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Cyclooxygenase-1 orchestrates germinal center formation and antibody class-switch via regulation of IL-17[1]

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Abstract

The cyclooxygenase (COX) enzymes are known modulators of innate immune cell function; however, their contributions to adaptive immunity are relatively unknown. We investigated the roles of COX-1 and COX-2 in the humoral immune response to infection with the Lyme disease pathogen, *Borrelia burgdorferi*. We report that *in vitro*, murine B cells constitutively expressed COX-1 and up-regulated expression of both COX-1 and COX-2 as well as their products PGE_2, $PGF_{2\alpha}$ and TXB_2 and their receptors following stimulation with *B. burgdorferi* or anti-CD40. *In vitro* inhibition of COX-1 and/or COX-2 in murine B cells resulted in decreased eicosanoid production, and altered antibody production. Importantly, infection of mice lacking COX-1, but not COX-2 activity resulted in a defect in immunoglobulin class-switching and a lack of *Borrelia*-specific IgG production. This defect correlated with decreased germinal center formation and IL-6 and IL-17 production, and could be partially recovered by restoration of IL-6, but fully recovered by IL-17. Furthermore, sera from COX-1 inhibitor-treated mice were dramatically less effective in killing *B. burgdorferi*, but borreliacidal activity was restored in COX-1 inhibitor-treated mice administered IL-17. We conclude that IL-17 plays a role in antibody production and immunoglobulin class-switching in response to infection and that COX-1 is a critical, previously unrecognized regulator of this response.

Introduction

A robust humoral immune response is crucial for host defense against many invading pathogens. Recognition of cognate antigens by naïve B cells and their subsequent production of antigen-specific antibodies is a tightly-regulated process that prevents improper antigen recognition and untoward immune responses. The expression of various growth factors, cytokines, chemokines, and their receptors in the B cell microenvironment, such as IL-6, CXCR4 and CXCR5, and their ligands, CXCL12 and CXCL13 (1) are essential for the progression of B cells through developmental stages and the eventual generation of

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pathogen-specific antibodies by mature B cells. The humoral immune response matures during successive rounds of B cell stimulation in the specialized structure of the germinal center (GC) within the secondary lymphoid follicles, during which affinity maturation and immunoglobulin class switching occurs in response to cytokine signals provided mainly by T-helper cells (2). Despite the well-known role of IL-6 and the hypothesized contribution of other cytokines, such as IL-17 (3) to GC development and immunoglobulin production, the mechanisms which regulate the activities of these cytokines are still poorly understood.

Products of the cyclooxygenase (COX) enzymes play important roles in the regulation of immunity. Two different COX isoforms exist and are considered to have differing biological roles. COX-1 is constitutively expressed by most cells, and is known to be involved in the regulation of platelet function and maintenance of gastric mucosa (4,5). The second cyclooxygenase isoform, COX-2, is normally undetectable in most healthy tissues and is induced as a key component of innate immune cell function during the inflammatory phase of the immune response (5–7). Although not as well characterized, the COX enzymes have also recently been recognized for their roles in T cell-mediated immunity. COX-2 was implicated in the regulation of lupus patient T cell apoptosis, thereby regulating lupus pathology (8), and in early thymocyte proliferation and the later maturation of $CD4^+$ T helper cells (9). COX-1 was traditionally considered to have little involvement in the immune response, but more recently has been shown to direct thymocyte progression from $CD4^-CD8^-$ double-negative to $CD4^+CD8^+$ double-positive cells (9). It was also integral for the induction of the signaling cascade subsequent to T cell receptor engagement, and COX-1 inhibition prevented the activation of the p38 kinase and down-stream transcription factors (10,11).

In comparison, studies examining roles for COX-1 or -2 in B lymphocyte activation and maturation have produced conflicting results. Early *in vitro* studies suggested that products of COX might be involved in the regulation of antibody production (12) and both COX isozymes have been implicated in antibody production *in vivo*, primarily in models of autoimmune disease. In experimental autoimmune encephalomyelitis (EAE), inhibition of COX-2 led to increased anti-myelin oligodendrocyte glycoprotein (MOG) antibody production (13), whereas non-specific COX inhibition during type-II collagen-induced arthritis significantly decreased collagen-specific IgG titers (14). Reports of immunization during COX inhibition are equivocal. One group demonstrated that mice lacking COX-2 failed to exhibit immunoglobulin class-switching when immunized with human papilloma virus virus-like particles (15), whereas another group demonstrated decreased class-switching during nonspecific COX inhibition in response to ovalbumin injection (16). Few reports have mentioned COX enzymes in the context of an antibody response to infection. We, and others, have previously reported that mice lacking COX-2 activity develop antibody responses comparable to control animals when infected with the bacterium, *Borrelia burgdorferi* (17,18). Although drugs that inhibit or alter COX activity are the most commonly used drugs in the world (19,20), a thorough understanding of their effects on immunity is lacking.

To address these issues we utilized a murine model of Lyme disease, caused by infection with the spirochete, *Borrelia burgdorferi*. The multi-systemic disease induced by *B. burgdorferi* infection of both humans and animals is the most common vector-borne disease in both the U.S. and in Europe (21). When untreated with antibiotics near the time of infection, 60% of individuals develop a severe arthritis, the intense pain and swelling of which is commonly treated with COX-specific inhibitors or traditional nonsteroidal anti-inflammatory drugs (tNSAIDs) (22). Here we show that murine B cells, in response to *B. burgdorferi* stimulation *in vitro*, expressed both COX isozymes, and inhibition of either isozyme affected B cell eicosanoid production. *In vivo* studies utilizing COX-1 or -2-specific

inhibitors or COX-specific knock-out mice demonstrated that COX-1 activity was required for the generation of a full anti-*Borrelia* IgG response. Further analysis demonstrated that COX-1 was necessary for the development of GC and the production of normal IL-6 and IL-17 levels in response to infection. Our results demonstrate a critical role for COX-1 in the regulation of GC formation and the generation of humoral immunity up-stream of IL-6 and IL-17 production during the response to infection. Additionally, these data suggest that commonly used NSAIDs may affect the ability of the host's immune system to effectively protect against pathogens.

Materials and Methods

Animals

Female C3H/HeJ (C3H) mice, 4–6 weeks of age, were purchased from The Jackson Laboratory (Bar Harbor, ME). COX-2 heterozygous mice (B6;129S7-*Ptgs2*^{tm1Jed}) were purchased as breeders, also from The Jackson Laboratory, and were backcrossed onto the C3H genetic background for ten generations. Heterozygous mice were then intercrossed to produce knockout and wild-type littermates. COX-1 knockout mice (B6;129P2-*Ptgs1*^{tm1Unc}) and wild-type controls were purchased from Taconic Farms (Germantown, NY). Animals were housed in a specific pathogen-free facility and given sterile food and water *ad libitum*. All studies were conducted in accordance with the guidelines of the Animal Care and Use Committee of the University of Missouri.

B lymphocyte isolation and stimulation

B lymphocytes were isolated from total splenocyte preparations using an AutoMacs B cell isolation kit (Miltenyi Biotech, Foster City, CA) according to the manufacturer's instructions for negative selection. B cells were found to be >99% $CD19^+$ and <1% $CD3^+$ or $CD14^+$ as determined by flow cytometry. Purified B cells were cultured in complete Dulbecco's Modified Eagle Medium (Invitrogen Life Technologies, Carlsbad, CA) supplemented with 1% Nutridoma-SP (Roche Applied Science, Indianapolis, IN) with or without stimuli for 8, 24, or 36 hours, or 7 days for mRNA, protein, eicosanoid, or immunoglobulin measurement, respectively. B cells were stimulated with rat anti-mouse CD40 (0.5 μg/mL; Southern Biotech), *B. burgdorferi* spirochetes at a multiplicity of infection (MOI) =1, *B. burgdorferi* total antigen (BbAg, 5μg/mL), arachidonic acid (10μM, Cayman Chemical, Ann Arbor, MI), or were untreated. The concentration of *B. burgdorferi* antigen used has been shown to activate B cells and induce their proliferation and differentiation into plasma cells (23). B cells were stimulated with arachidonic acid (AA) as a positive control for COX-1 stimulation (24). For the analysis of FP and TP receptor expression, B cells were stimulated with an MOI = 1 and collected at the indicated time points. For FP antagonism the FP antagonist AL-8810 (Cayman Chemical) was dissolved in 100% ethanol as a stock solution and stored at −20°C until dilution to the working concentration of 50 μM in cell culture medium. B cells were pre-incubated with vehicle or antagonist 30 minutes before the addition of stimulus and supernatants were harvested 7 days later. Cell viability was determined by trypan blue staining.

