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Acute *Plasmodium chabaudi* Infection Dampens Humoral Responses to a Secondary T-Dependent Antigen but Enhances Responses to a Secondary T-Independent Antigen

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High rates of coinfection occur in malaria endemic regions, leading to more severe disease outcomes. Understanding how coinfecting pathogens influence the immune system is important in the development of treatment strategies that reduce morbidity and mortality. Using the *Plasmodium chabaudi* mouse model of malaria and immunization with model Ags that are either T-dependent (4-hydroxy-3-nitrophenyl [NP]-OVA) or T-independent (NP-Ficoll), we analyzed the effects of acute malaria on the development of humoral immunity to secondary Ags. Total Ig and IgG1 NP-specific Ab responses to NP-OVA were significantly decreased in the *P. chabaudi*-infected group compared with the uninfected group, whereas NP-specific IgG2c Ab was significantly increased in the *P. chabaudi*-infected group. In contrast, following injection with T-independent NP-Ficoll, the *P. chabaudi*-infected group had significantly increased NP-specific total Ig, IgM, and IgG2c Ab titers compared with controls. Treatment with anti–IFN-γ led to an abrogation of the NP-specific IgG2c Ab induced by *P. chabaudi* infection but did not affect other NP-specific Ab isotypes or titers. IFN-γ depletion also increased the percentage of plasma cells in both *P. chabaudi*-infected and uninfected groups but decreased the percentage of B cells with a germinal center (GC) phenotype. Using immunofluorescent microscopy, we were able to detect NP* GCs in the spleens of noninfected mice, but there were no detectible NP* GCs in mice infected with *P. chabaudi*. These data suggest that during *P. chabaudi* infection, there is a shift toward an extrafollicular Ab response that could be responsible for decreased Ab responses to secondary T-dependent Ags.

In many parts of the world, especially in the tropics where mosquito-borne diseases and parasitic infections are common, there is a high frequency of coinfection (1, 2). Coinfection can lead to an exacerbation of one or both of the diseases because of the skewing of the immune response away from the optimal response needed for clearance of infection (3, 4). Globally, there are more than 225 million new cases of malaria each year with the greatest burden on children under 5 y of age (5). In malaria endemic regions, there is a significantly higher rate of “all-cause” morbidity and mortality that is greater than the effect of increased malaria infection in the population (6), suggesting a greater impact of malaria than infection alone. In these regions, it is common for people to have subpatent malaria infection (7), and therefore, it is important to understand how an established malaria infection could inhibit immunity to a newly acquired infection or vaccine. Evidence for a role of malaria in affecting vaccine efficacy is demonstrated by increased mortality of children living in endemic regions despite increased vaccination coverage (8). For example, seroconversion rates for the poliovirus vaccine are reduced by 30% in African children compared with their North American counterparts (9). In addition, malaria is thought to be the causative agent behind impaired Ab responses for vaccines such as tetanus (10), pertussis (10), *Haemophilus* (11), and typhoid (12). Understanding the effects of malaria on immunity to secondary pathogens or vaccines is important to develop treatments that will diminish the morbidity and mortality caused by high rates of coinfection in malaria endemic regions.

Blood stage malaria infection is systemic, at which time the host mounts a Th1 response characterized by the production of high levels of TNF-α following infection with *Plasmodium falciparum* (13) and IFN-γ following infection with *Plasmodium chabaudi* (14). B cells and Ab are crucial for the control of malaria parasites; and clearance of parasitemia requires a switch from the early Th1 response to a Th2 response as seen from studies done in the *P. chabaudi* mouse model (15, 16). The IgG subclass produced in response to infection can dramatically affect the ability of humoral immunity to confer protection against disease (17, 18). The Abs made in response to soluble *Plasmodium* vaccine candidate proteins are heavily skewed toward the IgG3 isotype in humans (19, 20), which is equivalent to IgG2b in mice (21). In most experimental mouse models of *Plasmodium* infection, there is a heavy skewing toward an IgG2a or IgG2c isotype during in vivo infection (22). IgG2c production is highly associated with increased levels of IFN-γ and decreased levels of IL-4 (23).
Coinfection models are complex, and the effects of immune system dysregulation can be difficult to differentiate from the effects of the interactions between the infecting agents. The humoral immune response against 4-hydroxy-3-nitrophenyl (NP) acetyl-hapten coupled to carrier protein or polysaccharide has been extensively studied and well characterized (24). The use of NP-carrier Ag to mimic a coinfection response during malaria has the advantages of a lack of competition for resources between the infecting agents, greater reproducibility between experiments, and the ability to use widely available reagents to monitor the immune response to the NP-hapten. The prototypical response to soluble protein or carbohydrate Ags are of the IgG1 or IgG3 subclasses in mouse models (25). The predominant Ab isotype made during immune responses to the model Ag NP-OVA is of the IgG1 subclass (26). High levels of IL-4 and low levels of IFN-γ characterize Th2 responses that are associated with the NP-OVA response (27, 28).

