B Lymphocytes Provide an Infection Niche for Intracellular Bacterium *Brucella abortus*

Radhika Goenka,[a] **Patrick D. Guirnalda,**[a] **Samuel J. Black, and Cynthia L. Baldwin**

Department of Veterinary and Animal Sciences, University of Massachusetts, Amherst, Massachusetts

Background. *Brucella* spp. are intracellular bacteria that establish lifelong infections whose mechanisms of chronicity are poorly understood. Notably, B cells facilitate the establishment of the high infection plateau that persists for months.

Methods. We evaluated the contribution of murine B cells toward providing infection niches for *Brucella* by using flow cytometry and microscopy and by determining live bacterial counts associated with B cells both in vivo and in vitro.

Results. Herein we demonstrate that immunoglobulin M and complement-opsonized *Brucella abortus* infects and survives inside primary murine B cells protected from bactericidal effects of gentamicin. The entry was dependent on microfilaments for internalization and subsequently brucellae reside in a late endosomal/lysosomal compartment. Throughout the infection, 10% of colony-forming units from infected mice was associated with B cells, and these cells transferred disease to naive hosts. Furthermore, *Brucella*-positive cells were positive for transforming growth factor (TGF) β1, and about 10% of such cells were B cells, similar to rates found for other intracellular pathogens that induce their hosts cells to produce TGF-β1.

Conclusions. To conclude, infected B cells contribute to chronic bacterial infections by providing an intracellular niche that may exert an immunoregulatory role. Although professional phagocytic cells harbor intracellular bacteria including *Brucella*, infection of lymphocytes by bacteria has not been previously appreciated.

Brucella abortus is an intracellular bacterium that establishes lifelong infections in livestock and humans and whose mechanisms of chronicity are poorly understood. *Brucella* spp., the causative agents of brucellosis, are facultative intracellular bacteria that inhabit membrane-bound compartments within professional phagocytic cells, such as macrophages and dendritic cells, as well as nonprofessional phagocytic cells, such as trophoblasts in the uterus of pregnant ruminants thus leading to abortion [1–3]. It is generally agreed that macrophages are the primary host cell responsible

for the chronic infection, because *Brucella* creates a niche inside these cells that supports bacterial replication [4, 5]. It does so by virulence factors that enable circumvention of the otherwise potent microbicidal properties of the macrophage and expression of genes, such as the type IV secretion system *virB*, that are necessary for the bacteria to survive intracellularly [6, 7]. Nevertheless, some bacteria are killed by phagocytes after appropriate activation. Studies conducted in murine experimental models have shown a critical requirement of type I immunity, particularly interferon γ, for control of infection (reviewed in [8]).

Experimental murine brucellosis is characterized by a high infection plateau, which persists for 2–3 months and is followed by a slow protracted clearance. Recently, we have shown that B cells, but not antibodies, facilitate the establishment of this high infection plateau, because the absence of B cells elicits rapid clearance between 10–14 days after infection [9]. Further investigation revealed that this rapid clearance of brucellae from B cell–deficient mice correlated with a decrease in their production of regulatory cytokines, including TGF-β1, which limits induction of protective

type 1 immune responses. Although the presence of TGF-β1+ B cells has been demonstrated in vitro, their existence in vivo has been uncertain (reviewed in [10]). Interestingly, TGF-β1+ B cells appeared as early as 1 week after brucella infection and were present during the plateau phase of the infection [9]. Moreover, even macrophages (the primary niche) were TGF-β1+ [9]. Because pathogens induce TGF-β1 production by infected host cells as a way to dampen host inflammatory immune responses (reviewed in [10]), this finding raised the possibility that Brucella spp. might use the B-cell niche for survival or expansion and/or to down-regulate protective immune responses.

B cells are not recognized as an infection niche for pathogenic bacteria, perhaps because of their low phagocytic index [11–14]. Nevertheless, in vitro, transformed B cells internalize whole Mycobacterium spp. [13], and primary B cells can internalize immunoglobulin (Ig) M–opsonized whole Staphylococcus aureus [11]. Furthermore, an in vivo study conducted using B cell–deficient mice has implicated B cells in the dissemination of mycobacteria from lungs to the periphery [12], raising the possibility that the bacteria survive within B cells. Herein, using an in vitro infection system, we demonstrate that IgM and complement-opsonized B. abortus infects and survives inside primary murine B cells in a compartment that protects them from the bactericidal effects of extracellular gentamicin. In vivo, 10% of the total infection was associated with B cells. All Brucella infected cells were TGF-β1+, and 10% of such cells were B cells. Thus, the results presented here demonstrate that Brucella infects B lymphocytes and imparts a regulatory character to them.

