Splenic Innate B1 B Cell Plasmablasts Produce Sustained Granulocyte-Macrophage Colony-Stimulating Factor and Interleukin-3 Cytokines during Murine Malaria Infections

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ABSTRACT The physiopathology of malaria, one of the most deadly human parasitic diseases worldwide, is complex, as it is a systemic disease involving multiple parasitic stages and hosts and leads to the activation of numerous immune cells and release of inflammatory mediators. While some cytokines increased in the blood of patients infected with Plasmodium falciparum have been extensively studied, others, such as granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3), have not received much attention. GM-CSF and IL-3 belong to the β common (βc/CD131) chain family of cytokines, which exhibit pleiotropic functions, including the regulation of myeloid cell growth, differentiation, and activation. GM-CSF can be secreted by multiple cell types, whereas IL-3 is mostly restricted to T cells, yet innate response activator (IRA) B cells, a subset of innate B1 B cells, also produce significant amounts of these cytokines during bacterial sepsis via Toll-like receptor 4 (TLR4)/MyD88 sensing of lipopolysaccharides. Herein, using murine models of malaria, we report a sustained production of GM-CSF and IL-3 from IgM+ and IgM+/IgG+CD138+Blimp-1+ innate B1b B cell plasmablasts. IgM+ B1b B cells include IRA-like and non-IRA B cells and express higher levels of both cytokines than do their IgG+ counterparts. Interestingly, as infection progresses, the relative proportion of IgM+ B1 B cells decreases while that of IgG+ plasmablasts increases, correlating with potential isotype switching of GM-CSF- and IL-3-producing IgM+ B1 B cells. GM-CSF/IL-3+ B1 B cells originate in the spleen of infected mice and are partially dependent on type I and type II interferon signaling to produce both cytokines. These data reveal that GM-CSF and IL-3 are produced during malaria infections, initially from IgM+ and then from IgG+ B1b B cell plasmablasts, which may represent important emergency cellular sources of these cytokines. These results further highlight the phenotypic heterogeneity of innate B1 B cell subsets and of their possible fates in a relevant murine model of parasitic infection in vivo.

KEYWORDS B1 B cells, GM-CSF, IL-3, IRA B cells, interferon gamma, Plasmodium, malaria, plasmablasts, type I interferon

Malaria is caused by the protozoan of the genus Plasmodium, among which P. falciparum is the deadliest species. Malaria remains prevalent worldwide, with 216 million cases and 445,000 deaths in 2016, primarily in children (1). Immunity against malaria involves both humoral and cell-mediated immune mechanisms that target the liver and blood stages of the infection (2), and multiple immune cell subsets contribute to either improve or worsen clinical symptoms (3). The onset of acute blood-stage malaria and severe clinical symptoms are associated with increased blood levels of inflammatory mediators and immune cell activation in human patients, as well as in mouse models (4–8). Levels of the proinflammatory cytokines tumor necrosis factor...
TNF), gamma interferon (IFN-γ), interleukin-6 (IL-6), IL-8, IL-12, IL-1β, and IL-18 are augmented and correlate with the control of parasite growth but at the cost of infection severity (6, 9, 10). TNF and IFN-γ can promote phagocyte activation to clear infected red blood cells and effectively kill parasites, yet they might also contribute to deleterious inflammation (11–14). As immunity is gained upon recurrent exposure, anti-inflammatory regulatory cytokines, like IL-10 and transforming growth factor beta (TGF-β), are reported to be generally increased, allowing for a less inflammatory and more controlled antiparasitic immune response (7, 15). While the prior cytokines have been investigated across many studies, some reports have also measured in the blood of *Plasmodium falciparum*-infected patients the presence of the granulocyte-macrophage colony-stimulating factor (GM-CSF) (16) and IL-3 (17), which both belong to the beta common chain (βc/CD131) family of cytokines (18). Further evidence using mice genetically deficient for GM-CSF infected with chronic *Plasmodium chabaudi* suggested that GM-CSF contributes to the control of parasite growth and rebounds (19). Interestingly, mice lacking IL-3 better resisted *Plasmodium berghei*-induced cerebral malaria, suggesting that IL-3 may impair mechanisms of protective immunity in this model (20). GM-CSF and IL-3 exhibit pleiotropic functions at steady state, as well as during inflammatory processes and immune defenses, which include promoting monocyte/neutrophil and megakaryocyte/mast cell/basophil production and activation (18, 21).

