

B Cell–Specific Expression of Inducible Costimulator Ligand Is Necessary for the Induction of Arthritis in Mice

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Objective. Inducible costimulator (ICOS)–ICOSL interactions are necessary for activation of T_H17 and follicular helper T (T_H) cells. ICOSL is expressed on B cells, macrophages, and dendritic cells and can be induced on nonhematopoietic cells. The aim of this study was to determine whether expression of ICOSL on B cells is necessary for the development of proteoglycan (PG)–induced arthritis (PGIA).

Methods. PGIA was initiated by immunizing wild-type and ICOSL-deficient (ICOSL^{-/-}) or B cell–specific ICOSL^{-/-} chimeric BALB/c mice with human PG in adjuvant. The onset and severity of arthritis were monitored over time. CD4⁺ T cell proliferation and CD4⁺ T cell cytokine production were measured *in vitro* after the cells were restimulated with PG. Germinal center (GC) B cells, plasma cells, T_H cells, and Treg cells were identified by staining with specific antibodies.

Results. Arthritis progression was completely inhibited in both ICOSL^{-/-} mice and B cell–specific ICOSL^{-/-} chimeric mice. Production of the T_H17 cell–produced cytokines interferon- γ and interleukin-17 (IL-17) and the antiinflammatory cytokine IL-4 was suppressed. The reduced percentages of GCs and T_H cells and the decreased production of IL-21 correlated with a decrease in the anti-mouse PG antibody response. However, the percentage of plasma cells was not reduced despite a reduction in IgG responses.

Conclusion. These data indicate that the signals provided by ICOSL-expressing B cells to T_H17 cells and T_H cells are necessary for the development of arthritis. Thus, therapeutic blockade of ICOSL–ICOS inter-

actions may be an effective strategy for the treatment of rheumatoid arthritis.

In rheumatoid arthritis (RA), autoreactive T cells are implicated in the disease process, based on genetic linkage between disease susceptibility and HLA alleles and evidence derived from experimental models of arthritis (1). T cell activation requires T cell receptor ligation in addition to secondary and tertiary signals provided by costimulator molecules and cytokines. The interaction between CD28 on T cells and CD80/CD86 on antigen-presenting cells (APCs) is the most prominent of these costimulatory interactions (2). Successful therapeutic intervention with the CD28 costimulatory antagonist CTLA4-Ig (abatacept) demonstrates the efficacy of specific inhibition of T cell interactions in RA (3,4).

Inducible costimulator (ICOS) is a member of the CD28 superfamily of costimulatory molecules. ICOS is up-regulated on activated T cells, while the expression level of ICOS on effector and memory T cells is similar to that of activated T cells. ICOS engages the cognate ligand ICOSL (also known as B7-H2, B7RP-1, B7h, GL50, and LICOS), which is expressed on B cells, macrophages, and dendritic cells and can be induced on some nonhematopoietic cells (5–8). The interaction between T cells and APCs through ICOS–ICOSL is necessary for several different T cell functions. ICOS is important in Th1 and Th2 effector responses promoting the production of interferon- γ (IFN γ) and interleukin-4 (IL-4) and, *in vivo*, by controlling viral or helminth infections (8–10). For Th17 cell differentiation, ICOS is not critical during priming but plays an important role in maintaining the Th17 cell response (11). The generation and function of Treg cells also require the expression of ICOS (12,13). ICOS plays a major role in T cell–dependent antibody responses. Follicular helper T (T_H) cells, a T cell population located in the germinal center (GC), express ICOS, IL-21, and the transcription factor Bcl-6 (14). In the absence of ICOS (e.g., ICOS^{-/-} mice),

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GC formation is defective, and T cell-dependent antibody responses are impaired (15,16). Specific ablation of ICOSL on B cells leads to reduced numbers of Tfh cells, indicating that Tfh cells are dependent on signaling from B cells (17).

In autoimmune disease models such as collagen-induced arthritis, disruption of ICOS-ICOSL interactions with antibodies specific for ICOSL inhibits disease (18–20). However, in experimental autoimmune encephalomyelitis (EAE), antibody blockade early in the induction phase exacerbates disease, whereas later treatment inhibits disease. In ICOS^{-/-} mice, EAE is exacerbated (9,11,21). Excessive ICOS activation in sanroque mice, which display a genetic mutation for a RING-type ligase that represses ICOS, leads to spontaneous GC formation in the absence of foreign antigen, increased numbers of Tfh cells, and spontaneous development of autoantibodies (22,23).

