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Journal

GASTROENTEROLOGY, 169(1)

ISSN

0016-5085

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Publication Date

2025-05-03

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Inflammatory Bowel Diseases, 2026, 00(00), 1–12
https://doi.org/10.1093/ibd/izag198
Original Research Article - Basic Science

B cells regulate T cell transfer colitis by partially reconstituting luminal IgA and modulating the colonic microbiota

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Abstract

Loss of immune tolerance to commensal microbial antigens is a central driver of inflammatory bowel disease. While regulatory T cells are well recognized in maintaining intestinal homeostasis, the contribution of B cells—particularly IgA-secreting cells—remains incompletely defined. In the T cell transfer colitis model, cotransfer of splenic B cells attenuates disease, an effect largely attributed to IL-10–producing regulatory B cells (Bregs). However, alternative B cell–dependent mechanisms have not been fully explored. Here, we investigated whether splenic B cells regulate T cell transfer colitis through IgA-dependent mechanisms independent of Breg-associated cytokines. We show that splenic B cells from wild-type C57BL/6 (B6) mice reconstitute bone marrow and peripheral B cell compartments in RAG1$^{-/-}$ recipients, undergo class switching, migrate to the colonic lamina propria as sIgA^{+} B lineage cells, and partially restore luminal secretory IgA. B cell cotransfer significantly attenuated clinical and histological colitis without increased expression of canonical Breg-associated cytokines, including interleukin-10, transforming growth factor β, and interleukin-35 subunits. In contrast, splenic B cells from IgA-deficient mice failed to attenuate colitis despite comparable peripheral B cell reconstitution. Restoration of secretory IgA was associated with increased bacterial IgA coating, reduced fecal bacterial burden, and remodeling of the colonic microbiota, including enrichment of beneficial commensal taxa. Importantly, because IgA-deficient B cells failed to attenuate colitis despite comparable peripheral B cell reconstitution, these findings support an important role for IgA in mediating the protective effect of B cell transfer. Collectively, these findings identify IgA-mediated modulation of the microbiota as an alternate mechanism by which B cells attenuate T cell transfer colitis, highlighting a Breg-independent regulatory pathway with potential relevance to inflammatory bowel disease pathogenesis.

Key words: IgA, IBD mouse models, colitis, B cells

Introduction

Loss of immune tolerance to yet unidentified antigens from the commensal microbiota has been implicated in the chronic dysregulated immune response that characterizes inflammatory bowel disease (IBD).[1] This is supported by the detection of several antibacterial antibodies in patients with Crohn's disease (CD), particularly those with ileal involvement and complicated disease course. Specifically, anti-*Saccharomyces cerevisiae* antibodies have been associated with strictures and fistulas. Anti-OmpC antibodies, which target the outer membrane porin C of *Escherichia coli*, are associated with ileitis and an aggressive disease course. Similarly, antibodies against bacterial flagellin (CBir1 antigen) (anti-CBir1) are associated with fibrostenotic ileitis. Finally, anti-I2 antibodies that target *E. coli*–associated bacterial antigen (I2) are also more common in ileal CD.[2] Those findings point to the presence of upregulated, humoral, B cell derived responses present in patients with IBD, particularly in CD.[3]

B cells are often considered positive regulators of immune responses, as they optimize CD4^{+} T cell activation and produce antibodies. However, B cell subsets that dampen immunological

Received: February 2, 2026. **Accepted:** September 14, 2026
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Key messages

What is already known?

- Naïve CD4$^+$T cell transfer (after Treg depletion) induces colitis in RAG1/2-deficient or SCID mice.
- B cell cotransfer alleviates colitis, mainly via Bregs producing anti-inflammatory cytokines.
- Splenic B cells also mature into ASCs and restore luminal IgA.

What is new here?

- We investigate whether IgA contributes to attenuation of colitis by splenic B cells.
- B cell cotransfer restores B cells in bone marrow and periphery of RAG1-deficient mice, reducing colitis.
- These B cells mature into sIgA$^+$ B lineage cells, home to the colon, restore luminal SIgA, and modulate microbiota.
- B cells from IgA-deficient mice fail to mitigate colitis or alter the microbiota.
- IgA is an additional mediator of B cell–driven attenuation of T cell transfer colitis.

How can this help patient care?

- Targeting B cell pathways—not just suppressing them—could correct dysbiosis and offer novel therapies for chronic inflammation in IBD patients.

responses in immune-mediated diseases have also been recognized.[4] These regulatory B cells (Bregs) exert their suppressive function through production of anti-inflammatory cytokines, primarily IL-10 and transforming growth factor β (TGF-β), although interleukin (IL)-35/27–producing Bregs have been shown to ameliorate colitis by inhibiting the proliferation of effector T cells. Additionally, Bregs in mesenteric lymph nodes (MLNs) may recruit T regs, CD8$^+$ T cells, and natural killer T cells.[5]

Our laboratory and others have recently shown that B cells are critically dependent on integrin $\alpha4\beta7$-MAdCAM-1 interactions for intestinal migration, as $\beta7$-deficiency and anti-MAdCAM-1 antibody blockade rendered the lamina propria (LP) of IL-10$^{-/-}$ mice B cell/IgA deficient, resulting in a luminal IgA deficit, decreased bacterial IgA coating, changes in microbiota composition, and acceleration of colitis.[6,7] These findings confirm the known critical role of B cell–derived secretory IgA (SIgA) for the maintenance of homeostasis between the microbiota and its host.[3] The interrelationship between the microbiota and IgA is highlighted by the fact that gnotobiotic mice have marked IgA deficiency[8] and IgA is rapidly restored upon bacterial colonization.[9] SIgA controls the proliferation of pathobionts within gut microbial communities, and in its absence pathobionts proliferate.[10,11] Furthermore, mice lacking the enzyme activation-induced cytidine deaminase, which mediates IgA class switch recombination and somatic hypermutation,[12] develop bacterial overgrowth with expansion of *Clostridial* species, including segmented filamentous bacteria.[13,14] Other studies have shown that IgA-coated bacteria isolated from patients with IBD confer the greatest susceptibility to colitis, when transplanted into germ-free mice,[15] suggesting a pathogenic role for this subset.

Although B cell–derived SIgA is one of the main mechanisms through which homeostasis between microbiota and its host is maintained, our understanding of the interface between B cells, IgA, and commensal microflora during initiation or progression of IBD is limited. The T cell transfer colitis model is a widely used subacute model of IBD, generated by transfer of regulatory T cell (Treg)–depleted naive (CD45RB^{high}) CD4$^+$ T cells into T/B cell deficient RAG1,2$^{-/-}$ or SCID mice. Cotransfer of Treg-containing CD45RB^{low} cells attenuates colitis through their production of TGF-β and IL-10.[16] In this model, unfractionated B cells also attenuated colitis, an effect that has been attributed to Breg-derived IL-10 and IL-35/27.[5,17,18]

Here, we examined whether B cells may additionally regulate T cell transfer colitis through a mechanism independent of Bregs. First, we demonstrate the capacity of splenic B cells from B6 mice to reconstitute the B cell compartment of RAG1$^{-/-}$ mice, class switch to IgA, migrate to colonic LP, and partially restore luminal SIgA. Next, we show a protective effect of cotransferred B cells on the severity of colitis in recipient mice that is independent of putative Breg-derived cytokines (ie, TGF-β, IL-10, IL-35/27 subunits [IL-12A/p35, EBI3]). In contrast, a potential regulatory role for SIgA was demonstrated, as transfer of IgA-deficient B cells did not attenuate colitis. Finally, we provide evidence for microbiota-mediated mechanisms, as bacterial IgA coating, fecal bacterial counts, and microbial community composition was substantially different between recipient mice that received wild-type C57BL/6J (B6) B cells or IgA-deficient B cells.

Methods

Mice

Rag1$^{-/-}$ on C57BL/6 background (stock #2216) and C57BL6/J (B6) mice (stock #664) were purchased from the Jackson Laboratory and bred in-house. IgA-deficient mice, also on B6 background, were first described by Mbawuike[19] and were a kind gift from Lars Eckmann (University of California San Diego). Homozygosity for the mutations was confirmed via genotyping (Transnetyx), flow cytometry, and serum/stool IgA enzyme-linked immunosorbent assay. All animal studies were performed under Institutional Animal Care and Use Committee–approved protocols.

