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The Loss and Gain of Marginal Zone and Peritoneal B Cells Is Different in Response to Relapsing Fever and Lyme Disease

**Borrelia**

Susan Malkiel, Christopher J. Kuhlow, Patricio Mena, and Jorge L. Benach

T cell-independent Abs are protective against Lyme disease and relapsing fever, illnesses caused by *Borrelia* spirochetes with distinct blood-borne phases of infection. To understand this protective response, we characterized splenic and peritoneal B cell compartments during infection using flow cytometry and immunohistochemistry. In the spleen, early after infection, *Borrelia crocidurae*, a relapsing fever species, induced a striking loss of marginal zone (MZ) B cells from the MZ, while *Borrelia burgdorferi*, the agent of Lyme disease, induced the expansion of this subset. At the same time, no significant changes were observed in follicular B cells in response to either species of *Borrelia*. In the peritoneal cavity, a further loss was demonstrated early in response to *B. crocidurae* in the B1b, B1c, and B2 cell subsets, but B1a cells were not significantly altered. The loss of B1c and B2 cells was sustained through subsequent peaks of spirochetaemia, suggesting these subsets may be important in resolving relapsing episodes. In contrast, an early and significant increase in peritoneal B1a, B1b, and B1c cells, but not B2 cells, occurred in response to *B. burgdorferi*. Later in the course of infection, both species of *Borrelia* induced the selective expansion of peritoneal B1b cells, suggesting that B1b cells may participate in long-lasting immunity to Lyme and relapsing fever spirochetes. Our data demonstrate that different *Borrelia* can activate the same B cell subsets in distinct ways and they each elicit a complex interplay of MZ and multiple peritoneal B cell subsets in the early response to infection. *The Journal of Immunology, 2009, 182: 498–506.

Rapid Ab responses to T cell-independent Ags are critical to the host defense. The conventional B cell response, which requires T cell help, is typically not fast enough to control infection in its early stages when it encounters foreign Ag for the first time. T cell-independent B cell responses can also become important when the invading pathogen undergoes antigenic variation within the host. How the various B cell subsets contribute to this response and how they are activated and regulated following infection is not completely understood.

Lyme disease and relapsing fever are world-wide tick-borne borrelioses that share clinical symptoms, including arthritis, carditis and neurologic disease (1, 2). A major distinction, however, is that Lyme disease lacks the massive and recurrent episodes of bacteremia that are characteristic of relapsing fever. *Borrelia burgdorferi* produces an early, mild transient blood-borne phase that facilitates dissemination and leads to systemic disease (3). Another important distinction is that, unlike relapsing fever spirochetes, *B. burgdorferi* can survive in multiple tissues in the mammalian host for years. Some of these differences may be explained by the mechanisms used by the *Borrelia* to evade immunity. In relapsing fever, each episode of bacteremia depends on the growth of spirochetes expressing an antigenically distinct surface Ag, produced by gene conversion involving the insertion of a silent variable major protein gene into an active expression locus (4). A prompt Ab response targeting the dominant variable surface Ag results in the rapid clearance of the spirochetes from the bloodstream. Meanwhile, an Ab response to an outer surface protein that does not undergo antigenic variation, the complement factor H-binding protein, is proposed to ultimately defeat the immune evasion tactics used by *Borrelia hermsii*, a relapsing fever species (5). Although Ab responses are also clearly protective against *B. burgdorferi*, they are outmaneuvered by numerous evasion strategies. These mechanisms include the down-regulation of outer surface lipoproteins, such as OspC, and antigenic variation of the surface lipoproteins vlsE and OspE (6–15). In contrast to relapsing fever spirochetes, the use of regenerative regeneration of vlsE produces a very large repertoire of variants at a given time (13–15). In addition to these mechanisms used by *Borrelia*, the mechanisms by which the Ag-specific Abs are generated may account for the final demise of *Borrelia* in the host.