Plasmodium chabaudi chabaudi AS. is a natural rodent pathogen that allows for the study of the blood stage of malaria in C57BL/6 mice (29). Previous studies using the P. chabaudi model of malaria infection in mice demonstrate that Ab responses to a secondary unrelated Ag (e.g., of NP-chicken gamma globulin [CGG]) are of a lower avidity when injection occurred at the same time as infection and that P. chabaudi infection had no effect on an already established humoral memory response to the secondary Ag (30, 31). These studies examined the effects of P. chabaudi on secondary injection of Ag prior to the establishment of infection but not during acute infection. In this study, we analyzed the humoral immune response to model T-dependent (TD) or T-independent (TI) secondary Ags during acute P. chabaudi chabaudi (AS) infection. Our findings suggest that P. chabaudi shifts immunity to secondary Ags toward predominantly extrafollicular humoral responses.

Materials and Methods

Animals/ethics statement

Male C57BL/6 mice (8–10 wk old) were obtained from The Jackson Laboratory (Bar Harbor, ME) or bred at State University of New York (SUNY) Upstate Medical University. Animals were maintained under specific pathogen-free conditions. All research involving animals has been conducted as required by the Animal Welfare Act, Public Health Service Guidelines, and New York State regulations with respect to husbandry, experimentation, and welfare. Mouse protocols were approved by the SUNY Upstate Medical University Committee on the Humane Use of Animals.

Experimental P. chabaudi infection and Ag injections

P. chabaudi chabaudi AS was a gift from Dr. J. Stoute (Pennsylvania State University, Hershey, PA). Parasitized RBCs (pRBCs) were stored at −80°C in glycerolyte solution until use. Prior to experimental use, frozen-infected RBCs were thawed and injected into donor mice for one passage to propagate P. chabaudi. Naïve C57BL/6 mice were injected i.p. with 5 × 10^7 pRBCs. Parasitemia was determined at selected days postinfection by microscopic examination of Giemsa-stained thin blood smears.

C57BL/6 mice were injected i.p. with 50 μg NP-OVA adsorbed onto alum or 10 μg NP-Ficol (Bissell Technologies, Novato, CA). NP conjugates were prepared as described previously (26). To determine the acute effects of P. chabaudi infection on secondary Ag responses, mice were injected 5 d postinfection (dpi). The effects of a prior infection on secondary Ag were determined by injecting mice 30 d following infection with P. chabaudi. IFN-γ depletion was performed by retro-oval i.v. injection of rat anti-INF-γ Ab (clone R4-6A2; BioXXCell, West Lebanon, NH) or 1 mg control rat IgG1 (Jackson ImmunoResearch Laboratories, West Grove, PA) at 4, 5, 9, and 13 dpi with P. chabaudi.

ELISA

Sera were collected from mice at indicated time-points and assayed by ELISA to determine NP-specific Ab responses. Briefly, ELISA plates (Nunc, Rochester, NY) were coated with NP-BSA in carbonate coating buffer (pH 9.6) and blocked with 1% BSA (Sigma-Aldrich, St. Louis, MO) in PBS containing 0.05% Tween 20 (Sigma-Aldrich). Total Ig, IgG1, IgG3, IgG2b, and IgG2c NP-specific responses were determined using appropriate peroxidase-conjugated goat anti-mouse Abs (Southern Biotechnology Associates, Birmingham, AL) and developed using 3,3’,5,5’-tetramethylbenzidine substrate (eBioscience, San Diego, CA). The reaction was stopped by addition of 1 M H_2PO_4 (Sigma-Aldrich), and the absorbance was read at 450nm.