CD45Rb^{high} colitis induction and B cell cotransfer

Murine splenocytes were isolated via mechanical dissociation of whole spleen, followed by red blood cell (RBC) lysis using RBC lysis buffer (1X) (BioLegend) according to the manufacturer's protocol. Splenic CD4$^+$ T cells from B6 mice and CD19$^+$ B cells from B6 and IgA$^{-/-}$ mice were enriched by positive selection with anti-mouse CD4 microbeads (130-117-043; Miltenyi Biotec) or enriched with negative selection using a B Cell Isolation Kit (130-090-862; Miltenyi Biotec) and further enriched with positive selection using anti-mouse CD19 microbeads (130-121-301;

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Miltenyi Biotec) respectively, as per the manufacturer's instructions. B6 $CD4^+$-enriched T cells (≥95% purity) were incubated with anti-CD4 (FITC/Clone GK1.5) and anti-CD45RB (PE/Clone C363.16A) for 30 minutes and washed, and $CD4^+$ $CD45Rb^{high}$ cells (top 10%-15% of total) were isolated by flow cytometric sorting using a FACSAria Sorter (BD Biosciences). Isolated cells were injected intraperitoneally into $RAG^{-/-}$ recipients (2.5×10^5). Magnetically enriched $CD19^+$ B cells from B6 or $IgA^{-/-}$ mice (≥95% purity; 2×10^6), were suspended in 200 µL of PBS and injected intraperitoneally into 8-week-old $RAG^{-/-}$ recipients that also received naive $CD4^+$ T cells. Tissues were harvested once mice receiving $CD4^+$ T cells had lost 20% of initial body weight (around 6 weeks) as per the University of California San Diego Institutional Animal Care and Use Committee–approved protocol. Mouse weights and temperatures were recorded weekly after cell transfer.

Tissue collection and histological evaluation

Tissues were prepared for histological analysis after fixation in 10% neutral buffered formalin (Thermo Fisher Scientific) for 24 hours, followed by 70% ethanol (VWR); embedded in paraffin; cut into vertical 4-μm-thick sections; and stained with hematoxylin and eosin and histological assessment of inflammation was performed using a standardized semiquantitative scoring system[20] by a pathologist (P.J.), blinded to the experimental conditions. Results were not separated for female and male mice, as we have observed no differences in disease severity between the sexes.

Fecal/serum IgA determination

Blood was obtained via submandibular vein bleed or via cardiac puncture bleed during necropsy into EDTA-containing vials. Plasma was removed after centrifugation and stored at −80° C for later analyses.

Fecal pellets were vortexed in 0.5 mL of phosphate-buffered saline (PBS) for 10 minutes and centrifuged at 12 000 *g* for 10 minutes twice, and supernatant was collected for fecal Ig level determination. Plasma was diluted at 1:10 000 and fecal supernatant was diluted at 1:100 for analysis. Fecal IgA levels were normalized per mg of total fecal protein content, as determined by Bradford Protein Assay (Bio-Rad). Absorbances were read using a BioTek Synergy Mx Microplate Reader and analyzed using the BioTek Gen5 software.

LP mononuclear cell isolation

Colonic LP cells were obtained as previously described.[6] In brief, colonic tissues were washed for 15 minutes at room temperature in 15 mL Hanks' balanced salt solution (HBSS) (Thermo Fisher Scientific), followed by 3 washes in HBSS + 1 mM EDTA (Hoefer), followed by one wash in HBSS, to remove the epithelial cells. Tissues were then subject to mechanical dissociation and digestion for 15 to 45 minutes, in a solution containing complete RPMI + 1.5 mg/mL collagenase VIII and 1 μM DNase (Sigma-Aldrich). Resulting cell suspensions were filtered and utilized for downstream analyses.

Flow cytometry

Splenocytes were isolated via mechanical dissociation of whole spleens from experimental mice, followed by RBC lysis using RBC lysis buffer (1X) (BioLegend) according to the manufacturer's protocol. Lymphocytes were obtained from MLNs through mechanical disruption. The resulting cell suspensions were filtered through a 70 µm cell strainer (Corning) to remove debris and centrifuged at 650 *g* for 6 minutes at 4°C in RPMI Medium 1640 (1X) (Gibco). Cells were subsequently used for downstream analyses.

Viability staining was conducted using fixable Viability Dye eFluor 780 (Thermo Fisher Scientific) according to the manufacturer's instructions, and antibody staining was performed using the following antibodies: CD4 FITC (clone GK1.5), CD19 Alexa Fluor 700 (clone 6D5), IgM BV510 (clone RMM-1), IgD BV650 (clone 11-26c.2a) (BioLegend), and IgA PE (clone mA-6E1) (Invitrogen). Following staining, cells were fixed for later acquisition using 4% paraformaldehyde solution diluted to 2% using 1× PBS (Thermo Fisher Scientific) and acquired on a Cytek Northern Lights cytometer. Analyses were performed using FlowJo v10.1 (TreeStar).

Definition of B cell subsets

B lineage populations were defined based on CD19 expression, with near-complete concordance observed between CD19 and B220 in our datasets (Figure S1). Surface IgD^+ and IgM^+ populations were classified as non–class-switched B cells. IgA^+ populations were identified based on surface IgA expression and may represent antibody-secreting/plasmablast-enriched populations. We note that classical plasma cell markers such as CD138 and $B220^{low/-}$ were not used to strictly define terminally differentiated plasma cells; therefore, we use conservative terminology when referring to these populations.

Immunofluorescence

The 4-μm paraffin sections were deparaffinized and rehydrated, followed by antigen retrieval in citrate buffer using a Biocare Medical decloaking chamber. After cooling and PBS-T (phosphate-buffered saline-TWEEN-20) washes, sections were mounted in Shandon cover plates and blocked with 5% normal donkey serum containing 0.3% Triton X-100 in PBS for 1 hour at room temperature. Slides were incubated overnight at 4°C with goat anti-mouse IgA (1:4000; SouthernBiotech; Cat: 1040-01) and rat anti-mouse CD4 (1:100; Thermo Fisher Scientific; Cat: 14-9766-82) in PBS-T. After washing, sections were incubated for 1 hour at room temperature with donkey anti-goat IgG Cy5 (1:500; Jackson ImmunoResearch; Cat: 705-175-147) and donkey anti-rat IgG Alexa Fluor 555 (1:500; Thermo Fisher Scientific; Cat: A48270). Nuclei were counterstained with Hoechst (1:1000 in PBS, 10 mg/mL stock) for 5 min. Fluorescent bleaching was performed in 4.5% H_2O_2 and 24 mM NaOH under light exposure, and slides were mounted using ProLong Gold Antifade Mountant (without DAPI) and laid flat in the dark for at least 24 h. Image acquisition was performed with a Zeiss LSM780 confocal microscope.

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Quantification of fecal bacteria

A Bacteria Counting Kit for flow cytometry (Cat: B7277; Thermo Fisher Scientific) was used according to the manufacturer's guidelines. Events were acquired on a Cytek Northern Lights cytometer. Analyses were performed using FlowJo v10.1.

Microbiota analysis

Analyses of fecal microbiota composition were performed by the Transnetyx Microbiome service. Stool pellets were collected in sample collection tubes provided with DNA stabilization buffer and genomic DNA was extracted with the Qiagen DNeasy 96 PowerSoil Pro QIAcube HT extraction kit and protocol. Library preparation was done with KAPA HyperPLUS preparation protocol and the sequencing was done with Illumina NovaSeq instrument and protocol (depth of 2 million 2×150 bp read pairs). Sequencing data were uploaded and analyzed using One Codex.[21] Genome count tables from One Codex were filtered to OTUs operational taxonomic units at >0.1% abundance in at least 5% of all samples (across all transfer groups) leaving 251 genomes. Ordination was performed by computing Aitchison distances followed by principal coordinates analysis using the ape package.[22] Differential abundance was fit with DESeq2 c (v1.48.2)[23] on raw counts with design ~'transfer group' and performing pairwise contrasts between groups. Figures of the relative abundance of bacterial taxa at the order level and beta diversity using principal coordinate analysis were performed and colors modified using R27 (v4.4.0)[24] and RStudio28 (v2024.4.0.735)[25] with the ggplot2 package (v3.5.1).[26]

Real-time quantitative polymerase chain reaction

Colonic tissues were harvested and stored at −80 °C in RNAlater solution (Thermo Fisher Scientific). RNA was isolated using the RNeasy Mini Kit. cDNA was generated using the high-capacity cDNA reverse transcription kit (Applied Biosystems). Gene expression was examined using the TaqMan probes (Thermo Fisher Scientific) TGF-β (Tgif1, Mm01227699_m1), EBI3 (Mm00469294_m1), and IL12A (Mm00434169_m1), and normalized to GAPDH (Mm99999915_g1).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 software (GraphPad Software). Column statistics tests were used to assess parametric distribution of datasets. For comparison of two groups, the Wilcoxon matched-pairs signed rank test was utilized, or the Mann-Whitney test for unpaired samples. For comparison of multiple groups, analysis of variance was utilized for parametric datasets, followed by Tukey's multiple comparison test, and the Kruskal-Wallis test was used for nonparametric datasets, followed by Dunn's multiple comparison test. Descriptive statistics are displayed as mean ± SD in all figures. Significance is defined as P values of $<.05$, and resulting statistical significances of difference are indicated in figures as $P < .05$, $P < .01$, $P < .001$, $P < .0001$.