In RA, B cells are critical to the disease process, as indicated by the efficacy of B cell depletion therapy with anti-CD20 antibody (rituximab). Autoantibody responses are inhibited by B cell depletion; however, clinical responses do not always correlate with antibody levels (24,25). Thus, along with B cell depletion, other antibody-independent mechanisms contribute to reducing clinical symptoms of disease. Our studies in proteoglycan-induced arthritis (PGIA) demonstrated that the specific interaction between T cells and B cells through the costimulator molecules CD28 and CD80/CD86 is necessary for the induction of autoreactive T cells that drive the development of arthritis (26,27). Therefore, we sought to determine whether specific ICOS-ICOSL interactions between B cells and T cells are also required for the initiation of PGIA. Here, we show that ICOSL^{-/-} mice are resistant to PGIA. This resistance was associated with reduced cytokine production by T cells, reduced percentages of GCs and Tfh cells, and reduced autoantibody titers. Importantly, a specific deficiency of ICOSL selectively on B cells and not on other APCs similarly inhibited arthritis, reduced T cell cytokine production, and decreased the numbers of GCs and Tfh cells.

MATERIALS AND METHODS

Mice, antigen, and assessment of arthritis. Wild-type (WT) BALB/c mice were obtained from the National Cancer Institute. ICOSL^{-/-} C57BL/6 mice were a gift from Dr. Andrew Welcher (Amgen). ICOSL^{-/-} mice were backcrossed to BALB/c mice for 9 generations and then intercrossed to obtain WT and ICOSL^{-/-} littermates. In all experiments, WT mice and ICOSL^{-/-} littermates were used. B cell-deficient BALB/c mice (J_HD) were provided by Dr. Mark

Shlomchik (Yale University). Female WT mice and ICOSL^{-/-} littermate mice (age >3 months) were immunized intraperitoneally with 150 µg of human proteoglycan (PG) in 2 mg of dimethyldioctadecylammonium bromide (DDA) adjuvant (Sigma-Aldrich) and boosted twice at 3-week intervals, as previously described (28). Human cartilage PG obtained from patients undergoing joint replacement surgery was purified by procedures approved by the Institutional Review Board of Rush University Medical Center, as previously described (29). All animal experiments were approved by the Animal Care and Use Committee at Rush University Medical Center. The mice were assessed in a blinded manner 3 times per week. The paws were scored for arthritis (extent of erythema and swelling) on a scale of 0 to 4, where 0 = normal, 1 = mild erythema and swelling of several digits, 2 = moderate erythema and swelling, 3 = more diffuse erythema and swelling, and 4 = severe erythema and swelling of complete paw with ankylosis. Each mouse received a cumulative score ranging from 0 to 16.

T cell proliferation and assessment of T cell cytokines. Splenic CD4⁺ T cells obtained from immunized mice were isolated by negative selection using microbeads and an auto-MACS separator (Miltenyi Biotec). CD4⁺ T cells (2.5×10^5) were cultured with mitomycin C-treated splenic APCs from nonimmunized mice (2.5×10^5), with or without PG (10 µg/ml), for 5 days. Proliferation was measured by ³H-thymidine incorporation. Supernatants were removed from similarly established cultures on day 4, and the expression of IFNγ (BD Biosciences), IL-17, IL-4, IL-10, IL-21, IL-6, and tumor necrosis factor α (R&D Systems) was analyzed by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

Detection of anti-PG antibodies by ELISA. Mice were bled from the orbital plexus at the time when they were killed, and serum was obtained. Anti-mouse PG and anti-human PG antibodies in serum samples were determined by ELISA. Individual mouse serum samples and internal standard samples (pooled sera from arthritic mice) were serially diluted (phosphate buffered saline and 0.5% Tween 20) in enzyme immunoassay tissue culture Costar 96-Well Half-Area plates (Corning) that were coated overnight with 0.5 µg human PG or 0.75 µg native mouse PG in carbonate buffer. Known concentrations of plate-bound unlabeled murine IgG1 and IgG2a antibodies (Southern Biotechnology) without plate-bound PGs were used as standard curves. Unlabeled plate-bound antibodies in standard curve wells or serum antibodies bound to PG-coated wells were detected using horseradish peroxidase-labeled anti-mouse IgG1 or IgG2a (Zymed) secondary antibodies with *o*-phenylenediamine. Spectrophotometer readings at 490 nm were used to determine colorimetric changes and serum concentrations of anti-PG antibodies.

Detection of cell populations by flow cytometry. Spleen cells were harvested from immunized mice, and single cell suspensions were stained. Germinal center B cells were identified using allophycocyanin (APC)-conjugated anti-CD19, fluorescein isothiocyanate (FITC)-conjugated anti-GL7, and phycoerythrin (PE)-conjugated anti-CD95 antibodies; plasma cells (PCs) were identified using PerCP-Cy5.5-conjugated anti-B220, FITC-conjugated anti-CD38, and PE-conjugated anti-CD138 antibodies; Tfh cells were identified using APC-Cy7-conjugated anti-CD4, PE-conjugated anti-CXCR5, FITC-conjugated anti-CD62L, APC-conjugated anti-ICOS,