Inhibition of cyclooxygenase-1 or -2

Celecoxib (LKT Laboratories, Inc, St. Paul, MN) and SC-560 (Cayman Chemical) were dissolved in 100% ethanol/0.01% Tween-20 or 100% ethanol alone, respectively, as stock solutions and stored at −20°C until dilution to the working concentration of 1 μM in cell culture medium. Treatment of cells with COX inhibitor concentrations greater than 10μM increased cell death in dose-response studies. B cells were pre-incubated with inhibitors or vehicle for 30 minutes before the addition of stimuli. For *in vivo* inhibition of COX-2, celecoxib was incorporated into a normal laboratory diet (Research Diets, New Brunswick,

NJ) as described (25). Animals were fed celecoxib chow beginning day -1 of infection with *B. burgdorferi*, and control animals were fed normal rodent chow (Purina PicoLab 5053, Purina Mills, St. Louis, MO). For *in vivo* COX-1 inhibition, dilutions of SC-560 were mixed daily in 200μL sterile PBS and animals were treated once daily by oral gavage for a final dosage of 10 mg $\cdot$ kg^{-1} $\cdot$ day^{-1}.

RNA and RT-PCR

Total RNA was extracted with TRIzol reagent (Invitrogen Corp, Carlsbad, CA) according to the manufacturer's protocol. One-step RT-PCR was performed using the EZ RT-PCR kit (Applied Biosystems, Foster City, CA) and 100ng of total RNA with the ABI Prism 7700 Sequence Detection System (Applied Biosystems). The mouse *Nidogen* gene, a single copy gene, was used as an endogenous control as described previously (26). COX-1 and -2 primer sequences were described previously (17). RT-PCR conditions were: 50°C for 15 min, 60°C for 30 min, 95°C for 10 min, and 45 cycles of 95°C for 30 sec and 60°C for 1 min..

SDS-PAGE/Western analysis

Total protein was isolated in 40μl modified radio-immunoprecipitation assay buffer (RIPA; 50mM Tris HCl pH=7.4, 150mM NaCl, 1mM PMSF, 1mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS). 30 micrograms was fractionated on a 10% SDS-PAGE gel and transferred electrophoretically to PVDF membrane (Millipore, Bedford, MA). Membranes were incubated with a 1:1000 dilution of rabbit anti-mouse COX-1, COX-2, $PGF_{2\alpha}$ receptor (FP), or TXB_2 receptor (TP) (Cayman Chemical, Ann Arbor, MI) or GAPDH (Bethyl Laboratories, Montgomery, TX) primary antibody. Protein detection was performed using the VectaStain ABC-AmP kit (Vector Corp, Burlingame, CA) according to manufacturer's instructions.

Flow cytometric detection of cyclooxygenase-2

Following stimulation, B lymphocytes were collected and washed with 5% fetal bovine serum in phosphate-buffered saline (FBS-PBS) and stained at a density of 1×10^6 cells/100 μl. Surface staining was performed with fluorescein (FITC)-conjugated rat anti-mouse CD19 antibody (eBioscience, San Diego, CA) in 5% FBS-PBS for 15 minutes at room temperature. Intracellular staining was performed for COX-2 protein using a phycoerythrin (PE)-labeled mouse anti-human IgG_1 antibody that displays cross-reactivity with mouse COX-2 (43) or the isotype control (Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and a Fix and Perm kit (Caltag Laboratories, Burlingame, CA) according to manufacturer's instructions. Flow cytometric data was collected on a FACScan flow cytometer and analyzed using CellQuest software (BD Biosciences).

Bacteria and infections

A virulent, low-passage, clonal isolate of the *B. burgdorferi* N40 strain (a kind gift from J. Weis, University of Utah) was used for all infections. Frozen stocks were placed in 7.5 mL of Barbour, Stoenner, Kelly (BSK) II medium (Sigma-Aldrich, St. Louis, MO) with 6% rabbit serum (Sigma-Aldrich) and grown to log phase by incubation for 5–6 days at 32°C. Spirochetes were enumerated using dark field microscopy and a Petroff-Hausser counting chamber (Hausser Scientific, Horsham, PA). Spirochete dilutions were made in sterile BSK II medium and mice were inoculated in both hind footpads with 2.5×10^5 *B. burgdorferi* organisms in 50 μl of medium, a concentration which reliably produces arthritis in susceptible animals and is a typical inoculum (17,27).

Determination of antibody levels

B. burgdorferi-specific IgM and IgG levels in the sera of infected animals were determined by ELISA using alkaline phosphatase-conjugated anti-mouse IgM and IgG as a modification of Bolz, et al. (28). Immulon 2B ELISA plates (Nalgene, Rochester, NY) were coated with 0.5 μg/mL of *B. burgdorferi* antigen in coating buffer (0.1 M bicarbonate buffer, pH 9.4). Two columns on each plate were reserved for the standard curve. These wells were coated with rat anti-mouse IgG (20 μg/mL; AnaSpec, San Jose, CA) or goat anti-mouse IgM (20 μg/mL; Southern Biotech, Birmingham, AL) in coating buffer. For total non-specific immunoglobulin from sera or cell culture supernatant, the plate was coated with rat anti-mouse IgG or goat anti-mouse IgM. Standard curves were created by 1:3 dilution of purified mouse total IgG (Equitech Labs, Kerrville, TX) or IgM (Bethyl Laboratories). Cell culture supernatants or sera of individual animals were diluted in BSA/PBS and incubated for 2 hours at room temperature. Alkaline phosphatase-conjugated donkey anti-mouse IgG (Jackson Immuno Research, West Grove, PA), or rat anti-mouse IgM (Southern Biotech) was applied at 1:1000 dilution. Plates were washed and read at 409 nm after addition of the phosphatase substrate (Sigma 104 tablets, Sigma-Aldrich).

Measurement of eicosanoids from cell culture

B lymphocyte culture supernatants were analyzed by enzyme immunoassay (EIA; Cayman Chemical) for the presence of PGE_2, $PGF_{2\alpha}$, and TXB_2 (as a measure of TXA_2) after 36 hours of incubation in the presence of stimulus and/or inhibitor(s).

Measurement of eicosanoids from spleen tissue

Tissues were snap-frozen in liquid nitrogen and stored at −80° until extraction. Spleens were pulverized and the resulting powder placed in 3 mL of 50% ethanol in water and then weighed. 10 μl of antioxidant cocktail (0.2 mg/mL butylated hydroxytoluene, 0.2 mg/mL EDTA, 2 mg/mL triphenylphosphine, 2 mg/mL indomethacin in a solution of 2:1:1 methanol:ethanol:H_2O) was added to each sample before incubation at −20°C for 72h. Samples were then centrifuged at 3500 × g for 30 minutes, the clear ethanolic supernatant removed to a new tube, and dried under nitrogen gas. Samples were reconstituted with 2 ml of 10% methanol and supplemented with 50 μL of 50 pg/μL (2.5 ng total) of deuterated internal standards all from Cayman Chemical. Samples were sonicated for 30 seconds, purified by solid phase extraction (SPE) as previously described (29) and stored at −20°C until analysis. Samples were evaporated and then reconstituted in 50 μL of LC Solvent A [water-acetonitrile-acetic acid (70:30:0.02; v/v/v)] immediately before analysis. The analysis of eicosanoids was performed by LC-MS/MS, based on previously published methodology (29). Eicosanoids were separated by reverse-phase LC on a Synergy C18 column (2.1 mm × 250 mm, 4u; Phenomenex, Torrance, CA) at a flow rate of 300 μl/min at 50°C. The column was equilibrated in Solvent A [water-acetonitrile-acetic acid (70:30:0.02; v/v/v)], and 40 μl of sample was injected using a 50 μl injection loop and eluted with 0% solvent B [acetonitrile-isopropyl alcohol (50:50; v/v)] between 0 and 1 min. Solvent B was increased in a linear gradient to 25% solvent B until 3 min, to 45% until 11 min, to 60% until 13 min, to 75% until 18 min, and to 90% until 18.5 min. Solvent B was held at 90% until min 20, dropped to 0% by 21 min and held until 25 min.