While GM-CSF can be secreted by multiple distinct cell types, the production of IL-3 seems more restricted to T cells. Yet, a series of relatively recent reports have identified a subset of innate B1a B cells, the innate response activator (IRA) B cells, as an important source of GM-CSF and IL-3 during bacterial sepsis, pneumonia, and atherosclerosis (22–25). IRA B cells contribute to host responses by enhancing inflammation, producing polyreactive IgM, and promoting extramedullary hematopoiesis. However, whether such a B cell population exists and plays a role in other microbial infections or pathological processes remains unknown. More generally, B1 B cells arise early during development and can differentiate into natural IgM antibody (Ab)-secreting cells (ASCs) and cytokine-producing B cells (26–28). They represent the major producers of natural IgM which play a critical role in microbial pathogen protection and in preventing autoimmunity. Peritoneal and pleural cavities are enriched for B1 B cells; however, spleen and bone marrow represent by far the major sites for natural IgM production by the B1 B cells (28). There is an important heterogeneity within the B1 B cell-derived ASCs, with some expressing classical markers of B1 B cells while others express those of terminally differentiated plasma cells exhibiting high levels of cell surface CD138 and intracellular Blimp-1 (27, 29, 30).

Here, using a surrogate murine model of acute blood-stage malaria infection, we report that IgM⁺ and IgM⁻ CD138⁺ innate B1b B cell plasmablasts that include IRA-like and non-IRA B cells exhibit sustained production of GM-CSF and IL-3 during blood parasitemia. We found that the production of GM-CSF by the B1 B cell plasmablasts is partially dependent on both cell-extrinsic type I (IFN-α/β) and cell-intrinsic type II (IFN-γ) interferon signaling but is independent of MyD88 signaling, a key cytosolic adaptor of most TLR signaling. Given that GM-CSF and IL-3 are reported to play opposite roles in malaria infection outcomes in mouse models (19, 20), we speculate that GM-CSF and IL-3 from these subsets of B1 B cell plasmablasts could represent important contributors to clinical malaria in humans.

**RESULTS**

**B cells produce GM-CSF and IL-3 in the course of malaria infections.** To investigate if GM-CSF and IL-3 are produced during malaria and whether B cells are a source of these cytokines, we inoculated wild-type (WT) C57BL/6 (B6) mice with the nonlethal rodent strain of *Plasmodium, Plasmodium yoelii* 17XNL, and monitored the production of both cytokines by splenic B cells during the course of the infection (Fig. 1A and B). While GM-CSF- and IL-3-producing B cells could be detected in the spleens of uninfected mice, the frequency of GM-CSF- and IL-3-producing splenic B cells increases up to 20 times and reaches peak production ~6 to 7 days postinfection; at
that time infection progresses with infected red blood cell (iRBC) proportion over 2% before undergoing a decline at 12 to 15 days postinfection. Both of these cytokines are detected from B cells *ex vivo* upon direct intracellular staining with no need of further restimulation or *in vitro* incubation, suggesting steady and sustained production by the B cells. The peak production of GM-CSF and IL-3 occurs prior to the peak of parasitemia and diminishes after blood parasitemia starts decreasing, suggesting a correlation with blood parasite elimination kinetics.

We next asked whether B cells also produced these cytokines after infection with other rodent strains of *Plasmodium*, namely, *P. yoelii* 17X YM and *P. chabaudi* chabaudi AS, which induce lethal and short chronic infections, respectively (Fig. 1C and D; see 

