Supplementary Materials and Methods

Mice

All mice were on C57BL/6J genetic background and housed in the specific pathogen-free animal barrier facility at the University of Arizona BIO5 institute. Wild-type (WT) C57BL/6J, B6.129X1-H2-Ab1tm1Koni/J,[1] Villin-Cre mice (strain B6.SJL-Tg [Vil-Cre] 997Gum/J,[2] and Rag1$^{-/-}$ mice were originally obtained from Jackson Laboratory (Bar Harbor, ME). Conditional knockout of MHCII in intestinal epithelium was generated by crossing B6.129X1-H2-Ab1tm1Koni/J mice with Villin-Cre mice (B6.Cg-Tg(Vil1-cre)997Gum/J), resulting in Cre^{+} I-Ab$^{\Delta IEC}$ (mutant) or Cre^{-} I-Ab^{WT} (control) mice. Mice were used between 7 and 10 weeks of age. To perform the adoptive T-cell transfer experiments, I-Ab^{WT} and I-Ab$^{\Delta IEC}$ were crossed with Rag1$^{-/-}$ mice to develop Rag1$^{-/-}$I-Ab^{WT} and Rag1$^{-/-}$I-Ab$^{\Delta IEC}$ recipient mice. Sentinel mice were routinely monitored and determined as free from common murine pathogens (mouse hepatitis virus, mouse parvovirus, minute virus of mice, Theiler's murine encephalomyelitis virus, *Mycoplasma pulmonis*, Sendai, epizootic diarrhea of infant mice, murine norovirus, and ecto- and endoparasites). All animal protocols and procedures were approved by the University of Arizona Animal Care and Use Committee.

Single Cell Preparation From MLNs and Colonic LP

Single cell suspensions from MLN were prepared, counted and cultured in RPMI 1640-Glutamax media (61870–036; Life Technologies, NY, NY) supplemented with penicillin and streptomycin (15140–122; Life Technologies), nonessential amino acids (SH30853.01; Hyclone Laboratories, Logan, Utah), β-mercaptoethanol (21985–023; Life Technologies) and 10% fetal bovine serum (FBS) (SH30910.03; Hyclone Laboratories) in 96-well round-bottom plates (82.1582.001; Sarstedt, Newton, NC) at a concentration of 2×10^5 cells per well and cultured in the presence of Dynabeads T activator CD3/CD28 (2021–07–31; ThermoFisher, Waltham, MA) for 72 hours. In the last 6 hours, Brefeldin A (555029; BD Biosciences, San Jose, CA) was added to the culture. Cells were harvested at the end of 72 hours and subjected to intracellular staining for IFN-γ. In experiments in which secreted cytokines were to be measured in culture supernatant, Brefeldin A was not added. Colonic LP cells from mice were isolated using the method described previously.[3] To obtain an enriched population of leukocytes, the LP single cells were subjected to CD45 enrichment using positive magnetic selection kit (130–052–301; Miltenyi Biotec, Bergisch Gladbach, Germany). The single cells were subjected to fluorescence-activated cell sorter (FACS) staining.

Flow Cytometry

The cells from colonic LP and MLN were stained with Zombie Aqua to distinguish between Live-Dead cells (423101; Biolegend, San Diego, CA) followed by blocking with anti-mouse CD16/CD32 for 15 minutes (14–0161–85; Thermo Fisher). The cells were then stained in the dark for 30 minutes at 4°C with the following fluorochrome-conjugated anti-mouse antibodies: CD4, CD44, CD62L, FoxP3, CD25, IL17A, and IFNγ (Supplementary Table 1). To phenotype plasma cells from Peyer's patches and small intestinal LP, fluorochrome-conjugated anti-mouse antibodies for CD138 and IgA were used (Supplementary Table 1). For intracellular staining for FoxP3, the surface staining was followed by fixation and permeabilization at 4 °C overnight using fixation/permeabilization buffer (00–5523–00; Life Technologies, Carlsbad, CA). Data were acquired using LSR Fortessa flow cytometer (BD Biosciences) and analyzed using FlowJo 10.0.06 (Tree Star Inc, Ashland, OR).