Eicosanoids were analyzed using a tandem quadrupole mass spectrometer (ABI 4000 Q Trap®, Applied Biosystems) via multiple-reaction monitoring in negative-ion mode. The electrospray voltage was −4.5 kV and the turbo ion spray source temperature was 525°C. Collisional activation of eicosanoid precursor ions used nitrogen as a collision gas. Eicosanoids were measured using the following Precursor→Product MRM pairs: (d_4) 6k $PGF_{1\alpha}$ (373→167), (d_4) TxB_2 (373→173), (d_4) $PGF_{2\alpha}$ (357→197), (d_4) PGE_2 (355→275), (d_4) PGD_2 (355→275), (d_4) PGJ_2 (337→275), (d_4) 15d PGD_2 (337→275), (d_4) 15d PGJ_2

(319→275), 6k $PGF_{1\alpha}$ (369→163), TxB_2 (369→169), $PGF_{2\alpha}$ (353→193), PGE_2 (351→271), PGD_2 (351→271), PGJ_2 (333→271), 15d PGD_2 (333→271), 15d PGJ_2 (315→271). Quantitative eicosanoid determination was performed by the stable isotope dilution method (30). A standard curve was prepared by adding 2.5 ng of each internal (deuterated) eicosanoid standard to the following amounts of eicosanoid (non-deuterated) primary standard: 0.1, 0.3, 1, 3, 10, 30 and 100 ng.

Measurement of serum cytokines

Serum concentrations of IL-6 and IL-17 were determined by ELISA according to manufacturer's instructions (eBioscience, San Diego, CA).

Quantification of germinal centers (GCs) in the spleen

Each spleen was cut into four serial sections 100 μm apart. Composite overview pictures of each spleen were produced using multiple image alignment in the Olympus MicroSuite Five software (Olympus America Inc., Center Valley, PA) and Adobe Photoshop software (Adobe Systems Inc., San Jose, CA) was used to equalize brightness and contrast across aligned panels. Total spleen area and $Ki67^+$ area were measured using the same software. Spleen area was determined by averaging the measurements of all four sections. Average GC areas were determined across all four sections of each individual spleen. Images were acquired with an Olympus DP71 camera attached to an Olympus BX40 light microscope using the MicroSuite Five software.

Quantification of GC B cells

Flow cytometry was used to determine the percentage of GC B cells in the spleens of WT or COX-1° mice 10 days following infection with *B. burgdorferi*. Single-cell suspensions of splenocytes were made and red cells removed by hypotonic lysis. B cells were stained with PE-B220 and AF647-GL7 (eBioscience, San Diego, CA). Percentages of stained cells were determined in a live gate and using CellQuest software (BD Biosciences).

Administration of IL-6 and IL-17

Recombinant mouse IL-6 (rmIL-6, Biomyx, San Diego, CA) and IL-17 (rmIL-17, R & D Systems, Minneapolis, MN) were reconstituted in sterile Dulbecco's PBS (Invitrogen) and administered subcutaneously at doses of 4 μg/mouse/day or 0.5 μg/mouse/day, respectively, in a total volume of 100 μL PBS (31,32). Vehicle-treated controls received 100 μl of PBS alone. Animals were treated beginning d0 of infection and once daily thereafter though d7 of infection.

Borreliacidal antibody assay

The determination of borreliacidal activity was performed as described previously with some modifications (33,34). Sera from *B. burgdorferi*-infected mice were diluted 1:5 in PBS, heat-inactivated at 55°C for 1 hour, and filter-sterilized through a 0.22 μm pore-size filter. Spirochetes were grown to log phase at 32°C and then diluted to a final concentration of 1×10^8/mL in BSK media. 100 μl of diluted *B. burgdorferi* was added to wells of a 96 well round-bottom microtiter plate. 10 μl of filter-sterilized guinea pig serum (active complement; Innovative Research, Novi, MI) was added to each well and then 100 μl of serum from individual animals was added. Sera from naïve mice were used as controls. Wells containing only *B. burgdorferi* were used to determine basal levels of dead bacteria. The plate was gently mixed and incubated at 32°C for 16 hours. Assays were performed in duplicate. After incubation, the wells were washed with serum-free BSK and bacterial viability was determined using the LIVE/DEAD *Bac*Light bacterial cell viability kit according to manufacturer's instructions (Invitrogen). At least 100 spirochetes total were

counted as either live or dead and the final percentage of killed *Borrelia* was determined by subtracting the basal percentage of dead bacteria present in the untreated control *Borrelia* wells. Values are presented as the percent borreliacidal activity of sera from different treatment groups as compared to the vehicle-treated control.

Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), Student's *t*-test or Mann-Whitney Rank Sum test using the SigmaStat 3.5 software (Systat Software Inc., Chicago, IL). Differences with *P* values equal to or less than 0.05 were considered significant. Error bars represent ± standard deviation.

Results

Murine B cells constitutively express COX-1 and up-regulate both COX-1 and COX-2 in response to stimulation with B. burgdorferi

Most cell types express COX-1 constitutively and many can induce COX-2 expression upon stimulation (5,7); however, their expression in normal murine B cells has not been demonstrated. Splenic B cells were isolated from C3H/HeJ mice and exposed to various stimuli for 8 hours *in vitro* to determine if murine B cells expressed COX-1 or COX-2 mRNA or protein in response to stimulation. Figure 1A shows low levels of constitutive COX-1 and COX-2 mRNA expression in naïve B cells that were increased upon activation with *B. burgdorferi* or anti-CD40 stimulation. Anti-CD40 is a non-specific activator of B cells, and *B. burgdorferi* has been shown to have B cell mitogenic effects (23,35).

Although traditionally considered to be constitutively expressed, post-transcriptional regulation of COX-1 protein expression has been shown in some disease conditions (36). Conversely, COX-2 transcription and translation are both positively and negatively regulated by numerous signals, with most regulation occurring post-transcriptionally (6). Western blot analysis (Figure 1B) demonstrated constitutive expression of COX-1 protein in unstimulated murine B cells, and this expression was greatly increased upon stimulation with *B. burgdorferi*. In contrast, stimulation of B cells with anti-CD40 had only a minor effect on the expression levels of COX-1 protein. COX-2 protein levels were undetectable in unstimulated or anti-CD40 stimulated B cells; however, in response to *B. burgdorferi* stimulation COX-2 protein levels were also greatly increased (Figure 1C). To specifically demonstrate the B cells and not some other contaminating cell type were expressing COX-2 protein, we used flow cytometry and set the gates on $CD19^+$ cells. Figure 1D demonstrates that >95% of the $CD19^+$ cells contained detectable COX-2 protein after stimulation, indicating that BbAg effectively up-regulated COX-2 protein expression in B cells. Thus, murine B cells constitutively express COX-1 and are able to up-regulate expression of both COX-1 andCOX-2 under certain stimulatory conditions.

Stimulation of murine B cells induces the production of prostaglandins and expression of their receptors

We subsequently assayed the ability of murine B cells to produce representative COX-1 and -2 products, PGE_2, $PGF_{2\alpha}$, and TXB_2, *in vitro* in response to co-culture with *B. burgdorferi*. Unstimulated B cells produced low levels of $PGF_{2\alpha}$, TXB_2, and PGE_2 (Figure 2A). Exogenous arachidonic acid (AA) was used as a positive control for COX-mediated eicosanoid production and thus represents the capacity of the cell cultures to produce eicosanoids. Incubation of naïve B cells with live *B. burgdorferi* induced production of $PGF_{2\alpha}$, TXB_2, and low levels of PGE_2. Anti-CD40 stimulation induced small but significant ($P < 0.01$) increases $PGF_{2\alpha}$ and TXB_2 production, but had no effect on the production of PGE_2. Previous studies have shown that murine B cells respond to PGE_2 *in vitro* via the

PGE_2 receptors EP_2 and EP_4 (37). To address the possibility that $PGF_{2\alpha}$ and TXB_2 may also act in an autocrine manner, *Borrelia*-stimulated B cells were assayed for expression of the $PGF_{2\alpha}$ receptor FP and the TXB_2 receptor TP. No basal production of FP or TP proteins was detected; however, protein for both receptors was up-regulated after just 30 minutes of incubation with *B. burgdorferi* (Figure 2B). Whereas FP expression remained high throughout the time course, TP protein expression appeared to peak at about 6–12 hours post-stimulation and had returned to baseline levels by 72 hours. These data imply that murine B cells may be subject to autocrine regulation by these eicosanoids, perhaps with different kinetics, within the B cell microenvironment.

