diets, mice were grouped according to the day they were sacrificed; under pABA(-) dietary conditions, mice were grouped according to the peak of parasitaemia (between days 10 and 14). Subsequently, parasitaemia decreased progressively, and the mice recovered. The variability in parasitaemia levels within each group is expressed as the standard error (vertical bars). b)

Parasite multiplication rates (mr = % parasitaemia on day N/ % parasitaemia on day N-1) were determined in synchronous infections, by injecting 10^3 mature schizonts into the caudal vein of two mice fed a purified pABA-deficient diet (red line) and two mice fed the same diet supplemented with pABA (blue line). The progression of the infection, monitored daily, showed no significant differences between the two groups of mice as calculated by the ANCOVA test (95% confidence intervals; t=-0.537, P=0.6). c) Survival curves of two groups of mice infected with *Plasmodium berghei* ANKA; each group represents the sum of four independent experiments. Mice in Group 1 (n=22) were fed a purified diet supplemented with pABA (pABA(+)), while those in Group 2 (n=37) received the same diet without pABA supplementation (pABA(-)). All infected mice fed a pABA(+) diet developed severe malaria and were euthanized upon the onset of simptoms, whereas 95% of the mice fed a pABA(-) diet survived.





b



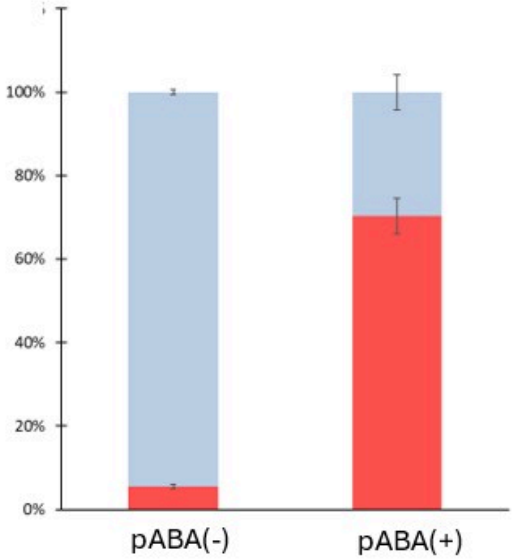


Figure 2

Phenylhidrazine-induced oxidative stress in mouse erythrocytes. a) Electron microscopy image of mouse erythrocytes treated with phenylhydrazine (left panel). Alterations in membrane shape (echinocytosis) are visible as spiny projections. The hystograms (right panel) show the percentage of echinocytes in the blood of mice fed a pABA(+) diet and treated in vitro with increasing doses of phenylhydrazine. b) Electron microscopy images (left panels) of erythrocytes from mice fed a diet with

or without pABA and treated with phenylhydrazine (200µM). The histograms (right panel) show a significant difference in the percentage of echinocytes between the two erythrocyte populations : 94,5 % in the pABA(-) diet and 30 % in the pABA(+) diet (one-sided T-test, 95% confidence interval, P<0.05).