Abs against *Borrelia* do not require T cell help to prevent infection, nor do they require complement to exert a bactericidal effect. The protection provided by T cell-independent Abs in Lyme disease has been demonstrated by the passive immunization of naive or SCID mice with immune sera from mice genetically deficient in T cells, or MHC class II- and CD40L-deficient mice, which have impaired T cell-dependent Ab responses (16–18). In relapsing fever, mice deficient in T cells resolve bacteremia similar to wild-type mice and are resistant to reinfection (19–21). IgM is the predominant isotype associated with T cell-independent responses, and it is critical for the clearance of relapsing fever *Borrelia* in the host (19, 22–25). Both polyclonal and monoclonal IgM confer passive protection, while spirochetaemia persists in secreted IgM-deficient mice (19, 22–26). An unusual property of this IgM is that it does not require complement to destroy the spirochetes, in

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contrast to its typical use of complement in facilitating phagocytosis or in recruiting the membrane attack complex to the surface of the invading microbe. Mice deficient in complement components C1q, C3, or C5 are not impaired in their ability to clear relapsing fever spirochetes (24, 26, 27). In vitro, CB515, a monoclonal IgM derived from an infected mouse and specific for a 19 kDa variable small protein, was observed to kill relapsing fever spirochetes in the absence of complement by causing direct structural damage to the outer membrane of the bacteria (26). The bactericidal activity of this Ab was found to reside in its variable region, as single chain variable fragments retained the function of the parental IgM (28). Other complement-independent bactericidal mAbs have been described against B. burgdorferi and B. hermsii (29–36). Meanwhile, the sources of these protective Abs are not completely defined.

Marginal zone (MZ)³ and B1 B cells provide an initial and prompt Ab response, mainly to T cell-independent Ags. These B

³Abbreviations used in this paper: MZ, marginal zone; FO, follicular; NF, newly formed; dpi, days postinfection.
FIGURE 2. Infection with B. crocidurae results in the egress of MZ B cells from the MZ. Frozen spleen sections of uninfected (0 dpi) and infected mice (4, 6, 7, 8, and 40 dpi) were stained with CD169 (red) to detect marginal metallophilic macrophages and IgM (blue) to detect MZ B cells. Data are representative of three mice per time point. Bar, 200 μm. Spirochetemia is shown in Fig. 1C.

cells participate in a first line of defense against bacterial, viral, and parasitic infections (19, 37–45). MZ B cells encounter invading blood-borne pathogens early based on their anatomical localization in the marginal sinus of the spleen. B1 cells are predominantly localized in pleural and peritoneal cavities and are comprised of B1a and B1b subsets. B1a cells are generally known to be responsible for the constitutive secretion of Ab, while both B1a and B1b cells can produce induced Ab responses. B1b cells can also elicit inducible and long-lasting memory (20). A third subset of Mac1⁺ CD5⁺ B cells has been described as a unique population and termed B1c cells, although they can develop into B1a cells (46, 47). Their role during infection has not been studied. Follicular (FO) B cells, also referred to as B2 or conventional B cells, constitute the majority of recirculating B cells in the body. Although they produce the bulk of the canonical Ab response to protein Ags and require T cell help to generate high-affinity, isotype-switched, somatically mutated Abs and B cell memory, they can also respond to Ags without T cell help. Moreover, FO B cell responses can be enhanced through innate signals, such as TLRs (48, 49). Recently peritoneal B2 cells were distinguished from their splenic counterpart and peritoneal B1 cells in terms of cell surface expression of B220, CD80, and CD43 and several genes (50). They resemble peritoneal B1 cells in their capability to survive in vitro and to spontaneously secrete IgM in vitro and in vivo and were shown to transition into B1b-like cells when transferred into naive SCID mice (50).

MZ B cells are important in controlling early bacteremia following infection with either B. hermsii or B. burgdorferi (19, 37, 38). Mice selectively depleted of MZ B cells produce less Ag-specific Ab in response to both Borrelia and have increased spirochete burdens (37, 38). However, they are not sufficient to clear B. hermsii as splenectomized mice do resolve both the initial and subsequent peaks of bacteremia (19). Meanwhile, primed B1b cells provide protection against challenge with B. hermsii but are mostly or completely protective only after a long period of time (20). We questioned how B cell responses may differ to other Borrelia and whether peritoneal B1b cell subsets are involved following B. burgdorferi infection. Flow cytometry and immunohistochemistry were performed to assess changes in the spleen and/or peritoneal cavity following infection with Borrelia crocidurae and B. burgdorferi B31. Our data demonstrate that early after infection, both Borrelia activate MZ B cells and peritoneal B cells but do not significantly affect splenic FO B cells. The mechanisms by which they activate these B cells appear distinct as B. crocidurae induces the loss of B cells from their compartments, while B. burgdorferi induces their expansion. In addition, the magnitude of the total B cell response and expansion of IgM plasmablasts is much greater following infection with B. crocidurae than with B. burgdorferi. The sustained loss of peritoneal B1c and B2 cells suggest that these subsets may be important in eliminating recurring episodes of relapsing fever spirochetemia. Both Borrelia induce the expansion of B1b cells later after infection, suggesting that B1b cells contribute to long-lasting immunity to both Lyme and relapsing fever spirochetes.

