Infection-induced plasmablasts are a nutrient sink that impairs humoral immunity to malaria

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*Plasmodium* parasite-specific antibodies are critical for protection against malaria, yet the development of long-lived and effective humoral immunity against *Plasmodium* takes many years and multiple rounds of infection and cure. Here, we report that the rapid development of short-lived plasmablasts during experimental malaria unexpectedly hindered parasite control by impeding germinal center responses. Metabolic hyperactivity of plasmablasts resulted in nutrient deprivation of the germinal center reaction, limiting the generation of memory B cell and long-lived plasma cell responses. Therapeutic administration of a single amino acid to experimentally infected mice was sufficient to overcome the metabolic constraints imposed by plasmablasts and enhanced parasite clearance and the formation of protective humoral immune memory responses. Thus, our studies not only challenge the current model describing the role and function of blood-stage *Plasmodium*-induced plasmablasts but they also reveal new targets and strategies to improve anti-*Plasmodium* humoral immunity.

Following either vaccination or microbial infection, humoral immunity generally consists of temporally and spatially layered B cell activation events. Early humoral responses are associated with extrafollicular B cells that proliferate and rapidly differentiate into short-lived antibody-secreting cells (ASCs), which are also referred to as plasmablasts. T cell-dependent follicular responses take longer to develop and involve antigen-specific B cells forming a germinal center (GC) where they engage in sustained interactions with CD4+ T follicular helper cells (Tfh cells). GC B cells subsequently differentiate into either memory B cells (MBCs) or long-lived plasma cells (LLPCs) that secrete high-affinity, class-switched antibodies. It is thought that extrafollicular, short-lived plasmablasts limit pathogen dissemination until LLPC and MBC responses develop, which act to resolve the primary infection and mediate resistance to subsequent pathogen exposures.

*Plasmodium* infections caused an estimated 219 million cases of malaria and resulted in approximately 435,000 deaths in 2017. Both clinical and experimental studies identify *Plasmodium*-specific antibodies as critical for both limiting disease severity and promoting the clearance of blood-stage parasites. Although durable immunity to either *P. vivax* or *P. falciparum* has been reported in travelers and individuals from areas of relatively low transmission intensity, in regions of high *P. falciparum* transmission, parasite-specific LLPCs and MBCs are not efficiently induced, and sterilizing immunity against blood-stage *P. falciparum* is seldom acquired, even following repeated infections.

Multiple mechanisms have been postulated to explain the short-lived nature of *Plasmodium*-specific humoral immune responses, including preferential expansion of CXCR3+ (Tfh,1-like) Tfh cells, regulatory T cells and atypical MBCs, as well as the dysregulation of chemokines and cytokines and induction of immune checkpoints that may delay or impair the acquisition of humoral immunity against malaria. Although these studies have identified numerous mechanisms that may contribute to the lack of durable immunity against malaria, our past studies showed that genetic manipulations that either expand or constrain the *Plasmodium*-specific GC response are associated with substantially blunted or elevated plasmablast responses, respectively (J.J.G. and N.S.B.; unpublished observations). These data raise the possibility that blood-stage *Plasmodium* infections may preferentially induce immunosuppressive plasmablast populations that reduce the development of GC B cell responses and the induction of long-lived humoral immunity. Herein, we used combinations of clinical trials and experimental rodent malaria models to define the dynamics of infection-induced plasmablast populations and interrogate their contribution to anti-*Plasmodium* immunity. Our data show that clinical and experimental blood-stage *Plasmodium* infection preferentially expands short-lived plasmablast populations and that during experimental malaria these cells may function as a metabolic sink that constrains GC-derived humoral immune reactions, thereby identifying a previously unknown mechanism by which *Plasmodium* parasites subvert host immunity.

Results

Plasmablasts dominate the response to *Plasmodium* infection. To examine the kinetics and magnitude of splenic ASC/plasmablast responses during experimental malaria, we infected wild-type C57BL/6 mice with *Plasmodium yoelii* strain 17XNL...
parasitized red blood cells (RBCs). This infection is normally non-lethal to wild-type C57BL/6 mice and it mimics aspects of severe anemia and hyperparasitemia associated with *P. falciparum* infection of malaria-naïve individuals. We quantified activated infection-induced splenic GC (B220^+^{glutaminase-}\textsuperscript{−}^{CD95^+}) B cell responses by analyzing the kinetics of splenic CD138^hi IgD^- B cells, representative of n = 6 biologically independent experiments with similar results. To determine the numbers of splenic plasmablasts (CD138^hi IgD^-) and GC B cells (GL7^+^{Fas^+}) and to examine the kinetics of parasite burden (% of infected RBCs) during *P. yoelii* infection, we quantified parasite-specific IgM and IgG antibodies secreted by splenic CD138^hi IgD^- plasmablasts isolated on day 10 post-infection. Data are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 6 wells (media only) and n = 12 wells (CD138^hi IgD^-). Numbers of parasite-specific antibody-secreting CD138^hi IgD^- plasmablasts isolated on day 10 post-infection. Data are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 8 IgG and n = 11 mice (IgM). Transmission electron micrographs of the indicated cells isolated on day 10 post-infection. Data are representative of n = 3 biologically independent experiments with similar results using n = 100 cells for each population and one mouse per experiment. Scale bars: 2 μm. Yellow arrows show the rough endoplasmic reticulum. FLICA staining in CD138^hi IgD^- plasmablasts (green) and naive B cells (red) on day 10 post-infection. Data are representative of n = 2 biologically independent experiments similar results using n = 6 mice per time point. Confocal micrograph of spleen at day 10 post-infection, showing CD4 T cells (gray), total B cells (red), GC B cells (blue) and CD138^hi plasmablasts (green). Data are representative of n = 2 biologically independent experiments using n = 3 mice. Scale bar: 300 μm.
suggesting that their disappearance from the spleen and blood may be linked to apoptosis and was probably not due to rapid migration of CD138hi plasmablasts to the bone marrow.

The spleen contains a heterogeneous population of B lymphocytes that include follicular (CD21+CD23+) and marginal zone (CD21loCD23hi) B cells (Extended Data Fig. 1h). Splenic CD138hi plasmablasts are reported to differentiate from either follicular or marginal zone B cells27. We found that CD138hi plasmablasts did not exhibit characteristics of follicular B cells and only a minor proportion (~15%) exhibited characteristics of marginal zone B cells, whereas blood-stage \textit{P. yoelii} infection-induced activated (CD138hiIgDlo) B cells and the bulk of resting (CD138loIgDhi) B cells displayed characteristics of either follicular or marginal zone B cells (Extended Data Fig. 1i). CD138lo plasmablasts also did not express markers of GC B cells (GL-7+ and CD38hi) (Extended Data Fig. 1j). In agreement with this surface phenotype, the majority of blood-stage \textit{Plasmodium} infection-induced CD138hi plasmablasts localized outside of B cell follicles (Fig. 1h). Together, these data support that blood-stage \textit{Plasmodium} infection is associated with transient yet substantial accumulations of CD138loIgDlo plasmablasts that localize outside of follicles.

CD138hi plasmablasts constrain anti-\textit{Plasmodium} humoral immunity. The substantial expansion and accumulation of CD138hi plasmablasts suggests they may play a critical role in mediating host protection against blood-stage \textit{Plasmodium} infection. To test this hypothesis, we first transferred CD138hi plasmablasts purified from \textit{P. yoelii}-infected donors to \textit{P. yoelii}-infected recipient mice 5d before the peak of the endogenous CD138hi plasmablast response in recipients (Extended Data Fig. 2a). Notably, the transfer of CD138hi plasmablasts resulted in higher parasite burdens (Fig. 2a) compared with recipients receiving either activated (CD138hiIgDlo) B cells or phosphate-buffered saline (PBS). Recipients of CD138hi plasmablasts also exhibited lower titers of parasite lysate-specific IgG2b (Fig. 2g). Importantly, \textit{P. yoelii} and selectively delete Prdm1 a genetic approach focused on post-infection (day 16 post-transfer) (Fig. 2b). Next, we employed a genetic approach focused on Prdm1. We generated competitive mixed bone marrow chimeric mice in which we could conditionally and selectively delete Prdm1 from the B cell compartment (Extended Data Fig. 2b). Tamoxifen treatment of \textit{P. yoelii}-infected Cre+ chimeric mice abrogated CD138hi plasmablast response (Fig. 2c) and enhanced parasite control (Fig. 2d). Genetic blockade of plasmablast development also resulted in eight- to tenfold increases in the number of GC B cells (Fig. 2e) and GC TFH-like cells (Fig. 2f) on day 21 post-infection. Anti-CD40L treatment of DTX-treated mice also exhibited fivefold higher basal respiration compared with the other two populations analyzed (Fig. 2f). Notably, our RNA-seq data also showed that CD138hi plasmablasts exhibit gene signatures consistent with induction of the unfolded protein response pathway that is induced upon plasma cell formation26,28 (Extended Data Fig. 4c and Supplementary Table 4), further supporting that blood-stage \textit{P. yoelii} infection-induced CD138hi plasmablasts are bona fide ASCs.