METHODS

Mice and Bacterial Strains

All animal experiments were approved by the University of Massachusetts Institutional Animal Care and Use Committee, and work with B. abortus was approved by the Institutional Biosafety Committee and the Centers for Disease Control and Prevention (registration number C20041019-0289). BALB/c WT and Jh−/− mice (B6–IgH–Jtm1Dhu N?+2) (Taconic) were housed in the ABSL3 facility and handled according to aseptic techniques. B. abortus 2308 pBBRMCS6-Y (designated as green fluorescent protein [GFP]–Brucella) [15] and B. abortus 2308 were expanded on Schaedler blood-agar (BBL; BD Biosciences) at 37°C and 5% carbon dioxide and stored in liquid nitrogen.

In Vivo Infection

Mice 7–14 weeks old were administered 5×10^4 total colony-forming units (CFU) of B. abortus 2308 in phosphate-buffered saline (PBS) intraperitoneally. The exact dose and splenic CFU counts were enumerated on Brucella agar (BBL). The total CFU count associated with CD19+ cells was accounted for by adjusting the CFU count obtained from the aliquot of positively sorted CD19+ cells relative to the total number of CD19+ cells.

In Vitro Infection

Splenic B lymphocytes were purified using CD19 microbeads (Miltenyi). Brucella was incubated with serum or IgM or IgG antibodies for 30 minutes at 37°C, washed in PBS, and used to infect B lymphocytes at a 1:10 ratio. The gentamicin protection assay was performed as described elsewhere [4]. For the phagocytosis inhibition assay, the B lymphocytes were treated with 2 μmol/L cytochalasin D or 5 μmol/L nocadazole (Sigma–Aldrich) for 30 minutes at 37°C before infection. The drugs were dissolved in dimethyl sulfoxide, and the concentrations used were not toxic to the B lymphocytes or Brucella (data not shown). For inhibition of the classic complement pathway, nonimmune serum was treated with 0.01 mol/L magnesium ethylene glycol tetraacetic acid (Mg/EGTA) during the opsonization process, as described elsewhere [16].

Antibody Purification

IgM antibody was purified from 1–2 weeks immune or from nonimmune serum on a mannose binding lectin column according to the manufacturer's protocol (Pierce). Immune IgG antibodies were purified from 4.5-week-postinfection immune serum by sequential purification on protein G and A columns according to the manufacturer's protocol (Amersham Biosciences). Nonimmune IgG was purchased from Southern Biotech. IgM and IgG antibody titers were determined by indirect enzyme-linked immunosorbent assay using heat-killed Brucella as antigen. Serum samples were incubated 2 hours, room temperature, and developed using horseradish peroxidase–conjugated goat anti-mouse IgG or IgM (Southern Biotechnology) with ABTS substrate (Sigma).

Immunofluorescence Analysis

Staining was performed as described elsewhere [9] with the following reagents: cholera toxin (Invitrogen), anti–lysosome-associated membrane protein 1 (LAMP1) (clone 1D4B; Pharmingen), biotinylated anti–TGF-β1 (clone A75-3; Pharmingen), anti-CD19 (clone MB19-1; ebioscience), anti-Brucella rabbit serum (BBL), goat anti-rabbit IgG (H + L) conjugated to fluorescein isothiocyanate (Southern Biotech) or phycoerythrin (Abcam) and streptavidin–allophycocyanin–cyanine 7. Flow cytometric acquisition was conducted with an LSRII flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (TreeStar). Microscopy was conducted with an laser scanning microscope (LSM) 510 Meta microscope using an LSM Image Browser version 4.0.0.241 (Zeiss).

Electron Microscopy

Cells were fixed overnight at 4°C with 2.5% gluteraldehyde in PBS containing 7.5% sucrose (PBS-sucrose). For scanning

electron microcopy, fixed cells were applied to a nucleopore filter (1.0-μm pore) and washed with PBS-sucrose. The cells were postfixed with 1% osmium tetroxide in PBS-sucrose for 1 hour at room temperature and dehydrated by increasing concentrations of ethanol: 30%, 50%, 70%, 80%, 95%, and 100%. The cells were critical point dried with carbon dioxide (Balzers CPD-030, Balzers) using ethanol as the transitional solvent. The filter disks were mounted onto 15-mm-diameter aluminum scanning electron microscope supports and coated with gold-palladium in a Polaron E5100 sputter coater (Quorum Technologies). The samples were observed and imaged on a scanning electron microscope (JEOL JSM-5400; JEOL).