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**FIG 1** GM-CSF and IL-3 are produced by B cells during malaria infections. Wild-type (WT) C57BL/6 (B6) mice were inoculated with infected red blood cells (iRBCs) of the indicated murine *Plasmodium* strain (*P. yoelii* 17XNL, *P. yoelii* 17X YM, or *P. chabaudi*) or injected with the same numbers of uninfected RBCs (uninfected). At indicated times postinfection, spleen cells were harvested and stained for cell surface CD45 and CD19 and intracellular GM-CSF or IL-3. (A) Intracellular GM-CSF or IL-3 staining among CD19^+^ B cells, gated on CD45^+^ live cells in the different experimental groups. Fluorescence minus 1 (FMO) is shown for the intracellular cytokine staining. (B) Kinetics of GM-CSF and IL-3 production by CD19^+^ B cells and blood parasitemia following *P. yoelii* infection. (C) GM-CSF and IL-3 production from B cells after infection with indicated *Plasmodium* strains. (D) Average proportion of GM-CSF- or IL-3-producing B cells among CD45^+^ splenic cells 6 (*P. yoelii* 17X YM) or 7 (*P. yoelii* 17XNL, *P. chabaudi*) days postinfection (dpi) across experiments (*n* = 3 to 12 mice). In all experiments, representative FACS dot plots of 2 to 5 independent replicate experiments with at least 3 mice are presented. Graphs show the average of the results from each experiment along with the standard error of the mean (SEM).
also Fig. S1 in the supplemental material). Similar to *P. yoelii* 17XLN infection, we found substantially increased proportions of GM-CSF- and IL-3-producing B cells in the spleen at peak parasitemia in both of these malaria infections, extending our observations to other models of murine malaria.

**GM-CSF**⁻ and **IL-3**⁺ **B cells induced during malaria infections are IgM⁺ and IgM⁻/IgG⁺ CD138⁺ Blimp-1⁺ B1 B cell plasmablasts.** To determine if GM-CSF and IL-3 are produced by specific subsets of B cells, we conducted an extensive flow cytometry characterization of the B cells producing these cytokines in uninfected versus *Plasmodium yoelii* 17XLN-infected mice after staining for cell surface CD19, IgM, CD93, CD23, CD43, and CD5 and intracellular GM-CSF, IL-3, IgG, and Blimp-1 (Fig. 2 and S2). GM-CSF⁻ and IL-3⁺ cells are CD23⁻ CD93⁺ (or CD93⁻) CD43⁺ CD5low, identifying them as B1b B cells (Fig. 2A and B and S2). GM-CSF⁺ and IL-3⁺ B1 B cells, respectively, represent between ∼0.2% (naive) and 1.5 to 4% (*P. yoelii*-infected) of hematopoietic (CD45⁺) cells, which account for ∼0.35% (naive) and 3.5 to 6.5% (infected) of the B (CD19⁺) cells (Fig. 2B to D). Approximately half of the GM-CSF⁻ or IL-3⁻ cells in naive or infected mice express cell surface IgM, while the other half do not. Within the cytokine-producing IgM⁺ B cells, 50 to 70% express CD93, reminiscent of the IRA B cell phenotype (25) (Fig. 2A to D and S2A). At the peak of parasitemia (day 15), CD93⁺ cells represented the main producers (>70%) of GM-CSF and IL-3, regardless of IgM expression (Fig. 2C and D). Interestingly, both IgM⁻ and IgM⁺ GM-CSF- and IL-3-producing counterparts express high levels of cell surface CD138 and the Blimp-1 transcription factor, consistent with plasmablast features (Fig. 2E and S2B). Of note, GM-CSF⁺ and IL-3⁺ IgM⁺ plasmablasts expressed high levels of intracellular IgG. These cells are also enlarged (forward scatter [FSC]) in infected compared to uninfected mice and all B cells. Thus, these results establish that a population of B1b B cells producing GM-CSF and IL-3 is found in the spleen of naive WT B6 mice, and they can expand ∼10 to 20 times upon malaria infection and acquire phenotypic features of plasmablasts.