Results

Cotransfer of splenic B cells and naive CD4$^+$ T cells partially reconstitute the peripheral and mucosal B cell compartments in recipient RAG1$^{-/-}$ mice

Spleen-derived CD19$^+$ B cells (near CD19/B220 concordance shown in Figure S1) restore luminal SIgA and bacterial IgA coating in RAG1-deficient mice that lack functional T or B cells. However, the specific antibody-secreting cell (ASC) subsets that repopulate the peripheral and colonic LP compartments after splenic B cell transfer were not examined. In these studies, we specifically looked for the ASC subsets in recipient mice after B cell transfer via flow cytometry. Because T cell transfer inflammation is colitis predominant without appreciable ileitis, we focused LP analyses on the colon, the principal affected site. We found that surface IgD$^+$ (sIgD$^+$) and sIgM$^+$ B cell populations were partially restored (~5% of CD19$^+$) in the spleen of T/B cell–reconstituted RAGs, compared with robust populations present in immunocompetent B6 mice (~32%-34%) (Figure 1A). However, these cells were rare in the colonic LP of RAG mice, in which only sIgA$^+$ B lineage cells were detected within the CD19^{neg} fraction, consistent with progressive differentiation toward ASCs/plasma cells (Figure 1B). B lineage cells downregulate CD19 and B220 as they differentiate toward mature IgA-secreting plasma cells. Immunofluorescent staining for IgA (magenta) in colonic segments confirmed the presence of sIgA$^+$ cells in LP of cotransferred RAG1$^{-/-}$ mice (Figure 1D). The sIgD$^+$ population likely represents naive or non–class-switched B cells. To confirm that partial reconstitution of LP sIgA B lineage cells leads to variable reconstitution of SIgA in the intestinal lumen, we measured SIgA levels in stool or T/B cell cotransferred RAG1$^{-/-}$ mice via enzyme-linked immunosorbent assay. We found a wide range of IgA levels clustering in the lower range (5 ng/mg) compared with ~16 ng/mg in B6 mice (Figure 1C). Of note, the stool concentration of IgA in ileocolitic TNFΔARE mice was several fold higher than that of noncolitic B6 mice, which have a normal complement of B cells (not shown). Cotransfer of T cells was required for LP and luminal IgA reconstitution, as transfer of splenic B cells alone did not restore peripheral or colonic ASCs, nor luminal SIgA (not shown). CD19/B220$^+$ B cells were reconstituted in secondary lymphoid organs (ie, spleen, MLNs) and in bone marrow (Figures S2A-S2C). Taken together, our findings show that B6 splenic B cells that are cotransferred with naive CD4$^+$ cells to RAG1$^{-/-}$ mice contain B cell populations capable of bone marrow, peripheral, and mucosal reconstitution, class switching to IgA ASCs and migrationto the colonic LP. Once in LP, they partially restore sIgA$^+$ B lineage cells and luminal SIgA.

Cotransfer of B cells attenuates the severity of T cell transfer colitis without tissue upregulation of Breg-derived cytokines

Prior studies have shown that cotransfer of splenic B cells and peritoneal regulatory B10 cells (which produce IL-10) attenuated

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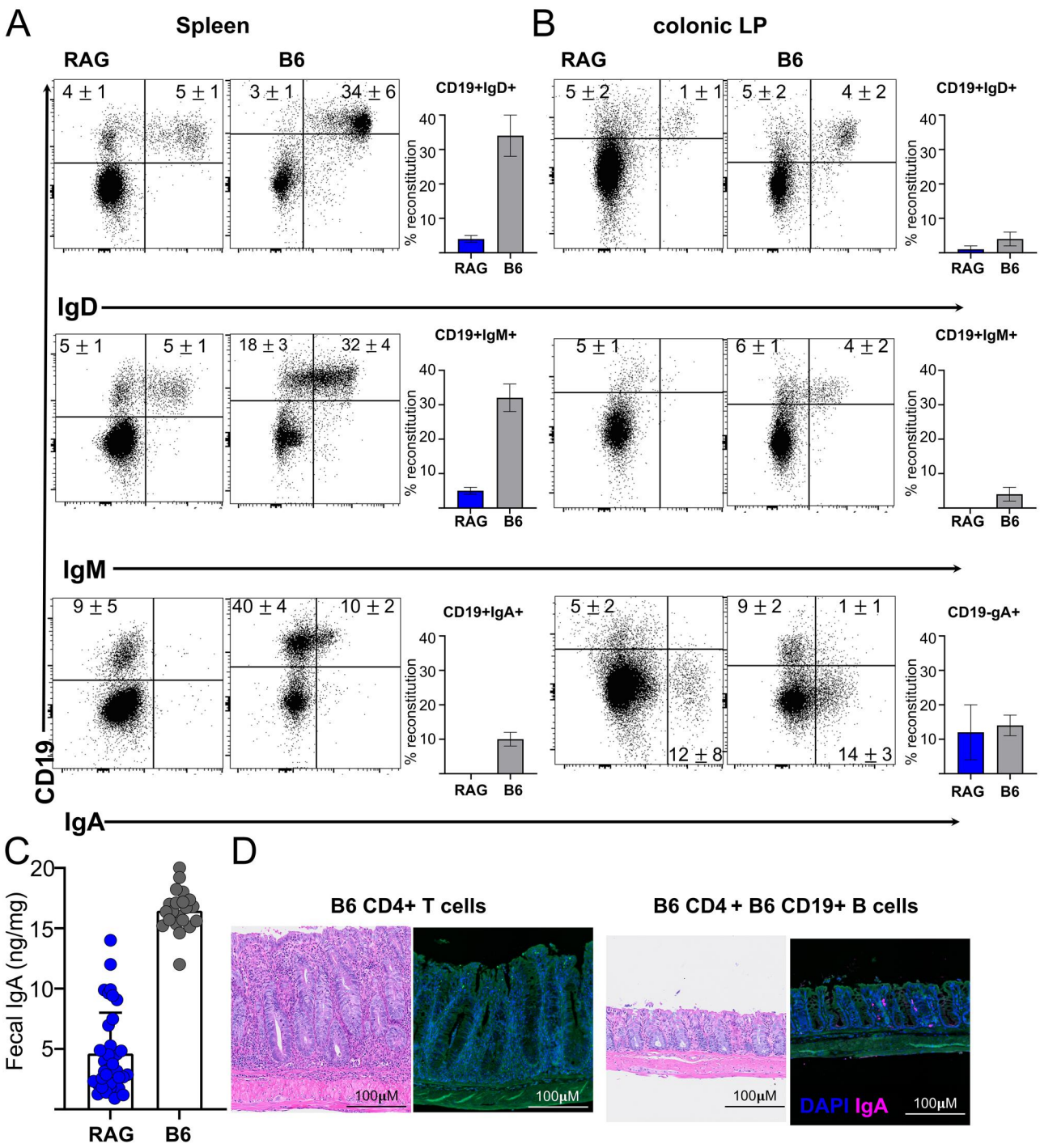


Figure 1 Splenic CD19$^+$ B cell cotransfer partially reconstitute peripheral and colonic surface IgD/M , IgA$^+$ B lineage cells in the lamina propria (LP) and luminal secretory IgA in RAG1-deficient mice. (A, B) Representative flow cytometry dot plots and quantification of (A) spleen and (B) colonic LP cells and surface IgD/M/A$^+$ B lineage cells isolated from RAG-1$^{-/-}$ mice compared with immunocompetent B6 controls as indicated. (C) Fecal IgA levels measured by enzyme-linked immunosorbent assay in reconstituted RAGs and B6 controls. (D) Representative micrographs (hematoxylin and eosin 10× magnification) and immunofluorescence staining of colonic tissue show architectural changes and confirmed presence of LP IgA$^+$ antibody-secreting cells. Data are representative of two independent experiments with n ≥ 5 mice per group and are shown as mean ± SD.