For transmission electron microscopy, fixed cells were enrobed in 2% agarose (Type IX, Ultralow-gelling; Sigma–Aldrich) and postfixed in 1% osmium tetroxide in PBS-sucrose for 30 minutes at 22°C and dehydrated in a graded ethanol series (30%, 50%, 70%, 80%, 95%, and 100%). Then the samples were changed to 100% dry acetone, infiltrated, and embedded with Spurr's epoxy resin (Electron Microscopy Sciences). Sections were cut at 60 nm, mounted on copper mesh grids, and stained with 2% uranyl acetate (aqueous) for 15 minutes at room temperature, followed by alkaline lead citrate (5 mg/mL in 0.1 N sodium hydroxide) for 5 minutes,

and then observed and photographed on a transmission electron microscope (JEOL JEM-100S).

Statistical Analysis
Data represent means ± standard errors and were tested for statistical significance at $\alpha = 0.05$ in a 2-tailed t test.

RESULTS

Immune Antibodies and Infection of B Cells In Vitro
In vitro experiments were performed to determine whether B. abortus can infect B cells and what the mechanism of infectivity might be. The studies employed B. abortus 2308 engineered to constitutively express GFP (designated as GFP-Brucella) [15]. The bacteria were opsonized by incubating with pooled immune serum collected from BALB/c mice 1–3 weeks after infection with strain 2308 or with nonimmune serum as a control. At 2 hours after infection, flow cytometry revealed that a greater proportion of B cells were GFP-Brucella positive when immune serum was used for opsonization compared with nonimmune serum (Figure 1A). Further analyses showed that immune serum harvested 2 weeks after infection led to maximal B-cell infectivity (Figure 1B). To determine whether this effect was due to Brucella-specific antibodies, we harvested immune serum samples at 1 week after infection from B cell/antibody–deficient Jh$^{-/-}$ mice [17]. Serum samples from Jh$^{-/-}$ mice had a 10-fold lower capacity to support GFP-Brucella infection compared with WT controls (n = 4 donors per mouse strain) (Figure 1C and 1D), indicating that antibodies were key for maximal infection.

IgM and Complement and Phagocytic Uptake of Brucella
IgM is the predominant isotype present in the immune serum of Brucella-infected mice up to 2 weeks after infection (Figure 2A; [18]), thus we hypothesized that it is key in facilitating infection of B cells. We purified IgM and IgG from immune serum and from control serum obtained from naive mice. Addition of immune IgM to serum collected from either infected Jh$^{-/-}$ mice (Figure 2B) or naive WT mice (Figure 2C) greatly increased their capacity to facilitate infection of B cells to a level comparable to that seen with whole immune serum obtained from WT mice (Figure 2B and 2C). In contrast, addition of nonimmune IgM, immune IgG or nonimmune IgG antibodies to Jh$^{-/-}$ serum did not facilitate uptake of brucellae into B cells (Figure 2B). Furthermore, inhibition of the classic pathway of complement activation by addition of Mg/EGTA [19] abrogated the opsonic ability of the immune IgM-supplemented nonimmune serum (Figure 2C). Although components of the complement cascade are efficient opsonins, they are also potent microbicidal agents; however, they are largely ineffective in killing smooth strains of Brucella, such as B. abortus 2308 (reviewed in [8, 20]). Thus, complement
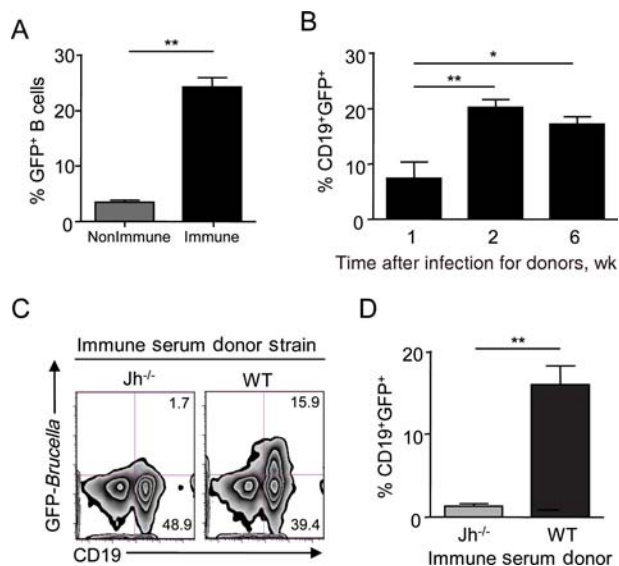