Murine B cells produce prostaglandins via both COX-1 and COX-2

This eicosanoid profile generated *in vitro* implicated both COX-1 and -2 involvement in B cell responses to live *B. burgdorferi*. We therefore examined the response of purified murine B cells to various stimuli in the presence or absence of the COX-1-specific inhibitor, SC-560 (38); the COX-2-specific inhibitor, celecoxib (Clx); or both inhibitors in combination, SCX (Figure 3A). Arachidonic acid was added as the positive control and represents the maximum capability of the cells to produce each eicosanoid under the given conditions. The lack of an effect by Clx on the AA-stimulated cells likely reflects the low levels of COX-2 present in naïve B cells without additional stimuli. Anti-CD40-stimulated B cells produced very little PGE_2 or TXB_2, but were capable of producing $PGF_{2\alpha}$ via both COX-1 and COX-2 pathways. In response to *B. burgdorferi* stimulation, naïve B cells were capable of making high levels of all three eicosanoids tested, and the production of each could be reduced by blocking either COX pathway. These results demonstrate that both COX-1 and COX-2 are active in stimulated B cells and are capable of producing inflammatory eicosanoids.

Mice deficient in COX-1 activity produce less pathogen-specific IgG

Our *in vitro* data indicated that both COX-1 and COX-2 could produce prostaglandins in response to B cell stimulation. Addition of PGE_2 to *in vitro* cultures of B cells has been reported to increase antibody production (39), while *in vitro* inhibition of human B cell COX-2 activity can decrease both IgM and IgG production (40). However, the specific contribution of COX-1 to antibody production has not been described. To determine the relative *in vivo* contribution of the two COX isoforms to the overall composition of the humoral response during a primary infection, we examined the effect of COX-1- and COX-2-specific inhibition on the ability of C3H mice to mount a humoral response to *B. burgdorferi* infection. C3H/HeJ mice were infected with *B. burgdorferi* and treated daily with either a COX-2 inhibitor, COX-1 inhibitor, or both in combination. Sera were collected at time points that correlated to the peak (day 14) and the resolution phases (day 24) of disease and were assayed for *B. burgdorferi*-specific IgM and Bb-specific-total IgG levels. At day 14 of infection, only the dual inhibition of both COX-1 and COX-2 had a significant effect ($P \leq 0.05$) on decreasing IgM production (Figure 4A), an effect that was abrogated by day 24 post-infection. Total *Borrelia*-specific IgG levels were similarly decreased by dual inhibition of COX-1 and COX-2 at day 14 post-infection. Total IgG subset levels were also minimally affected by either enzyme alone. Dual inhibition significantly decreased IgG2b levels at day 14 and 24, and IgG3 levels at day 24 (data not shown). Thus inhibition of COX-1 or COX-2 did not appear to cause a global Th1/Th2 skewing of the immune response. In contrast to IgM, however, *Borrelia*-specific IgG levels were significantly decreased by inhibition of COX-1 alone at day 24 of infection ($P \leq 0.05$), while inhibition of COX-2 activity had no effect. Thus, pathogen-specific IgG production was susceptible to *in vivo* inhibition of COX-1 alone or both isozymes in concert, but not COX-2 alone. These data suggested that COX-1 predominated over COX-2 in the regulation of antibody production *in vivo*.

To further define the role of either isozyme *in vivo*, we infected COX-1° or COX-2° mice with *B. burgdorferi* and measured *Borrelia*-specific antibodies at d14 and d24 post-infection. Levels of total IgG at d0 were not different between wild-type and COX-1° or COX-2° mice (Figure 4B and 4C), and *B. burgdorferi*-specific IgG levels were undetectable at this time point. Constitutive (day 0) IgM levels in COX-2° mice were comparable to their wild-type littermates (Figure 4B), as were the *B. burgdorferi*-specific IgM and total IgG responses at days 14 and 24 post-infection. Similar results were seen in COX-2-deficient mice on a DBA/2J background (data not shown), which is a mouse strain resistant to the development of Lyme arthritis. These findings demonstrate that a pathogen-specific immunoglobulin response can be mounted in the context of a complete lack of COX-2 activity, which suggests that COX-1 function must predominate in regulating the humoral response to infection.

To confirm the role of COX-1 in the generation of humoral immunity, we examined the ability of COX-1° mice to develop a *B. burgdorferi*-specific humoral response (Figure 4C). COX-1° mice demonstrated significantly higher basal levels of IgM, as well as significantly higher *Borrelia*-specific IgM levels at both d14 and d24 post-infection. Although *Borrelia*-specific total IgG was not different between knock-out and wild-type animals at day 14 post-infection, *Borrelia*-specific IgG was significantly lower at d24 post-infection in the COX-1° mice ($P < 0.05$). Thus, COX-1 deficiency resulted in increased *Borrelia*-specific IgM levels and decreased *Borrelia*-specific IgG production following infection with *B. burgdorferi*. These data are consistent with a regulatory mechanism for pathogen-specific antibody production that is dependent upon COX-1 activity, and thus susceptible to its inhibition.

COX-1 deficient mice have defective germinal center formation

Germinal center (GC) formation is central to the development of a pathogen-specific humoral immune response. For instance, animals lacking Bcl-6 or CXCR5 demonstrated lower or absent antigen-specific antibody production correlative with altered splenic architecture and decreased GC size or number (41,42). Since pathogen-specific IgG levels were most affected by a lack of COX-1 activity, we hypothesized that COX-1 was affecting the development of humoral immunity by altering the development of GC. Spleens from COX-1° and wild-type control animals were evaluated for development of germinal centers 10 days after *B. burgdorferi* infection. Ki67 staining for proliferating cells (43) demonstrated few GC in the spleens of uninfected (day 0) WT or COX-1° mice (Figure 5A). However, by day 10 post-infection abnormalities were apparent in the distribution of proliferating cells in the spleens of COX-1-deficient mice compared to controls. Composite photographs of representative H&E-stained spleens from WT and COX-1° (Figure 5B) mice illustrated the altered splenic architecture in COX-1° animals, particularly the lack of well-defined light and dark zones. Although the number of GC per splenic section was not altered and the difference in spleen area only inclined toward an increase in size in COX-1° mice, morphometric analyses demonstrated a significant decrease in the size of COX-1° GC ($P = 0.014$) and consequently, the percentage of the total splenic area that was occupied by GC ($P = 0.005$) as shown in Figure 5C. Flow cytometry was used to determine the number of GC B cells (Figure 5D&E). Spleens from COX-1° mice had a decrease of approximately 36% in GC B cells at 10 days post-infection. These data suggested that COX-1 was governing the production of a key regulator or regulators of the GC development process and ultimately the production of pathogen-specific IgG molecules.

Mice deficient in COX-1 activity produce decreased levels of prostaglandins and cytokines

Our *in vitro* data indicated that murine B cells were capable of producing several prostaglandins that could play a role in antibody responses. While PGE_2 is most commonly linked with lymphocyte responses, the involvement of other eicosanoids has not been

investigated. We therefore took a lipidomics approach to quantify changes in eicosanoid profiles in the spleens of wild-type and COX-1° mice following infection with *B. burgdorferi*. Spleens from *B. burgdorferi*-infected COX-1° mice were removed at 10 days post-infection and levels of eicosanoids were quantified by liquid chromatography-tandem mass spectrometry (LC/MS/MS) (29). Levels of prostanoids (PGs and Tx) in spleens from wild-type and COX-1° mice are shown in Table 1. Levels of other eicosanoids were mostly unchanged and are not shown. We found significant ($P \leq 0.01$) decreases in several prostaglandins in spleens of COX-1° versus wild-type mice. These include 6-keto $PGF_{1\alpha}$ (a stable derivative of PGI_2), TXB_2, $PGF_{2\alpha}$, and PGJ_2, while levels of PGE_2 and PGD_2 were reduced by 50% compared to WT mice, but were not statistically significant. Levels of other prostaglandins were unchanged. These data suggest that prostaglandins other than PGE_2 may also have a role in B cell activation and in regulating antibody responses. To investigate this possibility we isolated B cells from uninfected mice and treated them with SC-560 or AL-8810 (an FP antagonist) 30 min prior to their in vitro stimulation with *B. burgdorferi*. Treatment of B cells with either SC-560 or AL-8810 resulted in a significant decrease ($P < 0.05$) in B cell activation as measured by IgM and IgG production (Figure 6A and B). These results suggest that B cell COX-1 production of $PGF_{2\alpha}$ may play an important role in regulating B cell activation.