Materials and Methods

Mice

The 6- to 8-wk-old C3H/HeN female mice were obtained from Charles River Breeding Laboratories or Taconic Farms.

Infection of mice

B. crocidurae was obtained from A. G. Barbour (University of California, Irvine, CA). Frozen aliquots were passaged through a C3H/HeN donor mouse, and the spirochetes were harvested by cardiac puncture during peak spirochetemia. The B. burgdorferi low-passage B31 MI strain was grown to logarithmic phase in complete Barbour-Stoenner-Kelly-H medium (Sigma-Aldrich) from frozen stock. Mice were inoculated intradermally with 2 × 10⁶ spirochetes in 100 μl of Barbour-Stoenner-Kelly medium. The course of spirochetemia following B. crocidurae infection was measured daily using darkfield microscopy, whereas the dissemination of B. burgdorferi following infection was determined by quantitative PCR. Animal procedures were done by protocols approved by the institutional review board.

Flow cytometry

Single-cell suspensions were obtained from the spleens and peritoneal cavities of individual mice. Contaminating splenic RBC were lysed using a 0.14 M ammonium chloride buffer, after which the spleen cells were washed twice in Invitrogen DMEM. Cells were resuspended at a final concentration of 2 × 10⁶ cells/ml in 0.2% BSA in PBS with 0.09% NaN₃ and preincubated for at least 5 min with CD16/CD32 Fc Block (clone 2.4G2) to minimize background staining. Spleen cells were stained with CD19-allophycocyanin (clone 1D3), CD21-PE (clone 7G6), CD23-FITC (clone B3B4) to identify MZ, FO and newly formed (NF) B cells, and IgM-allophycocyanin (clone II/41), Mac1-PerCP-Cy5.5 (clone M1/70), and CD5-PE to identify the B2 and B1 cell subsets. All the Abs were purchased from BD Pharmingen. Per the manufacturers’ recommendation, 1 μg of Ab was used for 1 × 10⁶ cells. Cells were incubated for 30
min on ice and then washed with staining medium. If they were not analyzed immediately, they were fixed in 1% formalin. At least 10,000 lymphocytes were acquired using a BD FACSCalibur (BD Biosciences) and Cell Quest software (BD Biosciences) and were analyzed using WinList (Verity Software House).

**Immunofluorescent staining of spleen sections**

Spleen tissue was collected, fixed in 1% formalin for 1 h at 4°C, and then transferred into 30% sucrose in PBS for an overnight incubation at 4°C. The tissue was embedded in Neg-50 Frozen Section Medium (Richard-Allan Scientific) by flash freezing in isopentane cooled with liquid nitrogen and stored at −80°C. The blocks were cut into 5-μm sections, which were fixed in acetone for 30 s and rehydrated in PBS. To visualize the metallophilic MZ macrophages, indirect staining was used and involved three steps. Sections were blocked in 5% normal horse serum (Pierce) for 5 min at room temperature and washed. The sections were then incubated with a 1/100 dilution of primary Ab, rat anti-mouse CD169 (clone 3D6.112) (Sero-tect), and washed. The secondary Ab, biotin-SP-conjugated goat anti-rat-IgG (Jackson ImmunoResearch Laboratories), was added at a 1/500 dilution. Finally, the sections were washed and incubated with streptavidin-PE (BD Pharmingen) at 1/100 dilution and washed. To visualize the IgM⁺ B cells, sections were then blocked with 5% normal goat serum (Pierce) and incubated with goat anti-mouse IgM Alexa Fluor 647 (Invitrogen Molecular Probes) at a 1/50 dilution and washed. All serum and Abs were diluted.