Next, we sought to investigate factors associated with the increased metabolism of CD138hi plasmablasts. B cell activation and proliferation and increases in biomass require an ample supply of biomolecules such as N, S, P, O, and C and the acquisition of amino acids, especially L-glutamine26,28. This requirement raised the possibility that substantial numbers of CD138hi plasmablasts triggered by blood-stage \textit{P. yoelii} infection may deplete local metabolic resources in the spleen. In support of this hypothesis, targeted analysis of our RNA-seq data set revealed that multiple amino acid and nutrient
transporters were differentially expressed among the splenic B cell populations (Fig. 4c and Supplementary Table 5). Ingenuity pathway analysis (IPA) revealed broad activation of genes encoding solute carrier (SLC) transporters that are involved in amino acid acquisition, particularly l-glutamine (Fig. 4f). The activation of these pathways was also associated with CD138hi plasmablast metabolic hyperactivity and expression of Blimp-1, which is known to promote the expression of amino acid transporters43. We confirmed the upregulation of a set of l-glutamine SLCs, including messenger RNA (mRNA) sequences for Slc1a4 and Slc1a5, which correlated positively with Prdm1 and Xbp-1 and inversely with Pax5 mRNA expression (Fig. 4g). Additionally, we confirmed elevated mRNA and protein expression of Slc7a5 (CD98), another key SLC transporter that mediates l-glutamine uptake42,43 (Fig. 4g–i). Together, these data support the hypothesis that metabolically hyperactive CD138hi plasmablasts utilize SLC protein family members to increase the uptake of l-glutamine, which may in turn deplete key splenic metabolic resources required to sustain GC responses.

**l-glutamine enhances anti-*Plasmodium* humoral immunity.** Studies of *Plasmodium*-infected non-human primates and patients in malaria-endemic areas revealed substantial reductions in plasma l-glutamine concentrations during the acute phase of infection, which correlated with poorer outcomes and increased parasite burdens44. Similarly, splenic l-glutamine concentrations were reduced in *P. yoelii*-infected mice (Extended Data Fig. 5a). Given their numerical over-representation and increased expression of machinery required to uptake l-glutamine, we hypothesized that blood-stage *P. yoelii* infection-induced CD138hi plasmablasts impede GC reactions by acting as a nutrient sink, effectively incapacitating their numerical over-representation and increased expression of l-glutamine is transported by SLC1A5. Importantly, the protective effects of l-glutamine supplementation required the presence of essential branched-chain amino acid l-alanine or the non-essential amino acid l-valine (Extended Data Fig. 5b,c), which like l-glutamine is transported by SLC1A5. Importantly, the protective effects of l-glutamine supplementation required the presence of CD4+ T cells (Fig. 5c), functional Tfh cells (Fig. 5d) and secreted antibody responses (Fig. 5e). Disruption of GC reactions via anti-CD40L also abrogated parasite control and GC B and Tfh cell responses in l-glutamine-treated mice (Extended Data Fig. 5d,f). Thus, enhanced parasite control in l-glutamine-treated mice was...
directly linked to improved GC reactions and humoral immunity, and not simply impacting on the general physiology of either the host, host microbiome or parasite itself.

Consistent with the requirements for secreted antibody and TFH-like cells for enhanced parasite control following L-glutamine treatment, we observed higher-magnitude GC B cell responses that appeared with accelerated kinetics (Fig. 5f and Extended Data Fig. 5g), as well as elevated numbers of class-switched GC B cells in the spleen as early as day 12 post-infection (Extended Data Fig. 5h). While the numbers of CD138lo plasmablasts were not affected (Extended Data Fig. 5h), the accelerated and enhanced expansion of plasmablasts (day 10 post-infection) (Extended Data Fig. 5n). Notably, L-glutamine could enhance parasite control when administered as late as day 6 post-infection (Extended Data Fig. 5n,o), but the protective effects were not observed when L-glutamine administration was initiated after the peak numerical expansion of plasmablasts (day 10 post-infection) (Extended Data Fig. 5p,q). Collectively, these data are consistent with the notion that CD138lo plasmablasts, by virtue of their metabolic hyperactivity and their potential to uptake L-glutamine, transiently impair the metabolic and optimal functions of other splenic B cell populations. Thus, L-glutamine, when delivered either prophylactically or therapeutically, may relieve GC B and TFH-like cells from nutrient deprivation to enhance their numbers and functions.

Plasmablast deletion and L-glutamine are functionally redundant. The deletion of plasmablasts and L-glutamine treatment both reduce parasitemia, increase the GC response and boost the activation of B cells, as measured by OCR. To test whether these phenotypes are connected, and to determine whether CD138lo plasmablast deletion and L-glutamine administration functionally overlap, we administered L-glutamine to DTX-treated CD138-DTR chimeric mice (Extended Data Fig. 6). As expected, both L-glutamine and DTX treatment independently enhanced parasite control (Fig. 6a) and elevated the number of GC B cells (Fig. 6b) and GC TFH-like cells
Fig. 4 | Plasmablasts are metabolically hyperactive. a, Gating strategy used for sorting plasmablasts and activated (act) and resting B cell populations from P. yoelii-infected mice on day 10 post-infection that were used for bulk RNA-seq. b, c, Principal component analysis (PCA) depicting the clustering of the transcriptomic dataset from the three different B cell populations (b) and heat maps showing the relative expression levels of different genes involved in the glycolytic pathway (c; left) and citrate cycle pathway (c; right). d, Data comparing basal respiration of the three splenic B cell populations (left) and citrate cycle pathway (right). e, Heat map showing the relative expression levels of various glucose transporter (GLUT) molecules (e) and a network of the various amino acid transporters (f). g, mRNA expression levels (relative to Hprt) of plasmablast-specific genes (Prdm1 and Xbp1s), a B cell lineage gene (Pax5) and genes encoding the l-glutamine transporter molecules (Slc1a4, Slc1a5 and Cd98) (Slc3a2 and Slc7a5). Data are means ± s.e.m. for n = 4 mice, representative of n = 2 biologically independent experiments with similar results using n = 2 technical replicates and analyzed by two-tailed Mann–Whitney U-test. h, i, Frequency (h) and geometric mean fluorescence intensity (gMFI) (i) of CD98 expression on the indicated B cell populations isolated on day 10 post-infection. Data are means ± s.d., representative of n = 2 biologically independent experiments with similar results using n = 4 mice and analyzed by one-way ANOVA (false discovery rate < 0.05; 2 > fold-change > –2).

(Fig. 6c). However, these effects were not further enhanced when l-glutamine and DTX were combined (Fig. 6a–c). Furthermore, the metabolic activity of activated splenic B cells was elevated 8- to 12-fold in mice treated with either l-glutamine or DTX, yet the basal oxygen consumption was only minimally enhanced (~10%) when these treatments were combined (Fig. 6d). Taken together, these data suggest that CD138<sup>hi</sup> plasmablast deletion and l-glutamine supplementation are functionally redundant, which additionally supports the model that CD138<sup>hi</sup> plasmablasts may serve as a key glutamine sink during blood-stage Plasmodium infection.