IL-6 is necessary for GC development and therefore, antibody production (2) and IL-6 production by some cell types is regulated by $PGF_{2\alpha}$ and TXB_2 (44,45). We therefore hypothesized that COX-1 was affecting the humoral immune response via the regulation of IL-6 production. We assayed levels of IL-6 in the serum of *B. burgdorferi*-infected mice at 24 days post-infection treated with a COX-1 or COX-2 inhibitor, and found that serum IL-6 levels were significantly decreased only in animals treated with the COX-1 inhibitor ($P = 0.004$; Figure 7A). IL-6 was also significantly decreased at 24 days post-infection in mice deficient in COX-1 ($P < 0.05$; Figure 7B). Additionally, sera from COX-1 inhibitor-treated mice at day 24 post-infection were assayed for the production of IL-17, which has been shown to regulate IL-6 production (46) and more recently, has been proposed as a regulator of the immune response in autoantibody-mediated disease (3). Serum levels of IL-17 were also significantly decreased in COX-1 inhibitor-treated animals as compared to vehicle-treated controls ($P = 0.03$; Figure 7C). These data suggest that products of COX-1 regulate the development of humoral immunity by operating up-stream of IL-6 and IL-17 production.

Addition of IL-17 to COX-1-inhibited mice restores pathogen-specific IgG production

To address whether the decrease in pathogen-specific IgG production in mice lacking COX-1 activity was a direct result of decreased IL-6 and/or IL-17 production, we first determined if administration of exogenous IL-6 would restore to control levels the production of *B. burgdorferi*-specific IgG in COX-1 inhibitor-treated animals. Mice were infected with *B. burgdorferi*, treated daily with vehicle or SC-560, and rmIL-6 was administered once daily on days 1 though 7 post-infection and *B. burgdorferi*-specific antibodies measured at day 24 post-infection. Administration of rmIL-6 resulted only in a partial restoration of *B. burgdorferi*-specific IgG levels ($P = 0.2$ versus vehicle-treated animals; $P = 0.1$ versus SC-560-treated animals; Figure 8A), which led us to examine whether delivery of IL-17, either alone or in combination with IL-6, would fully restore pathogen-specific antibody production in COX-1 inhibitor-treated mice. Animals infected with *B. burgdorferi* were treated with vehicle or COX-1 inhibitor, and were administered rmIL-17 alone, or rmIL-17 and rmIL-6 on days 1 to 7 post-infection (Figure 8B). By day 24 of infection, exogenous IL-17 alone fully restored *B. burgdorferi*-specific IgG production in mice treated with SC-560. Thus, products of COX-1 appear to be required for the generation of pathogen-specific IgG via regulation of IL-17 production.

Antibody-mediated killing of spirochetes is decreased during COX-1 inhibition and is restored upon treatment with IL-17

Although pathogen-specific IgG antibody levels were significantly decreased in the absence of COX-1 activity, we wanted to ascertain whether this correlated to a functional defect in humoral immunity. Borreliacidal antibody levels correlate with the resolution of disease and provide protection against challenge with the Lyme disease pathogen (33,34). To provide additional evidence for the necessity of COX-1 to the humoral immune response, we assayed the borreliacidal activity of serum at day 14 post-infection from *B. burgdorferi*-infected mice treated with vehicle, COX-1 inhibitor, or COX-1 inhibitor plus exogenous IL-17 (Figure 8C). Borreliacidal activity of sera from vehicle-treated animals was set at 100% killing activity. Sera from COX-1 inhibitor-treated animals was <50% as effective in killing *Borrelia* spirochetes as vehicle-treated sera. Administration of rmIL-17 during COX-1 inhibition restored borreliacidal activity of the sera, consistent with the increase in anti-*Borrelia* antibody production. Thus, the decrease in anti-*Borrelia* antibodies affected by COX-1 inhibition resulted in a dramatic decrease in bacterial killing, further confirming the functional importance of COX-1 regulation of humoral immunity.

Discussion

Products of the COX enzymes, particularly PGE_2, significantly affect immune cell function and participate in the pathogenesis of several autoimmune diseases (36,47–50). The discovery of COX-2, which is induced by inflammatory stimuli, led to the belief that the constitutive isoform, COX-1, had little to no involvement in regulating the immune response (7,38). However, recent studies suggest that COX-1 may be actively involved in immunoregulation (36). In this report, we demonstrate that COX-1 products fulfill an unsuspected critical role in the humoral immune response by promoting isotype switching and the efficient development of pathogen-specific IgG via IL-17 production.

Increasing evidence indicates that IL-17 plays a significant role in antibody production. IL-$17^{-/-}$ mice developed lower levels of anti-TNP antibodies in a model of contact hypersensitivity (51), and GC development and isotype-switching were impaired in a model of autoimmune arthritis (3). Studies investigating the role of PGE_2 in the regulation of immunity with regard to IL-17 have been conducted primarily *in vitro* and have utilized exogenous PGE_2. Incubation of naïve human or mouse T cells with PGE_2 concomitant with activation led to an increased number of cells differentiated to the T helper (T_H) 17 phenotype with enhanced IL-17 production per cell (52,53). Because PGE_2 production is responsive to IL-17, and vice versa, it is tempting to conclude that as the “pro-inflammatory” COX isoform, COX-2 is necessary for governing this response. However, experiments in which PGE_2 production was inhibited in the context of T cell IL-17 production have utilized indomethacin, a non-specific inhibitor of COX activity, leaving open the question of which COX isoform was in fact responsible for modulating IL-17 production.

To date, no studies have examined the differential activity of COX-1 and COX-2 in B cells. Our data demonstrated that normal murine B cells express both COX-1 and COX-2, and are capable of producing PGE_2, as well as $PGF_{2\alpha}$ and TXB_2 via either enzyme. B cells have the capacity to respond to these eicosanoids in an autocrine manner, since both FP and TP were expressed upon stimulation. These data build upon earlier work, where $PGF_{2\alpha}$ was demonstrated to affect DNA synthesis and excision repair in murine splenocytes (54), implying that this lipid may be essential for immunoglobulin isotype switching. Blockade of FP resulted in a significant decrease in IgM and IgG *in vitro*, equivalent to the suppression seen using the COX-1-specific inhibitor SC-560, demonstrating that products of COX-1 other than PGE_2, such as $PGF_{2\alpha}$ and TXB_2 may influence the immune response by a number

of pathways, one of which could be autocrine regulation of Ig production. Although we have demonstrated that murine B cells produce $PGF_{2\alpha}$ and TXB_2 *in vitro*, further studies are needed to determine which cells of the splenic microenvironment produce these eicosanoids *in vivo* and whether other cells of the germinal center, such as follicular dendritic cells or T cells, are regulated by these same eicosanoids in an autocrine manner, subsequently regulating IL-6 and IL-17 production.

Neither COX-1 nor COX-2 have been linked to GC formation; however, COX-2 has been mentioned tangentially as a contributor to humoral immunity, with the assumption that the effect was mediated via alteration of the T_H1-T_H2 axis (29). Our data demonstrate that animals deficient in COX-1 demonstrated abnormal splenic architecture and failed to develop normal GC following infection with *B. burgdorferi*. Additionally, although COX-1 activity was necessary for the production of normal serum IL-6 and IL-17 levels, the addition of exogenous IL-17 alone fully restored pathogen-specific IgG production and borreliacidal activity in mice treated with a COX-1-specific inhibitor, supporting the hypothesis that IL-17 functions in the normal humoral immune response to infection. Furthermore, although previous studies have demonstrated that IL-17 can increase COX-2 mRNA production and, conversely, that the COX product PGE_2 can increase IL-17 production, this is the first study to identify COX-1 as the isozyme that influences IL-17 *in vivo*. Additionally, although studies have focused on the contribution of PGE_2 as the master eicosanoid involved in modulation of the acquired immune response, lipidomic analyses revealed copious production of several other COX products in the spleen, chiefly PGF_{2a} and TXB_2, illustrating the unexplored potential for other *in vivo* lipid mediators of IL-17-mediated antibody production.

Since dual inhibition *in vivo* had the earliest effect on pathogen-specific IgG production, this implies that compensation may be a factor when inhibition of only one COX isozyme is employed. This also indicates that drugs that inhibit both isozymes equally (tNSAIDs) may have a greater clinical effect with regard to antibody production by blocking compensatory activity of COX-2 during COX-1 inhibition. The use of COX-1° mice clearly illustrated the pivotal role of the COX-1 isozyme in the development of pathogen-specific antibody responses, as these animals demonstrated increased IgM and decreased *B. burgdorferi*-specific IgG, indicative of a class-switching defect.