**FIGURE 3.** Kinetic analysis of peritoneal B cell subsets in *B. crocidurae*-infected mice. As in Fig. 1, C3H/HeN mice were infected with 2 × 10⁵ *B. crocidurae* intradermally. A, Representative histograms of flow cytometry analysis for 0, 7, and 40 dpi. a, B cells were first gated on lymphocytes (plots not shown) and identified as B1 or B2 cells by the expression of IgM and IgD. b, B1 cells were further resolved by Mac1 expression. c, B1a and B1b cells were identified by CD5 expression on the Mac1⁺ B1 cells. d, B1c cells were identified by CD5 expression on the Mac1⁺ B1 cells. B, Graphs of absolute numbers and percentages of B cell subsets at indicated time points postinfection. Mean values (±SD) for four to five mice per time point are shown. Day 0 represents the uninfected controls used in each experiment (*n* = 6), *p* < 0.05; and ***, p < 0.01 between infected and uninfected mice. C, Spirochetemia is shown only for the mice used in the corresponding peritoneal cell analysis for 4, 5, 6, 14, and 20 dpi. On other days, spirochetemia is shown for the mice used at the subsequent time point in the peritoneal cell analysis.
in PBS and incubations performed for 30 min at room temperature, except where noted. The sections were washed for 3–4 min/110035 changes of PBS.

The sections were mounted in Opti-Mount and viewed by a Zeiss LSM confocal.

Quantitative PCR of B. burgdorferi DNA

DNA was isolated from mouse ear, heart, and bladder tissue, and from cultured B. burgdorferi, using the DNeasy kit (Qiagen) according to the manufacturers’ protocol. Previously published primers were used to amplify the B. burgdorferi recA gene (nTM17.F: 5′-GTG GAT CTA TTG TAT TAG AGG CTC TCG-3′ and nTM17.R: 5′-GCC AAA GTT CTG CAA CAT TAA CAC CTA AAG-3′) and the mouse nidogen gene (nido.F: 5′-CCA GCC ACA GAA TAC CAT CC-3′ and nido.R: 5′-GGA CAT CTG CTG CCA TC-3′) (51). The PCR was performed and analyzed on an ABI 7500 Real Time PCR System (Applied Biosystems) in 96-well optical reaction plates (Applied Biosystems). Each reaction included 10 ng of template DNA, 200 nM of primers (Midland Certified Reagent), and 25 ng/μl BSA in a final volume of 25 μl. Reactions were done in duplicate, and a standard curve was run on every plate consisting of either 10-fold serial dilutions for recA or 5-fold serial dilutions for nidogen. The standard thermocycler conditions were used (1 cycle of 95°C 10 min, 40 cycles of 95°C × 15 s, and 60°C × 1 min) and dissociation curves generated using 95°C × 15 s, 60°C × 1 min, and 95°C × 15 s.

Statistical analysis

Data were analyzed by the one-way ANOVA Dunnett multiple comparisons test using GraphPad Instat3 software. A probability value of <0.05 was considered significant when comparing infected mice to uninfected controls.

Results

Infection with B. crocidurae induces reductions of MZ B cells and peritoneal B cells

To characterize the B cell response to relapsing fever, C3H/HeN mice were infected intradermally with B. crocidurae and the participating B cells quantitatively analyzed by flow cytometry at several time points postinfection. Total B cells in the spleen were first identified by CD19 expression and then resolved into subsets based on their differential expression of CD21 and CD23, as previously described (52). MZ B cells were identified as CD21high...
CD23−, FO B cells as CD21lowCD23+, and transitional NF B cells as CD21−CD23+ (52). The involvement of MZ B cells was further examined by staining spleen sections with IgM and CD169, a marker expressed on marginal metallophilic macrophages that defines the inner rim of the marginal zone (53). Spirochetemia was first detected on day 3, after which it began to rise rapidly, peaking on day 6, and then declining to very low levels on day 8. Relapses immediately followed and continued until spirochetemia was no longer detectable on day 20 (Fig. 1C). Although MZ and FO B cell populations in the spleen remained constant when spirochetemia was only beginning to rise on day 4 (Fig. 1), MZ B cells appeared to have already migrated out of the MZ (Fig. 2). Although no significant changes were observed by flow cytometry in MZ and FO B cell numbers early after infection, a 2-fold reduction in MZ B cells was concurrently observed with an increase in IgM+ plasmablasts on day 6, the day before the drop in spirochetemia (Fig. 1B). At the same time, intense IgM staining was seen outside the MZ in the red pulp (Fig. 2). As B cells shed many of their surface receptors when differentiating into plasma cells, our data imply that MZ B cells become activated soon after infection with B. crocidurae and migrate out of the MZ where they differentiate into extrafollicular IgM plasma cells. Interestingly, the variation in MZ B cell numbers on day 8 correlated with the level of spirochetemia observed before clearance, with a reduced number of MZ B cells corresponding to a greater bacterial burden (data not shown). To determine whether the reduction of MZ B cells was due to cell death, the splenic B cell subsets were labeled with 7-氨基-actinomycin D. No significant differences were observed at the time points examined (data not shown). The approximate 2-fold increase in FO B cell numbers on day 6 suggests that they may participate in the early response as well (Fig. 1B). Meanwhile, the significant increase in total B cells observed from days 6 to 8 occurred simultaneously with the large influx of NF B cells and increased plasmablast populations (Fig. 1, A and B). Long after bacteremia was cleared (at day 40), FO B cells were the only B cell subset in the spleen significantly increased (Fig. 1B).