colitis induced by colitogenic Treg-depleted naive CD4$^+$ T cells.[17] Furthermore, B cells from IL-10$^{-/-}$ mice did not protect from colitis, suggesting that IL-10-producing Bregs were responsible for attenuation.[5,17] Here, we confirmed the ability of unfractionated splenic CD19/B220$^+$ B cells to attenuate clinical signs of colitis, such as weight loss ($P < .0001$) and hypothermia, ($P < .01$), compared with mice that received only naive CD4$^+$ T cells (Figures 2A and 2B). Blinded histological evaluation of colonic segments further confirmed attenuation of colitis with decreased acute (eg, neutrophilic), chronic (lymphocyte/monocyte), and total (sum of

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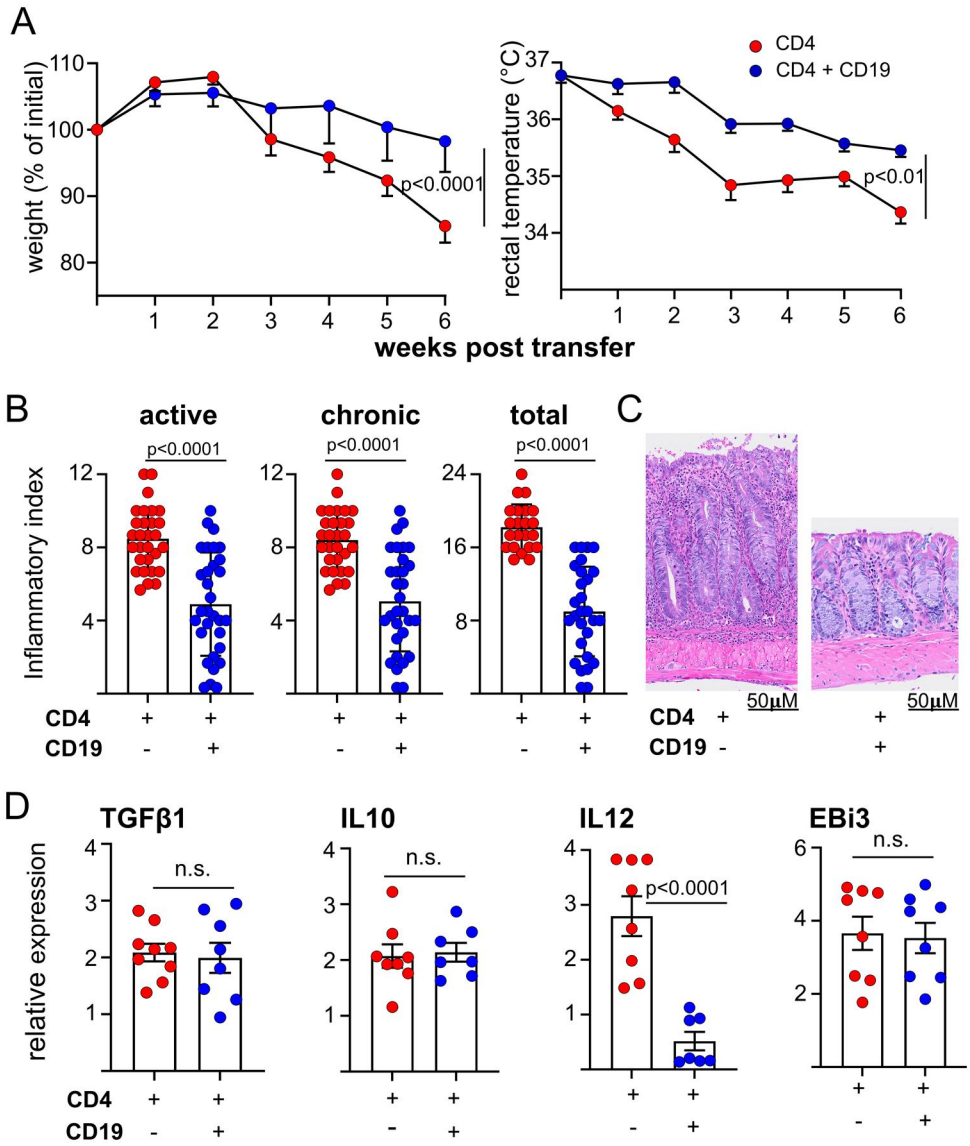


Figure 2 Cotransfer of B6 CD19^{+} B cells attenuated clinical signs and histological severity of T cell–induced colitis without upregulation of putative regulatory B cell–derived cytokines. (A) Body weights and rectal temperatures were monitored weekly in RAG1$^{-/-}$ mice transferred with naive CD4^{+}CD45RB^{Hi} (CD4) T cells, with or without wild-type CD19^{+} B cells ($n \geq 10$ per group). (B) Inflammatory indices of colitis assessed by blinded histological evaluation of hematoxylin and eosin–stained colon tissue ($n \geq 20$ per group, pooled from 3 independent experiments). (C) Representative micrographs (hematoxylin and eosin–stained tissue, 10× magnification) illustrate colonic architecture and inflammatory infiltrates. (D) Relative messenger RNA expression of regulatory cytokines (transforming growth factor β [TGF-β], interleukin [IL]-10) and shared subunits of IL-27/IL-35 (EBI3) and P35 (shared by IL-12/IL-35) quantified by quantitative real-time polymerase chain reaction in colonic tissue ($n \geq 8$ per group). Each data point represents an individual mouse. All data are shown as mean ± SD. Statistical significance was determined by analysis of variance followed by Tukey's multiple comparison test. n.s., not significant.

acute and chronic) inflammatory indices in mice cotransferred with B6 B cells, compared with mice receiving only T cells (all *P* values $<.0001$) (Figure 2B). We also observed decreased hypertrophy of both mucosa and muscularis in mice receiving B6 B cells (Figure 2C; Figure S1). Induction of colitis by naive CD4^{+} T cells and attenuation of colitis by B cells are dependent on engraftment of both CD4 and CD19 in bone marrow, from where they migrate to peripheral sites such as the spleen and MLNs. Thus, we examined T and B cell reconstitution in bone marrow and peripheral lymphoid sites (ie, spleen, MLNs) as internal controls. As expected, we found only CD4^{+} in mice receiving sorted naive CD4, whereas we additionally found CD19^{+} B cells in bone marrow, spleen, and MLNs after cotransfer of splenic B cells (Figure S1). Thus, transferred splenic T and B cells engraft in recipient mice and reconstitute bone marrow and peripheral lymphoid compartments, from which they populate secondary lymphoid tissues and give rise to sIgD^{+} and sIgM^{+} B cell populations as well as sIgA^{+} antibody-secreting/plasmablast populations. The IgA^{+} ASCs also acquire a homing program, migrate to colonic LP, and partially reconstitute luminal SIgA.

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To examine the contribution of Breg-derived cytokines to the modulation of colitis, we quantified whole tissue colonic messenger RNA (mRNA) transcripts of putative cytokines known to mediate Breg function (Figure 2D). These include TGF-β, IL-10, the EBI3 subunit shared by IL-27 and IL-35, and the P35 subunit shared by IL-12 and IL-35, all previously implicated in attenuation of colitis by Bregs.[18] Neither TGF-β nor IL-10 mRNA transcripts were increased in mice receiving B cells under our experimental conditions. Similarly, EBI3 transcripts were not affected, whereas mRNA transcripts of the P35 subunit of IL-12 were decreased in mice receiving B6 B cells, consistent with decreased IL-12 in the setting of attenuated colitis. Overall, these data show that cotransfer of B cells attenuate the clinical and histological severity of colitis without tissue upregulation of putative Breg-derived cytokines under our experimental conditions. Thus, the current findings may suggest that modulatory mechanisms other than Breg-derived cytokines contribute to the attenuation of T cell transfer colitis by B cells.