**Proportions of IgM⁻/IgG⁺ GM-CSF⁻ and IL-3⁺ B1b B cell plasmablasts increase while that of the IgM⁺ counterpart decreases as infection progresses.** Since in both naive and *P. yoelii*-infected mice, about half of the GM-CSF⁺ or IL-3⁺ splenic B cells are IgM⁺ while the other half are not, we next asked if the IgM⁺ subset may convert into the IgM⁻/IgG⁺ subset or simply expand from those found at steady state (Fig. 3). We monitored the kinetics of IgM⁺ and IgM⁻ GM-CSF- and IL-3- or noncytokine-producing B cell expansion after *P. yoelii* infection, hypothesizing that the proportion of IgM⁻ GM-CSF/IL-3⁺ B cells should increase concomitantly to the decrease of IgM⁺ counterparts if IgM⁺ converts to IgM⁻/IgG⁺ B cells (Fig. 3A). As reported in prior studies using both *P. yoelii* and other murine models of malaria, the overall proportion of all splenic IgM⁺ B cells decreases (factor of ∼2), while that of IgM⁻ (IgG⁺) increases (factor of ∼10) as infection progresses and robust parasite-specific germinal center reactions and IgG responses are taking place (31, 32). Interestingly, in our model, the frequencies of IgM⁺ GM-CSF⁺ and IL-3⁺ B cells among B cells increase to peak by ∼7 to 8 days postinfection and then steadily diminish. IgM⁻/IgG⁺ GM-CSF⁺ B cells then augment comparatively to reach a plateau before contracting with kinetics similar to that of total splenic IgM⁻ B cells (Fig. 3A). Thus, this finding correlates with the idea that IgM⁺ GM-CSF⁺ and IL-3⁺ B1 B cell plasmablasts could undergo isotype switching during the infection. Levels of cell surface CD19 are also lower in IgM⁻ but not IgM⁺ GM-CSF⁺ and IL-3⁺ B cells from infected spleens, consistent with a possible long-lived plasma cell fate of switched cells (Fig. S2C). We also note that GM-CSF and IL-3 staining ratios in the IgM⁺ fraction are, respectively, ∼3- and 1.2-fold higher than that of the IgM⁻ plasmablasts, and their ratios remain sustained over time, supporting the idea that GM-CSF⁺ and IL-3⁺ IgM⁺ and IgM⁻ B1 B cells are functionally different (Fig. 3B). Collectively, our results show that in the course of malaria infection, as GM-CSF- and IL-3-producing IgM⁺ splenic B1 B cell proportions decrease, that of the IgM⁻/IgG⁺ counterpart increases, correlating with possible isotype switching and plasma cell differentiation as they expand in the spleen of *P. yoelii*-infected mice.
FIG 2  B1b B cell plasmablasts produce GM-CSF and IL-3 during *P. yoelii* infection. WT B6 mice were inoculated with *P. yoelii* 17XNL iRBCs or injected with the same numbers of uninfected RBCs (uninfected). (A to C) Spleens from uninfected or day 7.5 *P. yoelii*-infected mice were harvested and the cells stained for cell surface CD45, CD19, IgM, CD23, CD43, and CD93 and intracellular GM-CSF or IL-3. (A and B) Dot plots are shown with the successive gating strategy used (FO, follicular; T1/T2/T3, transitional 1/2/3; MZP, marginal zone precursor; MZ, marginal zone; PC, plasma cell; IRA, innate response activator). GM-CSF^+^ (blue) or IL-3^+^ (fuchsia) cells are overlaid on the indicated populations. (C and D) Bar graphs summarize the frequency of GM-CSF^+^ or IL-3^+^ IgM^+^ cells among indicated cell populations (left) and the proportion of GM-CSF^+^ or IL-3^+^ IgM^+^ cells that express CD93 or not (right) in uninfected versus *P. yoelii*-infected mice. (E) Spleen cells from uninfected or day 7.5 *P. yoelii*-infected mice were stained for cell surface CD45, CD19, IgM, CD23, and CD138 and intracellular GM-CSF, IgG, and Blimp-1. In all experiments, representative FACS dot plots of 1 mouse across 5 replicate experiments are shown (n = 8 to 14). Graphs show the average of the results from each experiment, with each dot representing 1 individual mouse or with the mean ± SEM. max, maximum.
FIG 3 Expansion and contraction of IgM⁺ GM-CSF/IL-3⁺ B1b B cell plasmablasts inversely correlate with those of the IgM⁻/IgG⁺ counterparts. WT B6 mice were inoculated with *P. yoelii* iRBCs or injected with the same amount of uninfected RBCs (uninfected). Spleens from uninfected or *P. yoelii*-infected mice were harvested at the indicated days, and cells were stained for cell surface CD19, IgM, CD23, CD43, and CD93 and intracellular GM-CSF or IL-3 in some but not all experiments. (A) Top left graph shows the proportion of indicated IgM⁺ or IgM⁻ CD19⁺ B cells over time postinfection. The middle and lower graphs on the left show the proportions of the indicated IgM⁺/GM-CSF⁺ or IgM⁻/IL-3⁺ cell populations among B cells over time postinfection. (B) Gated on CD45⁺ CD19⁺ cells. Representative dot plots of intracellular GM-CSF or IL-3 staining of IgM⁺ or IgM⁻ B cells from spleen of day 7.5 *P. yoelii*-infected mice. Bar graphs show the corresponding average mean fluorescence intensity (MFI) for indicated intracelular cytokine. The right graphs depict the ratios of GM-CSF or IL-3 MFI in IgM⁺ versus IgM⁻ B cells over time after infection. Two to 5 independent replicate experiments with at least 3 mice are presented. Graphs show the average of the results from each experiment along with the SEM. *P* values are indicated when applicable.
GM-CSF$^+$ and IL-3$^+$ B cells originate from the spleen but not the peritoneal cavity. While we would expect GM-CSF/IL-3$^+$ plasmablasts to originate in the spleen, IRA B cells are reported to come from peritoneal B1a B cells during bacterial sepsis (25). To determine whether GM-CSF/IL-3$^+$ B1 B cells originate from the spleen or the peritoneal cavity of P. yoelii-infected mice, we isolated CD4$^+$CD8$^+$CD11b$^+$TER119$^+$IgD$^+$CD45.1$^+$ cells from either the spleen or peritoneal cavity of donor mice, and adoptively transferred them to WT CD45.2$^+$ recipient mice that we further inoculated with P. yoelii-iRBCs the next day (Fig. 4A). Six and a half days later, spleens were harvested and cells stained to track GM-CSF$^+$ and IL-3$^+$ transferred B cells (Fig. 4B to D). We detected in the transferred B cells whether they originated from the spleen or the peritoneal cavity of donor mice, yet only those originating from the spleen but not from the peritoneal cavity stained positive for GM-CSF and IL-3. Thus, in contrast to...
what is reported during bacterial sepsis, in the course of malaria infection, GM-CSF- and IL-3-producing B1 B cells seem to expand from a local splenic progenitor.