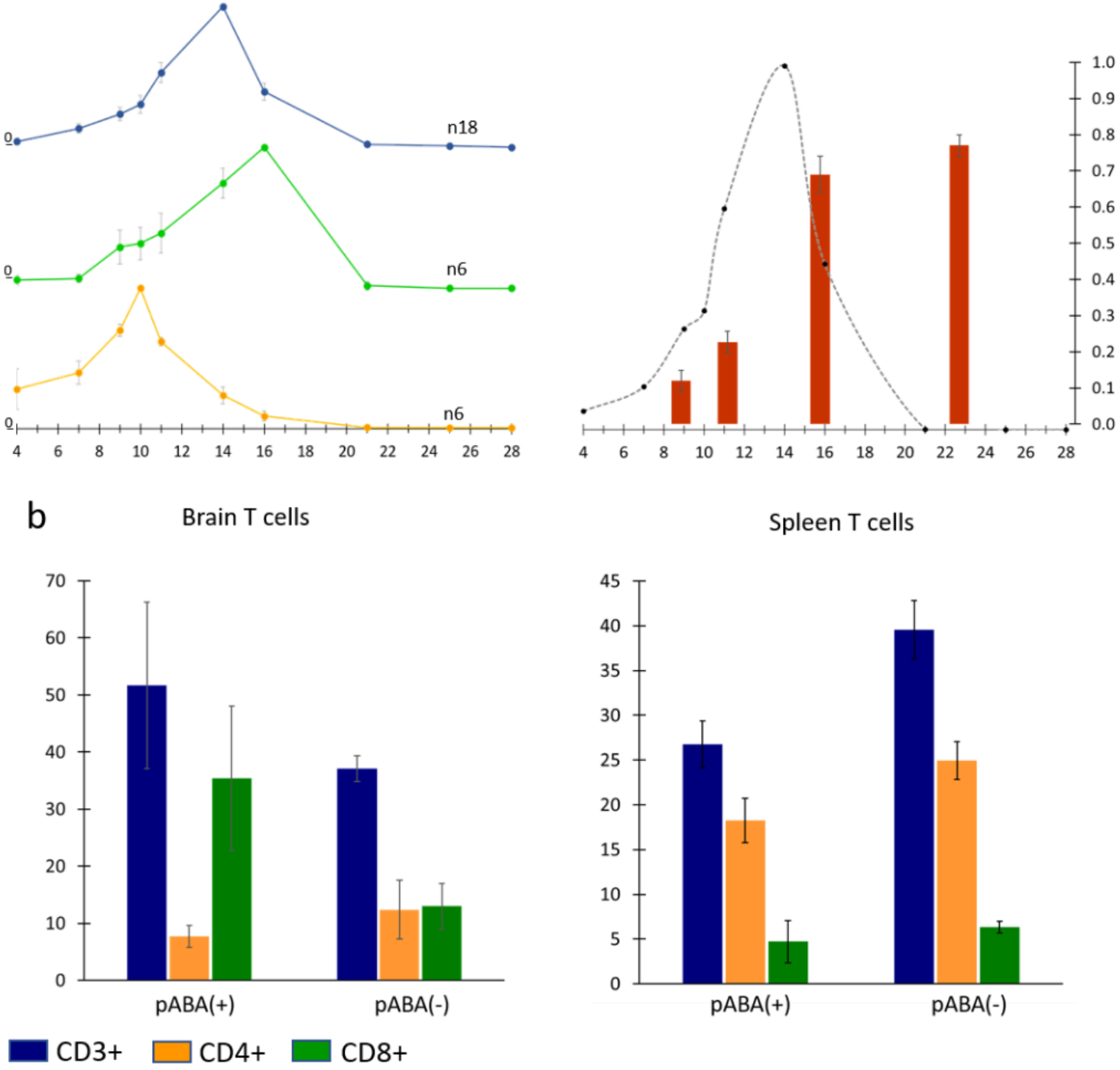


Figure 3

Immune response to *P. berghei* infection in mice fed a pABA(-) or pABA(+) diets. a) Data from 30 mice fed a pABA(-) diet were grouped based on similarities in infection progression (left panel); days post infection (dpi). Parasitemia values were normalized by setting the maximum value to 1. Infection profiles

were drawn by plotting median normalized parasitemia values ± standard errors. The number of mice in each group is indicated in the three stacked profiles. Infection peaks were reached between days 10 and 16. The humoral response during infection (right panel) was monitored by ELISA (1:1000) on sera obtained from tail blood samples collected from groups of infected mice on day 9 (experiment 2) , day 11 (experiment 1), day 16 (experiment 1 and 2) and day 23 (experiment 3). ELISA data were normalized to the mean value of two sera from hyperimmune control mice. The mean normalized ELISA values ± standard errors are represented as histograms. The median normalized parasitaemia of the 30 mice is indicated by a dashed line. b) quantification of T lymphocytes in brain-infiltrates or the spleen, expressed as a percentage of total leukocytes ($CD45^+$ cells) in groups of mice fed a pABA(+) or pABA(-) diets. In the brain, the CD8+/CD4+ ratio is approximately 5 in mice fed a pABA(+) diet and 1 in mice fed a pABA(-) diet (P=0.02). In the spleen of mice fed either a pABA(-) or pABA(+) diet, the CD8+/CD4+ ratio was 0.25 in both groups (P=0.95). P-values were obtained using a two-tailed Welch's test with a 95% confidence interval.