Histology

Spleens were harvested from mice and placed in a mold containing OCT compound (Sakura, Torrance, CA). The tissues were immediately frozen over liquid nitrogen and kept at −80°C until used. Frozen tissue blocks were brought to −18°C, and thin cross-sections (7 μm) were cut in a cryostat (Leica CM1850; Leica Microsystems, Buffalo Grove, IL) and placed onto poly-l-lysine-coated slides. Slides were dried at room temperature for ~1–2 h and stored at −80°C until used. Slides were brought to room temperature, fixed in acetone/ethanol (75/25, v/v) for 10 min, and washed three times in PBS to remove the OCT. Slides were then blocked with 10 μg/ml purified Fc block Ab (BioXCell) in PBS containing 5% normal mouse serum and normal donkey serum [1:1] for 30 min before being probed with FITC-conjugated GL-7 (10 μg/ml; BD Biosciences, San Jose, CA) and NP-allophycocyanin (0.1 μg/ml) in a humidified chamber for 30 min. After washing three times with PBS, slides were incubated with Alexa Fluor 488-conjugated anti-FITC (20 μg/ml; Invitrogen, Grand Island, NY) for an additional 30 min. Slides were washed again three times with PBS and mounted with AquaMount (Polysciences Warrington, PA). Images were captured with a ×20 dry objective using a Leica TCS SP5II confocal microscope and analyzed using the Leica LAS AF software version 2.2.1 (Leica Microsystems). Contrast and brightness were adjusted using the Fiji software.

Lymphocyte isolation and flow cytometry

Spleens were aseptically collected from infected and/or immunized mice at various times. Spleens were mechanically dissociated in PBS and passed through a 70-μm nylon mesh screen yielding a single-cell suspension. RBCs were lysed by addition of ammonium chloride lysis buffer. Total and live cells were enumerated using trypan blue (Sigma-Aldrich) exclusion with a hemacytometer. Cells were stained in PBS containing 1% BSA and 0.1% sodium azide on ice with mAbs against the following cell surface molecules: AA4.1, B220, IgM, CD38 (eBioscience), CD19, CD23, CD21, (BioLegend, San Jose, CA), and CD138 (BD Biosciences, San Diego, CA). FCRs were blocked using 2.4G2 mAb. Peanut lectin (agglutinin) (PNA)-FITC (Vector Laboratories, Burlingame, CA) was used to identify germinal center (GC) phenotype B cells. Cells undergoing apoptosis were determined using an AnnexinV staining kit (BD Biosciences), according to the manufacturer’s instructions. NP-binding cells were visualized using NP-allophycocyanin as previously demonstrated (32) or NP-PE (Biosearch). Following staining, cells were analyzed using a LSRll analyzer (BD Biosciences) capable of 10-color analysis. Data were analyzed using FlowJo software (Tree Star, Ashland, OR).

Results

Acute P. chabaudi infection leads to decreased Ab titers to a secondary NP-OVA immunization and a shift in NP-specific Ab isotype

To determine whether P. chabaudi infection impacts the generation of Ab to secondary Ags, we evaluated the humoral response to NP-OVA. This approach allowed us to evaluate Ab responses to a single, well-defined TD immunogen. We first evaluated whether the timing of the injection of NP-OVA relative to the stage of P. chabaudi infection altered the humoral response to NP-OVA. C57BL/6 mice were infected with P. chabaudi by injecting 5 × 10^7 pRBCs, and then, mice were injected with the TD Ag NP-OVA alone or during acute P. chabaudi infection at 5 dpi or during resolved infection at 30 dpi. Giemsa-stained blood smears were used to determine the parasitemia. At 5 dpi, the parasitemia was on average 12% infected RBC, and at 30 dpi, the parasitemia ranged from 0.01 to 0.5% infected RBC. Serum was collected to assess the production of NP-specific Ab by ELISA 12 d post–NP-OVA injection to examine the TD response after the initiation of the GC response. Mice injected with NP-OVA during the acute phase of P. chabaudi infection exhibited significant reductions in the production of NP-specific total Ig (H+L) Abs compared
with noninfected NP-OVA–injected mice (Fig. 1A). In contrast, mice infected with *P. chabaudi* for 30 d prior to NP-OVA injection had similar levels of NP-specific Abs compared with mice injected with only NP-OVA (Fig. 1A). The lack of an effect when NP-OVA injection was given at 30 dpi suggests that the acute phase of infection plays a crucial role in diminishing the NP-specific Ab response. In addition, previous studies have shown that there is no effect if secondary Ag is injected before or at the same time as secondary Ag (30, 31). Therefore, we focused on the 5 dpi secondary immunization time point throughout the rest of our study. We next examined the NP-specific IgG isotypes induced following injection during acute *P. chabaudi* infection. We observed that NP-specific IgG1 Ab titers were significantly lower in the *P. chabaudi*–infected mice compared with uninfected NP-OVA–injected mice. Levels of NP-specific IgG2b and IgG3 were unaffected by *P. chabaudi* infection (Fig. 1B). In addition, although NP-specific total Ig was reduced, *P. chabaudi*–infected NP-OVA–injected mice had higher levels of NP-specific IgG2c in their sera compared with uninfected NP-OVA–injected animals (Fig. 1B). The increased NP-specific IgG2c Ab production following NP-OVA injection of *P. chabaudi*–infected mice suggests that the defect in total Ig may be primarily a result of a loss of the IgG1 response.