**Generation of B1 B cell plasmablasts and their production of GM-CSF are partially dependent upon interferon signaling.** We next assessed if major innate sensing or cytokine signaling pathways controlled (i) the generation of splenic B1 B cell plasmablasts and (ii) their induced production of GM-CSF and IL-3. Mice lacking the major intracellular sensing adaptor MyD88 (Myd88−/−), the type I interferon (Ifnar−/−) receptor, the type II interferon (Ifngr−/−) receptor, both interferon receptors (Ifnar−/− Ifngr−/−), or control WT counterparts were inoculated with Plasmodium yoelii 17XNL-infected iRBCs, and 7.5 days later, spleens were harvested and stained for cell surface CD45, CD19, IgM, IgD, and CD43 and intracellular GM-CSF. (A) Representative FACS dot plots across 2 to 3 independent replicate experiments are shown. (B and C) The average result across all experiments is shown, with each dot representing an individual mouse (n = 4 to 19 for knockout [KO] mice, n = 44 for WT mice).

**FIG 5** Type I and II interferon signaling enhances the generation of B1 B cell plasmablasts and their production of GM-CSF during malaria infection. Indicated knockout or WT mice (all B6 background) received 2 × 10⁷ Plasmodium yoelii 17XNL-infected iRBCs, and 7.5 days later, spleens were harvested and stained for cell surface CD45, CD19, IgM, IgD, and CD43 and intracellular GM-CSF. (A) Representative FACS dot plots across 2 to 3 independent replicate experiments are shown. (B and C) The average result across all experiments is shown, with each dot representing an individual mouse (n = 4 to 19 for knockout [KO] mice, n = 44 for WT mice).
capacity is promoted by interferon signaling in both subsets of plasmablasts, suggesting that these processes are regulated through distinct yet overlapping mechanisms.

To further determine whether cell-intrinsic or cell-extrinsic interferon signaling accounted for our observations, we adoptively cotransferred splenocytes from $\text{Ifnar}^{-/-}$ or $\text{Ifngr}^{-/-}$ and control WT mice into congenically distinct WT recipient mice, which we subsequently inoculated with $\text{P. yoelii}$ and tracked the differentiation and GM-CSF/IL-3 production of transferred B cells 7.5 days postinfection (Fig. 6A). We found that the generation of CD43$^+$ IgM$^+$ B1 B cells is impaired by the lack of direct, cell-intrinsic type
I or II interferon signaling, while that of the IgM− counterparts is less affected (Fig. 6B), consistent with results in the full-knockout mice (Fig. 5A and B). Interestingly, we also reveal that disrupting cell-intrinsic IFN-γ signaling significantly decreases GM-CSF production by both IgM+ and IgM− B1 B cells (Fig. 6C). In contrast, we observed only a mild impact in B cells lacking type I IFN signaling, suggesting that type I interferon largely promotes GM-CSF production through cell-extrinsic mechanisms. In conclusion, both type I interferon and IFN-γ signaling enhance the generation of CD43+ IgM+, but not IgM+/IgG+, B1 B cells, largely through direct, cell-intrinsic mechanisms. Moreover, cell-intrinsic IFN-γ and cell-extrinsic type I interferon signaling contributes to enhancing the production of GM-CSF by IgM+ and IgM− B1 B cells.