IgA-deficient B cells do not attenuate T cell transfer colitis

B cells mature into professional ASCs and plasma cells in the intestinal LP, where they produce large quantities of IgA. LP IgA binds to PIgR and is transcytosed to the lumen as SIgA, a fraction of which is retained within the extensive mucus layer, serving as a primary local barrier that keeps bacteria away from intestinal epithelium (immune exclusion). This way, IgA (or equivalent molecules in lower vertebrates) is critical for the maintenance of a homeostatic communication between vertebrates and their commensal microbiota. Our current and prior findings in IL10$^{-/-}$ mice[6,27] show that both integrin β7 deficiency and MAdCAM-1 blockade hastened colitis by interfering with ASC migration to intestinal LP, resulting in a LP sIgA^{+} B cell deficit, decreased luminal SIgA, bacterial overgrowth, and dysbiotic bacterial communities.[6]

To directly assess the role of IgA on the attenuation of transfer colitis by B cells, we compared the effects of cotransfer of IgA-sufficient or IgA-deficient B cells on the clinical and histological severity of colitis (Figure 3). Mice receiving naive T cells developed clinically evident colitis by 5 to 6 weeks post-transfer, reflected clinically by weight loss and hypothermia. Splenic B cells from B6 mice exhibited less clinical signs of colitis (Figures 3A and 3B) compared with mice receiving only T cells. By contrast, these were not different from mice receiving only T cells, if B cells were isolated from IgA-deficient mice (Figures 3A and 3B). Clinical evaluation paralleled blinded histopathological evaluation of colitis, as we observed attenuation of active, chronic, and total indices in mice receiving B6 B cells but not in mice that received IgA-deficient B cells (Figures 3C and 3D). Similarly, the distorted colonic architecture (mucosal and muscularis hypertrophy), which is partially restored by B6 cells, was similar between mice receiving T cells only and those cotransferred with IgA$^{-/-}$ B cells (Figure 3D). Ineffective attenuation of colitis was not due to inadequate reconstitution of CD19^{+} cells in spleen, where the percentage of IgA-deficient CD19^{+} cells was comparable to that of mice receiving B6 B cells (Figure 3E). As expected, colonic sIgA^{+} B cells and luminal and serum IgA were absent in mice receiving IgA$^{-/-}$ B cells (Figures 3E and 3F). Taken together, under our experimental conditions, SIgA appears to be a critical molecular effector for the B cell–mediated attenuation of T cell transfer colitis.

Intact IgA production is required for beneficial remodeling of intraluminal microbiota

Prior studies have shown that colonization with a restricted colonic microbiota and administration of oral antibiotics attenuated T cell transfer colitis.[28–30] Thus, the microbiota appears to play a fundamental role on the initiation and perpetuation of colitis in this model. Given the known role of SIgA for the control of intestinal microbiota, we next examined the effects of splenic B cells isolated from control B6 or IgA-deficient mice on IgA coating of fecal bacteria, bacterial counts by flow cytometry, and stool microbiota composition via shotgun metagenomic analyses. As expected, whereas around 10% to 30% of stool bacteria were IgA bound after B6 B cell cotransfer, we found no IgA-coated fecal bacteria in RAG1$^{-/-}$ mice receiving only CD4^{+} T cells or cotransferred with splenic B cells from IgA-deficient mice (Figures 4A and 4B). Similarly, while B6 B cells induced a decrease in stool bacterial counts, IgA-deficient B cells did not modulate bacterial overgrowth in colitic RAG-1$^{-/-}$ mice (Figure 4C). Importantly, the microbiota of mice receiving IgA-deficient B cells closely resembled that of mice receiving CD4^{+} T cells alone, indicating that IgA-deficient B cells do not significantly modulate microbial composition in this model.

We then analyzed microbial community composition via shotgun metagenomics in recipient mice that received B cells from different donors. We showed that mice receiving B6 B cells had a vastly different microbiota from that of mice receiving only T cells, whereas the microbiota of recipients of IgA-deficient B cells was not significantly altered (Figures 4D and 4E).

Metagenomic analyses of stool from RAG1$^{-/-}$ mice receiving only CD4^{+} T cells revealed distinct microbial communities across groups (PERMANOVA, $R^2 = 0.31$, $P = .0002$) (Figure 4D). Cotransfer of B6 B cells increased overall diversity, which was significantly higher than in CD4^{+} T cell–only recipients (Mann-Whitney *U*, $P = .0087$) and mice receiving IgA$^{-/-}$ B cells (Mann-Whitney *U*, $P = .024$) (Figure 4E). Differential abundance analysis (DESeq2) identified an expansion of 32 *Eubacteriales* species and 30 *Lachnospirales* species in mice cotransferred with B6 B cells (Figures S3A and S3B). Within these orders, Eubacteriaceae and Oscillospiraceae showed marked increases (10.6-fold and 10.2-fold, respectively), both of which are probionts linked to butyrate production and mucosal homeostasis (Figure 4F).

Thus, our findings support the hypothesis that splenic B cells, reconstitute intestinal sIgA^{+} B lineage cells and luminal SIgA, which in turn leads to modulation of bacterial counts and remodeling of microbial communities, selectively enriching for beneficial commensals in colitic RAG1$^{-/-}$ mice. In turn, these beneficial effects on microbiota composition appear to play a major role on the attenuation of T cell transfer colitis by splenic B cells.

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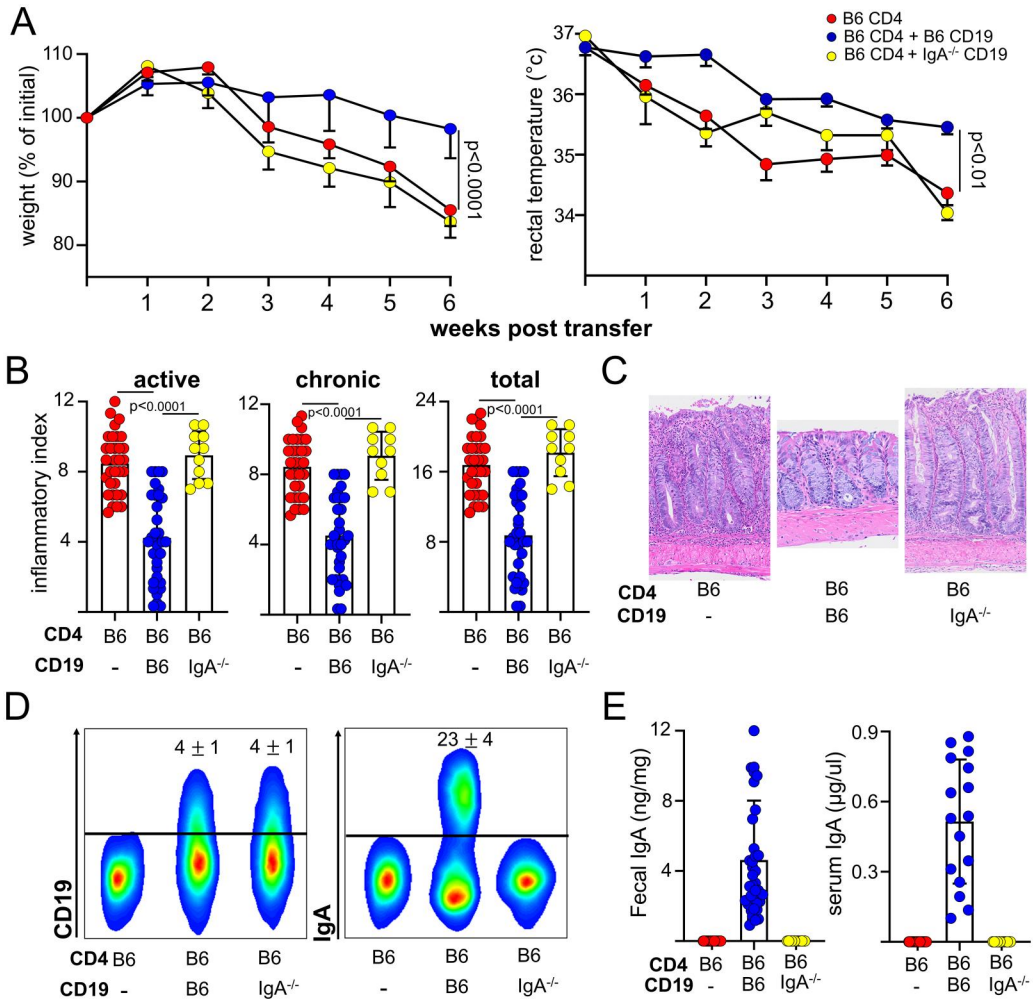