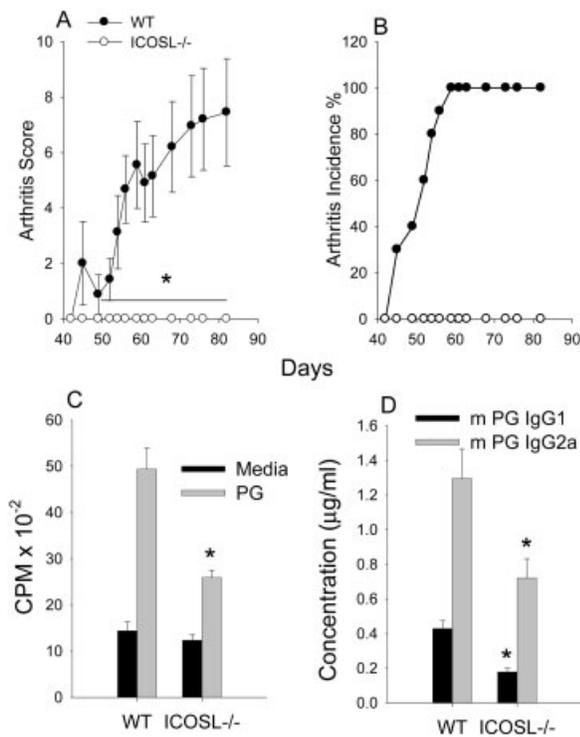


Figure 1. Arthritis, T cell activation, and antibody production are diminished in inducible costimulator ligand-deficient (ICOSL^{-/-}) mice. Wild-type (WT) BALB/c mice and ICOSL^{-/-} littermates received 3 intraperitoneal immunizations with proteoglycan (PG) in dimethyldioctadecylammonium bromide, and the development of arthritis was monitored over time. **A** and **B**, Arthritis severity (**A**) and incidence (**B**) at various time points after immunization. Values in **A** are the mean \pm SEM ($n = 10$ – 12 mice per group) and are representative of 2 independent experiments. **C**, CD4⁺ T cell proliferation in spleens harvested from mice 12 weeks after immunization. CD4⁺ T cells were isolated and stimulated in the presence of antigen-presenting cells, with or without PG (10 μ g). Stimulation was measured by ³H-thymidine incorporation during the last 24 hours of the 5-day culture. **D**, Concentrations of anti-mouse PG (mPG) antibodies in the sera of WT mice and ICOSL^{-/-} littermates, as determined by enzyme-linked immunosorbent assay. Values in **C** and **D** are the mean \pm SEM ($n = 5$ mice per group) and are representative of 2 independent experiments. * = $P < 0.05$ versus WT.

and PE-Cy7-conjugated anti-programmed death 1 (PD-1) antibodies; and Treg cells were identified using PerCP-Cy5.5-conjugated anti-CD4, APC-conjugated anti-CD25, and PE-conjugated anti-FoxP3 antibodies (all from BD Biosciences). Cell analysis was performed using a FACSCanto II system with FACSDiva software (BD Immunocytometry Systems).

Generation of bone marrow chimeras. Female BALB/c mice (8–10 weeks of age) were given acidified water 1 week before irradiation and bone marrow reconstitution. On the day of cell transfer, the mice were switched to trimethoprim/sulfamethoxazole-treated water, and this was continued for 1 month. Mice were lethally irradiated with 2 separate 450-rad doses 4 hours apart using a ¹³⁷Cs source followed by reconstitution with bone marrow cells. Bone marrow was depleted

of red blood cells and then depleted of Thy1.2 (CD90)-expressing T cells using anti-CD90 microbeads and an auto-MACS separator (Miltenyi Biotec). To generate B cell: ICOSL^{-/-} chimeric mice, WT mice were reconstituted with B cell-deficient bone marrow plus ICOSL-deficient bone marrow. Recipient WT mice were reconstituted with a mixture of bone marrow from B cell-deficient mice and ICOSL^{-/-} mice to generate positive control chimeras. Bone marrow-reconstituted mice were allowed to recover for 4 months before arthritis was induced.

Statistical analysis. The Mann-Whitney U test was used to compare nonparametric data. Two-tailed P values less than 0.05 were considered significant.