Ex Vivo Organoid Culture from Colons and IFN-γ stimulation

The organoids were prepared and cultured from the mouse colons as described by Miyoshi et al.[4] The colons were dissected, and opened longitudinally and cut into small (~2 mm) pieces. The pieces of tissue were then suspended in phosphate-buffered saline (PBS), and pipetted up and down and allowed to settle by gravity with supernatant being gently aspirated off. This step was repeated multiple times until the supernatant was clear. The tissue was then dissociated with PBS with 2 mM EDTA for 20 minutes in a shaking incubator at 37° C. The dissociated tissue was then resuspended in PBS with 0.1% bovine serum albumin and allowed to settle by gravity. The supernatant was then aspirated and filtered to obtain crypts, which were then resuspended in Matrigel (CB-40230C, Growth Factor Reduced Basement Membrane Matrix; Corning, Corning, NY). Next, a droplet of Matrigel/crypt mix was placed in the center of each well of a 24-well plate. After 30 minutes of polymerization, 400 μL of conditioned medium (advanced Dulbecco's modified Eagle's medium/F12 supplemented with 20% FBS recovered from L-WRN cells) was added to the culture, along with ROCK inhibitor Y-27632 (SCM075; Millipore Sigma, Bedford, MA) and ALK4/5/7 inhibitor (A83–01, Millipore Sigma). The medium was changed every 2 to 3 days. To passage, the organoids were trypsinized and transferred into fresh Matrigel. Organoids from I-Ab^{WT} or I-Ab$^{\Delta IEC}$ mice were treated with 100 U/mL of recombinant mouse IFNγ (BMS326; Thermo Fisher Scientific) for 18 hours, after which RNA was extracted using standard TRIzol method.

Fecal Microbial DNA Extraction and Microbiome Analysis

The hypervariable V4 region of the 16S ribosomal RNA (rRNA) gene was amplified from each sample using barcoded 806R primers and 515F primer and MyFi Mix (Bioline, London, UK). After assessing the quality and quantity of amplicons, pooled library was created by combining 240 ng of DNA from each sample. The pooled library was cleaned using UltraClean PCR Clean-Up Kit (12500; MoBio, Carlsbad, CA) and quantified against a standard curve using the qPCR-based KAPA Library Quantification Kit. Due to the

limited sequence diversity among 16S rRNA amplicons, 5% of the PhiX control library (Illumina, San Diego, CA) made from phiX174 was added to the run. Final concentration of 6.5 pM of the pooled library was subjected to the paired-end sequencing using 2 × 150 base pairs MiSeq Reagent Kit V2 (Illumina) using custom primers.[5] For sequencing, the samples from this study were combined with samples from another study. Sequencing was performed on the Illumina MiSeq (SN M02149, with the MiSeq Control Software v 2.5.0.5). After de-multiplexing (*idemp* script, https://github.com/yhwu/idemp), filtering, dereplication, sample inference, chimera identification, and merging of paired-end reads was done with a reference-free Divisive Amplicon Denoising Algorithm 2 (Dada2) R package.[6] The ASV taxonomy was assigned using RDP classifier against SILVA database release 132.[7] The vegan package[8] was used as a tool for diversity analysis, ordination methods, for the analysis of dissimilarities, and statistical analysis. The obtained results were visualized with ggplot2 package.[9]

The *Citrobacter rodentium* quantification was confirmed by qPCR by using primers for espB gene of the bacteria (espB-F: ATGCCGCAGATGAGACAGTTG, espB-R: CGTCAGCAGCCTTTTCAGCTA) and universal 16S rRNA primers for normalization (926-F: AAACTCAAAKGAATTGACGG, 1062-R: CTCACRRCACGAGCTGAC). qPCR was performed using LightCycler 480 SYBRGreen I Master Mix (Roche, Basel, Switzerland) and the LightCycler96 (Roche) system. The final volume of each reaction was 10 μL containing 300 nM primers and 1 μL DNA. The qPCR reaction started with a single 5-minute denaturation step at 95°C followed by 45 cycles of 95°C for 10 seconds, 60°C for 20 seconds, and 72°C for 20 seconds.