Both IL-6 and IL-17 are mediators of the adaptive immune response to *Borrelia* infection. IL-6° mice infected with *Borrelia burgdorferi*, *Listeria monocytogenes*, or vesicular stomatitis virus, among others, have significantly lowered serum levels of antigen-specific IgG (55,56). More recently the contribution of IL-17 as a modulator of the humoral immune response has been explored in models of autoimmunity. Decreased levels of IL-17 correlated with decreased autoantibody production in several animal models of autoimmune disease (57,58), which has subsequently been linked to the regulation of GC generation (3). Although these previous studies indicated a role for IL-17 in the genesis of autoantibodies, the current study is the first to demonstrate that IL-17 plays a role in the generation of antibodies during the normal humoral immune response to infection. The connection between COX-1, IL-17, and humoral immunity was confirmed in the borreliacidal activity assay. The ability of antibodies to kill *Borrelia* is commonly used as a diagnostic tool to determine the efficacy of clinical treatments for Lyme disease (34,59). We therefore utilized this assay to demonstrate that the decreased antibody levels induced by COX-1 inhibition lead to a clear functional decrease in the ability of antibodies to kill *Borrelia* spirochetes. This defect was recovered in animals treated with exogenous IL-17, restoring borrreliacidal activity in animals treated with COX-1 inhibitors and establishing a clear role for COX-1 activity in the generation of a functional humoral immune response.

The use of the *B. burgdorferi* infection model demonstrates that in response to a clinically-relevant pathogen, products of COX-1 govern the humoral response via regulation of IL-6 and IL-17 production, and the generation of GC. The description of this previously unappreciated role for COX-1 not only expands our understanding of the regulation of humoral immunity, but comes at a time when the use of alternatives to COX-2-specific inhibitors is on the rise (60) in addition to the already prevalent use of tNSAIDs (19,20). Although it is predicted that patients using COX-1-specific inhibitors or tNSAIDs may be more susceptible to infectious agents or have decreased responses to vaccine preparations, this study also reveals COX-1 as a potential therapeutic target in conditions caused by pathologic antibody production.

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Abbreviations used in this paper

COX	cyclooxygenase
FP	F prostanoid receptor
GC	germinal center
TP	T prostanoid receptor
TX	Thromboxane
LC	liquid chromatography
MS/MS	tandem mass spectrophotometry
AA	arachidonic acid
BbAg	*B. Burgdorferi* antigen
CLX	celecoxib
SCX	celecoxib and SC-560
tNSAIDs	traditional non-steroidal anti-inflammatory drugs

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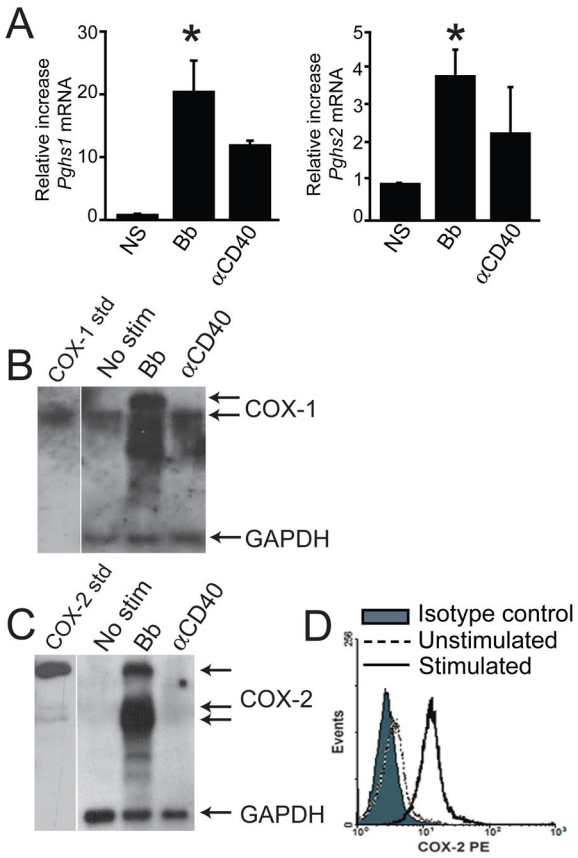

Figure 1.

Murine B cells constitutively express COX-1 and up-regulate both COX-1 and COX-2 expression in response to co-culture with *B. burgdorferi*. B cells isolated from spleens were analyzed by real-time PCR **(A)** or Western blot **(B&C)** in the absence (NS) or presence of *B. burgdorferi* or anti-CD40. Recombinant COX-1 and COX-2 were used as positive controls in the Western blots. **(D)** COX-2 expression by flow cytometry. Cells were gated on $CD19^+$ cells. The filled histogram indicates the isotype control, dashed line indicates unstimulated cells, and the solid line indicates cells stimulated with BbAg. Data are from pooled splenocytes of four mice **(A-C)** or two mice **(D)** and are representative of three or four experiments. Bars in **(A)** are means ± s.d. *$P < 0.05$ by Student's *t*-test.

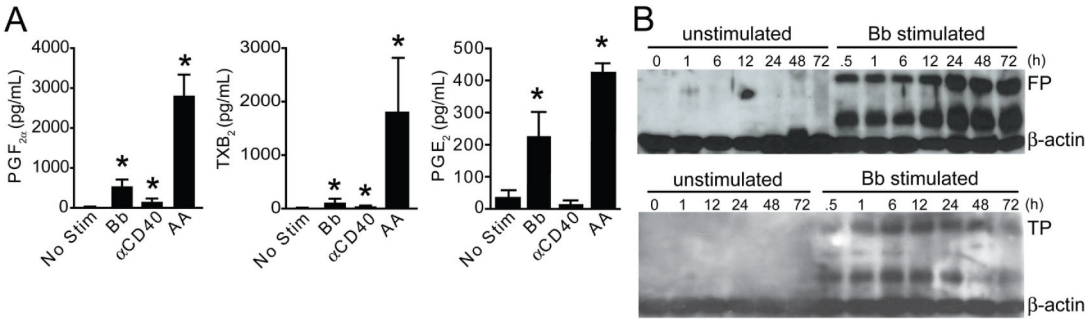

Figure 2.

Stimulated murine B cells produce $PGF_{2\alpha}$, TXB_2, and PGE_2 and the receptors FP and TP. **(A)** Naïve B cells were stimulated with live *B. burgdorferi* (Bb), anti-CD40, or arachidonic acid (AA) for 36 h and the levels of $PGF_{2\alpha}$, TXB_2, and PGE_2 were measured from culture supernatants. Values represent the means ± s.d. of four mice. **P* < 0.01 versus unstimulated controls. Data are representative of three experiments. **(B)** The $PGF_{2\alpha}$ receptor, FP, or the TXB_2 receptor, TP, expression in unstimulated B cells or B cells stimulated with *B. burgdorferi* at an MOI of 1. Protein expression by pooled B cells from four mice was measured by Western blot after 0.5 to 72 h of culture. Data are representative of three separate experiments.

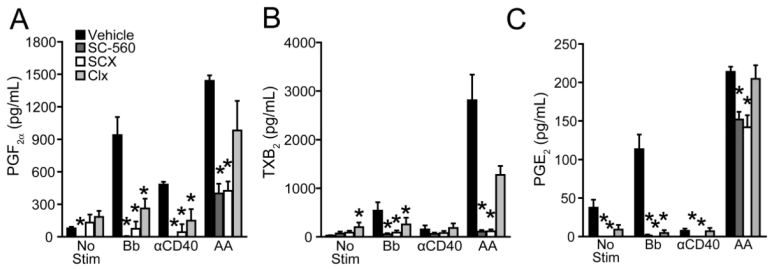

Figure 3.

Inhibition of *in vitro* B cell eicosanoid production by specific COX inhibitors. Naïve B cells were pre-incubated with vehicle or SC-560 (1μM), celecoxib (CLX, 1 μM), or both (SCX) 30 min before stimulation with *B. burgdorferi*, anti-CD40, arachidonic acid, or no stimulation. **(A)** $PGF_{2\alpha}$, **(B)** TXB_2, and **(C)** PGE_2 were measured from cell supernatants after 36 h by EIA. **P* < 0.05 compared to vehicle controls. Values are mean ± s.d. Data are representative of four separate experiments.

