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Supplementary Materials for

A specific gene-microbe interaction drives the development of Crohn's disease–like colitis in mice

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The PDF file includes:

Materials and Methods
Fig. S1. Skip lesions in older Tac-fostered *Nod2*$^{-/-}$*Cybb*$^{-/-}$ mice.
Fig. S2. Kinetics of intestinal inflammation in doubly deficient *Nod2/Cybb* mice.
Fig. S3. Cellular infiltrates in the large intestine of Tac- and Jax-fostered mice.
Fig. S4. CD-like inflammation in *Nod2*$^{-/-}$*Cybb*$^{-/-}$ mice is characterized by T_H1 immune responses.
Fig. S5. Characterization of Tac-fostered doubly deficient *Nod2/Cybb* mice before the development of colitis.
Fig. S6. Analysis of the gut microbiota in Tac- and Jax-fostered doubly deficient *Nod2/Cybb* mice and quantification of mucus-dwelling bacteria in fostered mice.
Fig. S7. Increased abundance of *Mucispirillum* in inflamed colonic areas in *Nod2*$^{-/-}$*Cybb*$^{-/-}$ mice.
Fig. S8. ROS production after microbial stimulation.
Fig. S9. Immunoglobulin production in fostered mice.
Fig. S10. *Mucispirillum*-specific colonization and Ig production in $J_H^{-/-}$, *IgA*$^{-/-}$ and *FcRn*$^{-/-}$ mice.
Fig. S11. *Mucispirillum*-specific IgA and IgG cooperate in conferring protection against colitis in doubly deficient *Nod2/Cybb* mice.
Fig. S12. Specificity of *Mucispirillum*-induced intestinal inflammation in doubly deficient *Nod2/Cybb* mice.
Fig. S13. Efficiency of B cell depletion in doubly deficient *Nod2/Cybb* mice.
References (*69*–*73*)

Other Supplementary Material for this manuscript includes the following:

(available at immunology.sciencemag.org/cgi/content/full/4/34/eaaw4341/DC1)

Table S1 (Microsoft Excel format). The abundances and LEfSe data of individual OTUs in Jax- and Tac-fostered mice.

File S1 (Microsoft Excel format). Data in tabular format.

Materials and Methods

Lamina propria mononuclear cell preparation

LPMCs were prepared as described (*69*). LPMCs (2×10^5 cells/well) were suspended in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2-β-mercaptoethanol (50 μM), L-glutamine (2 mM), sodium pyruvate (1 mM), MEM non-essential amino acids and penicillin-streptomycin (all from Gibco) (complete RPMI medium). For measurement of cytokine production, total LPMCs were seeded in 96-multiwell plates in triplicate, incubated for 12 hr in 5% CO2 incubator and then cell-free supernatant were subjected to ELISA analyses. For characterization of cellular infiltrates, total LPMCs were stained and analyzed by flow cytometry. For analysis of cytokine expression, total LPMCs were used for RNA extraction. LP neutrophils were prepared using a described protocol (*69*). Briefly, after digestion of cecal and colonic tissues in Hanks' balanced salt solution (HBSS, Gibco) containing 400 U/ml collagenase type 3 and 0.1 mg/ml DNase I (Worthington Biochemical), digested samples were spun on a discontinuous Percoll gradient (45%, 50%, 55%, 62%, 81%) (GE Healthcare-Life Sciences) at 600 *g* for 30 min at 10°C. Cells from the 62%-81% interface were recovered and used for DNA extraction and qPCR. Cells were more than 90% $CD11b^+Ly6C^-Ly6G^+$ by flow cytometry.

Luminal neutrophil preparation

Intraluminal cells were isolated from cecal and fecal samples of fostered mice. The luminal content was filtrated sequentially through 100 μm, 70 μm, and 40 μm cell strainer and then centrifuged at 1,000 rpm for 20s to remove debris. Luminal neutrophils were isolated by centrifugation on a Percoll gradient (45%, 50%, 55%, 62%, 81%) at 600 *g* for 30 min at 10°C.