To determine whether the reduction in NP-specific Ab was transient or sustained, we injected control and *P. chabaudi*–infected mice with NP-OVA at 5 dpi and examined serum Ab titers at 90 dpi. There was still a significant reduction in NP-specific total Ig at 90 dpi compared with levels of NP-OVA Ig in mice injected with NP-OVA alone (Fig. 1C). These data indicate that TD secondary Ag exposure during acute *P. chabaudi* infection resulted in significantly reduced Ab responses at day 17 and that those reduced levels were sustained out to 90 dpi.

**P. chabaudi infection enhances the Ab response to the TI Ag NP-Ficoll**

The next set of experiments addressed whether *P. chabaudi* infection had any impact on the humoral response to a TI Ag such as NP-Ficoll (33). Mice were infected with *P. chabaudi* and at 5 dpi injected with NP-Ficoll. NP-specific serum Ab titers were determined 7 d post–NP-Ficoll injection to examine the TI immune response at its peak and before potential contributions from follicular B cells (34). *P. chabaudi* infection significantly increased the serum NP-specific Ab titers for total Ig, IgM, IgG2c Abs, and to a lesser degree IgG1 (Fig. 2). These results suggest that *P. chabaudi* infection enhances a TI Ab response while simultaneously suppressing a TD Ab response.

**IFN-γ depletion abrogates NP-specific IgG2c production**

*P. chabaudi* induces high levels of serum IFN-γ, which skew toward an IgG2c response (35), suggesting that the observed increase in IgG2c was a cytokine-mediated effect. To test whether IFN-γ was necessary for the skewing of the NP-OVA response from IgG1 to IgG2c, we depleted the cytokine using anti–IFN-γ Abs. *P. chabaudi*–infected mice were injected with pRBCs at day 0, and then, control and infected mice were immunized with NP-OVA/alum at 5 dpi. Anti–IFN-γ Ab or control rat IgG was given i.v. at 4, 5, 9, and 13 dpi. On day 17, ELISAs were performed to measure NP-specific Ab titers in serum. Depletion of IFN-γ did not affect the levels of NP-specific total Ig (Fig. 3A), IgG1 (Fig. 3B), or IgM (Fig. 3D) when compared with controls. However, IFN-γ depletion did result in significantly reduced NP-specific IgG2c in the *P. chabaudi*–infected mice such that the levels were similar to the uninfected group (Fig. 3C). These results suggest that IgG2c skewing occurs as a result of the increased serum IFN-γ levels and that IFN-γ was not responsible for decreasing the total Ig and IgG1 NP–specific responses.
The percentage and total number of NP-specific B cells is significantly lower in spleens of P. chabaudi–infected mice

To determine whether the reduced NP response in P. chabaudi–infected groups was the result of a decrease in NP-specific B cells, we assayed the percentage of NP+ B cells by flow cytometry. In addition, we examined the effects of IFN-γ depletion on the ability to produce NP+ B cells at 17 dpi. The total number of splenocytes isolated from P. chabaudi–infected mice was significantly higher in all treatment groups compared with NP-OVA alone controls (Fig. 4B). The percentage and total number of NP+CD19+ B cells in the total lymphocyte population in the NP-OVA alone group was significantly higher when compared with the group immunized with NP-OVA during acute P. chabaudi infection in both the rat IgG control and anti–IFN-γ groups (Fig. 4A, 4C). The depletion of IFN-γ did not lead to significant differences in NP+ B cells in the P. chabaudi or uninfected NP-OVA alone mice (Fig. 4A, 4C).