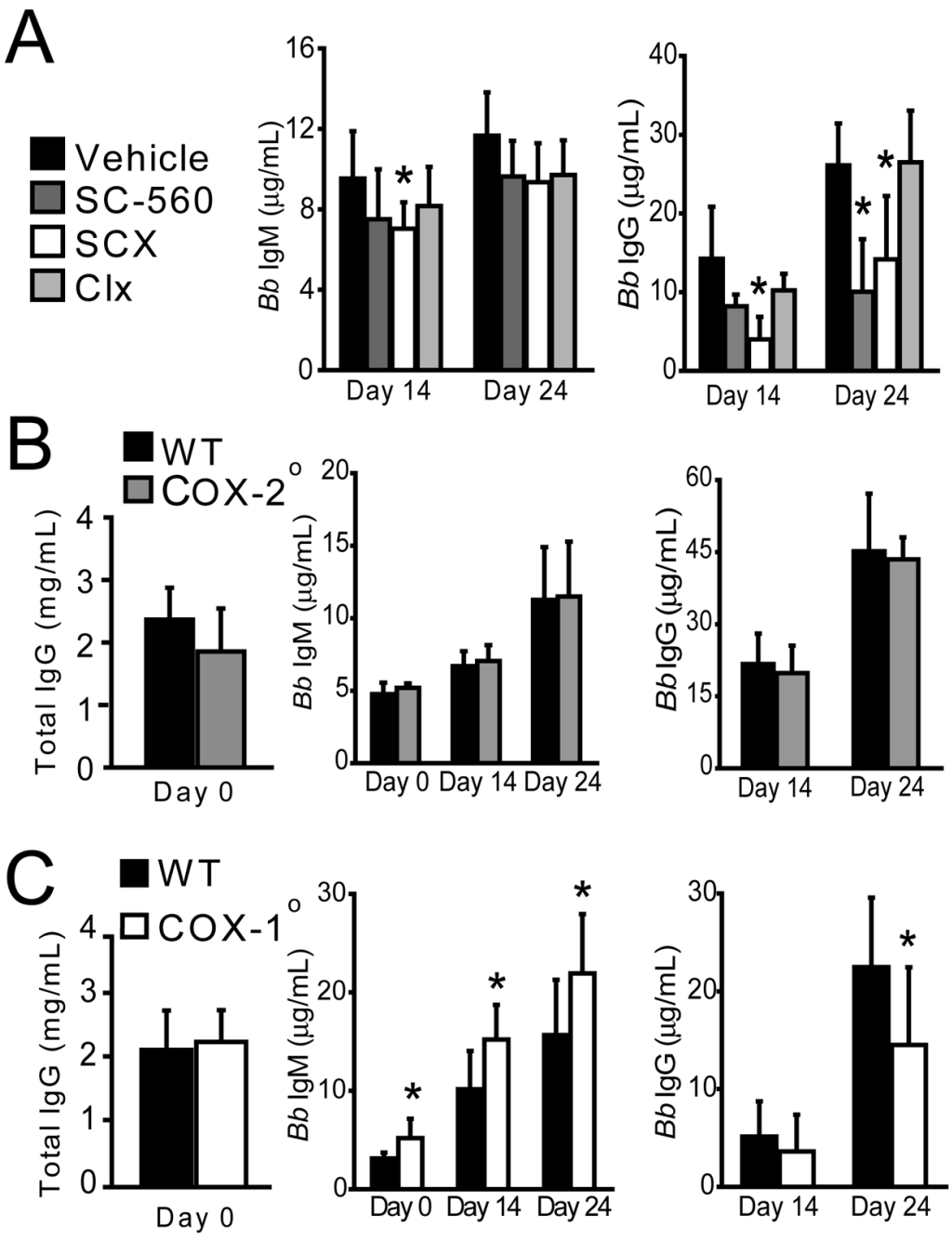

Figure 4.

Borrelia-specific antibody production during inhibition or deficiency of COX enzymes. **(A)** IgM and total IgG levels in sera from mice infected with *B. burgdorferi* and treated with vehicle, SC-560 alone, SC-560 plus celecoxib (SCX), or celecoxib (CLX) alone at days 14 and 24 post-infection. Antibody levels in sera from uninfected (day 0) or *B. burgdorferi*-infected **(B)** COX-2° and **(C)** COX-1° mice at various days post-infection. **P* ≤ 0.05. Values are means ± s.d. n = 10 mice per group. Data are representative of two separate experiments.

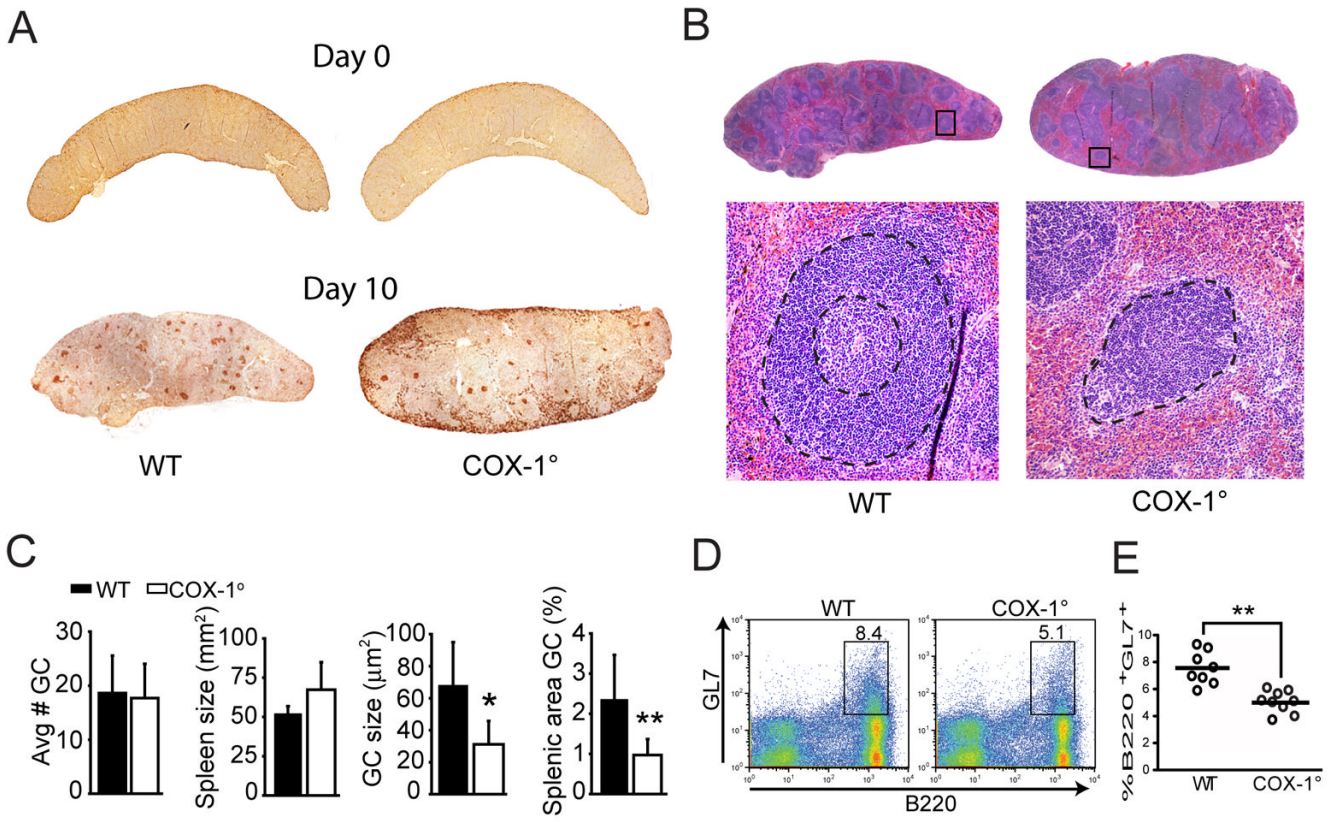

Figure 5.

COX-1 is required for normal germinal center development in the spleen. **(A)** Sections were stained with anti-Ki67 antibody to identify GC and proliferating cells in spleens from WT or COX-1° mice on days 0 or 10 post-infection with *B. burgdorferi*. **(B)** H&E stained sections from the same WT or COX-1° day 10 spleens, with dotted lines outlining GC under higher magnification. **(C)** Average number of GC per spleen, total spleen size, average individual GC size, and percentage of splenic area occupied by GC from WT and COX-1° mice. Flow cytometry analysis dot plot **(D)** or percentage **(E)** of GC B cells ($B220^+$, $GL7^+$) in spleens from WT or COX-1° at 10 days post-infection. **P* < 0.02 or **P < 0.01 versus WT controls. n = 5–9 mice per group. Data are representative of two experiments and are means ± s.d.