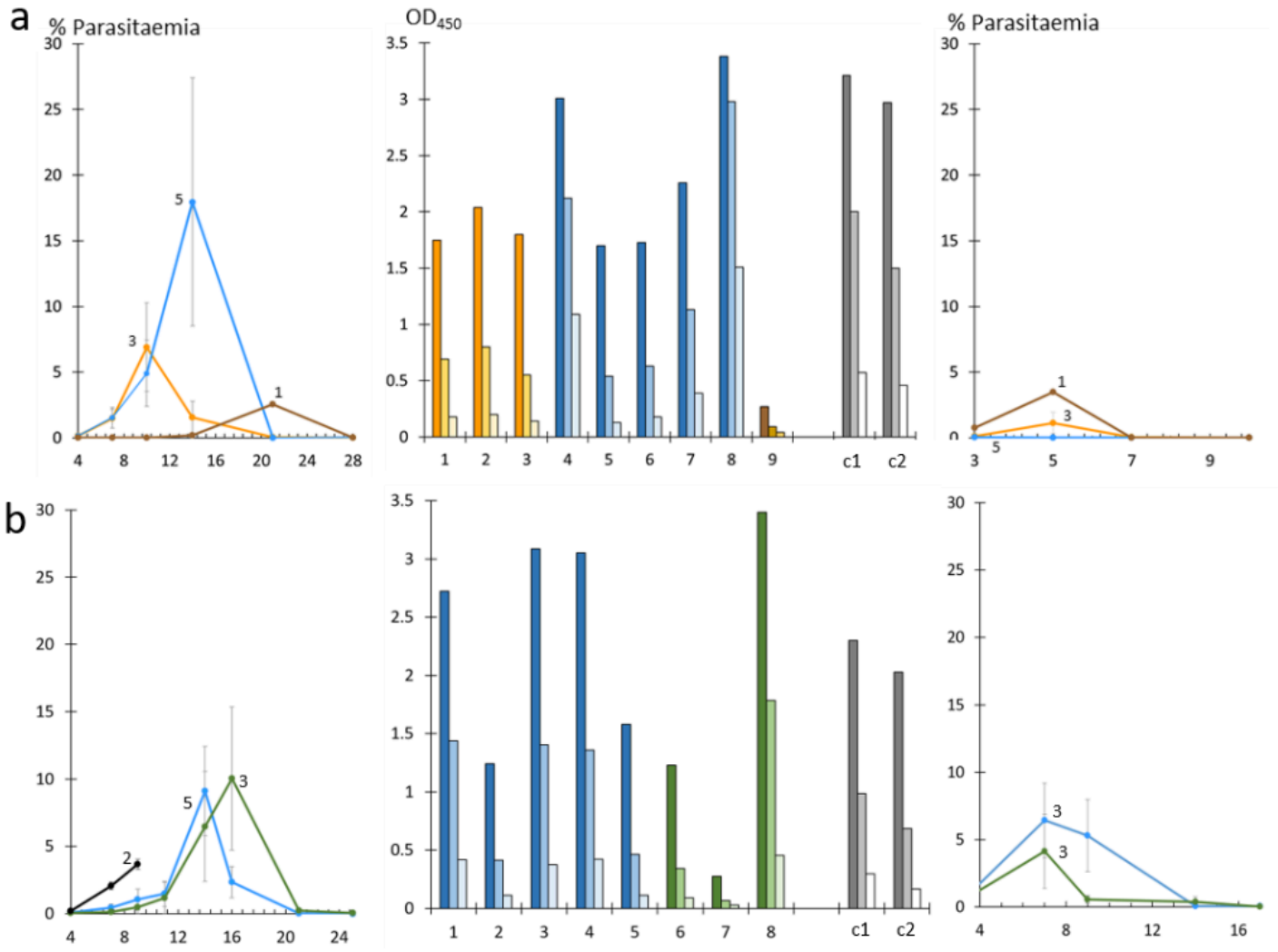


Figure 4

Humoral response against *P. berghei* in mice fed a pABA(-) diet. To determine whether mice fed a pABA (-) diet that recovered from a primary infection were protected against reinfection, two experiments were conducted: a) a group of 9 mice (experiment 4) was reinfected 3 months after the initial inoculation; b) a group of 8 mice (experiment 3) was reinfected 10 months after the initial inoculation. Two days prior to the second inoculation, the humoral response of each mouse was evaluated by ELISA and compared with the antibody levels of two hyperimmune control mice. The histograms display the OD values across three serial serum dilutions (1:1000; 1:5000; 1:25000). Infections resulting from the primary (left panel) and secondary (right panel) inoculations were grouped according to the peak of parasitemia; mean values per group ± standard errors are reported. At three months, all mice had survived, whereas at 10 months, 6 out of 8 had survived.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalGrasso.pdf