Figure 1. Brucella-specific antibodies facilitate uptake of Brucella abortus into B cells. A, CD19$^+$ cells were incubated with green fluorescent protein (GFP)–Brucella opsonized with pooled nonimmune or immune serum for 2 hours. B, Splenocytes were infected with GFP-Brucella opsonized with immune serum harvested from wild-type (WT) mice (n = 3) at various times after infection. C, D, Splenocytes were infected with GFP-Brucella opsonized with 1-week-postinfection immune serum obtained from WT or Jh$^{-/-}$ mice (n = 4), with representative staining shown in C. Results are representative of 2 experiments. *.01 < P < .05; **P < .01.
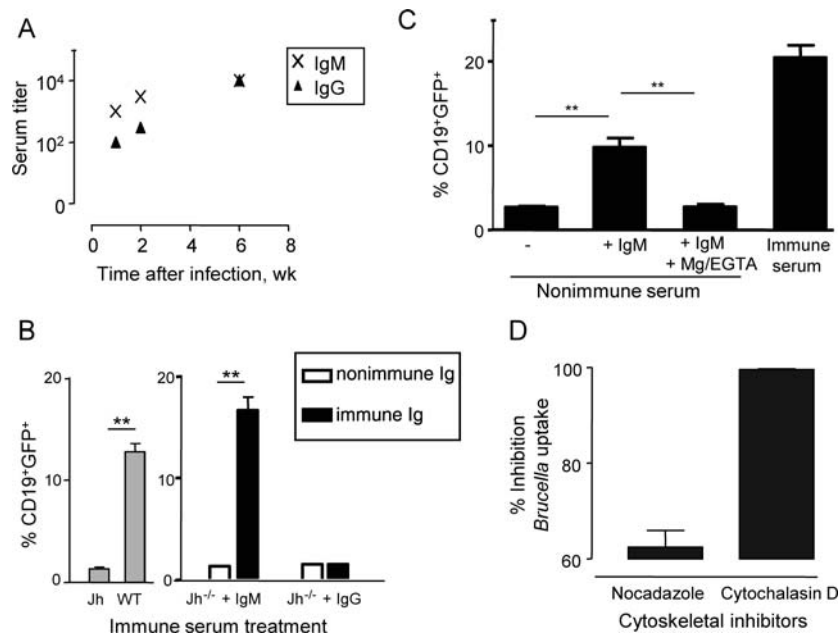
Figure 2. Immune immunoglobulin (Ig) M and bioactive complement facilitate uptake of *Brucella abortus* into B cells. *A,* Anti-*Brucella* IgM and IgG titers in infected wild-type (WT) mice (n = 3). *B,* Splenocytes were infected with green fluorescent protein (GFP)–*Brucella* opsonized with Jh$^{-/-}$ immune serum alone (Jh) or supplemented with 0.25 μg of nonimmune or immune IgM antibodies (Jh$^{-/-}$ + IgM), nonimmune or immune IgG antibodies (Jh$^{-/-}$ + IgG), or WT immune serum alone (WT). *C,* Purified immune IgM (0.5 μg) was added to nonimmune serum harvested from WT mice with or without Mg/EGTA (Mg/Ethylene glycol tetraacetic acid) and used for opsonization. *D,* B cells were incubated with inhibitors 30 minutes before infection and infected with opsonized *Brucella.* After 1 hour incubation with gentamicin, B cells were lysed to release the intracellular brucellae and CFU counts determined. Data represent the percentage of inhibition of uptake relative to dimethyl sulfoxide control. Results are representative of 2 experiments. **$P < .01$.

activated by IgM-*Brucella* complexes should not impact the viabiliy of *B. abortus* 2308. We confirmed this by determining the number of live brucellae inside B cells using a gentamicin protection assay. This assay distinguishes between intracellular and extracellular brucellae, because only the brucellae present inside the cell survive in the presence of the antibiotic [4]. After opsonization, we found live brucellae associated with B cells at 1 hour after infection, despite the presence of gentamicin (Figure 3D).