Plasmablast deletion and l-glutamine improve immune memory. GC responses result in the production of LLPCs and memory. GC responses result in the production of LLPCs and memory. GC responses result in the production of LLPCs and memory. Accelerated and higher-magnitude TFH and GC reactions in DTX- and l-glutamine-treated mice are thus predicted to translate into
increased numbers of MBCs and LLPCs and heightened resistance to a heterologous challenge with a lethal species of Plasmodium parasite, P. berghei ANKA. In support of this notion, kinetic analyses revealed higher frequencies and numbers of CCR6^CD38^ MBCs in both DTX- (Fig. 7a) and l-glutamine-treated (Fig. 7d) mice between days 10 and 60 post-infection. DTX- and l-glutamine-treated mice also exhibited elevated LLPC responses, whether these were measured using functional enzyme-linked immune absorbent spot (ELISpot) assays (Fig. 7b,c) or flow cytometry (Extended Data Fig. 7a,b). Mice treated with either DTX (Fig. 7c) or l-glutamine (Fig. 7f) during a primary P. yoelli infection were also more resistant to lethal P. berghei ANKA challenge compared with their control counterparts. Of note, radiation chimeras are more resistant to lethal P. berghei ANKA challenge, which may explain the extended survival among both PBS- and DTX-treated chimeric mice, relative to the H2O- and H2O + l-glutamine-treated mice. Finally, we observed that MBC (Extended Data Fig. 7c) and LLPC (Extended Data Fig. 7d) responses were not enhanced when l-glutamine was administered from days 16–21 post-infection, which is consistent with the therapeutic window during which l-glutamine functions to enhance parasite control (days 6–10 post-infection). Thus, the mechanisms by which l-glutamine promotes MBC and LLPC formation are probably linked to the enhanced function of the GC reaction and not strictly connected to enhancements in the post-GC survival of these cell subsets. Collectively, these data show that either CD138^hi plasmablast deletion or therapeutic administration of l-glutamine during an established blood-stage Plasmodium infection can be sufficient to accelerate the formation and increase the magnitude of GC reactions, which are further associated with enhancements in MBC, LLPC and protective immune memory responses.

Robust plasmablast expansions during acute human malaria. To identify potential parallels between CD138^hi plasmablast inductions in mice and plasmablast expansions during acute human malaria, we evaluated peripheral B cell responses in 40 humans in P. falciparum malaria-naive subjects were challenged with P. falciparum acute blood-stage parasites at ~20,000 parasites per ml, at day 8 post-infection. Importantly, acute blood-stage P. falciparum infection resulted in transient yet substantial increases in the frequencies of CD19^CD3^-CD27^CD38^ plasmablasts by day 14/15 post-infection, with some subjects exhibiting plasmablast responses that comprised greater than 15% of their entire peripheral B cell pool (Fig. 8a). Notably, the magnitude of plasmablast expansion was positively correlated with total parasite biomass (Fig. 8b). These clinical data show that plasmablast responses are robustly induced in malaria-naive humans at their first exposure to P. falciparum in a way similar to experimental murine models of malaria.

Together, our observations in P. falciparum-infected humans, in combination with our rodent data, support the hypothesis that in malaria-naive hosts the initial B cell activation events are numerically dominated by extrafollicular plasmablasts/CD138^hi B cells. Furthermore, our cellular, genetic and biochemical approaches support that plasmablasts functionally constrain GC responses and limit the induction of LLPC and MBC populations by acting as a metabolic sink that limits l-glutamine availability during experimental malaria.

**Discussion**

Rapidly forming, short-lived extrafollicular plasmablasts play complex roles in humoral immunity and host protection. Indeed, it is widely accepted that plasmablasts can provide an early source of protective antibody during bacterial infections. Similar to LLPCs, short-lived plasmablasts express Blimp-1 and are equipped with the machinery to secrete antibodies. However, there are also data suggesting that plasmablasts may either inefficiently prime or directly inhibit T_{H1} cell activation and function, which may limit autoreactivity and tune the selection of B cell clones expressing the highest-affinity receptors. While our data do not exclude the possibility that blood-stage Plasmodium infection-induced plasmablasts directly impair T_{H1} cell responses, our results show that either abrogation of plasmablast differentiation or deletion of plasmablasts during an established experimental Plasmodium infection resulted in enhanced clearance of the parasite, which was associated with tenfold increases in the magnitude of the GC response. Our observations in mice are further supported by our P. falciparum VIS trials where substantial plasmablast expansions occurred and were positively correlated with parasite burdens. Distinct from previous work showing immunosuppressive roles for either plasmablasts or plasma cells, our data reveal that CD138^hi plasmablast populations comprise the dominant proportion of the total activated B cell pool following blood-stage Plasmodium infections. Thus, their over-representation may.

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**Fig. 5 | l-glutamine supplementation enhances GC responses. a–n.** Mice of the indicated genotypes were either left untreated (H2O) or treated with l-glutamine-supplemented water (H2O + l-glut) starting on day 0 post-infection. a,b. Kinetics of parasite burden (a) and AUC as a measure of total parasite biomass (b). Data are means ± s.d., representative of n = 3 biologically independent experiments with similar results using n = 4 mice per group. Data in a were analyzed using a two-way ANOVA with Šidák’s multiple comparisons test (d.f. = 6; F = 6.542). Data in b were analyzed with a two-tailed Mann–Whitney test. c. Kinetics of parasite burden in wild-type mice treated with either GK1.5 or rIgG on days 5 and 7 post-infection. Data are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 6 mice (H2O + l-glut + GK1.5, H2O + rIgG and H2O + l-glut and H2O + rIgG) and n = 7 mice (H2O + l-glut + rG). d. Kinetics of parasite burden in CBA-Cre × B6CD6^l-glut^ mice. Data are means ± s.e., pooled from n = 2 biologically independent experiments with n = 7 mice per group. e. Kinetics of parasite burden in CBA-Cre × B6CD6^l-glut^ mice. Data are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 6 mice per group. f,g. Numbers of splenic GC B cells (f) and GC T_{H1}, like cells (g) on day 12 post-infection in P. yoelli-infected mice. Data are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 6 mice per group. h–i. Anti-MSPL1 IgG serum titers on days 12 (h) and 15 (i) post-infection. Data in h and i are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 6 (h), n = 9 (i) H2O mice or n = 8 mice per group (H2O + l-glut). j–m. Confocal micrographs (j: scale bars: 500 μm), numbers of B cell follicles (k: n = 8 mice for H2O; n = 7 mice for H2O + l-glut), number of GL7^hi B cell follicles (l: n = 7 mice per group) and surface area of B cell follicles per focal area (m: n = 58 foci for H2O; n = 78 foci for H2O + l-glut) in the spleens of mice on day 12 post-infection. Data in k–m are means ± s.e.m., pooled from n = 2 biologically independent experiments. n,o. OCRs (n) and basal respiration (o; minutes 0–20) of plasmablasts and activated B cells isolated on day 10 post-infection. Data in n and o are means ± s.e.m., representative of n = 2 biologically independent experiments with similar results using n = 5 (plasmablasts with H2O and activated B cells with H2O + l-glut) and n = 6 (activated B cells with H2O and plasmablasts with H2O + l-glut) technical replicates using 2.5×10^5 cells per well sorted from n = 3 mice per group. Symbols in b, f, g, i, k and l represent individual mice. Symbols in m and o represent technical replicates. Data in m were analyzed using a two-tailed Mann–Whitney test. PB, plasmablasts.
be a cardinal feature of blood-stage *Plasmodium* infections and potentially other infections of the blood that are associated with systemic inflammation, eryptosis, hemolysis, anemia and impairments in durable humoral immunity.