To create a standard curve for espB gene, the concentration of bacterial culture (colony-forming units [CFU] per mL) was determined by plating 10-fold serial dilutions on the LB agar plates. Six replicates of 1 mL of culture with known CFUs were used for DNA extraction. qPCR was performed as described previously and the standard curve was created by plotting the Ct values against the CFU/mL of serially diluted *C rodentium*, as previously described.[10] The standard curve showed linearity within tested range (from 4×10^5 CFU/mL to 4×10^1 CFU/mL, $R^2 = 0.9976$) with a calculated efficiency of 90%. The C_t values from collected fecal samples were compared with the constructed standard curve and results expressed as CFU/mL.

Induction of Experimental Colitis Using Adoptive T-Cell Transfer

$Rag1^{-/-}$ I-Ab^{WT} and $Rag1^{-/-}$I-$Ab^{\Delta IEC}$ mice were adoptively transferred with naïve $CD4^+CD45RB^{Hi}$ T cells as described previously.[11] Splenocytes were isolated from C57BL/6J WT donors followed by $CD4^+$ T cell enrichment using negative selection kit and magnetic columns (130–104–454; Miltenyi Biotec). $CD4^+$ cells were then fluorescently labeled with anti-mouse antibodies for CD4 and CD45RB (Supplementary Table 1). Subsequently, $CD4^+CD45RB^{hi}$ population was sorted using BD FACS Aria II at the University of Arizona Flow Cytometry Core Facility. Cells were counted using a cell counter and a suspension of 10^6 cells/mL was prepared. A suspension of 5×10^5 cells in 500 μL PBS was intraperitoneally injected into recipient mice. Control mice received 500μL PBS injection, body weights were recorded at the same time and monitored closely thereafter.

Preparation of C rodentium and Infection in Mice

C rodentium DBS100 (51459; ATCC, Manassas, VA) were grown overnight in LB medium followed by dilution with PBS to an optical density at 600 nm of 1.5. Mice were infected as previously described.[12] Four days before *C rodentium* inoculation, the mice were administered metronidazole (155710; MP Biomedicals, LLC, Solon, OH) at 750 mg/L in drinking water. Mice were then switched back to regular drinking water for 24 hours, after which *C rodentium* suspensions were delivered orally, in 100 μL of PBS containing 2 to 3×10^8 CFU.

Quantitative RT-PCR

Total RNA was isolated from mouse proximal and distal colon using TRIzol followed by reverse-transcription using the Universal Transcriptor cDNA synthesis kit (Roche). Real-time qPCR was performed on these samples to evaluate mucosal expression of relevant cytokines and targets using LightCycler96 Thermocycler (Roche). Cq values were obtained using LightCycler96 software (version 1.1.0.1320) and were analyzed using the comparative Ct method as the means of relative quantification, normalized to TBP as the housekeeping gene and relative to a calibrator (normalized Ct value obtained from control mice) and expressed as $2^{-\Delta\Delta Ct}$ (Applied Biosystems [Foster City, CA] User Bulletin #2: Rev B "Relative Quantification of Gene Expression").

Histological Examination

At the end of experiments, sections were excised from proximal and distal ends of colon and fixed in 10% formalin (Fisher Scientific, Fair Lawn, NJ) and embedded in paraffin. Samples were cut into 5-μm-thick sections and stained with hematoxylin-eosin (H&E). H&E-stained slides were blindly assessed by a veterinary pathologist blinded to the experimental group assignment, based on lesion scoring system for mouse intestinal lesions as modified from Burich et al.[13] Some slides were processed further for immunofluorescence.