Classic phagocytosis via Fcγ receptors and complement receptors requires microfilaments and microtubules [16]. To determine whether uptake of opsonized brucellae by B cells occurred via classic phagocytosis, B cells were treated with the microfilament- or microtubule-disrupting drugs cytochalasin D or nocadazole, respectively, before infection with immune serum–opsonized brucellae. Preincubation of B cells with nocadazole partially inhibited uptake of bacteria, as evidenced by a 63% reduction in recovered CFU counts (Figure 2D). In contrast, cytochalasin D resulted in 99% inhibition of uptake of brucellae into B cells (Figure 2D), consistent with results seen with uptake of polystyrene beads [14]. Scanning electron micrographs revealed that multiple opsonized brucellae were associated with individual B cells as early as 5 minutes after in

vitro infection and were engulfed by either membrane veils (indicated by thick arrows) or small pseudopodia (indicated by thin arrows) (Figure 3A). Transmission electron micrographs revealed that *Brucella* (indicated by black arrows) was found inside B cells by 2 hours after infection (Figure 3B). Interestingly, confocal microscopy revealed that infected GFP-*Brucella*–positive B cells exhibited polarized CD19 expression, compared with neighboring uninfected B cells (Figure 3C), indicating that *Brucella* infection perhaps leads to activation of B cells.

Evaluation of Survival and Replication in B Cells

To determine whether *Brucella* can survive and/or replicate inside B cells, we maintained gentamicin in extracellular medium. Interestingly 10% of the internalized brucellae survived inside B cells when evaluated at 24 hours (Figure 3D). However, unlike what occurs in macrophages infected with brucellae in vitro, the infected B cells did not support bacterial replication and the number of viable internalized bacteria therefore remained constant between 24 and 48 hours after infection (Figure 3D) [4]. Thus, the B-lymphocyte niche supports survival with minimal to no growth of the brucellae.
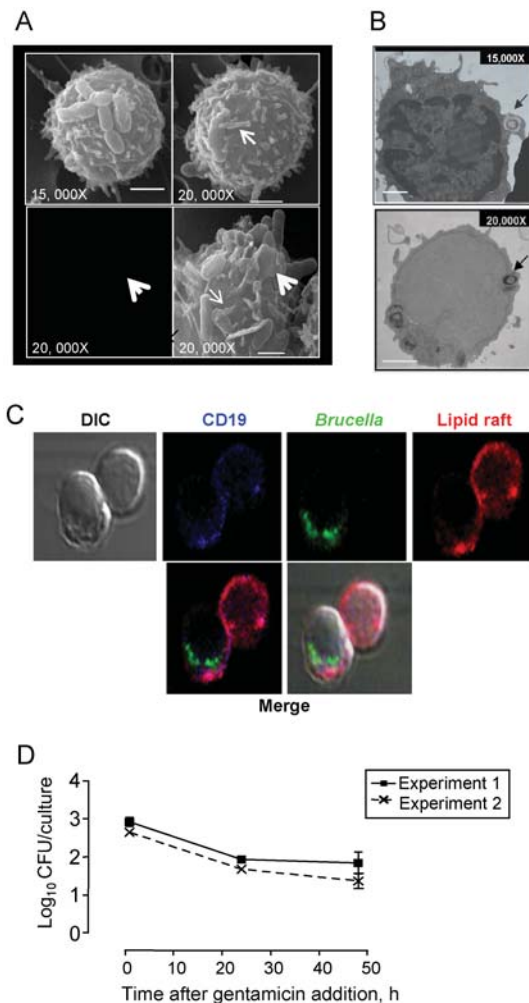
Figure 3. *Brucella abortus* survives inside B cells. CD19+ cells were infected with *Brucella* opsonized with 1–2-week-postinfection immune serum. *A, B,* Purified CD19+ cells were infected for 5 minutes (*A*) or 2 hours (*B*) with immune serum–opsonized *Brucella*. The cells were washed to remove free brucellae and fixed with 2.5% glutaraldehyde in phosphate-buffered saline–sucrose for scanning (*A*) or transmission (*B*) electron microscopy. Scale bar equals 1 μm, and arrows indicate the presence of *Brucella*. *C,* Confocal micrographs depicting the presence of green fluorescent protein–*Brucella* inside CD19+ cells after in vitro infection. Cholera toxin was used to stain lipid rafts. The flourescent images were merged with differential interference contrast (DIC) image. *D,* CD19+ cells infected with opsonized *Brucella* were lysed at 1, 24, or 48 hours after addition of gentamicin to determine colony-forming unit (CFU) counts. In panel A, membrane veils are indicated by thick arrows while small pseudopodia are indicated by thin arrows. In panel B, arrows indicate brucella.