RESULTS

Effect of ICOSL on PGIA and T cell and B cell responses. We previously reported that a CD80/CD86:CD28 costimulatory interaction between B cells and T cells is necessary for autoreactive T cell activation and induction of arthritis (26). To determine whether interaction between ICOS and ICOSL is required for the development of arthritis, female WT BALB/c mice and ICOSL^{-/-} littermate mice were immunized with PG/DDA and boosted twice at 3-week intervals. Arthritis began to develop in WT mice after the third injection

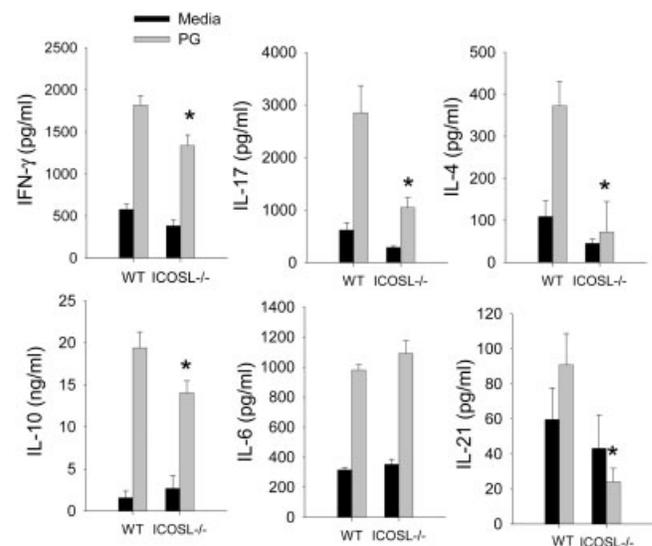


Figure 2. Cytokine production in ICOSL^{-/-} mice is reduced after immunization with PG. Wild-type BALB/c mice and ICOSL^{-/-} littermates were immunized with PG/dimethyldioctadecylammonium bromide, and the spleens of these mice were harvested 12 weeks later. Interferon- γ (IFN γ), interleukin-17 (IL-17), IL-4, IL-10, IL-6, and IL-21 production in supernatants of CD4⁺ T cells, with or without PG, was measured by enzyme-linked immunosorbent assay. Values are the mean \pm SEM ($n = 5$ mice per group) and are representative of 2 independent experiments. * = $P < 0.05$ versus WT. See Figure 1 for other definitions.