Immunofluorescence

Sections of 5 μm were deparaffinized and rehydrated, followed by washing and blocking for 30 minutes in TBST containing 5% FBS. Immunostaining was carried out by using appropriate dilutions of purified rat anti-mouse I-A/I-E antibody (1:200, 556999; BD Biosciences) at 4°C overnight followed by incubation with the secondary antibody in blocking solution for 30 minutes at room temperature. Tissues were mounted using Prolong Gold with 4′,6-diamidino-2-phenylindole (DAPI) (P36971; Invitrogen, Carlsbad, CA). Images were obtained using a Leica (Wetzlar,

Germany) DMI6000 microscope and Leica LAS-X software to capture bright field images. For immunofluorescence, sections were imaged using an Olympus (Tokyo, Japan) FluoView 1200 confocal microscope with a ×60, 1.43 NA oil immersion objective. Identical imaging parameters were used within a single experiment.

Sample Preparation for Fecal Lipocalin 2 and Serum Amyloid A Detection

The fecal samples were weighed and suspended in 1 mL PBS, followed by vortexing at 400 rpm to disperse the pellets. The samples were then centrifuged, and the clear supernatant was separated and stored at −20°C until use. For serum amyloid A detection, serum was separated from blood samples within 2 hours of collection and stored at −80 °C until use.

Colonic Explant Culture and xMAP Assays

Colon segments were opened longitudinally and shaken vigorously for 10 minutes in PBS to remove fecal content. Tissue was then weighed, minced with scissors, and distributed to the wells (∼100 mg of tissue per well) of a 24-well tissue culture plate (3524; Corning Costar, Lowell, MA) and incubated in 1 mL of RPMI 1640 medium containing 5% heat-inactivated FBS, pen-strep, and nonessential amino acids, at 37 °C for 24 hours. They were collected and stored at −80 °C until assayed. Selected cytokine concentrations were evaluated by an xMAP assay (Millipore, Billerica, MA) and Luminex 100 platform (Millipore, Danvers, MA). Data are presented as pg/mL supernatant per gram of colon explant.

Analysis of the IgA-coated Bacteria

To prepare bacteria for flow staining, ileum and colon were harvested from mice. Both ileal and colonic washings were collected separately in a buffer containing PBS and protease inhibitor cocktail (P8340; Millipore Sigma). For each sample, the fecal material was resuspended by vortexing followed by centrifugation at 800*g* for 2 minutes to pellet down larger aggregates. The supernatant was then centrifuged at 8000*g* for 7 minutes to collect bacteria from the fecal suspension. After washing the pellet twice with PBS, *Bac*light Red (B-35001; Thermo Fisher Scientific) was added and incubated at room temperature for 15 minutes. Then, rat normal serum was added for another 15 minutes at 4°C, followed by centrifugation at 8000*g* for 10 minutes. Anti-IgA fluorescein isothiocyanate was added at this point to the pellet and incubated for 30 minutes at 4°C. After 2 washes in PBS, the bacteria were fixed in paraformaldehyde and acquired on LSR Fortessa (BD Biosciences).

C rodentium–*IgA Binding Assay*

C rodentium DBS100 was cultured as described in the preceding section "Preparation of *C rodentium* and Infection in Mice." Approximately 2×10^8 bacteria were incubated with luminal washings from ileum of I-Ab^{WT} and I-Ab$^{\Delta IEC}$ mice adjusted to contain either 10 ng/mL or 100 ng/mL of SIgA (as quantified by SIgA ELISA), at 4°C for 1 hour. The incubation was followed by FACS staining as described in the previous section.