Figure 3 Cotransfer of IgA-deficient ($IgA^{-/-}$) $CD19^+$ B cells did not attenuate clinical signs or histological severity of colitis, despite similar reconstitution of peripheral B cells. (A) Body weights and serial temperatures monitored weekly in mice transferred with naive $CD4^+$ T cells, with or without B6 or $IgA^{-/-}$ $CD19^+$ B cells ($n \geq 10$ per group; $IgA^{-/-}$ group, $n = 10$). (B) Histopathological indices of colitis severity assessed by blinded histological analyses of hematoxylin and eosin–stained colon tissue (mean ± SD, $n \geq 10$ to 20 mice (pooled from 3 independent experiments). (C) Representative micrographs of indicated experimental groups (hematoxylin and eosin, 10× magnification). (D) Flow cytometry dot plots of $CD19^+$ B cell and surface IgA^+ B lineage cell reconstitution in spleen (left) and IgA^+ antibody-secreting cell reconstitution in the colonic lamina propria (right) ($n \geq 5$ per group). (E) Fecal and serum IgA levels were measured by enzyme-linked immunosorbent assay in $RAG1^{-/-}$ mice transferred with the indicated cell types (mean ± SD, $n \geq 10$ to 20 mice per group (pooled from 3 independent experiments). Each data point represents an individual mouse. Statistical significance was determined by analysis of variance followed by (A, B) Tukey's multiple comparison test or (E) Student's *t* test.

Discussion

In this study, we identified an IgA-dependent mechanism by which B cells attenuate T cell transfer colitis in immunodeficient $RAG1^{-/-}$ mice. We show that splenic B cell cotransfer restores bone marrow B cells, mucosal $sIgA^+$ B lineage cells partially reconstitutes luminal SIgA, and is associated with reduced disease severity and remodeling of the intestinal microbiota. Importantly, this protective effect occurs without sustained upregulation of canonical Breg-associated cytokines, supporting a regulatory pathway distinct from previously described IL-10– or IL-35–mediated mechanisms.[17,18]

The T cell transfer colitis model has been instrumental in defining immune regulatory pathways in intestinal inflammation.[16] In this system, colitis induced by naive $CD4^+CD45RB^{high}$ T cells can be suppressed by regulatory T cells through IL-10– and TGF-β–dependent mechanisms.[31,32] Prior studies have also shown that unfractionated splenic or peritoneal B cells attenuate colitis, an effect largely attributed to IL-10–producing Bregs, including peritoneal B10 cells.[17,29] Additional work implicated IL-27/IL-35 signaling in B cell–mediated induction of Tr1 cells.[5,18,33] Our data suggest that B cell–mediated protection is not limited to these pathways. Because IL-10–, TGF-β–, and IL-35/IL-27–related transcripts were measured in bulk colonic tissue, these data cannot exclude cell-specific Breg activity or transient cytokine induction at earlier time points. Rather, they indicate that canonical Breg-associated cytokines were not detectably upregulated in whole colonic tissue at the endpoint under our experimental conditions. In contrast, the failure of IgA-deficient B cells to attenuate colitis, together with IgA-dependent

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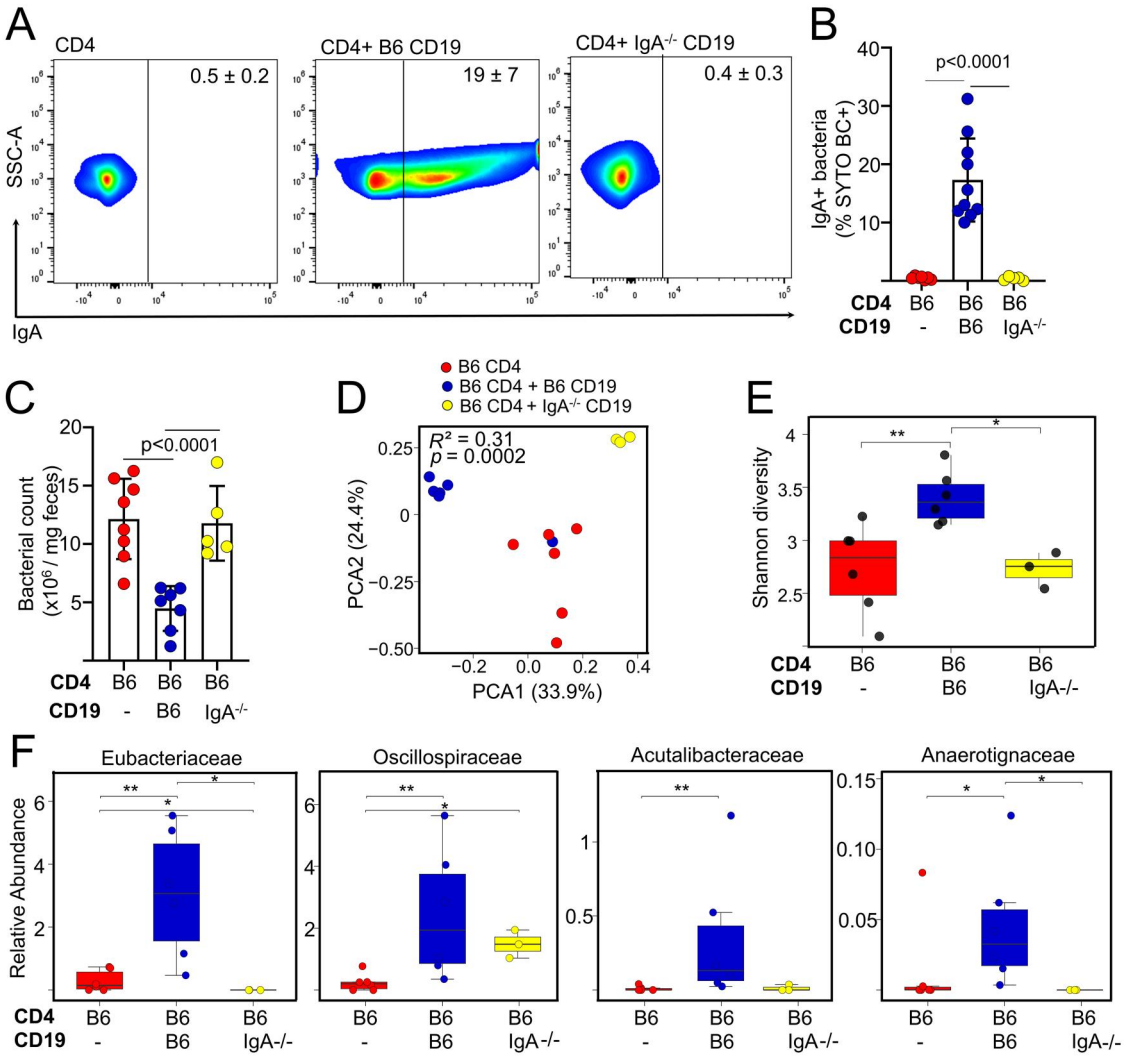


Figure 4 IgA-sufficient but not IgA-deficient B cells restored bacterial IgA coating, decreased bacterial counts, and modulated stool microbial communities. (A, B) Representative dot plots and quantification of IgA-coating from fecal bacteria from indicated mouse cohorts assessed by flow cytometry. (C) Bacterial counts in indicated mouse cohorts quantified via flow cytometry. (D) Principal component analysis (PCA) of Bray-Curtis distances showing distinct community structures across groups. (E) Shannon diversity indices demonstrate IgA-dependent increases in diversity with wild-type B cell transfer. (F) Relative abundance (%) of major Eubacteriales families within recipient mice. *$P < .05$; **$P < .01$. All data are presented as mean ± SD, from n = 3 to 5 mice per group. Each data point represents an individual mouse. Statistical significance was determined using 1-way analysis of variance or Mann-Whitney *U* test. SSC-A, side scatter area.

restoration of bacterial coating and remodeling of microbial communities, supports an additional IgA-dependent mechanism of B cell–mediated protection. Although IgA-deficient B cells reconstituted peripheral CD19⁺ B cell compartments, we did not comprehensively assess whether IgA deficiency altered differentiation or recruitment of IgA⁻ plasma cell subsets to the colonic LP. Thus, we cannot exclude effects of IgA deficiency on plasma cell fate or homing, although the absence of luminal IgA and bacterial IgA coating supports a requirement for intact IgA-mediated effector function.