Brucella Infection of Marginal Zone Versus Follicular B Cells

To determine whether *Brucella* infected specific subsets of B cells, we infected splenocytes with immune serum–opsonized GFP-*Brucella* and tracked the infection in mature B-cell subsets by flow cytometry. We gated on CD19+ AA4.1−

mature B cells that were further parsed into marginal zone B (MZB) (IgM^{hi}IgD^{lo}) and follicular B (FOB) (IgM^{lo}IgD^{hi}) cell subsets (representative gating strategy shown in Figure 4). Within these subsets of mature B cells, we showed that a greater proportion of MZB cells were infected with *Brucella*, compared with FOB cells (Figure 4), indicating that MZB cells are more susceptible to *Brucella* infection.

B. abortus Survival Inside B Cells In Vivo

To evaluate whether brucellae associated with B cells in infected mice are viable, splenic CD19+ cells were isolated, washed, lysed, and plated to determine CFU counts. Live brucellae (approximately 7.2% ± 5% of total CFU counts) could be cultured from purified CD19+ cells as early as 1 week after infection and until ≥6 weeks after infection, with a significant increase in CFU counts associated with B cells occurring as the infection progressed (Figure 5A). Furthermore, we determined whether the CD19+ cells from infected mice transferred the disease into naive mice. Three weeks after the adoptive transfer, CFU counts were determined in the recipient mice that received CD19+ or CD19− cells as a control. The CD19+ cells transferred the disease as efficiently as the CD19− fraction (Figure 5B). Thus, infected B cells were competent for perpetuating infection of the host.

To confirm that *Brucella* were present inside B cells in infected mice and not just associated with their cell surface, we stained for the late endosomal and lysosomal marker, LAMP1, because in infected macrophages brucellae traffic through a LAMP1+ compartment [1, 5, 21]. Staining with anti-*Brucella* antibody along with fluorochrome-conjugated secondary antibody was used to identify the infected cells, whereas anti-CD19 antibodies were used to identify B cells. Confocal microscopy revealed that brucellae mainly colocalize inside the CD19+ cells with LAMP1 (Figure 5C), as transiently occurs in infected macrophages [1, 5]. Furthermore, CD19 was polarized on B cells infected in vivo (Figure 5C), indicative of the activated state of B cells [22] and consistent with findings of in vitro infection studies (shown above). Together, these data show that brucellae are present inside activated B cells.

B. abortus–Infected B Cells Produce TGF-β1

Pathogens induce their hosts to produce TGF-β1 to facilitate immune evasion [23, 24]. In murine brucellosis, absence of B cells (even when antibodies have been adoptively transferred into the mice), limits the ability of *Brucella* to establish the high infection plateau. This correlates with reduced production of regulatory cytokines, including B cell–derived TGF-β1, early in the infection [9]. Thus, we determined whether *Brucella* are associated with B cells that produce TGF-β1 during the course of the infection in BALB/c mice. Splenocytes were harvested from *Brucella*-infected mice at 4 weeks after infection or from uninfected control mice and were stained for intracellular
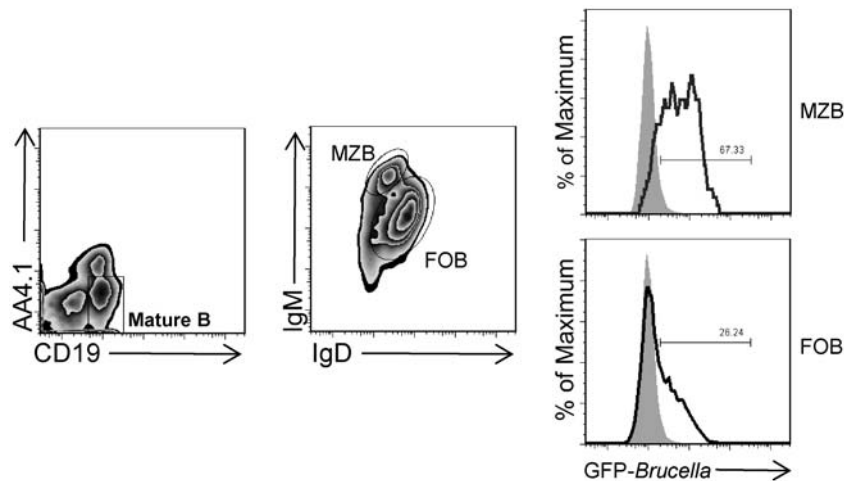
Figure 4. Marginal zone B (MZB) cells are more permissive to *Brucella* infection than follicular B (FOB) cells. Splenocytes were infected with immune serum–opsonized green fluorescent protein (GFP)–*Brucella*. To identify mature B-cell subsets, we gated on CD19$^+$ AA4.1$^-$ that were further parsed into MZB (immunoglobulin [Ig] M^{hi}IgD^{lo}) and FOB (IgM^{lo}IgD^{hi}) cells. Representative histogram overlay of GFP expression in MZB (*top panel, gray line histogram*) and FOB (*bottom panel; black line histogram*) cells shown from 2 independent experiments. Percentages of GFP-*Brucella*–positive cells in each subset are shown in the gate, compared with background of uninfected splenocytes (*filled histogram*). Results are representative of 2 independent experiments.