IFN-γ depletion leads to increased plasma cell production and decreased GC B cells

To determine whether reduced NP-specific Ab during P. chabaudi infection is the result of diminished differentiation of responding B cells to plasma cells and GC B cells, we examined the phenotype of cells within the spleen. The low percentage of NP+ B cells restricted our ability to determine whether there were NP+ GC B cells during P. chabaudi infection. Interestingly, the IFN-γ depletion significantly reduced the percentage of GC phenotype B cells in the spleen as defined by CD19+CD93+CD38+ PNA+ cells in both the NP-OVA alone and NP-OVA and P. chabaudi (Fig. 4A, 4E), but a large percentage of CD19+CD93+CD38+ PNA+ cells were present. The percentage of plasma cells was determined by gating on CD138+ cells. In addition, the depletion of IFN-γ led to significantly higher plasma cell percentages in both the NP-OVA alone and P. chabaudi– and NP-OVA–treated mice, but the total number of plasma cells was significantly higher in the P. chabaudi–infected groups for both rat IgG and IFN-γ–depleted mice (Fig. 4A, 4D). These results suggest that increased IFN-γ resulting from P. chabaudi infection is driving responding B cells toward GC differentiation rather than toward the generation of plasma cells.

NP-OVA injection during acute P. chabaudi infection results in there being no NP+ cells in GCs

To determine whether P. chabaudi infection influenced the development of NP-specific B cells, we used immunohistochemistry to examine the localization of NP+ B cells in the spleens of NP-OVA–injected mice. Fig. 5 shows that uninfected mice immunized with NP-OVA have NP+ cells present in the spleen that are colocalized with GL7+ GCs. In contrast, Plasmodium-infected mice immunized with NP-OVA possessed GL7+ GCs, but no GC-associated or extrafollicular NP+ cells were detected at 17 dpi. These data suggest the NP-specific GC response has been altered by P. chabaudi infection.

Discussion

Coinfections can often result in suboptimal responses to one or both of the pathogens involved (3, 4). Malaria is a systemic infection that results in a heavy skewing toward a proinflammatory Th1 environment (15). Using the P. chabaudi mouse model of infection, we tested the hypothesis that acute infection with P. chabaudi would alter the development of humoral immunity to model Ags. Our results demonstrate that the TD Ab response to NP was significantly reduced with regard to both total Ig and IgG1 Ab levels typically seen following injection with NP-OVA, whereas the NP-
specific IgG2c response was increased in an IFN-γ-dependent manner. In contrast, humoral immunity to the TI Ag NP-Ficoll was enhanced by acute P. chabaudi infection. Thus, the Ab response to a secondary Ag was altered during P. chabaudi infection in both the magnitude and Ab isotype induced.

In human malaria, it has been demonstrated using passive transfer of immune serum that Abs are critical in providing protection against blood stage disease (36). However, there is growing evidence that Ab responses are dysregulated following infection with Plasmodium. Several studies have shown that following infection, Ab responses to malarial Ags are short-lived (reviewed in Ref. 37). Similarly, impaired Ab responses also have been observed for several different vaccines in malaria endemic regions (8–12). Kinyanjui et al. (38) showed that IgG responses in Kenyan children following