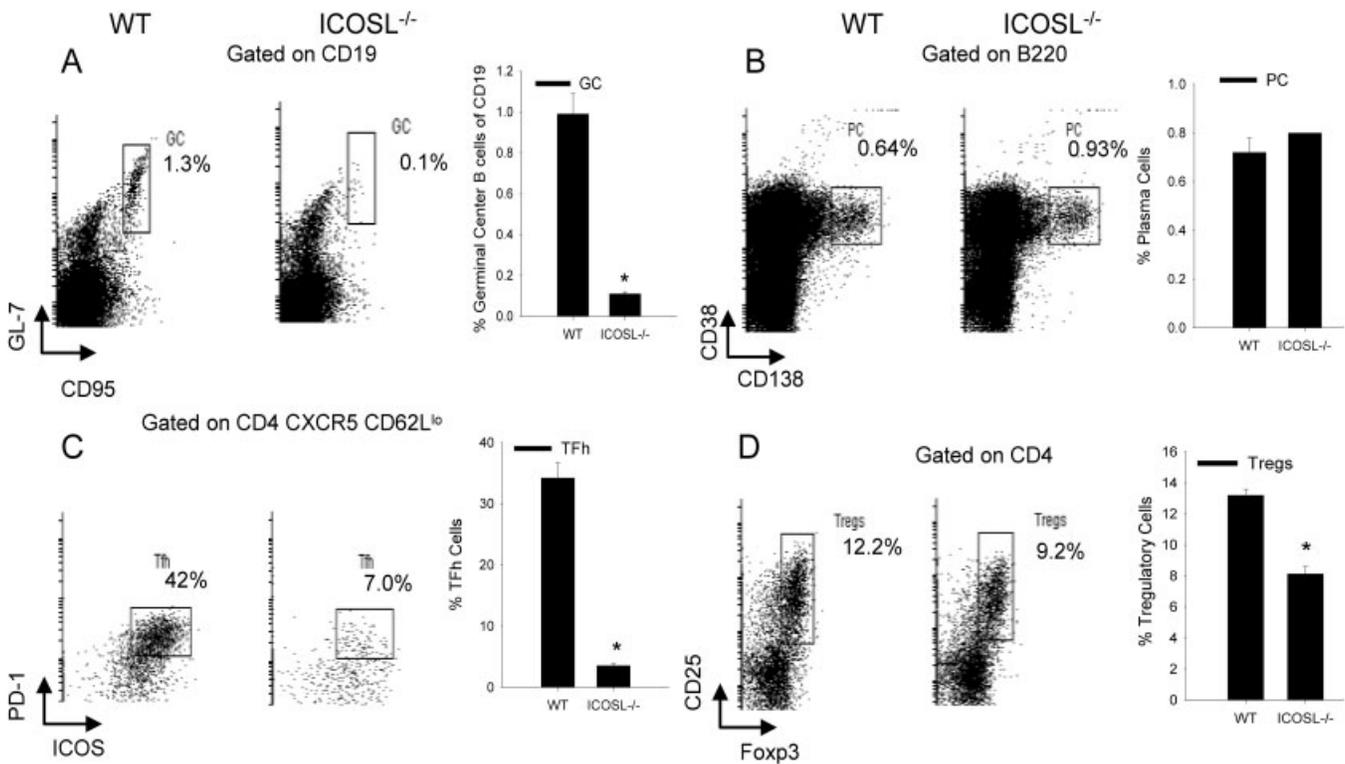


Figure 3. Germinal center (GC) B cells, follicular helper T (Tfh) cells, and Treg cells, but not plasma cells (PCs), are inhibited in ICOSL^{-/-} immunized mice. Spleens from WT and ICOSL^{-/-} mice were isolated 12 weeks after immunization with PG/dimethyloctadecylammonium bromide. **A**, Percentage of GC cells, as determined by gating on CD19 B cells and dual staining with GL7 and CD95. **B**, Percentage of PCs, as determined by gating on B220 B cells and dual staining for CD38 and CD138. **C**, Percentage of Tfh cells, as determined by gating on CD4, CXCR5, and CD62L^{low} T cells and dual staining for programmed death 1 (PD-1) and ICOS. **D**, Percentage of Treg cells, as determined by gating on CD4+ T cells and dual staining for CD25 and FoxP3. Bars show the mean \pm SEM ($n = 5$ mice per group) and are representative of 2 independent experiments. * = $P < 0.05$ versus WT. See Figure 1 for other definitions.

with PG/DDA, and the severity of arthritis usually stabilized around the 12-week time point. However, arthritis was completely inhibited in all ICOSL^{-/-} mice (Figures 1A and B). These data demonstrated that ICOSL-ICOS interactions are absolutely required for the development of PGIA.

Both T cells and B cells are necessary for the induction of arthritis. Therefore, we assessed antigen-specific T cell proliferation and PG-specific antibody responses. CD4+ T cells from immunized mice were stimulated in vitro in the presence of APCs, with or without PG. T cell proliferation in ICOSL^{-/-} mice was reduced by $\sim 50\%$ compared with that in WT mice (Figure 1C). The concentrations of anti-human PG-specific IgG1, anti-mouse IgG1, and anti-mouse IgG2a were reduced significantly but not completely (Figure 1D). These data demonstrated that ICOSL-ICOS interactions between T cells and B cells are required for the development of PGIA.

Inhibition of proinflammatory cytokines in ICOSL^{-/-} mice. We previously reported that PGIA is a Th1 cell-mediated arthritis that is dependent on IFN γ but not IL-17 for induction (30). Because ICOS is up-regulated on T cells after activation and may be necessary for maintaining T cell activation and cytokine production, we sought to determine whether T helper cell cytokines and other proinflammatory or anti-inflammatory cytokines were regulated by ICOSL interactions. CD4+ T cells from PG-immunized mice were stimulated in culture with PG for 4 days, and cytokines were measured in supernatants. Production of the T cell cytokines IFN γ , IL-17, IL-4, IL-10, and IL-21 was significantly inhibited, whereas IL-6 production was not inhibited (Figure 2).

Reduced percentage of GCs and Tfh cells, but not PCs, in ICOSL^{-/-} mice. It is known that ICOSL-ICOS interactions between GC B cells and Tfh cells are necessary for GC formation and the differentiation of Tfh