**DISCUSSION**

The current study characterizes a population of splenic innate B1 B cells that exhibits sustained production of the myeloid trophic and activating cytokines GM-CSF and IL-3 during murine malaria infection. These cells are B1b B cells (CD23− CD43+ CD5−) that express markers of plasmablasts (CD138(high) Blimp-1(high)). Interestingly, while the majority of these cells express surface IgM early on, as infection progresses, a population of IgM− IgG+ B1b B cell plasmablasts increases as IgM+ counterparts are lost, suggesting that IgM+ could be giving rise to the IgM−/IgG+ subset after isotype switching. Whether the GM-CSF/IL-3-producing IgG+ B1 B cells arise from the IgM+ subset or expand from a preexisting population, the two subsets appear to be functionally different; for instance, IgG+ plasmablasts exhibit lower intracellular staining for GM-CSF and IL-3 than do their IgM+ counterparts. Our results also establish that type I interferon signaling and type II interferon signaling are essential in the generation of IgM+ but not IgM− B1 B cell plasmablasts, via B cell-intrinsic mechanisms, and in the induction of GM-CSF production, via B cell intrinsic for IFN-γ and cell extrinsic for type I IFN signaling, from both IgM+ and IgM− B1 B cell plasmablasts during malaria infection.

Prior work has reported the characterization of a population of splenic GM-CSF- and IL-3-producing innate B1 B cells, the IRA B cells, in models of bacterial sepsis (24, 25) and pneumonia (23) and during atherosclerosis (22). In our malaria infection model, we observed that about 50% of GM-CSF- and IL-3-producing IgM+ B1 B cells are phenotypically identical to the IRA B cells (CD23− CD43+ CD93+ CD138+). However, these cells also show greater heterogeneity since half of them do not express the stem cell marker CD93, which is reported to be functionally important in antibody secretion and plasma cell maintenance in bone marrow (33). In addition, GM-CSF and IL-3 production in these cells is independent of MyD88, and they originate from a splenic B1b B cell precursor, all features that are distinct from that of IRA B cells, which are reported to (i) require MyD88 to initiate the production of GM-CSF and IL-3 and (ii) arise from B1a B cells residing in serosal sites (peritoneal or pleural cavity). Our data also show a clear role of cell-intrinsic IFN-γ and cell-extrinsic type I interferon signaling in enhancing GM-CSF and IL-3 secretion from these innate B1 B cells during malaria infection. Thus, despite a phenotypic heterogeneity, their common splenic origin and comparable mechanism of GM-CSF and IL-3 cytokine induction support the idea that the GM-CSF- and IL-3-producing B1 B cells in malaria infection have functional requirements distinct from those of the IRA B cells described previously. It is worth noting, however, that IRA B cells were discovered in an acute, short-term model of microbial sepsis in the peritoneal cavity, and the long-term fate of IRA B cells was not analyzed further. The kinetics and tropism of *Plasmodium* infection are clearly very different from those of acute sepsis and may well be accounting for the distinct origin, fates, phenotype, and mechanism of activation of these GM-CSF- and IL-3-producing B1 B cells.