Plasmablast numbers have stood as a surrogate for protective immunity\(^4^1\). However, our assays revealed that few (~1%) plasmablasts express receptors that detectably interact with parasite antigens and that plasmablasts constrain rather than promote humoral immunity. Our efforts to identify potential mechanisms by which CD138\(^{hi}\) plasmablasts constrain GC-dependent MBC and LLPC formation revealed links to their metabolism. From a cell biological perspective, *Plasmodium* infection-induced plasmablasts resemble a transient plasmablastic lymphoma similar to diffuse large B cell lymphoma characterized by downregulation of tumor suppressor genes and upregulation of genes that promote tumorigenesis. Moreover, tumor cells\(^4^2,4^3\) and plasmablasts express an array of SLC
molecules, probably to help meet the metabolic demands of their high rates of proliferation and increases in cellular biomass. It has also been reported that cancer cells are addicted to glutamine and are capable of acting as a metabolic sink. Thus, we hypothesized that the large numbers and increased metabolic demands of plasmablasts, together with their high expression of SLC molecules, may cause energetic and metabolic shortfalls for other effector B cells in the spleen, which may in turn functionally incapacitate and/or delay development of the GC response. Consistent with this hypothesis, our studies show that supplementing the drinking water of Plasmodium yoelii-infected mice with l-glutamine could relieve the apparent plasmablast-mediated state of nutrient deprivation that the large numbers and increased metabolic demands of plasmablasts, together with their high expression of SLC molecules, probably cause energetic and metabolic shortfalls for other effector B cells in the spleen, which may in turn functionally incapacitate and/or delay development of the GC response. Consistent with this hypothesis, our studies show that supplementing the drinking water of Plasmodium yoelii-infected mice with l-glutamine could relieve the apparent plasmablast-mediated state of nutrient deprivation and elevate GC, MBC, LLPC and LLPC responses. Our kinetic studies revealed a critical window during which l-glutamine supplementation can augment GC-dependent humoral immunity, which temporally overlaps with the expansion and maximal accumulation of Plasmodium blood-stage infection-induced plasmablasts. Thus, although both plasmablasts and GC B cells may exhibit similar metabolic requirements, the over-representation of plasmablasts probably depletes GC B cells of critical nutrients required for their development and function. Future studies will be required to determine whether plasmablast deletion and associated changes in the metabolic fitness of GC B cells also impact either B cell antigen receptor affinity maturation or epigenetic programming of MBCs and LLPCs. Although 13C-l-glutamine tracing studies designed to determine whether plasmablasts sink glutamine in vivo are warranted, these studies are confounded by the time required for tissue processing. Nevertheless, the composite of data support the model that plasmablasts serve as a key glutamine sink during blood-stage Plasmodium infection and the hypothesis that alternative, metabolism-based strategies may have clinically relevant applications for malaria.

Previous studies reported that dietary supplementation of l-arginine enhanced T cell responses in rodents during lethal Plasmodium infection and that inhibiting glutamine metabolism with 6-diazo-5-oxo-l-norleucine (DON) improved survival and prevented the onset of experimental cerebral malaria (ECM) in mice challenged with Plasmodium berghei ANKA. No mechanisms were identified in the l-arginine study and ref. 46 primarily attributed the effects of DON to inhibition of CD8+ T cell degranulation—a key
function that drives the onset of ECM. During experimental *P. yoelii* and *P. berghei* ANKA infections truncated with anti-blood-stage drugs, neither of which cause CD8⁺ T cell-mediated immunopathology and ECM, CD8⁺ T cells were robustly activated and contributed to host protection. Thus, our data do not exclude the possibility that an enhanced parasite-specific CD8 T cell response also contributes to the improvements in host resistance to *Plasmodium* during l-glutamine supplementation. We predict that strategies to specifically inhibit l-glutamine uptake by plasmablasts would yield results similar to those we observed when we either

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**Fig. 7 | Plasmablast deletion and l-glutamine enhance humoral immune memory.** a-c, *P. yoelii*-infected CD138-DTR bone marrow chimeras were treated with vehicle (PBS) or DTX on days 5 and 7 post-infection. The kinetics of splenic MBCs (a) and bone marrow ASCs (b) are shown. Data in a and b are means ± s.e.m., pooled from *n* = 2 biologically independent experiments with *n* = 4 (day 60) and *n* = 6 mice per group (days 10, 15 and 21). c, Survival of mice challenged with *P. berghei* ANKA 30 d after an initial *P. yoelii* infection. Data are pooled from two biologically independent experiments with *n* = 7 (DTX) and *n* = 8 mice (PBS). d-f, *P. yoelii*-infected wild-type mice were either left untreated (H₂O) or treated with l-glutamine-supplemented water (H₂O + l-glut) starting on day 0 post-infection. d, Kinetics of splenic MBC responses. e, Kinetics of bone marrow ASCs. Data in d and e are means ± s.e.m., pooled from *n* = 2 biologically independent experiments with *n* = 6 mice per group. f, Survival of mice challenged with *P. berghei* ANKA 30 d after an initial *P. yoelii* infection. Data are pooled from *n* = 2 independent experiments with *n* = 10 mice per group. Data in a, b, d and e were analyzed by two-tailed Mann-Whitney U-test. Data in c and f were analyzed by Mantel-Cox test.

**Fig. 8 | Positive correlation between parasite burden and plasmablasts in human malaria.** a, Plots (left; gated on CD19⁺CD3⁻ cells) and summary graph (right) showing the kinetics of plasmablasts in the peripheral blood of volunteers drawn on the indicated days during the course of the study. Boxplots show median values ± the interquartile range. Whiskers show the data range to 1.5 times the interquartile range. Data were analyzed by two-sided Wilcoxon matched-pairs signed rank test. b, Correlation graph showing the relationship between parasite burden on day 15 (AUC) and the increase in plasmablast numbers from day 0 to day 15 (ΔPB). Data were analyzed by two-tailed Spearman’s rho. Data in a and b derive from *n* = 36 men and *n* = 4 women from four studies across six independent cohorts.
abrogated plasmablast development or deleted these cells using genetic approaches. However, at present, we know of no way to block either glutamine uptake or glutaminolysis specifically in plasmablasts. Nevertheless, our data and the experiments with l-arginine and DON during experimental malaria highlight critical roles for immune cell metabolism and warrant the further exploration of strategies to modulate these cell biological processes in specific cell types.

In summary, our data support the model that during blood-stage Plasmodium infection, B cell differentiation is biased towards metabolically hyperactive, short-lived plasmablast responses that by their sheer numbers constrain GC reactions, probably due to glutamine deprivation. The preferential induction of extrafollicular, immunosuppressive plasmablasts represents an additional mechanism by which Plasmodium parasites subvert host protective immunity. Thus, the identification of an expansion of immuno-inhibitory plasmablasts during Plasmodium infection represents a conceptual shift with implications for either future immune- or metabolism-based strategies designed to limit plasmablast expansions, which may ultimately contribute to the development of durable humoral immunity against malaria.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41590-020-0678-5.

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Articles

Methods

VISs. *P. falciparum* blood-stage VISs, inoculum preparation, volunteer recruitment, infection, monitoring and treatment were performed as previously described. In brief, healthy malaria-naive individuals (n = 36 men; n = 4 women) underwent induced blood-stage malaria inoculation with 2,800 viable *P. falciparum* 3D7-parasitized RBCs, and peripheral parasitemia was measured at least daily by quantitative PCR as described previously. Participants were treated with antimalarial drugs at day 8 of infection. Blood samples from 40 volunteers (from four studies across six independent cohorts) were collected before infection (day 0), at peak infection (day 8) and 14 or 15 and 27–36 (EOS) days after infection (in the analyses, these time points are grouped as 0, 8, 14/15 and EOS). Plasma was collected from lithium heparin whole-blood samples according to standard procedures, snap-frozen in dry ice and stored at −70°C. B cells were analyzed from fresh whole blood at the time of collection. All studies were registered with the US National Institutes of Health (NIH) at https://clinicaltrials.gov/ (NCT02867059, NCT02783833, NCT02431650 (ref. 39)). Participants were healthy adults aged 18 and 55 years with no previous exposure to malaria or residence in malaria-endemic regions. Area under the curve (AUC) values were calculated using the trapezoidal method on serial log10 parasites/ml data from 4d post-infection to each of the three defined time points (8, 14/15 and EOS; similar to previously described). Equation 1 below describes the calculation, with t being each time point sampled, P being the log10 parasites/ml at that time and T0 being either 8d post-infection, 14/15d post-infection or EOS.

\[
\text{AUC}_{T_0} = \frac{1}{2} \sum_{i=1}^{n} (P_i + P_{i+1}) \times (T_i - T_{i-1})
\]

Samples for which parasitemia was not detected were substituted with 0 on the log10 scale. The samples collected between the four defined time points (ranging from daily to twice daily before treatment, ranging from daily to every 2h after treatment, and ranging from every 4d to daily between time point 14/15 and EOS) were used in the calculation of AUC but not in any other analyses.

Study approval. Written informed consent was obtained from all participants. Ethics approval for the VISs and the use of human samples was obtained from the Human Research and Ethics Committee of the QMIR Berghofer Institute of Medical Research and Alfred Human Research and Ethics Committee for the Burnet Institute.