Multiple lines of evidence from our study instead support a central role for IgA. First, cotransfer of splenic B cells resulted in selective repopulation of the colonic LP with class-switched CD19⁻ sIgA⁺ B lineage cells and partial restoration of luminal SIgA, consistent with prior reports demonstrating the capacity of splenic B cells to reconstitute mucosal IgA compartments in lymphopenic hosts.[34–36] Inclusion of additional markers such as CD138 and B220 would have further refined these populations and note this as a limitation. Second, splenic B cells isolated from IgA-deficient mice failed to attenuate colitis despite effective peripheral B cell reconstitution, demonstrating that B cell engraftment alone is insufficient for protection. Third, restoration of SIgA was accompanied by increased bacterial IgA coating, reduced fecal bacterial burden, and an altered microbial community composition.

The compartmentalization of B cell differentiation observed in this study is notable. While sIgM⁺ and sIgD⁺ B lineage cells predominated in secondary lymphoid tissues, sIgA⁺ B lineage cells selectively populated the colonic LP, consistent with tissue-specific differentiation and homing programs described in prior studies.[6,34] The absence of CD19/B220⁺ B cells in the LP of recipient mice likely reflects limited formation of inducible lymphoid

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follicles or tertiary lymphoid structures within the time frame of disease development, which typically require sustained chronic inflammation.[37] These findings emphasize the dominant contribution of IgA-secreting cells to mucosal immunity in this context.

Our microbiota analyses further support IgA-mediated regulation as a key downstream mechanism. A critical role for the microbiota in T cell transfer colitis is supported by earlier studies showing that restricted microbial colonization or antibiotic treatment attenuates disease severity.[28,30] In our model, cotransfer of IgA-sufficient B cells increased microbial diversity and promoted the expansion of taxa associated with mucosal homeostasis, including members of the Eubacteriales and Lachnospirales orders. These changes were absent in recipients of IgA-deficient B cells, indicating that IgA is required for both bacterial coating and selective enrichment of beneficial commensals. Although these data do not establish direct causality, they are consistent with extensive prior work demonstrating that SIgA constrains pathobiont expansion, promotes microbial diversity, and maintains host–microbiota homeostasis.[10,11,13,38] We acknowledge that IgA-coated bacteria may exhibit context-dependent pro- or anti-inflammatory properties, as described by Palm et al[15]; however, our findings support an overall protective role for IgA in this inflammatory setting. Although these findings support an IgA-dependent microbiota-modulatory mechanism, they do not identify the specific IgA-coated taxa or microbial products responsible for protection. Future studies using defined microbial consortia or transfer of IgA-coated bacterial fractions within the same T cell transfer framework may further clarify causality. Nevertheless, the failure of IgA-deficient B cells to attenuate colitis despite peripheral B cell reconstitution provides functional evidence that IgA is required for the protective effect of B cell cotransfer in this widely used colitis model.

Our findings integrate well with observations from other experimental models of IBD. In IL-10$^{-/-}$ mice, impaired IgA production or defective ASC homing leads to dysbiosis and exacerbated colitis.[6,27,39] Similarly, mice lacking activation-induced cytidine deaminase, which is required for IgA class switching, develop bacterial overgrowth and altered microbial communities.[13] Across two distinct inflammatory models, loss of IgA worsens disease, whereas restoration of B cell–derived IgA attenuates disease. Together, these studies support a model in which IgA availability becomes increasingly critical under inflammatory conditions, when regulatory demands exceed the capacity of baseline IgA synthesis.[40]

Clinically, B cells remain an underexplored component of IBD pathogenesis. Although alterations in B cell and plasma cell populations have been reported in patients with CD and ulcerative colitis,[41–46] therapeutic strategies targeting B cells have yielded mixed results. Notably, B cell depletion with rituximab failed to demonstrate efficacy in ulcerative colitis,[47] perhaps reflecting the loss of protective B cell functions, rather than a lack of B cell involvement. In humans, compensatory SIgM responses may partially offset IgA deficiency under homeostatic conditions,[19] but such compensation may be insufficient during chronic intestinal inflammation. A significant limitation of this study is the absence of human validation and the singular reliance on the T cell transfer model. Together with our prior findings in IL-10–deficient colitis,[6] in which impaired IgA ASC trafficking caused luminal IgA deficiency, dysbiosis, and worsened disease, the current data support a reciprocal model in which restoration of B cell–derived IgA remodels the microbiota and attenuates colitis.

In conclusion, we demonstrate that B cell cotransfer attenuates T cell transfer colitis through an IgA-dependent mechanism involving restoration of mucosal IgA-secreting cells, intraluminal SIgA, and IgA-mediated modulation of the intestinal microbiota. These findings expand the current paradigm of B cell–mediated regulation in intestinal inflammation and highlight IgA-secreting cells, alongside regulatory B cell subsets, as critical contributors to mucosal immune homeostasis in colitis.

Acknowledgments

The authors thank Shaila Yeasmin for technical assistance.

Author contributions

J.R.-N.: study concept and design; J.U., M.K., A.C., P.K., Z.S., M. H.: data generation; P.J.: histopathological analyses and critical review of manuscript; J.U., G.U., G.B., J.R.-N.: analysis, interpretation of data, and review of the manuscript.

Supplementary material

Supplementary data is available at *Inflammatory Bowel Diseases* online.

Funding

This work was supported by grants from the National Institute of Diabetes and Digestive and Kidney Diseases (R01DK132242, R01DK131532, K26DK138378, P30DK120515) and Veterans Administration Merit Award (BLRD-I01BX005951).

Conflicts of interest

The authors disclose no conflicts.

Data availability

The metagenomic sequencing data generated in this study have been deposited in the NCBI Sequence Read Archive and will be publicly released on publication. All other data underlying this article are available in the article and its online supplementary material.

References

1. Knights D, Lassen KG, Xavier RJ. Advances in inflammatory bowel disease pathogenesis: linking host genetics and the microbiome. *Gut*. 2013;62:1505-1510. https://doi.org/10.1136/gutjnl-2012-303954
2. Tyler AD, Milgrom R, Xu W, et al. Antimicrobial antibodies are associated with a Crohn's disease-like phenotype after ileal