After determining that MZ cells actively participate in the splenic B cell response to B. crocidurae, we next analyzed the contribution of the B cell subsets in the peritoneal cavity. B1 cells were identified as IgMhighIgDlow and B2 cells as IgMlowIgDhigh. The B1 cell subsets were further resolved by Mac1 and CD5 expression: B1a cells were Mac1+CD5+ and B1b cells were Mac1−CD5−. Mac1−CD5− B cells are referred to as B1c cells in this paper for simplicity. In this experiment, spirochetemia was first detected on day 3, peaked on day 6, and quickly dropped to very low or undetectable levels on day 7. Relapses immediately followed and continued until spirochetemia was no longer detectable on day 19 (Fig. 3C). Although bacteremia was rapidly increasing on day 4, a striking and significant loss of cells was found in every B cell subset within the peritoneal cavity with the exception of B1a cells, but their numbers were decreased as well (Fig. 3, A and B). The B2, B1a, and B1c cell subsets all had significantly reduced cell numbers up to and including the resolution of the first peak of spirochetemia on day 7 (Fig. 3). A large majority of the B2 and B1c cells disappeared during this period, up to 95 and 89%, respectively (Fig. 3). Both of these subsets remained depressed during a subsequent peak of spirochetemia on day 14 and when spirochetes were no longer detectable on day 20 (Fig. 3). Although B2 cell numbers were finally restored to normal levels on day 40, B1c cell numbers continued to be significantly decreased (Fig. 3, A and B). In contrast, B1b cell numbers returned and remained comparable to uninfected controls on days 14 and 20 and were significantly expanded at day 40 (Fig. 3, A and B).

The loss of the peritoneal B cells may be linked to the increase in the total number of B cells and plasmablasts in the spleen, both of which begin before the initial decline in bacteremia (Figs. 1 and 3). It should also be noted that the parameters used to define NF B cells in the spleen may have included recently migrated peritoneal B1 cells, which also do not express CD23 and express relatively high levels of CD8+ (54). Therefore, the large influx of NF B cells in the spleen may represent some of the lost B1 cells from the peritoneal cavity (Figs. 1 and 3).

Infection with B. burgdorferi induces an expansion of MZ B cells and peritoneal B1 cells.

B. burgdorferi becomes blood-borne within 4 days of intradermal inoculation and induces peak carditis and arthritis 2 to 4 wk after infection (55). As we were interested in the early B cell response, we evaluated time points through the second week of infection. As spirochetes are not present in great quantities in the bloodstream and are more abundant in tissues, we monitored infection of multiple organs by quantitative PCR. B. burgdorferi DNA was not detected in any of the organs analyzed until day 9, when it first appeared in the urinary bladders (Fig. 4B). It was additionally detected in the heart and ear tissue of all mice by day 15 (Fig. 4B).
Although *B. crocidurae* elicited a decrease in MZ B cells at the same time that IgM⁺ plasmablasts were expanded in the spleen (Fig. 1, A and B), *B. burgdorferi* induced the concomitant expansion of MZ B cell numbers and IgM⁺ plasmablasts when infection was first detected on day 9 (Fig. 4, A and B). IgM⁺ plasmablast numbers continued to be significantly expanded on days 11 and 17 (Fig. 4A). In the meantime, FO B cell numbers did not undergo any significant changes (Fig. 4A). The significant increase in total B cell numbers on day 9 must encompass the influx of NFB cells and the increase in MZ B cells and plasmablasts (Fig. 4A). As noted in the previous section, it may also include other B cells that have migrated to the spleen.