Animals, infections and parasitemia quantification. The University of Iowa Institutional Animal Care and Use Committee approved all of the experiments. C57BL/6 wild-type (CD45.2 and CD45.1), Blmpy1-1EYF (JAX stock; 008828), μMT (JAX stock; 002288) and Rosa26-ERT2/Cre (JAX stock; 008463) mice were purchased from The Jackson Laboratory. Aicda−/− mice were a gift from E. Lund (Oxford, UK). CD138−/− mice were generated via Cas9 editing at the University of Iowa Genome Editing Facility. Male and female mice were used in all of the experiments, sex-matched only for adoptive transfers and generating chimeras. *P. yoelii* (clone 17XNL; obtained from the Malaria Research and Reference Reagent Resource (MR4), American Type Culture Collection) stocks were generated by single passage in NIH Swiss Webster (NIHSC) mice. Infections in experimental mice were initiated by a serial intravenous inoculation of either recombinant MSP119 or parasite-infected RBC lysate. Plates were washed with PBS (3 min per wash) then stained with 1X PBS (3 min per wash). Sections were blocked with 1% bovine serum albumin (BSA) in 1X PBS for 2h at 25°C. After a 5-min wash in 1X PBS, sections were stained with the primary antibodies (1% BSA in 1X PBS) for 2h at 4°C. Sections were washed in 1X PBS (3 min per wash) then mounted using hard-set mounting medium for fluorescence (VECTASHIELD H-1000). Sections were stained with B220-AF488 (5 μg/ml; clone RA3-6B2; eBioscience) and CD4-AF647 (6.7 μg/ml; BD Pharmingen) and CD138- BV421 (1.67 μg/ml; clone 28-1; BioLegend). Imaging was done using a Zeiss LSM710 confocal microscope and images were processed using IMARIS x64 software (version 9.2.1).

Bone marrow chimeras. For the generation of Rosa26-ERT2/Cre Prdm1Δ15-mMT chimeras, wild-type recipient (CD45.1) mice were irradiated with 475rads twice, separated by 4h. Bone marrow cells from Rosa26-ERT2/Cre Prdm1Δ15 (CD45.2) and μMT mice (CD45.1) were mixed 1:1 and 1x10⁷ cells were injected intravenously. Mice were monitored every 2–3d post-infection. Monitoring and treatment were performed as previously described. Area under the curve (AUC) values were calculated using the trapezoidal method on serial log10 parasites/ml data from 4d post-infection to each of the three defined time points (8, 14/15 and EOS; similar to previously described). Equation 1 below describes the calculation, with t being each time point sampled, P being the log10 parasites/ml at that time and T0 being either 8d post-infection, 14/15d post-infection or EOS. Mice were maintained on a Uniprim diet (Envigo) for 2–3 weeks. Chimerism was assessed at 6 weeks and mice were infected with 1x10⁷ P. yoelii-parasitized RBCs. On days 5 and 7 post-infection, mice were treated orally using a gavage needle, with either 100μl tamoxifen (4mg per mouse) or corn oil. Depletion of plasmablasts was assessed on day 10.

For the generation of CD138−DTR chimeras, wild-type (CD45.2) mice were irradiated with 475rads twice, separated by 4h. Some 1x10⁷ bone marrow cells from CD138−DTR mice were injected intravenously. Mice were maintained on a Uniprim diet as above. Chimerism assessments, infections and tamoxifen treatments were as described above.

Tamoxifen and DTX preparation and treatment. Desiccated tamoxifen (1g; Sigma–Aldrich; T5648) was dissolved in 5ml of 200-proof ethanol, mixed with 20ml corn oil and stored at −20°C. On days 4, 5 and 6 after *P. yoelii* infection, mice were administered 100μl (4mg per mouse per dose) of the tamoxifen corn oil mixture via oral gavage. Lyophilized DTX (1mg; Sigma–Aldrich; D5634) was resuspended in 3ml sterile PBS to achieve a concentration of 0.33mg/ml. Mice were treated intraperitoneally with 250ng DTX on the indicated days.

RNA-seq and gene set enrichment analysis. The three different B cell populations (resting B cells, activated B cells and plasmablasts) were flow-sorted from four *P. yoelii* infected mice on day 10 post-infection and RNA was extracted using a Nucleospin RNA kit (Takara Bio USA) according the manufacturer’s protocol. RNA sample integrity and quantity were determined using a TapeStation Bioanalyzer (Agilent), with all samples showing RNA integrity numbers > 8. Total RNA (20ng) of was used for each library. A strand-specific RNA-seq library was created using the Illumina library preparation protocol. Libraries were pooled and sequenced using an Illumina NextSeq 550 sequencer using paired-end chemistry with a 50-base pair read length (Gene Expression Omnibus record: GSE134548). The quality of sequence reads was assessed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/) and they were aligned using STAR aligner to the mouse genome version mm10 and the corresponding isoforms, following read alignment, gene expression profiles were computed using featureCounts. Next, differentially expressed genes (2 > fold-change > −2) with an adjusted P value threshold of 0.05 were identified using Partek Genomics Suite software. Heat maps to enable the visualization of differentially expressed genes were generated using Partek Genomics Suite software. Furthermore, Ingenuity Pathway Analysis software (Qiagen Bioinformatics) was used to identify the underlying molecular pathways and interaction networks that involved the differentially expressed genes.

Quantitative real-time PCR and primers. The three different B cell populations (resting B cells, activated B cells and plasmablasts) were flow-sorted from four *P. yoelii* infected mice on day 10 post-infection and RNA was extracted using a Nucleospin RNA kit (Takara Bio USA) according the manufacturer’s protocol. RNA (2μg) was used for complementary DNA synthesis. Complementary DNA (1μl) was added to 19μl of PCR mixture containing 2x PowerUp SYBR Green Master Mix and 0.2μM forward or reverse primers. Amplification was performed in a QuantStudio 3 thermocycler (Applied Biosystems). Genes and forward and reverse primers are shown in Supplementary Table 6. Cycle threshold (Cq) values were normalized to those of the housekeeping gene hypoxanthine phosphoribosyltransferase (*Hprt*) using the following equation: ΔCq = Cqgene − CtHprt. All of the results are shown as a ration of *Hprt*, calculated as 2ΔCq.

ELISpot. White polylysine plates (Nunc MaxiSorp) were coated with 0.5μg ml−1 of either recombinant MSP1α or parasite-infected RBC lysate. Plates were washed with PBS and blocked for at least 2h with PBS/2.5% BSA/5% goat serum. Bone marrow cells or CD138−CD19−IgM− B cells sort-purified from *P. yoelii*-infected...
mice were used. Serial dilutions of cells in supplemented Iscoves modified Dulbecco’s media were added to each well for 20h at 37°C with 5% CO2. Following extensive washes with PBS/0.05% Tween 20, horseradish peroxidase-conjugated goat anti-mouse IgM and IgG was added overnight at 4°C. Spots were developed with 3-amin-o-naphthyl-4-blue-chloro-benzene.

Metabolic flux analysis. Flow-sorted B cell populations (resting B cells, activated B cells and plasmablasts) from at least three P. yoelii-infected mice (day 10 post-infection) were grown in 250,000 cells per well in a poly-lysis-coated Seahorse XF96 cell culture microplate. Cells were allowed to adhere for 30 min to 1h. The OCR was measured in modified Dulbecco’s modified Eagle medium containing 2 mM l-glutamine (XF media) under basal conditions in a 96-well extracellular flux assay using an XFe-96 (Seahorse Bioscience). In experiments comparing the metabolic activity of B cell populations sorted from mice maintained on either regular water or water supplemented with l-glutamine, the culture medium was devoid of l-glutamine.