The fact that the GM-CSF- and IL-3-producing B1 B cells we describe undergo functional maturation into plasma cells and could possibly also be undergoing isotype switching has also not been reported to happen for IRA B cells, though this has been suggested to occur in B1 B cells (30, 34). Their expression of the plasma cell surface marker CD138 and the transcription factor Blimp-1 is a feature consistent with plasma
cell functional differentiation. While our kinetic analysis is consistent with the possibility that, as infection develops, IgM⁻/⁺ B1 B cells become IgM⁻/IGG⁺, it still is possible that the IgM⁻/⁺/IGG⁺ population is specifically expanded and has a competitive edge on the IgM⁺ counterpart, and that no progeny relationship exists between these IgM⁺ and igM⁻ B1 B cells. Along these lines, B1 B cells can secrete natural IgG3 antibodies (Abs) (29) and may selectively expand during this parasitic infection. In patients, IgG3 Abs against the merozoite-derived protein MSP3 of Plasmodium falciparum have been strongly associated with long-term clinical protection (35) and opsonization (36), although the origin of the Plasmodium falciparum-specific IgG3 Abs was not investigated. The IgM−/IgG+ plasmablasts we characterize express high levels of the Blimp-1 transcription factor, which suggests that they are acquiring features of switched plasma cells and are different (or represent a more differentiated stage) from the previously described natural IgG3-producing B1 B cells. A tempting hypothesis given the current results is that infection-induced GM-CSF⁺ and IL-3⁺ IgM⁺ producing B1 B cell-derived plasmablasts contribute to a first wave of innate immune defense that is sustained through either the production of a progeny of IgM⁻ or P. yoelii-specific IgG-secreting plasmablasts or the expansion of IgM⁻ natural IgG3-producing plasmablasts. A recent study in human malaria proposed an interesting link of an IgG3 polymorphism in the neonatal Fc receptor that increases Plasmodium falciparum-specific IgG3 transplacental transfer and clinical protection in infants (37).

Similarly to bacterial sepsis, in which IRA B cells are reported to either promote (via IL-3) or protect (via GM-CSF) the host, a lack of GM-CSF or IL-3 has opposite outcomes during malaria infection, with GM-CSF-deficient mice exhibiting lower resistance to chronic P. chabaudi infection (19), while IL-3-deficient mice resisted better P. berghei-induced experimental cerebral malaria (ECM) (20). IRA B cell-derived GM-CSF increases resistance to bacterial sepsis through secreted IgM and subsequent activation of innate myeloid cell phagocytic capacity and bacterial clearance. In contrast, IRA B cell-derived IL-3 enhances extramedullary emergency hematopoiesis, the production and activation of phagocytes and their progenitors, and the subsequent release of inflammatory cytokines, such as TNF, IL-1β, and IL-6, all of which also take place during blood-stage malaria and may account for IL-3-deficient mouse-augmented resistance to ECM. However, none of the prior reports analyzing mice lacking GM-CSF or IL-3 explored if one or several cell subsets producing either of these cytokines account for the observed susceptibility or resistance phenotypes. Given the sustained production of both cytokines we found in innate B1 B cells in the course of murine malaria infections, it is possible that these cells indeed represent key actors of resistance to this significant parasitic disease. Along these lines, human subjects with genetic polymorphisms in the IL-3 gene exhibit increased resistance to malaria (38). While the cell surface phenotypes of the cells producing GM-CSF and IL-3 during malaria infection appear to be identical, despite multiple attempts, we have not been able to determine whether the same cells express both cytokines, largely due to technical limitations associated with the available reagents and associated fluorochromes. The proportion of GM-CSF⁺ B1 B cells is 3 to 5 times higher than that of IL-3 during the course of malaria infection; thus, it may be that GM-CSF⁺ versus IL-3⁺ B1 B cells represent a functional subspecialization, consistent with their presumably distinct functional roles described in the various infection models. Also, it is unclear what the contribution of other cell subsets that may produce GM-CSF and IL-3 during malaria, though we have not detected significant de novo sources other than that of the B1 B cells. T cells and innate lymphoid cells (ILCs), for instance, are known to be able to secrete both of these cytokines, but it is likely that specific conditions of ex vivo restimulation would have to be further tested. It is worth noting that a population of GM-CSF-producing B cells has been reported in human patients suffering from multiple sclerosis (39), yet these cells belong to the B2 B cell lineage, express markers of memory B cells, and are induced through CD40L⁻ and T cell-derived cytokines.

In conclusion, this work and current literature on GM-CSF- and IL-3-producing B cells further underline the important heterogeneity in their origin, phenotypes, and func-
tions. Many questions remain to be explored in order to further define (i) both the cell-intrinsic and -extrinsic cues that drive their differentiation into GM-CSF- and/or IL-3-producing cells, depending on their origin, (ii) their functional fates (whether they do undergo isotype switching and, if yes, of which predominant isotype), and (iii) at least for malaria, if and how they may contribute to resolving or promoting the pathogenesis of this deadly infection.