Brucella, intracellular TGF-β1 and expression of CD19. As a control for background staining, we also stained splenocytes from uninfected mice. Flow cytometry revealed that most *Brucella*-positive splenocytes make TGF-β1 and approximately 11% were CD19$^+$ B cells (Figure 5D).

DISCUSSION

This study demonstrates the capacity of B lymphocytes to act as an infection niche for *Brucella*. *Brucella* infection was associated with B cells both in vitro and in vivo, and infected B cells transferred disease to naive animals. Furthermore, in vivo, *Brucella* infected B lymphocytes produced TGF-β1, a regulatory cytokine, and consequently, have a regulatory B-cell phenotype [10]. Taken together, these findings demonstrate 2 unappreciated roles of B lymphocytes: their capacity to act as an infection niche for chronic bacterial infections and the ability of these infected B cells to produce TGF-β1 in vivo during the course of infection.

Consistent with previous findings [13], B cells had a low phagocytic index compared with professional phagocytic cells such as macrophages, in which multiple brucellae are present per cell [4]. Also, unlike in macrophages, the infected B cells had minimal to no capacity to support replication of brucellae. Despite these constraints, 10% of cells infected with *Brucella* in vivo were B cells and were responsible for harboring 10% of the bacteria in the infected mice. In addition, the activated state of the *Brucella*-positive B cells (indicated by capping of CD19) suggests the contribution of the B-cell niche toward

chronicity of intracellular bacterial infection, because activated B cells are known to have longer life spans.

The replication incompetence of *Brucella* in B lymphocytes could be due to the inefficient remodeling of the phagosomal compartment to construct a replicative niche as occurs in macrophages [1, 5] It has been reported that opsonized brucellae reside in a LAMP1$^+$ non–ER-derived replicative niche in macrophages [5], contrasting with their behavior under non-opsonizing conditions, when the brucellae are found in vacuoles that acquire ER markers [1]. In data presented here, we demonstrate that opsonized brucellae co-localize with LAMP1 in B lymphocytes. However, the lack of replication in B lymphocytes suggests that brucellae are unlikely to efficiently remodel the intracellular compartment to become a replicative niche, despite their ability to do so in macrophages after entry under opsonizing conditions [5]. There could be a lack of optimal phagosomal acidification needed for bacterial replication [25], a process critical for induction of virulence genes such as virB [26], even though endosomal compartments containing LAMP1 are expected to have an acidic pH. Interestingly, others have recently shown that *B. abortus* deficient in virB largely remains extracellular in mice deficient in B cells [27], raising the possibility that they might be internalized into B cells; however, it is much more likely that their extracellular localization is due to the lack of antibody-mediated opsonization for entry into macrophages, because transfer of either natural antibodies or immune antibodies or CD11b$^+$ cells (monocytes or macrophages) alone restored the ability of the B-cell/antibody–deficient mice to control the virB mutant, despite the lack of B cells [27].
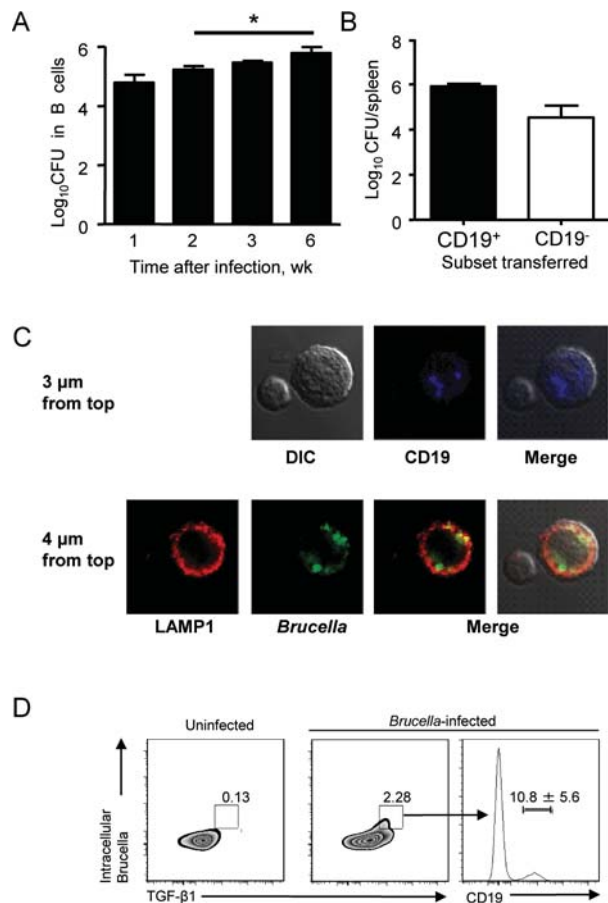
Figure 5. *Brucella abortus* infects B cells in vivo. *A,* Total *Brucella* colony-forming unit (CFU) counts associated with CD19+ cells in the spleens of wild-type (WT) mice (n = 3) at the indicated times after infection. Results are representative of 2 experiments. *B,* Splenic CD19+ cells (dose, 3.84 log10 CFU) or CD19− cells (dose, 2.94 log10 CFU) were obtained at 3 weeks after infection from WT mice and transferred intraperitoneally into naive mice (n = 4). CFU counts in recipient mice were determined at 3 weeks after infection *C,* Confocal micrographs of *Brucella*, CD19, and lysosome-associated membrane protein 1 (LAMP1) in splenocytes harvested 3 weeks after infection. *D,* Flow cytometric analysis of transforming growth factor (TGF) β1 and brucellae was conducted on splenocytes obtained from mice that were either uninfected or infected with *Brucella* for 4 weeks (n = 3) along with CD19. Results are representative of 2 experiments. *.01 < *P* < .05.