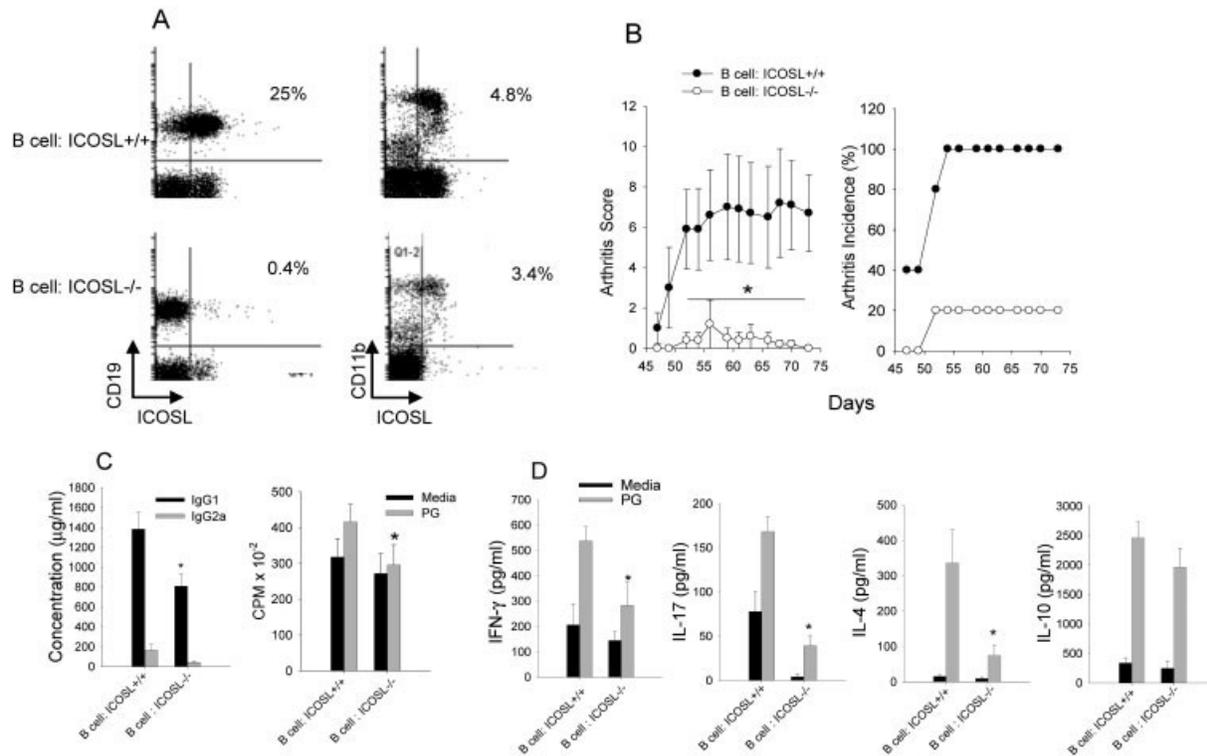


Figure 4. Arthritis is suppressed in mice with a B cell-specific defect in ICOSL expression. Mixed bone marrow chimeric mice were generated by transferring bone marrow from B cell-deficient mice and WT mice (B cell:ICOSL^{+/+}) or from B cell-deficient mice and ICOSL^{-/-} mice (B cell:ICOSL^{-/-}) into irradiated WT mouse recipients. Recipients were allowed to recover for 4 months before receiving 3 immunizations with PG/dimethyldioctadecylammonium bromide. **A**, Expression of ICOSL on spleen cells from B cell:ICOSL^{+/+} mice and B cell:ICOSL^{-/-} mice immunized at week 11, as determined by flow cytometry. **B**, Severity and incidence of arthritis in immunized B cell:ICOSL^{+/+} mice and B cell:ICOSL^{-/-} mice. Arthritis scores are shown as the mean \pm SEM (n = 5 mice per group). * = $P < 0.05$ versus B cell:ICOSL^{+/+}. **C**, Serum antibody concentrations in immunized B cell:ICOSL^{+/+} mice and B cell:ICOSL^{-/-} mice and T cell proliferation following in vitro restimulation in the presence of antigen-presenting cells, with or without PG (10 μ g). **D**, Concentrations of interferon- γ (IFN γ), interleukin-17 (IL-17), IL-4, and IL-10 in the supernatants of spleens activated in vitro with PG (10 μ g/ml). In **C** and **D**, values are the mean \pm SEM (n = 5 mice per group) and are representative of 2 independent experiments. * = $P < 0.05$ versus B cell:ICOSL^{+/+}. See Figure 1 for other definitions.

cells (17,31). To determine whether resistance to arthritis in ICOSL^{-/-} mice is associated with a reduction of GCs and PCs, we examined spleen cells from PG/DDA-immunized mice at the time point when WT mice were arthritic. CD19⁺ B cells were gated on CD95⁺GL7⁺ cells to identify GCs. We observed a significant reduction in the percentage of GCs in ICOSL^{-/-} mice compared with that in WT mice (Figure 3A). B220⁺ cells were gated on CD138⁺CD38⁺ cells to identify PCs (Figure 3B). The percentage of PCs in ICOSL^{-/-} mice was not reduced compared with the percentage in WT mice. Thus, the reduced anti-human and anti-mouse PG-specific IgG1 and IgG2a responses were not reflected in a reduction in plasmablast formation.

We next assessed whether there was a defect in Tfh cells in WT and ICOSL^{-/-} mice. CD4⁺

CXCR5⁺CD62L^{low} cells were gated on PD-1 and ICOS as markers for Tfh cells. The percentage of Tfh cells was dramatically reduced in ICOSL^{-/-} mice (Figure 3C). Because Treg cell differentiation is also dependent on ICOSL-ICOS interactions, we examined Treg cell percentages in immunized WT and ICOSL^{-/-} mice and observed a minor but significant reduction in the percentage of Treg cells. The reduction in IL-21 production (see Figure 2) mirrored the decline in the percentage of Tfh cells. These results suggested that defective interactions between ICOS-expressing T cells and GC B cells partly contribute to the reduction in arthritis incidence and severity.