MATERIALS AND METHODS

Mice. Wild-type (WT) C57BL/6J (B6), CD45.1+/+ (strain 2014; Jackson Laboratory), Myd88−/− (strain 009088; Jackson Laboratory), Ifnar−/− (kind gift of Jake Kolehieme, Emory Vaccine Center), Irf3−/− (kind gift of Thomas Moran, Mount Sinai School of Medicine), and Il6r−/− (strain 003288; Jackson Laboratory) mice were housed and bred in our specific-pathogen-free (SPF) animal facility for all experiments. The mice used for all experiments were 6 to 12 weeks old. This study was carried out in strict accordance with the recommendations by the Institutional Animal Care and Use Committee (IACUC) at the Albert Einstein College of Medicine (protocol number 20171113), which adheres to the regulations and guidelines set by the National Research Council. The Albert Einstein College of Medicine is accredited by the International Association for Assessment and Accreditation of Laboratory Animal Care.

Plasmodium infections and blood parasitemia. (i) Infections. Plasmodium yoelii (17XNL[1.1]) parasites (stock MRA-593), Plasmodium yoelii (17X) clone YM parasites (stock MRA-755), and Plasmodium chabaudi chabaudi AS parasites (stock MRA-748) were obtained from the Malaria Research and Reference Reagent Resource Center as part of the BEI Resources Repository (NIAID, NIH, Manassas, VA; deposited by D. Walliker). P. yoelii-infected red blood cells (iRBCs) from a frozen stock (stored in −80°C in Alsever’s solution, 10% glycerol) were intraperitoneally (i.p.) injected into a WT B6 mouse and grown for 4 days. When parasitemia reached 2 to 5%, P. yoelii iRBCs were diluted to appropriate concentrations with 1× phosphate-buffered saline (PBS) and injected intravenously into each experimental mouse, unless otherwise indicated.

(ii) Parasitemia. Blood parasitemia was determined by flow cytometry on 1 μl of blood obtained by cutting the tip of the tail with a sterile razor. Blood was fixed in 200 μl of 0.025% glutaraldehyde in 1× PBS containing 1 mM EDTA before being washed and permeabilized with 0.25% Triton X-100 in 1× PBS for 5 min. RBCs then were incubated in 1 mg/ml RNase A (Sigma) for 30 min at room temperature (RT), stained with 0.5 μM YOYO-1 dye (Invitrogen) for 30 min at RT, and directly analyzed on a BD FACSCanto II (Becton, Dickinson, CA). RBCs were gated based on forward and side scatter, and parasitemia was determined as the frequency of YOYO-1− cells among all cells. Eye counting of Giemsa-stained blood smears by microscopy confirmed that the determination of parasitemia using the above-described method gave comparable results.

Cell suspensions for flow cytometry and adoptive transfer. Spleens were dissociated on nylon meshes (100-μm pore size) and incubated at 37°C for 20 min in Hanks’ balanced salt solution (HBSS) medium containing 4,000 U/ml collagenase I (Gibco) and 0.1 mg/ml DNase I (Roche), and RBCs were lysed with NH4Cl buffer (0.83% [vol/vol]). Cell suspensions were resuspended in fluorescence-activated cell sorting (FACS) buffer (PBS, 1% fetal calf serum [FCS], 2 mM EDTA, 0.02% sodium azide) and used for the different analyses (see below).

Cell staining for FACS analysis. Cell suspensions were incubated with 2.4G2 Fc block for 15 min at 4°C and then stained with specified antibody cocktail (Table S1) in FACS buffer. For intracellular GM-CSF and IL-3 de novo staining, cells were fixed in intracellular (IC) fixation buffer (eBioscience) for 15 min at 4°C after extracellular staining and incubated with intracellular antibodies for 30 min in 1× Perm/Wash buffer (eBioscience). Stained cells were collected on either a FACS BD LSR II or a FACS Aria III. Data were analyzed using FlowJo version 9 (Tristar).

Statistics. Statistical significance was calculated using an unpaired Student’s t test with GraphPad Prism software, and two-tailed P values are given as *, P < 0.05; **, P < 0.01; ***, P < 0.001; ****, P < 0.0001; and ns, P > 0.05. All P values of 0.05 or less were considered significant and are referred to as such in the text.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/IAI.00482-19.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 2, PDF file, 0.4 MB.

SUPPLEMENTAL FILE 3, PDF file, 0.1 MB.

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