Metabolite measurements. Frozen spleens from naive and P. yoelii-infected mice (~40 mg) were homogenized and, after blocking total parasite lysate-coated Nunc plates and processed as described above. For the ex vivo CD138<sup>hi</sup> plasmablasts were sorted-purified from mice at day 10 post-infection and cultured in supplemented Iscoves modified Dulbecco’s media (10% FBS, 2 µg/ml emetine, 100 µM l- penicillin, 100 µg/ml streptomycin and 50 mM β-mercaptoethanol) in the presence of 5% normal goat serum. Supernatants were recovered after 3 d of culture. CD138<sup>+</sup> B cell supernatants were diluted 1:2 and added to previously coated with either 0.5% normal goat serum and resuspended at 10<sup>6</sup> cells per 100 µl. Fc receptors were blocked using Fc block/CD16/32 (clone 2.4G2) in FACS buffer (PBS+0.09% sodium azide +2% fetal calf serum) for 15 min at 4°C, followed by cell-staining protocol as described below. For detecting GC T<sub>eff</sub> like cells, cells were stained with purified rat or mouse CXC5R1 (1:100; clone 2G8; BD Biosciences) in T<sub>eff</sub> staining buffer (PBS+0.09% sodium azide +2% fetal calf serum +0.5% BSA +2% mouse serum) for 1 h at 4°C. Cells were washed in FACS buffer, spun at 300g for 5 min at 4°C and resuspended in Biotin-SP-conjugated AffiniPure Goat anti-rat IgG (H + L) F(ab)1 (1:100; Jackson ImmunoResearch) in T<sub>eff</sub> staining buffer and incubated for 30 min at 4°C. Cells were washed in FACS buffer, spun at 300g for 5 min at 4°C and resuspended in an antibody cocktail containing CD4 PerCP-Cy5.5 (1:300; clone GK1.5; BioLegend), CD44-AF700 (1:100; clone IM7; BioLegend), CD11a-IFC (1:300; clone M17/4; BD Biosciences), PD-1-PE or PE-Cy7 (1:300; clone RMP1-14; Biologend) and streptavidin-BV421 (1:500) and incubated for 30 min at 4°C. The cells were washed with FACS buffer as before and resuspended in FACS buffer before acquisition.

Cell staining and flow cytometry. For cellular analyses of splenic cells, mouse spleens were perforated through a 70-µm mesh to generate single-cell suspensions. The single-cell suspensions of splenocytes were subjected to RBC lysis, counted and resuspended at 10<sup>8</sup> cells per 100 µl. Fc receptors were blocked using Fc block/CD16/32 (clone 2.4G2) in FACS buffer (PBS+0.09% sodium azide +2% fetal calf serum) for 15 min at 4°C, followed by cell-staining protocol as described below. For detecting GC T<sub>eff</sub> like cells, cells were stained with purified rat or mouse CXC5R1 (1:100; clone 2G8; BD Biosciences) in T<sub>eff</sub> staining buffer (PBS+0.09% sodium azide +2% fetal calf serum +0.5% BSA +2% mouse serum) for 1 h at 4°C. Cells were washed in FACS buffer, spun at 300g for 5 min at 4°C and resuspended in Biotin-SP-conjugated AffiniPure Goat anti-rat IgG (H + L) F(ab)1 (1:100; Jackson ImmunoResearch) in T<sub>eff</sub> staining buffer and incubated for 30 min at 4°C. Cells were washed in FACS buffer, spun at 300g for 5 min at 4°C and resuspended in an antibody cocktail containing CD4 PerCP-Cy5.5 (1:300; clone GK1.5; BioLegend), CD44-AF700 (1:100; clone IM7; BioLegend), CD11a-IFC (1:300; clone M17/4; BD Biosciences), PD-1-PE or PE-Cy7 (1:300; clone RMP1-14; Biologend) and streptavidin-BV421 (1:500) and incubated for 30 min at 4°C. The cells were washed with FACS buffer as before and resuspended in FACS buffer before acquisition. For B cell staining, cell staining were stained with: rat anti-mouse B220-PerCP-Cy5.5 (1:300; clone RA3-6B2; BioLegend); rat anti-mouse CD19-APC (1:300; clone J55; BioLegend); rat anti-mouse IgD-Pac Blue or BV510 (1:500; clone 11-26 c.2a; BioLegend); rat anti-mouse CD138-APC or Pac Blue (1:250; clone 281-1; BioLegend) (for plasmablast staining); rat anti-mouse GL7-PE (1:300; BioLegend) and hamster anti-mouse CD95-FITC (1:300; clone Jo2; BD Biosciences) (for GC B cell staining); or rat anti-mouse CD23-PE Cy7 (1:300; clone B38; BioLegend) and rat anti-mouse CD21-APC (1:300; clone 7E5; BioLegend) (for marginal zone B cell staining). For human B cell staining, 200 µl blood was stained with surface antibodies to CD19 (FITC; clone HIB19), CD27 (AF700; clone M-T271) or CD38 (BV785; clone HLT2).

BrDU and FLICA staining. BrdU (Sigma–Aldrich) was injected intraperitoneally at 2 mg and supplemented in the drinking water at 0.8 mg ml<sup>−1</sup> from days 6–10 post-infection. FLICA reagent was prepared and added to cells according to the manufacturer’s instructions (Thermo Fisher Scientific). For BrdU staining, cells were permeabilized, treated with 5 µg ml<sup>−1</sup> DNase (Invitrogen) and subjected to intracellular staining with either anti-BrdU-FITC (clone PRB-1) or mouse IgG1-FITC.

Statistical analysis. All statistical analyses were performed using GraphPad Prism 6 software (GraphPad). Specific tests of statistical significance are detailed in the figure captions.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. Source data for Figs. 1–8 and Extended Data Figs. 1–3, 5 and 7 are presented with the paper. The data that support the findings of this study are available from the Gene Expression Omnibus (accession code GSE134548). All data are available from the corresponding author upon request.

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Author contributions. R.V., J.J.G. and N.S.B. conceptualized the project. J.J.G. performed the foundational studies and characterized the phenotype of plasmablasts in mice. R.V. characterized the expression repertoire of the plasmablasts and conceived and executed the adoptive transfer studies, chimeric studies, metabolism analyses and fluorescence at low concentrations.
assistance. C.R.E., J.S.M. and M.J.B. supervised the clinical studies. N.S.B. supervised the experimental rodent studies. R.V., J.J.G., H.-H.X., C.R.E., J.S.M., M.J.B. and N.S.B. designed the experiments. R.V., J.J.G., A.J.S., F.A.S., R.L.P., D.A., J.-A.C. and F.d.L.R. performed the experiments. R.V., J.J.G., A.J.S., F.A.S., B.R.S., J.-A.C., F.d.L.R., L.W., C.R.E., J.S.M., M.J.B. and N.S.B. analyzed the data. R.V. and R.R.S. performed the bioinformatics analyses. R.V. and J.J.G. made the figures. R.V. wrote the original draft. J.J.G., A.J.S., C.R.E., J.S.M., M.J.B. and N.S.B. reviewed and edited the manuscript and figures.