Cells from the 62%-81% interface were recovered. Cells were more than 95% $CD11b^{+}Ly6C^{-}$ $Ly6G^{+}$ by flow cytometry.

Bone Marrow Neutrophil isolation

Femurs and tibias were harvested and dissected free of adherent tissue. The ends of the bones were cut off and the marrow tissue flushed with PBS. Cells were filtered through a 40 μm cell strainer and then washed by centrifugation. Cells were spun on a discontinuous Percoll gradient (45%, 50%, 55%, 62%, 81%) at 600 *g* for 30 min at 10°C. Cells from the 62%-81% interface were recovered and red blood cells were lysed with RBC lysis buffer (Invitrogen). Cell purity was >80% via cytospin.

Chemiluminescence assay

NADPH oxidase activity was assayed using chemiluminescence enhanced by luminol. Bone marrow-derived neutrophils (BMNs) (2.5×10^5) were suspended in PBSG (PBS supplemented with 0.9 mM CaCl2, 0.5 mM MgCl2, 7.5 mM dextrose all from Sigma-Aldrich) in the presence of 50 μM luminol (Sigma-Aldrich) and preincubated at 37°C for 10 minutes. Cells were then stimulated with phorbol myristate acetate (PMA; final concentration 40mM, Sigma-Aldrich), without or with superoxide dismutase (SOD; final concentration 75 μg/mL, Sigma-Aldrich). Additionally, BMNs (2.5×10^5) were primed with LPS (1μm/mL, InvivoGen) for 60 min at 37ºC and then left untreated or stimulated with fMLP (1μM, Abcam). Horseradish peroxidase (HRP; final concentration: 20 U/mL, Sigma-Aldrich) was added and the relative light units (RLU) were monitored at 98-second intervals for up to 30 minutes at 37°C by the Long Kinetic module for

PMA stimulation and at 1-second intervals for up to 2.9 minutes at 37°C by the Fast Kinetic module for fMLP stimulation in an Lmax microplate luminometer from Molecular Devices (Sunnyvale, CA). Integrated RLU values were calculated by SOFTmax software (Molecular Devices).

Flow cytometry

Cell surface fluorescence was measured using a LSRII (BD Biosciences) instrument and the data analyzed using FlowJo software (Tree Star). LP dead cells were excluded with LIVE/DEAD staining (Invitrogen). Fc block was performed by incubating cells with purified anti-mouse CD16/CD32 antibody (eBioscience). Fluorescence-conjugated mAbs against CD3 (145-2C11), CD19 (Ebio1D3), CD8 (53-6.7), B220 (RA3-6B2), MHCII (M5/114.15.2), CD11b (M1/70), CD11c (N418) were from eBioscience. mAbs for CD45 (30-F11), CD4 (RM4-5), Ly6C (AL-21), and Ly6G (1A8) were purchased from BD Pharmingen. mAb for Gr1 (RB6-8C5) was purchased from BioLegend. For intracellular staining, LPMCs were incubated with 50ng/ml PMA (Sigma-Aldrich) and 1μg/ml ionomycin (Sigma-Aldrich) in the presence of GolgiStop (BD) in complete RPMI medium for 10 hr at 37°C. Intracellular staining was performed according to the manifacturer's protocol (BD). Fluorescence-conjugated mAb against IFNγ (XMG1.2) (BD Pharmingen), IL-17A (eBio17B17) (eBioscience) were used. Cells were further stained intracellularly with fluorescence-labelled mAB against T-bet (4B1O) (BioLegend) and IFNγ (XMG1.2) (BD Pharmingen) using Foxp3 staining/permeabilization buffer (eBioscience). Isotype-matched antibodies (eBioscience, BD Pharmingen and Biolegend) were used for control staining.