The B cell response to *B. burgdorferi* in the peritoneal cavity differed from the response to the relapsing fever *Borrelia* in that most of the peritoneal B cell subsets were significantly expanded, rather than diminished, following infection (Fig. 5). B1a cell numbers significantly increased on days 9 and 11 (Fig. 5). B1b cell numbers significantly increased on days 9, 11, and 17 (Fig. 5). B1c cells participated the earliest and showed significant expansion on day 5, before *B. burgdorferi* was detected in the skin, heart, or bladder (Figs. 4B and 5). Only peritoneal B2 cells were not significantly expanded (Fig. 5). Meanwhile, the expansion of each peritoneal B cell subset was less than that of the MZ B cells in the spleen.

Overall, infection with *B. crocidurae* produced 4 times as many B cells and 10 times as many IgM plasmablasts than infection with *B. burgdorferi*. Splenomegaly, which may be indicative of proliferating and infiltrating immune cells, was observed in mice infected with *B. crocidurae*, but not *B. burgdorferi* (data not shown).

**Discussion**

In both relapsing fever and Lyme disease, Abs are the most critical effectors of the host defense either by themselves or by opsonization followed by lethal Fc-receptor-mediated phagocytosis. The damaging Ab response to *Borrelia* may have propelled the development of the antigenic variation mechanisms of these spirochetes (2). In this study, we have shown that two species of closely related *Borrelia* induced markedly different B cell responses. The main pathological differences between the course of infection of relapsing fever and Lyme disease is the overt spirochetaemia in the former and persistent infection in multiple tissues in the latter, which may account, at least in part, for the different B cell responses elicited.

We demonstrated that infection with *B. crocidurae* resulted in a dramatic loss of B cells from the MZ and a marked reduction in the percentage of MZ B cells in the spleen. The expansion of IgM plasmablasts was observed only after the MZ B cells presumably migrated into the red pulp, indicating that egress preceded differentiation. MZ B cells are known to migrate following exposure to Ag to the T-B interface and then relocate as differentiated plasmablasts to the red pulp (56). They have only recently been found to proliferate, differentiate, and migrate out of the MZ in response to TLR agonists (57–59). Coordinate signaling through the TLRs and the BCR is required for the *B. hermsii*-specific IgM response and, therefore, may be critical in the activation of MZ B cells in response to relapsing fever spirochetes (60). Deficiencies in both TLR1, TLR2, and Myd88, a major adaptor in the TLR-signaling pathway, generate specific IgM against *B. hermsii* with delayed kinetics, demonstrating that the adjuvant-like properties of the TLRs can impact the expansion of the specific IgM response to relapsing fever *Borrelia* (60). However, deficiency of both Btk, which diminishes BCR signaling, and Myd88 were required to completely ablate the response (60). In contrast, infection with *B. burgdorferi* resulted in the concomitant expansion of MZ B cells and a significant, but less robust, increase in IgM plasmablasts. Meanwhile, TLRs do not seem to be required for the production of *B. burgdorferi*-specific IgM (61, 62). Instead, MyD88 negatively regulates IgM production in response to *B. burgdorferi* by an indirect, undefined mechanism (63). This mechanism is thought to safeguard the host against the production of autoreactive Abs, which can arise in response to a mitogenic stimulus. It would also seem to benefit *B. burgdorferi* in dampening and limiting the specific IgM response to itself and may contribute to the overall smaller B cell response when compared with the response to *B. crocidurae*. Other potential receptors which may activate MZ B cells in response to *Borrelia* are C3, which targets T cell-independent Ags to the MZ B cell surface, or CD1d. C3 may not be important in this setting as C3-deficient mice can clear relapsing fever *Borrelia* and produce an Ag-specific IgM repertoire similar to that of wild-type mice (24). Likewise, mice lacking the B cell complement receptors CD21 and CD35 are not impaired in the host response to *B. burgdorferi* infection and even produce increased levels of Ag-specific IgM (64). However, infection of C3-deficient mice with larger numbers of *B. burgdorferi* produce the opposite result (65). Signaling through CD1d has also been proposed to explain how this molecule enhances MZ B cell production of *B. hermsii*-specific Ab (38).