Prevention of arthritis, GCs, and Tfh cells with B cell-specific deletion of ICOSL. Although a complete deficiency in ICOSL prevents the development of arthritis, it is unclear whether the expression of ICOSL

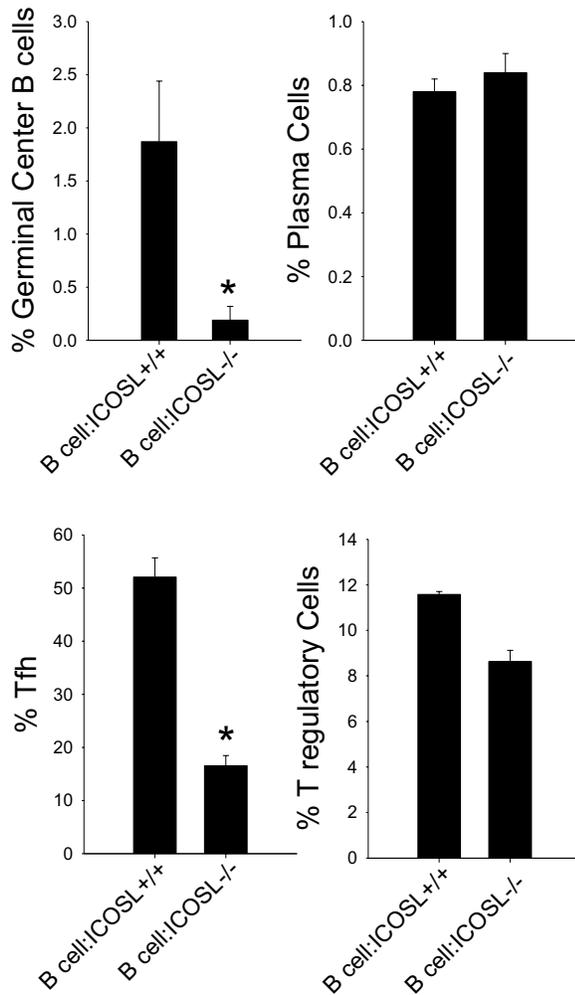


Figure 5. Production of germinal center (GC) B cells, follicular helper T (Tfh) cells, and Treg cells, but not plasma cells (PCs), is inhibited in chimeric mice with a B cell-specific defect in ICOSL expression (B cell:ICOSL^{-/-} mice). Spleen cells obtained from chimeric mice 11 weeks after immunization with PG/dimethyldioctadecylammonium bromide were stained with antibodies for specific markers to identify GC cells, PCs, Tfh cells, and Treg cells, as described in Figure 3. Values are the mean \pm SEM (n = 5 mice per group) and are representative of 2 independent experiments. * = $P < 0.05$ versus B cell:ICOSL^{+/+}. See Figure 1 for other definitions.

on B cells is essential for the initiation of PGIA. We previously showed that antigen-specific B cells that express CD80 and CD86 are important APCs for the activation of autoreactive T cells (26,27,32). To determine whether ICOSL expression on B cells is necessary for the induction of arthritis, we generated mixed bone marrow chimeric mice in which ICOSL^{-/-} mouse B cells coexist with other APCs that express ICOSL. Recipient WT mice were lethally irradiated and reconstituted with a mixture of bone marrow from B cell-deficient mice

and ICOSL^{-/-} mice (B cell:ICOSL^{-/-}). In these chimeric mice, the non-B cell APCs are derived from the B cell-deficient bone marrow and thus express ICOSL, whereas the B cells arise from the ICOSL^{-/-} mouse bone marrow. Positive control chimeras received a mixture of bone marrow from WT mice and B cell^{-/-} mice (B cell:ICOSL^{+/+}). Among the chimeric mice, expression of ICOSL on CD19⁺ B cells was significantly reduced in B cell:ICOSL^{-/-} mice compared with B cell:ICOSL^{+/+} mice (Figure 4A). The percentage of ICOSL-positive CD11b cells was not significantly different in B cell:ICOSL^{+/+} mice and B cell:ICOSL^{-/-} mice (Figure 4A).

To determine whether the expression of ICOSL on B cells is necessary for the development of PGIA, chimeric mice were immunized with PG/DDA. The incidence and severity of arthritis were monitored over time. In B cell:ICOSL^{+/+} mice, arthritis began to develop after the third immunization with PG/DDA, and disease severity progressed over time. However, arthritis developed in only one of the B cell:ICOSL^{-/-} mice and was very mild and did not progress (Figure 4B).

We next assessed whether ICOSL expression on B cells is required for the development of PG-specific antibody production. The serum concentrations of antibodies specific for human PG were significantly reduced in B cell:ICOSL^{-/-} mice compared with those in B cell:ICOSL^{+/+} mice (Figure 4C), as was T cell proliferation (Figure 4C). Concentrations of the proinflammatory cytokines IFN γ and IL-17 were also decreased in B cell:ICOSL^{-/-} mice (Figure 4D), whereas the production of IL-4 was inhibited in B cell:ICOSL^{-/-} mice, but the production of IL-10 was not inhibited. We further observed that the percentages of GCs and Tfh cells were significantly reduced in B cell:ICOSL^{-/-} mice (Figure 5), to levels similar to those observed in mice with a complete deficiency in ICOSL. In addition, the percentage of PCs was not reduced. These data suggested that blocking the interaction between T cells expressing ICOS and B cells expressing ICOSL is sufficient to reduce arthritis.