FIGURE 4. NP-OVA injection during acute P. chabaudi infection leads to reduced NP+ B cells and lower plasma cells percentages compared with NP-OVA alone. IFN-γ depletion rescues and enhances the plasma cell percentages in P. chabaudi–infected mice and leads to a reduction in GC B cells. (A) Representative flow cytometry of splenic cells taken from mice that were treated with NP-OVA/adum alone or NP-OVA and P. chabaudi in the presence of anti–IFN-γ Abs or control rat IgG. (B) The total cell number per spleen from each of the groups listed above. (C) The percentage of NP+ cells and total cell number per spleen as represented in the left column of (A). (D) Percentage of plasma cells of the total live cells and total cell number per spleen as demonstrated in the middle column of (A). (E) The percentage of mature B cells and total cell number per spleen of GC B cells (PNAhiCD38low) of the mature B cells (CD19+CD93lo) as represented in the right column of (A). n = 4–5 mice/group. *p < 0.05, **p < 0.001 determined by a Mann–Whitney U test.
an acute malaria infection exhibited a reduced half-life compared with the normal values for this Ab subclass. Furthermore, it has been shown that Ab levels against merozoite Ags are highest during periods of high parasite transmission and lowest during times of low transmission (39, 40). Despite these suboptimal Ab responses, several studies have demonstrated in humans (41) and mouse models (22) that memory B cell and plasma cell responses develop in response to infection. Weiss et al. (41) showed that human B cell memory and long-lived Ab levels expand gradually with repeated infections. In mice, although some studies have shown normal development of memory B cell responses during Plasmodium infection (22, 42), others have shown defects in memory B cell generation or maintenance (43, 44). Crompton and colleagues (45) also have suggested that P. falciparum drives short-lived plasma cell responses, which may explain why Ab levels are not maintained in the absence of repeated exposures, and because memory B cell and plasma cell responses are regulated independently of each other, the presence of memory B cells may not be required to maintain serum Ab levels.

The predominant IgG subclass induced during an infection can be critical to the generation of an effective immune response against that pathogen. Immunization with NP-OVA in alum produces predominantly IgG1, which is associated with a strong Th2 response (27). Our data showed that P. chabaudi infection resulted in a shift in the NP-specific Ab response from IgG1 to IgG2c. Of potential significance is that P. chabaudi–specific Abs have been shown to be predominantly of the IgG2c isotype in response to infection (22), thus suggesting that P. chabaudi infection resulted in skewing of the immune response to the secondary Ag. The increase in plasma cells in response to P. chabaudi in the presence of anti–IFN-γ Ab suggests that the Th1 program induced during infection is involved in the lack of a long-lived plasma cell response to secondary Ag. The IgG2c skewing and lack of long-lived plasma cells also may be indicative of TD extrafollicular responses, such as those reported against Salmonella (46) where high-affinity IgG2c was produced in the absence of GCs.

The skewing of IgG isotypes is dependent on the cytokines produced during infection (15). The production of IgG2c (IgG2a in BALB/c mice) is largely dependent on IFN-γ (47). High levels of IFN-γ have also been associated with depressed B cell responses and lower IgG1 Ab production (47). Our data demonstrate that when IFN-γ is depleted, there is a reduction in NP-specific IgG2c levels in infected mice. However, IFN-γ did not appear to affect the NP-specific total Ig or IgG1 titer compared with mice injected with control IgG instead of anti–IFN-γ. This suggests that IFN-γ produced during P. chabaudi infection is primarily skewing toward an increase in IgG2c but not inhibiting IgG1 because of there being no other significant effect on the production of Ab against secondary Ags following IFN-γ depletion. Interestingly, type I IFN previously has been shown to skew follicular B cell Ab responses against TI-2 Ags toward an IgG2c isotype (48). Type I IFN is produced during Plasmodium infection (49) and permits B cells to become responsive to IL-12 and become B effector 1 (Be1) cells (50). Sustained expression of IFN-γ and T-bet by Be1 cells is maintained through a signaling loop involving dendritic cells (DCs) producing IL-12 and IL-18 leading to expression of IFN-γ by Th1 cells and B cells (23, 51). Our findings together with these studies suggest that the Th1 cytokine program, as well as potential contributions from Be1 cells, is a critical component in instructing class switching to IgG2c.

The timing of P. chabaudi infection and exposure to secondary Ag was found to be an important factor as demonstrated by significant differences in NP-OVA responses only when injected during acute P. chabaudi infection but not when given at the same
time or after the infection was resolved. Previous studies found no effect on OVA-specific Ab responses when OVA was injected before *P. chabaudi* infection (31) or when NP-CGG was given at the same time as *P. chabaudi* infection (30). The effect of timing of the secondary Ag delivery during *P. chabaudi* infection could be the result of the immune system being primed early in malaria infection toward a Th1 response or because of the presence of a variety of parasite pathogen–associated molecular patterns that are capable of stimulating B cells (30, 52).