Similar to MZ B cells, we found that peritoneal B1 cells are lost early in response to *B. crocidurae* but are expanded in response to *B. burgdorferi*. As activated peritoneal B1 cells migrate into the spleen and other effector sites where they proliferate and differentiate into plasma cells, it seems likely that the loss of peritoneal B1 cells is an important step in generating immunity to *B. crocidurae* (66–68). Egress may be a crucial mechanism for peritoneal B cells in response to relapsing fever *Borrelia* in general, as B1b cells were similarly lost from the peritoneal cavity early after infection with *B. hermsii* (69). Relapsing fever *Borrelia* may trigger egress through a TLR signaling pathway, which can induce the down-regulation of multiple integrins and CD9 on B1 cells (68, 70). In contrast, B1 cells activated by *B. burgdorferi* either proliferate in the peritoneal cavity before they migrate or may not migrate at all. Inhibiting migration may be another immunomodulatory tactic of *B. burgdorferi*.

We found that B1a cells increase during infection with *B. burgdorferi* and may be the source of natural IgM that targets this organism while it is within the vector (71). In contrast, B1a cells are expendable in the host response to *B. hermsii* and were not significantly responsive to *B. crocidurae* in this study (20).

Our finding that B1b cells were the only expanded peritoneal B cell subset at the last time point examined in response to both *B. crocidurae* and *B. burgdorferi* suggests that B1b B cells may be a source of long-lasting immunity to various *Borrelia*. They may be a source of the high level of specific Abs to *B. burgdorferi* than can persist for years (72). Elevated serum IgM or IgG can be found in various stages of Lyme disease and may be the result of mitogenic or antigenic responses (72–74). We also found persisting immunity to *B. crocidurae*, as mice were resistant to re-challenge more than 2 mo after the initial infection (data not shown). B1b cells provide inducible T cell-independent memory and long-lasting protection against *B. hermsii*, and complement factor H-binding protein was recently identified as a target Ag for this protective response (5, 20).

We found that B1c cells were responsive to infection with *Borrelia*, although how they contribute to host immunity remains to be determined. B1c cells may be a heterogeneous population comprised of transitional cells that develop into B1a cells, splenic B1a cells that have just migrated into the peritoneum, or a unique subset (46, 47). The loss of B1c cells induced by *B. crocidurae* without
an associated increase in B1a cells in the peritoneal cavity may be explained if they migrate and then differentiate into either B1a cells or plasma cells outside the peritoneal cavity. Their sustained loss at day 40 suggests that their turnover rate is slow. Meanwhile, the transient increase of B1c cells induced by B. burgdorferi cannot be easily explained in the context of their role as progenitors of B1a cells in our study. If the expanded B1c cells were developing into B1a cells, an immediate increase in B1a cells would be expected to follow the increase in B1c cells. However, this was not evident.

We found that B. crocidurae induced significant early changes in peritoneal B2 cells, but not splenic FO B cells. This finding suggests that either the location of the B2 cell may influence its early response or supports intrinsic differences between peritoneal and splenic B2 cells. Meanwhile, a sustained loss of peritoneal B2 cells occurred throughout the course of spirochetemia, and splenic FO B cells became significantly increased after the clearance of bacteremia. This suggests that peritoneal B2 cells may contribute to the resolution of relapsing spirochetemia, and FO B cells may contribute to long-term immunity to B. crocidurae. B2 cells are not required to eliminate B. hermsii, as IL-7-deficient mice, which are deficient in both splenic and peritoneal B2 cells, are able to clear spirochetaemia as efficiently as wild-type mice (19). In contrast, the importance of FO B cells in B. burgdorferi immunity has not been directly determined. We found that neither B2 subset was significantly responsive to B. burgdorferi.

Our findings demonstrate the dynamics of different B cell populations after infection with Borrelia and show that Lyme and relapsing fever spirochetes use distinct pathways to activate MZ and splenic B2 cells. Meanwhile, a sustained loss of peritoneal B2 cells occurred throughout the course of spirochetemia, and splenic FO B cells became significantly increased after the clearance of bacteremia. This suggests that peritoneal B2 cells may contribute to the resolution of relapsing spirochetemia, and FO B cells may contribute to long-term immunity to B. crocidurae. B2 cells are not required to eliminate B. hermsii, as IL-7-deficient mice, which are deficient in both splenic and peritoneal B2 cells, are able to clear spirochetaemia as efficiently as wild-type mice (19). In contrast, the importance of FO B cells in B. burgdorferi immunity has not been directly determined. We found that neither B2 subset was significantly responsive to B. burgdorferi.

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Disclosures
The authors have no financial conflict of interest.

References