DISCUSSION

Here, we show that a deficiency in ICOSL leads to complete suppression of PGIA. Our results confirm the requirement for ICOSL-ICOS interactions observed in other models of arthritis, which demonstrated that blockade of ICOS-ICOSL interactions with anti-ICOS antibodies in collagen-induced arthritis and in glucose-6-phosphate isomerase-induced arthritis reduced disease severity (19,20). This study extends previous work

and shows that B cell-specific expression of ICOSL is necessary for the development of arthritis. Several mechanisms could account for the reduction in the incidence and severity of arthritis. It was originally reported that ICOS-knockout mice demonstrated a defect in T helper cell function (16). ICOSL-ICOS interactions are necessary for T cell activation, and we observed that antigen-specific T cell proliferation and cytokine production were substantially reduced in ICOSL^{-/-} mice. Because ICOS is essential for the survival and expansion of activated Teff cells, a reduction in the activity of the Teff cell population contributes to resistance to arthritis induction in ICOSL^{-/-} mice. We observed that the production of both IFN γ and IL-17 was suppressed. In EAE, ICOS deficiency leads to exacerbated disease (9). In ICOSL^{-/-} mice, IL-17 production was increased, and IL-10 production was decreased, which may account for the increase in susceptibility in EAE (9,11). In PGIA, the antiinflammatory cytokines IL-4 and IL-10 were suppressed; however, this did not lead to enhanced disease severity, presumably due to a lack of Th1 cell activation. Importantly, the reduction in T cell activation occurred whether ICOSL was deficient in all cells or was specifically deleted in B cells.

PGIA is dependent on the development of antibodies to human PG. Over time, after immunization with human PG, mice develop a cross-reactive antibody response to native mouse PG. We previously reported that PG-specific B cells are essential for the induction of arthritis, as both antibody-producing cells and antigen-specific APCs (33). ICOS was first identified on GC T cells, and it was demonstrated that ICOS^{-/-} mice have impaired GC formation and class-switch recombination to IgA, IgE, and some IgG isotypes. Later work demonstrated that this defect was attributable to an absence of Tfh cells (34). Similarly, we observed that complete deletion of ICOSL leads to a reduction in the number of Tfh cells and GCs, with a substantial but not complete reduction in the production of anti-human and anti-mouse PG antibodies.

We anticipated that PC formation would be reduced in ICOSL^{-/-} mice; however, the reduction in anti-mouse and anti-human IgG1 and IgG2a antibody concentrations was not caused by decreased PC formation. GC reactions create high-affinity class-switched, long-lived PCs (35). Early events in the antibody response are initiated by the interaction between Tfh cells and B cells, which occurs at the border between the T cell zone and B cell follicles (36,37). Following the initial interaction, B cells and T cells migrate to the follicle and form the GC (36). However, as an alternative to the GC

B cell fate, B cells may differentiate into low-affinity PCs and migrate to the red pulp border and form extrafollicular plasmablasts (38,39). Among MLR/*lpr* mice, in which a spontaneous extrafollicular response occurs, the percentages of plasmablasts in ICOSL^{-/-} mice were unchanged despite a reduction in the percentage of IgG isotype-expressing PCs (40). Thus, the PCs that are present in PG-immunized ICOSL^{-/-} mice may not be IgG-positive PCs, which is corroborated by the reduced percentage of IgG antibodies. Similar to what was observed in MLR/*lpr* mice, ICOS-ICOSL interactions may be dispensable for extrafollicular plasmablast generation in PGIA. In addition, the diminished IL-21 response, which is critical for IgG production, also contributes to a reduction in the PG-specific antibody response (17).

Although ICOSL is expressed on macrophages and dendritic cells, we show that specific expression of ICOSL on B cells is necessary for Teff cell activation as well as initiation or maintenance of GC and Tfh cell numbers and optimal antibody production. In RA, therapeutic depletion of B cells removes a major source of cells expressing ICOSL and other costimulatory molecules necessary for the activation and maintenance of various T cell populations.

In summary, we show that B cell expression of ICOSL is necessary for the development of PGIA. ICOSL-ICOS interactions between B cells and Teff cells control proinflammatory cytokine production, and interactions between B cells and Tfh cells regulate antibody production. Therefore, therapeutic blockade of ICOSL-ICOS interactions in RA may prove to be an effective strategy for the treatment of disease.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Finnegan had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Hamel, Finnegan.

Acquisition of data. Hamel, Cao, Olalekan.

Analysis and interpretation of data. Hamel, Olalekan, Finnegan.

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