**Competing interests**
The authors declare no competing interests.

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Extended Data Fig. 1 | Experimental malaria expands extrafollicular plasmablast populations. a, Gating strategy for identifying plasmablasts (CD138hiIgDneg), activated (CD138loIgDneg) and resting B cells (CD138loIgDhi). b, kinetics of PB in blood. Data are means ± s.d, representative of n = 2 biologically independent experiments with similar results using n = 3 mice/time point. c, CD138hiIgDneg plasmablasts were sort-purified from Py-infected mice on day 10 p.i., cultured for 20 hours and parasite lysate-specific IgM and IgG secreting ASCs were detected. Representative wells of ELISPOT assay. d, Relative CD19 expression by CD138hiIgDneg plasmablasts on days 7, 10, and 14 post Py infection. Data are representative of n > 5 experiments with similar results. e, BrdU incorporation in CD138hiIgDneg plasmablasts was assessed on day 10 p.i. Histogram represents BrdU staining, solid gray histogram is isotype (mouse IgG1). Data are representative of n = 2 experiments with similar results using n = 8 mice. f, Forward scatter and side scatter of CD138hiIgDneg plasmablasts examined on day 10 p.i. Data are representative of n > 5 experiments with similar results. g, Blimp-1/eYFP reporter mice were infected with Py. CD138hi Blimp-1/eYFP+ cells in bone marrow from naïve (left panel) or day 21 infected mice (right panel). Data are representative of n = 2 independent experiments with similar results using n = 4 mice/group. h, i, CD21 and CD23 expression by B cells in a naïve mouse (h) and day 10 Py-infected mouse (i) showing plasmablasts (green box) activated (blue box) and resting (red box) B cells. Data are representative of n > 5 experiments with similar results using n = 3 mice/group. j, Representative plots of the frequency and total numbers of GC B cells (GL7+CD95+) among plasmablasts, activated and resting B cell populations on day 10 p.i. Data are means ± s.e.m., representative of n = 3 experiments with similar results using n = 5 (Day 0 Total B cells) and n = 4 mice (each remaining group).
Extended Data Fig. 2 | Developmental abrogation of blood stage Plasmodium infection-induced plasmablast responses. a, Experimental design for adoptive transfers. b, Experimental design for Rosa26-ERT2/Cre × Prdm1<sup>fl/fl</sup> (CD45.2) : µMT bone marrow chimeric system. Eight weeks after engraftment, mice were infected with 1 × 10<sup>6</sup> Py and then treated with either corn oil or tamoxifen on days 4, 5, and 6 p.i. Data are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 6 mice/group. c, Gating strategy of TFH cells. d, Kinetics of parasite burden in Py-infected wild-type mice treated with tamoxifen or corn oil on days 4, 5, and 6 p.i. Data are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 6 mice/group. e, Experimental design for the three-way mixed bone marrow chimera. f, Eight weeks after engraftment, mice were infected with Py, treated with either corn oil or tamoxifen on days 4, 5, and 6 p.i. and the relative proportions (pie diagram) of Prdm1<sup>fl/fl</sup> (CD45.2) and wild-type (CD45.1) cells in the GC B cell compartment was analyzed. Data are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 4 mice/group. g, Evaluation of T<sub>H</sub>1 responses in PBS and tamoxifen-treated mixed bone marrow chimeric mice (as shown in b). Data are means ± s.d. representative of n = 2 biologically independent experiments with similar results using n = 5 (corn oil) and n = 4 mice (tamoxifen). Data in f,g were analyzed using two-tailed Mann-Whitney. Symbols and symbols represent individual mice.
Extended Data Fig. 3 | Deletion of blood stage Plasmodium infection-induced plasmablast responses. a, Experimental design for generating CD138-DTR chimeras. Eight weeks after engraftment, mice were infected with $1 \times 10^6$ Py and on days 5 and 7 p.i. treated with either DTx or PBS to delete plasmablasts. Data are means ± s.d., representative of $n = 2$ independent experiments with similar results using $n = 4$ mice/group. b-d, Py-infected wild-type mice were treated with either DTx or PBS on days 5 and 7 p.i. Kinetics of parasite burden (b), representative plots and summary data of GC B cells (c) and GC-Tfh cells (d) on 21 p.i. Data are means ± s.d., representative of $n = 2$ biologically independent experiments with similar results using $n = 4$ (PBS) and $n = 3$ mice (DTx). e-g, CD138-DTR chimeric mice were infected with $1 \times 10^6$ Py, plasmablasts were deleted with DTx and mice were subsequently treated with either MR-1 (anti-CD40L) or hamster IgG on days 8–11 p.i. Representative plots and summary data of GC B (e) and GC-Tfh (f) cells as measured on day 21 p.i. and kinetics of parasite burden (g). Data are means ± s.e.m., pooled from $n = 2$ biologically independent experiments with $n = 6$ mice/group. Symbols in c-f represent individual mice and data were analyzed using two-tailed Mann-Whitney.
Extended Data Fig. 4 | Differentially expressed genes among splenic B cell populations. a, Venn diagram showing differentially expressed genes assessed by RNA-seq among the three splenic B cell populations on day 10 p.i. Respective cell types were sort-purified from $n = 4$ Py-infected mice. Data were obtained from one RNA-Seq experiment. Two-tailed ANOVA was used for identifying differentially expressed genes. b, Heat map showing the relative expression of all annotated genes assessed using RNA-Seq. c, Heat map showing the relative expression of genes involved in the unfolded protein response pathway (UPR).
Extended Data Fig. 5 | See next page for caption.
Extended Data Fig. 5 | L-glutamine enhances GC responses during experimental malaria. a, L-glut concentrations in the spleens of naive and Py-infected wild-type mice on day 5 p.i. Data are means ± s.d. representative of n = 2 biologically independent experiments with similar results using n = 3 mice/group analyzed by a two-tailed unpaired t test (DF = 4; t = 5.933). b, c, Kinetics of parasite burden in mice treated with either L-alanine (b, H2O + L-ala), L-valine (c, H2O + L-val) or water starting on day 0 p.i. Data are means ± s.d., representative of n = 2 independent experiments with similar results using n = 3 mice/group. d–f, Py-infected wild-type mice were treated with L-glutamine (H2O + L-glut) or water starting day 0 p.i. and subsequently treated with MR-1 (anti-CD40L) or Hamster IgG antibody on days 8–11 p.i. Kinetics of parasite burden (d) and frequency of GC B cells (e) and GC-Tfh cells (f) on day 21 p.i. Data in d are means ± s.e.m, pooled from n = 2 independent experiments with similar results using n = 7 mice/group. Data in e,f are representative of n = 2 biologically independent experiments with similar results using n = 3 mice/group. g–m, Py-infected wild-type mice were treated with L-glutamine (H2O + L-glut) or water starting day 0 p.i. Kinetics of GC B cells (g), class-switched GC B cells (h), plasmablasts (i), and GC-Tfh-like cells (j). Data in g–j are means ± s.d., representative of n = 2 biologically independent experiments with similar results using n = 6 mice/group analyzed by two-tailed Mann-Whitney. k, gMFI of BCL6 on GC-Tfh-like cells. Data are means ± s.d., representative of n = 2 biologically independent experiments with similar results using n = 3 mice/group analyzed by two-tailed unpaired t test (DF = 4; t = 1.257). Number of TH1 cells (l, Ly6C+CXCR3+IFNg+) and gMFI of CD80 and CD86 expression on splenic dendritic cells (m, MHCII+CD11c+) on day 10 p.i. Data in l,m are means ± s.e.m, pooled from n = 2 biologically independent experiments with n = 6 (H2O) and n = 7 mice (H2O + L-glut) analyzed by two-tailed Mann-Whitney. n, o, Kinetics of parasite burden (n) and area under curve as a measure of total parasite biomass (o) in Py-infected wild-type mice treated with L-glutamine (H2O + L-glut) starting on day 6 p.i. Data in n are means ± s.d., representative of n = 2 biologically independent experiments with similar results using n = 4 mice/group analyzed using two-way ANOVA with Sidak’s multiple comparison (DF = 5; F = 5.728). Data in o analyzed with two-tailed Mann Whitney. p–q, Kinetics of parasite burden (p) and area under curve (q) as a measure of total parasite biomass in Py-infected wild-type mice treated with L-glutamine (H2O + L-glut) starting day on 10 p.i. Data in p,q are means ± s.d., representative of n = 2 independent experiments with similar results using n = 4 mice/group analyzed by two-tailed Mann Whitney. Symbols in a, l–q represent individual mice.
Extended Data Fig. 6 | Experimental design for treating CD138-DTR: WT chimeras with L-glutamine. Eight weeks after engraftment, mice were infected with Py and treated with L-glut or water starting on day 0 p.i. Mice were subsequently treated with either DTx or PBS on days 5 and 7 p.i. to delete plasmablasts.
Extended Data Fig. 7 | Post-GC administration of L-glutamine does not appreciably enhance LLPC and MBC responses.  

**a.** Gating strategy for long lived plasma cells (LLPCs) in bone marrow.  

**b.** Numbers of LLPCs in the bone marrow on day 60 p.i. from Py-infected mice treated with either L-glutamine (H2O + L-glut) or water (H2O). Data are means ± s.e.m., pooled from n = 2 biologically independent experiments with n = 7 mice/group analyzed by two-tailed Mann Whitney.  