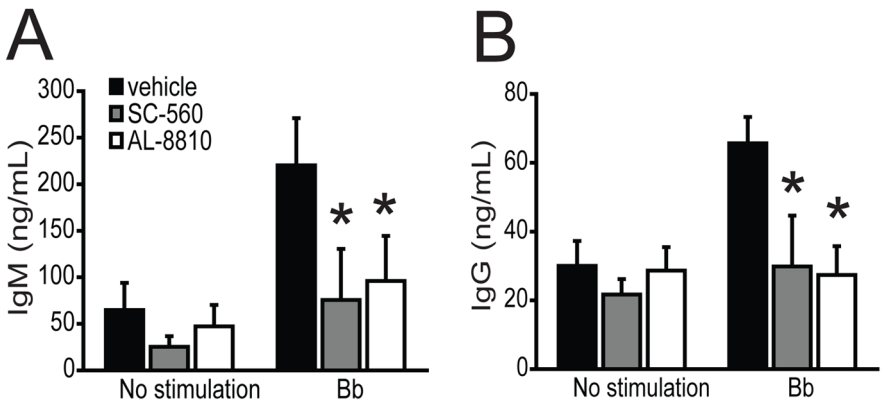

Figure 6.

Inhibition of *in vitro* antibody production by COX-1 inhibitor or FP antagonist. Naïve B cells were pre-incubated with vehicle, SC-560 (1 μM), or AL-8810 (50 μM) 30 min before stimulation with live *B. burgdorferi.* Total **(A)** IgM or **(B)** IgG were measured by ELISA from culture supernatants after 7 d of stimulation. Values represent the means ± s.d. of three mice. **P* < 0.05 versus vehicle-treated controls. Data are representative of three separate experiments.

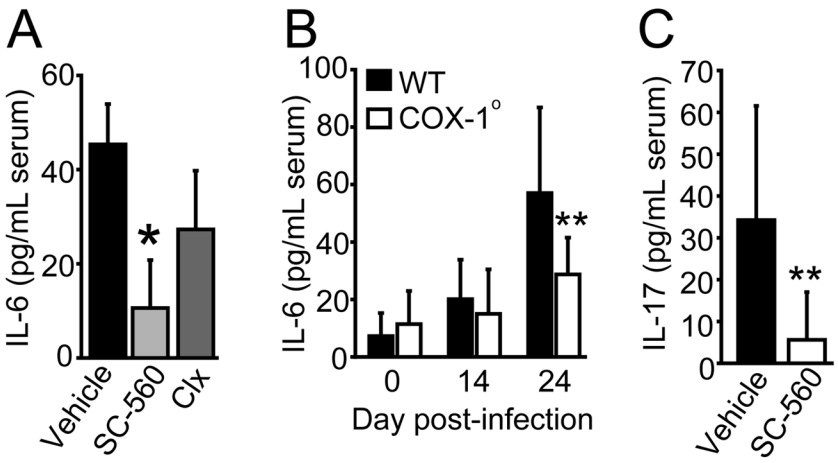

Figure 7.

Reduced IL-6 and IL-17 production *in vivo* in the absence of COX-1 activity. Levels of IL-6 **(A & B)** or IL-17 **(C)** from sera of mice infected with *B. burgdorferi*. Sera were collected at **(A)** day 24 post-infection from mice treated with vehicle, SC-560, or CLX; **(B)** at days 0, 14, and 24 post-infection from WT and COX-1° mice; or **(C)** at day 24 post-infection from mice treated with vehicle or SC-560. **P* ≤ 0.05 versus WT or vehicle controls. n = 4–8 mice per group. Data are means ± s.d. and are representative of three or four experiments.

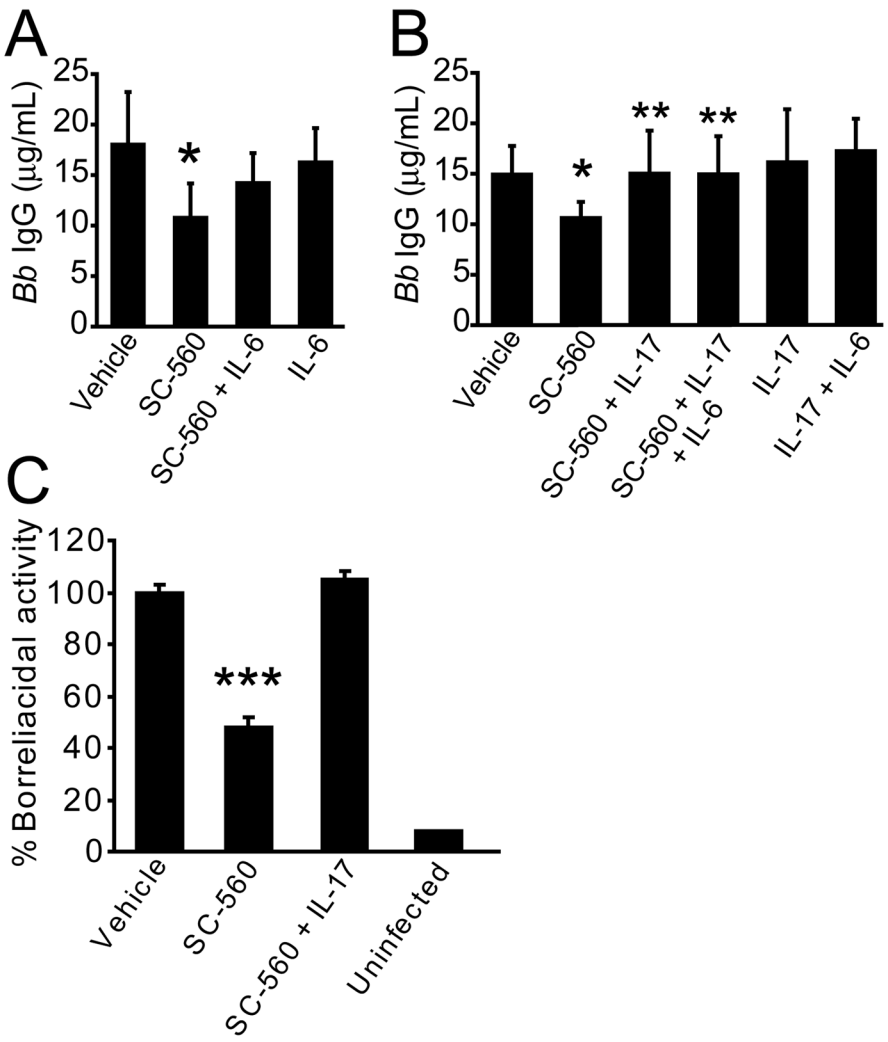

Figure 8.

Restoration of *Bb*-specific IgG production and borreliacidal activity by administration of IL-17. **(A)** Levels of *Bb*-specific IgG in sera of mice treated with vehicle, SC-560, SC-560 + IL-6, or IL-6 alone at 24 days post-infection. **(B)** Levels of *Bb*-specific IgG in the sera of mice treated with vehicle, SC-560, SC-560 + IL17, SC-560 + IL-17 + IL-6, IL-17 alone, or IL-17 + IL-6 at 24 days post-infection. **(C)** Borreliacidal antibody activity of sera from mice treated with vehicle, SC-560, or SC-560 + IL-17 at 24 days post-infection. Sera from uninfected mice were used as controls. **P* ≤ 0.05 versus vehicle-treated controls. ***P* ≤ 0.005 versus Sc-560-treated animals. ****P* ≤ 0.001 versus vehicle-treated controls. n = 5 mice per group. Data are the mean ± s.d. and are representative of **(A)** four or **(B & C)** two experiments.

Table 1

Levels of prostanoids in spleens from mice infected with *B. burgdorferi*[a]

	pg/mg tissue	
Eicosanoid	**WT**	**COX-1°**
6k $PGF_{1\alpha}$	5.5 ± 1.1	0.6 ± 0.1*
TXB_2	1.6 ± 0.4	0.4 ± 0.1*
$PGF_{2\alpha}$	2.4 ± 0.4	0.4 ± 0.3*
PGE_2	14.7 ± 4.7	9.7 ± 2.4
PGD_2	13.8 ± 4.3	8.4 ± 2.0
PGJ_2	0.3 ± 0.1	0.0 ± 0.0*
15d PGD_2	0.8 ± 0.3	0.8 ± 0.2
15d PGJ_2	1.4 ± 0.2	1.7 ± 0.4

[a] Spleens were harvested at day 10 post-infection Results are expressed as means ± s.e.

* $P \leq 0.01$