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pouch-anal anastomosis. *Clin Gastroenterol Hepatol*. 2012; 10:507-512.e1. https://doi.org/10.1016/j.cgh.2011.09.016
3. Brandtzaeg P, Carlsen HS, Halstensen TS. The B-cell system in inflammatory bowel disease. *Adv Exp Med Biol*. 2006;579: 149-167. https://doi.org/10.1007/0-387-33778-4_10
4. Jansen K, Cevhertas L, Ma S, et al. Regulatory B cells, A to Z. *Allergy*. 2021;76:2699-2715. https://doi.org/10.1111/all.14763
5. Mishima Y, Liu B, Hansen JJ, et al. Resident bacteria-stimulated IL-10-secreting B cells ameliorate T cell-mediated colitis by inducing Tr-1 cells that require IL-27-signaling. *Cell Mol Gastroenterol Hepatol*. 2015;1:295-310. https://doi.org/10.1016/j.jcmgh.2015.01.002
6. Tyler CJ, Guzman M, Lundborg LR, et al. Antibody secreting cells are critically dependent on integrin alpha4beta7/MAdCAM-1 for intestinal recruitment and control of the microbiota during chronic colitis. *Mucosal Immunol*. 2022;15: 109-119. https://doi.org/10.1038/s41385-021-00445-z
7. Verstockt B, Verstockt S, Veny M, et al. Expression levels of 4 genes in colon tissue might be used to predict which patients will enter endoscopic remission after vedolizumab therapy for inflammatory bowel diseases. *Clin Gastroenterol Hepatol*. 2020;18:1142-1151.e10. https://doi.org/10.1016/j.cgh.2019.08.030
8. Macpherson AJ, Gatto D, Sainsbury E, et al. A primitive T cell-independent mechanism of intestinal mucosal IgA responses to commensal bacteria. *Science*. 2000;288: 2222-2226. https://doi.org/10.1126/science.288.5474.2222
9. Benveniste J, Lespinats G, Salomon J. Serum and secretory IgA in axenic and holoxenic mice. *J Immunol*. 1971; 107:1656-1662.
10. Brandtzaeg P. Secretory IgA: designed for anti-microbial defense. *Front Immunol*. 2013;4:222. https://doi.org/10.3389/fimmu.2013.00222
11. Stephens WZ, Round JL. IgA targets the troublemakers. *Cell Host Microbe*. 2014;16:265-267. https://doi.org/10.1016/j.chom.2014.08.012
12. Chandra V, Bortnick A, Murre C. AID targeting: old mysteries and new challenges. *Trends Immunol*. 2015;36:527-535. https://doi.org/10.1016/j.it.2015.07.003
13. Fagarasan S, Muramatsu M, Suzuki K, et al. Critical roles of activation-induced cytidine deaminase in the homeostasis of gut flora. *Science*. 2002;298:1424-1427. https://doi.org/10.1126/science.1077336
14. Hosoe N, Miura S, Watanabe C, et al. Demonstration of functional role of TECK/CCL25 in T lymphocyte-endothelium interaction in inflamed and uninflamed intestinal mucosa. *Am J Physiol Gastrointest Liver Physiol*. 2004;286:G458-G466. https://doi.org/10.1152/ajpgi.00167.2003
15. Palm NW, de Zoete MR, Cullen TW, et al. Immunoglobulin A coating identifies colitogenic bacteria in inflammatory bowel disease. *Cell*. 2014;158:1000-1010. https://doi.org/10.1016/j.cell.2014.08.006
16. Powrie F, Leach MW, Mauze S, et al. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol*. 1993;5: 1461-1471. https://doi.org/10.1093/intimm/5.11.1461
17. Maseda D, Candando KM, Smith SH, et al. Peritoneal cavity regulatory B cells (B10 cells) modulate IFN-gamma + CD4+ T cell numbers during colitis development in mice. *J Immunol*. 2013;191:2780-2795. https://doi.org/10.4049/jimmunol.1300649
18. Shen P, Roch T, Lampropoulou V, et al. IL-35-producing B cells are critical regulators of immunity during autoimmune and infectious diseases. *Nature*. 2014;507:366-370. https://doi.org/10.1038/nature12979
19. Mbawuike IN, Pacheco S, Acuna CL, Switzer KC, Zhang Y, Harriman GR. Mucosal immunity to influenza without IgA: an IgA knockout mouse model. *J Immunol*. 1999;162:2530-7.
20. Burns RC, Rivera-Nieves J, Moskaluk CA, et al. Antibody blockade of ICAM-1 and VCAM-1 ameliorates inflammation in the SAMP-1/Yit adoptive transfer model of Crohn's disease in mice. *Gastroenterology*. 2001;121:1428-1436. https://doi.org/10.1053/gast.2001.29568
21. Minot SS, Krumm N, Greenfield NB. One codex: a sensitive and accurate data platform for genomic microbial identification. bioRxiv 027607. https://doi.org/10.1101/027607, September 28, 2015. preprint: not peer reviewed.
22. Paradis E, Claude J, Strimmer K. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*. 2004;20: 289-290. https://doi.org/10.1093/bioinformatics/btg412
23. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15:550. https://doi.org/10.1186/s13059-014-0550-8
24. R Core Team. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing; 2024.
25. RStudio. Version 2024.4.0.735. Posit Software; 2024.
26. Wickham H. *Ggplot2: Elegant Graphics for Data Analysis*, 3rd ed. Springer-Verlag New York; 2016.
27. Sun H, Kuk W, Rivera-Nieves J, et al. beta7 integrin inhibition can increase intestinal inflammation by impairing homing of CD25(hi)FoxP3(+) regulatory T cells. *Cell Mol Gastroenterol Hepatol*. 2020;9:369-385. https://doi.org/10.1016/j.jcmgh.2019.10.012
28. Aranda R, Sydora BC, McAllister PL, et al. Analysis of intestinal lymphocytes in mouse colitis mediated by transfer of CD4+, CD45RBhigh T cells to SCID recipients. *J Immunol*. 1997;158:3464-3473.
29. Bouaziz JD, Yanaba K, Tedder TF. Regulatory B cells as inhibitors of immune responses and inflammation. *Immunol Rev*. 2008;224:201-214. https://doi.org/10.1111/j.1600-065X.2008.00661.x
30. Reinoso Webb C, den Bakker H, Koboziev I, et al. Differential susceptibility to T cell-induced colitis in mice: role of the intestinal microbiota. *Inflamm Bowel Dis*. 2018;24:361-379. https://doi.org/10.1093/ibd/izx014
31. Powrie F, Carlino J, Leach MW, et al. A critical role for transforming growth factor-beta but not interleukin 4 in the suppression of T helper type 1-mediated colitis by CD45RB(low) CD4+ T cells. *J Exp Med*. 1996;183:2669-2674. https://doi.org/10.1084/jem.183.6.2669
32. Powrie F, Read S, Mottet C, et al. Control of immune pathology by regulatory T cells. *Novartis Found Symp*. 2003;252: 92-98; discussion 98-105, 106–114.
33. Apetoh L, Quintana FJ, Pot C, et al. The aryl hydrocarbon receptor interacts with c-Maf to promote the differentiation of type 1 regulatory T cells induced by IL-27. *Nat Immunol*. 2010;11:854-861. https://doi.org/10.1038/ni.1912

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34. Bunker JJ, Bendelac A. IgA responses to microbiota. *Immunity*. 2018;49:211-224. https://doi.org/10.1016/j.immuni.2018.08.011
35. Bunker JJ, Erickson SA, Flynn TM, et al. Natural polyreactive IgA antibodies coat the intestinal microbiota. *Science*. 2017;358:eaan6619. https://doi.org/10.1126/science.aan6619
36. Bunker JJ, Flynn TM, Koval JC, et al. Innate and adaptive humoral responses coat distinct commensal bacteria with immunoglobulin A. *Immunity*. 2015;43:541-553. https://doi.org/10.1016/j.immuni.2015.08.007
37. McNamee EN, Rivera-Nieves J. Ectopic tertiary lymphoid tissue in inflammatory bowel disease: protective or provocateur? *Front Immunol*. 2016;7:308. https://doi.org/10.3389/fimmu.2016.00308
38. Olm MR, Spencer SP, Takeuchi T, et al. Metagenomic immunoglobulin sequencing reveals IgA coating of microbial strains in the healthy human gut. *Nat Microbiol*. 2025;10:112-125. https://doi.org/10.1038/s41564-024-01887-4
39. Maharshak N, Packey CD, Ellermann M, et al. Altered enteric microbiota ecology in interleukin 10-deficient mice during development and progression of intestinal inflammation. *Gut Microbes*. 2013;4:316-324. https://doi.org/10.4161/gmic.25486
40. Keubler LM, Buettner M, Hager C, et al. A multihit model: colitis lessons from the interleukin-10-deficient mouse. *Inflamm Bowel Dis*. 2015;21:1967-1975. https://doi.org/10.1097/MIB.0000000000000468
41. Jinno Y, Ohtani H, Nakamura S, et al. Infiltration of CD19+ plasma cells with frequent labeling of Ki-67 in corticosteroid-resistant active ulcerative colitis. *Virchows Arch*. 2006;448:412-421. https://doi.org/10.1007/s00428-005-0136-7
42. Hosomi S, Oshitani N, Kamata N, et al. Increased numbers of immature plasma cells in peripheral blood specifically overexpress chemokine receptor CXCR3 and CXCR4 in patients with ulcerative colitis. *Clin Exp Immunol*. 2011;163:215-224. https://doi.org/10.1111/j.1365-2249.2010.04290.x
43. Tarlton NJ, Green CM, Lazarus NH, et al. Plasmablast frequency and trafficking receptor expression are altered in pediatric ulcerative colitis. *Inflamm Bowel Dis*. 2012;18:2381-2391. https://doi.org/10.1002/ibd.22962
44. MacDermott RP, Nash GS, Bertovich MJ, et al. Alterations of IgM, IgG, and IgA Synthesis and secretion by peripheral blood and intestinal mononuclear cells from patients with ulcerative colitis and Crohn's disease. *Gastroenterology*. 1981;81:844-852.
45. Wang X, Jiang Y, Zhu Y, et al. Circulating memory B cells and plasmablasts are associated with the levels of serum immunoglobulin in patients with ulcerative colitis. *J Cell Mol Med*. 2016;20:804-814. https://doi.org/10.1111/jcmm.12728
46. Wang X, Hao GL, Wang BY, et al. Function and dysfunction of plasma cells in intestine. *Cell Biosci*. 2019;9:26. https://doi.org/10.1186/s13578-019-0288-9
47. Leiper K, Martin K, Ellis A, et al. Randomised placebo-controlled trial of rituximab (anti-CD20) in active ulcerative colitis. *Gut*. 2011;60:1520-1526. https://doi.org/10.1136/gut.2010.225482

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