**c, d.** Py-infected wild-type mice were treated with either L-glutamine (H2O + L-glut) or water (H2O) at indicated time points p.i. and analyzed on day 30 p.i. Numbers of splenic memory B cells (c, CCR6+CD38+) and representative ELISPOT wells and summary data (d) demonstrating number of antibody-secreting LLPCs in the bone marrow. Data in **c, d** are means ± s.e.m, pooled from n = 2 biologically independent experiments with n = 5 (d, days 6–10) and n = 6 mice (remaining groups) analyzed by two-tailed Mann Whitney. Symbols represent individual mice.
Extended Data Fig. 8 | Experimental design for the volunteer infection study. Two thousand eight hundred viable *P. falciparum* infected RBCs were intravenously into malaria naïve healthy volunteers (*n* = 36 men, *n* = 4 women). On day 8 p.i., volunteers received anti-malarial drug treatment. On days 0, 8 (treatment day), 14/15 and 27/28/36 (end-of-study, EOS), blood samples were collected and plasmablasts were assessed by flow cytometry.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

☑ n/a: The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☑ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☑ The statistical test(s) used AND whether they are one- or two-sided
  *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☑ A description of all covariates tested
☑ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☑ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☑ For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  *Give P values as exact values whenever suitable.*
☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒ Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

**Data collection**

- Flow cytometry: BD FACS Diva Software v.8.0.1 [using BD LSR II or BD FACS Canto II] and BD FACS Suite software v1.0.5 [using BD FACS Verse].
- RNA Seq data: NextSeq 550 sequencer
- Microscopy: Zeiss LSM710 Confocal, JEOLJEM-1230 Transmission Electron Microscope. Metabolic flux: Seahorse XFe94
- ELISA: Biotek Microplate reader
- qPCR: QuantStudio 3 [Applied Biosystems]

**Data analysis**

- Flowjo v10.5.0 [Treestar]
- IMARIS x64 software v9.2.1 [Bitplane AG]
- Pnsm v7.0 [GraphPad]
- Excel v16.16.20.9 [Microsoft]
- STAR aligner v2.7
- R v3.6
- Partek Genomic Suite v10.13
- Ingenuity Pathway Analysis v1.12
- Biotek plate reader v5

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- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the study and summarized in the manuscript will be available from the corresponding author upon request.

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- Life sciences
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | For all studies samples size were defined on the basis of past experience in the laboratory. In vivo studies, between 3 and 5 mice per group were used, dependent on the experimental manipulation and additionally based on historical variance within each of the assays and the relevant readouts. For ex vivo studies, 3 mice/biological group were used as cell donors with technical replicates that encompassed at least 3 wells/mouse. No statistical methods were used to determine the sample size. |
| --- | --- |
| Data exclusions | No data were excluded. |
| Replication | As reported in the figure legends, all experiments were repeated at least twice as part of biologically independent studies. All experiments replicated successfully. For RNA seq data analysis and metabolite measurements, the experiments were conducted once with at least 4 mice/group. |
| Randomization | All mice were randomized, but within an experiment the mice were sex- and age-matched. For human studies, volunteers were randomized across the cohorts. |
| Blinding | Investigators were not blinded during data analysis. For most experiments, blinding was not necessary because comparisons were made within the same animal. For other experiments, blinding was not possible due to symptoms exhibited by parasite-infected mice. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
| --- | --- |
| n/a | n/a |
| ☒ Antibodies | ☒ ChIP-seq |
| ☒ Eukaryotic cell lines | ☒ Flow cytometry |
| ☒ Paleontology | ☒ MRI-based neuroimaging |
| ☒ Animals and other organisms | |
| ☒ Human research participants | |
| ☒ Clinical data | |

Antibodies

Antibodies used

For mouse flow cytometry:
- Purified rat anti-mouse CXCR5 (1:100; clone:2G8, Cat# 551961; Lot# 8183892; BD Biosciences)
- Biotin SP-conjugated AffiniPure Goat-anti-IgG (H+L) F(ab) (1:500; Cat# 112-065-167; Lot# 142795; Jackson Immunoresearch)
- CD4-PercP-Cy5.5 (1:100; clone:GK1.5; Cat# 100434; Lot# B276680; BioLegend)
- CD44-AF700 (Cat# 103026; Lot# B287924) or -FITC (Cat# 103006; Lot# B174943) (1:1000; clone:IM7; Cat# 103026; BioLegend)
- CD11a-FITC (1:300; clone:IM7/4; Cat# 101106; Lot# B285001; BioLegend)
- PD-1-PE (Cat# 109104; Lot# 200608) -APC (Cat# 109112; Lot# B247623) or -PE Cy7 (Cat# 109110; Lot# B271005) (1:300; clone: RMP1-30; BioLegend)
Animals and other organisms

Policy information about studies involving animals. ARRIVE guidelines recommended for reporting animal research.

Laboratory animals
C57BL/6 WT (CD45.2 and CD45.1), Blimp-1eYFP [JAX stock #008828], uMT [JAX stock #00288] and Rosa26-ERT2/Cre [JAX stock #008463], Prdm11fl/fl and Aicda-/-/naive-/-, CD138-DTR mice. Both male and female mice were used in all experiments except when the study design involved the use of tamoxifen, which in our experience is toxic to males. Within an experiment the mice were sex- and age-matched. Mice were used when between the ages of 6 (naive wild-type and gene knockout) and 13 weeks (chimeric mice).

Wild animals
This study did not involve the use of wild animals.

Field-collected samples
This study did not involve the use of samples collected from field.

Ethics oversight
The University of Iowa IACUC approved all experiments.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Human research participants

Policy information about studies involving human research participants.

Population characteristics
A total of 40 healthy, malaria naive volunteers (n = 36 men, n = 4 women), aged between 18 to 55 years were recruited to the study.

Recruitment
Recruited from a database of healthy volunteers maintained by QPharm, or by advertisement to students of Queensland universities and the general community. Screening medical histories and physical examinations were performed after written informed consent had been obtained. Inclusion and exclusion criteria are detailed in McCarthy, J.S et al., A Pilot Randomised Trial of Induced Blood-Stage Plasmodium falciparum Infections in Healthy Volunteers for Testing Efficacy of New Antimalarial Drugs. Plos One, 6, e21914 (2011). Participants were randomized across multiple cohorts within each independent study to mitigate
self-selection bias. There were no specific gender or ethnic restrictions for recruitment; apart from study eligibility requirements as listed in the relevant clinical trial registrations (NCT02867059, NCT02783833, NCT02431637, NCT0243165079). As in most other early phase clinical trials, it is not possible to randomly select from the population but rather it is necessary to recruit eligible subjects who typically are young college-age individuals.

Ethics oversight
The study was approved by the Queensland Institute of Medical Research Human Research Ethics Committee (QIMR-HREC) and reviewed by the MMV Global Safety Board. The study was conducted in accordance with the Declaration of Helsinki principles for the conduct of clinical trials and the International Committee of Harmonization Good Clinical Practice Guidelines as recognized by the Australian Therapeutic Goods Administration (TGA) (www.tga.gov.au/docs/pdf/guidlines/ICH/GCP electronic.pdf). The trial was conducted with regulatory oversight by the TGA scheme and registered at the ClinicalTrial.gov.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data
Policy information about clinical studies
All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration
All studies were registered with US NIH ClinicalTrials.gov (NCT02867059, NCT02783833, NCT02431637, NCT0243165079).

Study protocol
For the trial protocol please refer to McCarthy, I.S et al., A Pilot Randomised Trial of Induced Blood-Stage Plasmodium falciparum Infections in Healthy Volunteers for Testing Efficacy of New Antimalarial Drugs. Plos One, 6, e21914 [2011].

Data collection
Blood samples from 40 volunteers (n = 36 men, n = 4 women from 4 studies across 6 independent cohorts) were collected prior to infection (day 0), at peak infection (day 8) and 14 or 15 and 27-36 days (end of study, EOS) after inoculation (in analyses these time points are grouped as 0, 8, 14/15 and EOS). Plasma was collected from lithium heparin whole-blood samples according to standard procedures, snap frozen in dry ice and stored at -70°C. B-cells were analyzed from fresh whole blood at time of collection.

Outcomes
Samples where parasitaemia was not detected were substituted with 0 on the log10 scale. The samples collected between the 4 defined timepoints (ranging from daily to twice daily before treatment, ranging from daily to every two hours after treatment, and ranging from every four days to daily between timepoint 14/15 and EOS) were used in the calculation of AUC but not in any other analyses.

Flow Cytometry
Plots
Confirm that:
☑ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
☑ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
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☑ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology
Sample preparation
Single cell suspensions of splenocytes were obtained by dissociating in a mesh screen followed by RBC lysis via either ACK red blood cell lysis buffer or Vitlyse. Suspensions were filtered using 70 um strainer, counted and resuspended at 1e6 cells/100uL for subsequent staining.

Instrument
BD LSR II, BD FACSVerse, BD FACS Canto II

Software
Flowjo 10.5.0

Cell population abundance
While acquiring data for cellular phenotyping, at least 5000-10000 cells were included in the stopping gate. For cell sorting, a yield sort was performed to >95% purity

Gating strategy
Gating strategy for each experiment is provided as panels in supplementary figures.

☑ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.