Coinfections occurring alongside malaria can consist of many different types of Ag and pathogens. The ability of acute *P. chabaudi* to decrease TD and increase TI secondary Ag-specific Ab responses suggests that the nature of the Ag is critical during co-infection. Enhancement of TI Ab responses by *P. chabaudi* infection could be due in part to the cytokine milieu induced by acute infection. There is evidence that IL-12 signals are capable of enhancing Ab responses to polysaccharide TI vaccines (53), and IL-12 expression is important to immunity to *P. chabaudi* (54). In addition, B cell priming via TLR signaling or other pattern recognition receptors could lead to enhanced TI Ab responses (55). TI Ab enhancement could happen in addition to a productive TD Ab response and not necessarily instead of a robust TD response. It is likely that there are several different mechanisms in play during malaria infection that lead to changes in Ab responses to secondary Ags and pathogens.

The progression of B cells from naive cells to Ab-producing cells involves a tightly regulated process dependent on migration between niches within secondary lymphoid tissues. The kinetics of GC responses to NP-coupled TD Ags (e.g., OVA or CCG) were defined by Jacob et al. (24). Following treatment of mice with NP-CGG, GCs containing B cells undergoing somatic hypermutation appear in the spleen 8–10 d later and persist until at least 16 d postimmunization. GCs induced as a consequence of parasitic infections, including *P. chabaudi*, demonstrate similar GC kinetics (56, 57). However, it is clear that infection with *Plasmodium* affects the architecture of the spleen (30), which is a key immune induction site for blood-borne pathogens. Carvalho et al. (43) observed that GCs in the spleen lost their conventional structure following infection with *Plasmodium berghei* and reported extrafollicular development of plasmablasts. In addition, extralymphatic B cell activation has been reported for *P. chabaudi* (57) and other parasite infections (58). Disruption of the GC reaction has been shown to result in diminished production of class-switched, high-affinity Abs (59). In addition, *P. chabaudi* infection has been shown to affect B cell lymphopoiesis and the maturation of B cells into marginal zone or follicular B cells (60). These defects in B cell development could impact GC responses and the development of both B cell memory and long-lived Ab responses.

Extralymphatic B cell activation in the spleen is characterized by the generation of short-lived plasmablasts that secrete Abs with lower affinity for Ag (61). In the current study, we demonstrated that *P. chabaudi* is capable of inducing an increase in plasma cells at 17 dpi, and depletion of IFN-γ resulted in significantly increased plasma cell percentages in both NP-OVA control and *P. chabaudi*– and NP-OVA–injected mice. At present, it is unclear whether malaria modulates signals important to either the organization of the GC such as CXCL13 (62, 63) or lymphocyte migration signals involving sphingosine 1-phosphate (64) that are critical to the GC reaction. The lack of detectable NP cells in GCs following NP-OVA injection of *P. chabaudi*–infected mice, but the presence of NP-specific Ab suggests that the response to NP-OVA is extralymphatic during *P. chabaudi* infection. The enhancement of NP-specific Ab titters following TI Ag during *P. chabaudi* infection also suggests a shift to extralymphatic B cell responses. This work did not examine the effects of *Plasmodium* infection on T cell helper functions, which may ultimately affect the fitness of Ag-specific B cells and their ability to mount an effective humoral response. T cell function during *Plasmodium* infection has been studied in the context of TCR transgenic CD4 T cells specific for merozoite surface protein 1, which proliferated poorly near the peak of parasitemia (65). In addition, DCs isolated from the spleens of infected mice have been shown to transiently lose their ability to activate T cells (65), but timing is important because other groups have shown DC function is normal in *Plasmodium*-infected mice (66). Furthermore, human studies have demonstrated that CD4 T cells isolated from children with malaria express cell surface markers associated with exhaustion (67). Using our experimental model, future studies using NP-OVA as the experimental Ag will allow for the use of MHC class II tetramers to analyze the Th cells responding to OVA and further assess the effect of *Plasmodium* on the T cell response to NP-OVA immunization. Further work will need to be conducted to assess the contributions of Ag-presenting and T cells in this experimental model system.

In sum, the results of this study suggest that during an acute malaria infection, the Ab responses to secondary Ags/pathogens will be skewed toward an extralymphatic response that lead to decreased Ab titers or shift in the predominant Ab isotypes. The consequence of *P. chabaudi* infection to the host then would be a decreased Ab titer and shift in Ab isotype to a secondary Ag that could lead to a diminished protection. Understanding the weakened humoral immunity to secondary pathogens in malaria endemic regions is important to decrease the morbidity and mortality resulting from *Plasmodium*-associated coinfections.

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