Gene Microarray Analysis

RNA was isolated using TRIzol (Thermo Fisher Scientific) and further purified with the RNeasy PowerClean Pro CleanUp kit (Qiagen, Hilden, Germany). RNA integrity analysis (Bioanalyzer 2100; Agilent, Santa Clara, CA) resulted in RNA Integrity Number ≥ 9.0, and RNA concentrations were determined with Nanodrop (Thermo Fisher Scientific). Amplified and biotinylated sense-stranded DNA targets were generated from each of 12 unique RNA samples derived from I-Ab^{WT} and I-Ab$^{\Delta IEC}$ colons (n = 3 in each experimental group) using the GeneChip WT PLUS Reagent kit (Affymetrix, Santa Clara, CA), and subsequently hybridized to GeneChip Mouse Gene 2.0 ST arrays (Affymetrix). Microarray data analyses were performed using GeneSpring (Agilent, v. 14.9). Data were processed using the RMA16 summarization algorithm and normalized against the mean of control (I-Ab^{WT}) samples for visualization. Statistical analysis was performed using built-in tools, including moderated *t*-test ($P \leq .05$) with Westfall–Young multiple testing correction. Raw and normalized expression values can be viewed at the National Center for Biotechnology Information Gene Expression Omnibus microarray depository website (GEO accession no. GSE144952).

IgA Repertoire Analysis

IgA^{+}CD138^{+} cells were isolated from the small intestinal LP and PPs of I-Ab^{WT} and I-Ab$^{\Delta IEC}$ mice using flow sorting. Cells were lysed and RNA was extracted using NucleoSpin RNA XS kit (740902.50; Macherey-Nagel, Duren, Germany) per manufacturer's instructions. Complementary DNA, primed with IgA reverse-UMI-stdseq2 primer (Supplementary Table 2) was generated using Maxima Reverse Transcriptase (EP0743; ThermoFisher) per manufacturer's instructions. IgA immunoglobulin transcripts were amplified with first-round PCR using the following primers: msVHEstdseq1 forward primer and stdseq2 reverse primer (Supplementary Table 2). PCR products were cleaned using a gel/PCR DNA fragments extraction kit (IBI Scientific, Dubuque, IA), and then used as templates for a second round of PCR with the following primers: P5 forward-stdseq1 and P7 reverse-index-stdseq2 (Supplementary Table 2). Samples were pooled, gel purified, and then sequenced using the Illumina Miseq platform with the standard sequencing primers: stdseq1, stdseq2, and index seq (Supplementary Table 2). Sequencing data were extracted from fastq files, filtered, and assembled using a modified Illumina MiSeq 2 × 250 BCR mRNA workflow by pRESTO.[14] BCR repertoire information containing V, D, and J gene usages and the number of somatic hypermutations were then extracted using Migmap.