Brucella-specific IgM in conjunction with complement components was critical for infectivity. Because antigen-specific IgM is required, infection of B cells is unlikely to affect the establishment of infection until after the first week. Indeed, B-cell–deficient and intact mice have comparable infection kinetics until 10 days after infection [9]. The potential receptor for IgM-mediated uptake of brucellae by B cells perhaps involves Fcα or Fcμ receptors along with complement receptors, because both types are expressed on resting mature B cells [11, 28]. The greater propensity for infection of MZB cells, compared with FOB cells, might be due to higher known

expression of both these receptors on MZB cells [11, 28]. Because MZB cells are the primary responders to thymus-independent antigens or pathogens, such as *Brucella* spp. [29, 30], we speculate that they may be the predominant infection niche in the spleen.

The infected B cells may regulate the immune response by producing TGF-β1, thus acting as regulatory B cells (Bregs); TGF-β1 facilitates immune evasion by dampening the effects of interferon γ [8, 10, 23, 24]. It is reasonable to speculate that the "Breg" character of the *Brucella*-positive B cells may be especially important for formation of the chronic niche, because we have shown that B cells are critical for establishment of the high infection plateau phase of brucellosis and for increased regulatory cytokine production, including TGF-β1 [9]. *Brucella* components that induce TGF-β1 are not yet known and may involve lipopolysaccharide, as reported by in vitro studies conducted using endotoxin from pathogens [10]. Another likely candidate is proline racemase, which has been shown to be a B-cell mitogen and was required to establish chronic infection [31]. It is not yet known whether B cells respond to this mitogen after production within the infected B cells or respond to that secreted from infected macrophages. Recently, B-lymphoid–committed cells that potently suppress T-cell responses have been identified; these cells express dendritic cell surface markers on Toll-like receptor ligation [32]. It will be interesting to know whether *Brucella* components, including its DNA, stimulate such a response in infected B cells.

To conclude, invasion of B cells by *Brucella*, and possibly other bacterial pathogens, can induce immunoregulatory B cells which may facilitate chronic bacterial infection by dampening host immune responses that evoke clearance of infection, such as type 1 immune components. The mechanisms underlying survival of *Brucella* in infected B cells in the absence of replication remain to be resolved, as do the extent and mechanisms of any remodeling of *Brucella*-containing vacuoles in the infected B cells. The B cells may present a benign environment for intracellular *Brucella* akin to that of other nonprofessional phagocytes, such as trophoblasts that harbor *Brucella* [3], hepatocytes that harbor *Listeria* [33], and enterocytes that harbor *Shigella* [34]. Moreover, the infected B cells also provide an infection niche from which resident bacteria can invade other cells, thereby facilitating dissemination of infection. In conclusion, this study speaks to the ability of pathogens to use diverse cell types to promote their survival and perpetuation.

Notes

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