Supplementary References

1. Hashimoto K, Joshi SK, Koni PA. A conditional null allele of the major histocompatibility IA-beta chain gene. Genesis 2002;32:152–153.
2. Madison BB, Dunbar L, Qiao XT, et al. Cis elements of the villin gene control expression in restricted domains of the vertical (crypt) and horizontal (duodenum, cecum) axes of the intestine. J Biol Chem 2002;277:33275–33283.
3. Larmonier CB, Shehab KW, Laubitz D, et al. Transcriptional reprogramming and resistance to colonic mucosal injury in poly(ADP-ribose) polymerase 1 (PARP1)-deficient mice. J Biol Chem 2016;291:8918–8930.
4. Miyoshi H, Ajima R, Luo CT, et al. Wnt5a potentiates TGF-beta signaling to promote colonic crypt regeneration after tissue injury. Science 2012;338:108–113.
5. Caporaso JG, Lauber CL, Walters WA, et al. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. ISME J 2012;6:1621–1624.
6. Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: High-resolution sample inference from Illumina amplicon data. Nat Methods 2016;13:581–583.
7. Quast C, Pruesse E, Yilmaz P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 2013;41:D590–D596.
8. Oksanen J, Blanchett FG, Friendly M, et al. vegan: community ecology package. R package version 2.5–4. 2019. Available at: https://CRAN.R-project.org/package=vegan. Accessed September 14, 2020.
9. Wickham H. ggplot2: elegant graphics for data analysis. New York: Springer-Verlag, 2016.
10. Sagaidak S, Taibi A, Wen B, et al. Development of a real-time PCR assay for quantification of *Citrobacter rodentium*. J Microbiol Methods 2016;126:76–77.
11. Harrison CA, Laubitz D, Midura-Kiela MT, et al. Sexual dimorphism in the response to broad-spectrum antibiotics during T cell-mediated colitis. J Crohns Colitis 2019;13:115–126.
12. Wlodarska M, Willing B, Keeney KM, et al. Antibiotic treatment alters the colonic mucus layer and predisposes the host to exacerbated *Citrobacter rodentium*-induced colitis. Infect Immun 2011;79:1536–1545.
13. Burich A, Hershberg R, Waggie K, et al. *Helicobacter*-induced inflammatory bowel disease in IL-10- and T cell-deficient mice. Am J Physiol Gastrointest Liver Physiol 2001;281:G764–G778.
14. Vander Heiden JA, Yaari G, Uduman M, et al. pRESTO: a toolkit for processing high-throughput sequencing raw reads of lymphocyte receptor repertoires. Bioinformatics 2014;30:1930–1932.

Supplementary Table 1. Anti-Mouse Monoclonal Antibodies Used for Flow Cytometry

Antibody	Conjugated fluorophore	Clone	Source	Catalog no.
CD4	PE, FITC	RM4-5	BD Biosciences	553049; 561835
CD44	PE-Cy7	IM7	eBioscience	25-0441-81
CD62L	APC	MEL-14	eBioscience	17-0621-82
CD45 RB	FITC	16A	BD Biosciences	553100
FoxP3	PE	FJK16S	eBioscience	12-5773-82
CD25	APC	PC61.5	eBioscience	17-0251-81A
IFNγ	PE	XMG1.2	BD Biosciences	562020
CD138	PE	281.2	Biolegend	142504
IgA	DyLight 650, FITC	C10-3	Abcam; BD Biosciences	ab97014; 559354

APC, Allophycocianin; FITC, fluorescein isothiocyanate; PE, phycoerythrin

Supplementary Table 2. List of Primers Used in IgA Repertoire Analysis

Primer name	Primer sequence (5′ → 3′)
IgA reverse-UMI-stdseq2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTNNNNTNNNNTNNNNTGGGTAGATGGTGGGATTTC (UMI, unique molecular identifies, are represented by N)
msVHEstdseq1	ACACTCTTTCCCTACACGACGCTCTTCCGATCTGGGAATTCGAGGTGCAGCTGCAGGAGTCTGG
Stdseq2	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT
P5 forward-stdseq1	AATGATACGGCGACCACCGAGATCTACAC TCTTTCCCTACACGACGC
P7 reverse-index-stdseq2	CAAGCAGAAGACGGCATACGAGATXXXXXXXXGTGACTGGAG TTCAGACGTGTGTG (X represents a unique combination for barcoding purposes)
Stdseq1	ACACTCTTTCCCTACACGACGCTCTTCCGATCT
Index seq	AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC

Supplementary Table 3. List of Primers used in qRT-PCR

Target	Assay_Id
IFNγ	Mm00801778_m1
TNF	Mm00443258_m1
IL1β	Mm00434228_m1
IL6	Mm00446190_m1
IL12b	Mm00434174_m1
IL17A	Mm00439618_m1
IL22	Mm01226722_g1
IL23A	Mm01160011_g1
REGIIIγ	Mm00441127_m1
TBP	Mm00446971_m1
I-A^b	Mm00439216_m1
CIITA	Mm00482914_m1

NOTE. Source: Thermo Fisher Scientific