Quantitative Real-Time PCR

RNA was extracted with E.Z.N.A. Total RNA Kit (Omega Biotek) according to the manufacturer's instructions. RNA was reverse transcribed using the High Capacity RNA-to-cDNA Kit (Applied Biosystems). Quantitative real time RT-PCR (qPCR) was performed using a SYBR green PCR master mix and StepOne Real-time PCR system (Applied Biosystems). The following primer sets were used for amplification: TNFα-F; 5′-GCCTCCCTCTCATCAGTTCT-3′, TNFα-R; 5′-CACTTGGTGGTTTGCTACGA-3′, IFNγ-F; 5′-TCAAGTGGCATAGATGTGGAAGAA-3′, IFNγ-R; 5′-TGGCTCTGCAGGATTTTCATG-3′, IL-17A-F; 5′-GGACTCTCCACCGCAATGA-3′, IL-17A-R; 5′-GGCACTGAGCTTCCCAGATC-3′, IL-17F-F; 5′-CCCCATGGGATTACAACATCAC-3′, IL-17F-R; 5′-CATTGATGCAGCCTGAGTGTCT-3′, IL-1β-F; 5′-GATCCACACTCTCCAGCTGCA-3′, IL-1β-R; 5′-CAACCAACAAGTGATATTCTCCATG-3′, CXCL1-F; 5′-TGTTGTGCGAAAAGAAGTGC-3′, CXCL1-R; 5′-TACAAACACAGCCTCCCACA-3′, CXCL2-F; 5′-GAGCTTGAGTGTGACGCCCCCAGG-3′, CXCL2-R; 5′-GTTAGCCTTGCCTTTGTTCAGTATC-3′, GAPDH-F; 5′-CACCATCTTCCAGGAGCGAG-3′, GAPDH-R; 5′-GCCTTCTCCATGGTGGTGAA-3′, *Mucispirillum*; 5′-GGCTGGGTGTTAATGGCAGA-3′ and 5′-CTGCCTGTCGCCTTTGTTTG-3′, *SFB;* 5′-GACGCTGAGGCATGAGAGCA-3′ and 5′-GACGGCACGGATTGTTATTC-3′, 16S-8F; 5′-AGAGTTTGATCCTGGCTCAG-3′, 16S-338R; 5′-TGCTGCCTCCCGTAGGAGT-3′, *Akkermansia muciniphila;* 5'-AGAGGTCTCAAGCGTTGTTCGGAA-3' and 5'-TTTCGCTCCCCTGGCCTTCGTGC-3', Firmicutes; 928F-Firm 5'-TGAAACTYAAAGGAATTGACG-'3 and 1040firmR, 5' ACCATGCACCACCTGTC '3, *B.uniformis* (dnLKV2); 5'-*AGTCAGAAACCGACCAAAACAC*-3′ *and* 5'-

GATAAAATGTAGTGTGTGGAGG-3′ . Relative mRNA expression was calculated by the ΔΔCt method and normalized to the expression of either GAPDH or to the universal 16S rRNA gene.

DNA extraction and 16S rRNA gene sequencing and sequence curation.

Genomic DNA was extracted from fecal pellets, colonic mucus scrapings, spleen and liver using the E.Z.N.A stool DNA kit (Omega Biotek). We generated amplicons of the V4 region within the 16S rRNA gene and sequenced the fragments using an Illumina MiSeq instrument (*70*) . The sequences were curated using Mothur (v.1.35) (*70, 71*) and sequences were binned into OTUs at >97 % sequence level. Taxonomic assignments, α- and β-diversity and NMDS plot of θ_{YC} values were all determined using Mothur (*71*). LEfSe Linear discriminant analysis (LDA) was performed by the Mothur command using all OTU reads. The OTUs that were present in >0.1 % abundance at maximum were selected and shown in Fig. 3D and fig S6A. The OTU abundance ratio was calculated from means of indicated groups after error suppression by replacement of zero value with minimal detection threshold values. The bacteria under detection (zero read) in 16S rRNA gene Illumina MiSeq analysis in denominator groups are indicated by asterisks (Fig. 3D and fig S6A). The OTUs shown in the LEfSe panels are those whose abundance was statistically different with $p<0.05$ (Fig. 3D and fig S6A). For LefSe analysis of microbiota of Tac-DKO NI *versus* Jax-DKO, the OTUs that also showed different abundance between Tac-WT mice and Jax-WT mice with the same polarity were removed from the list shown in fig S6A.

Administration of anti-TNFα Antibody

Anti-TNFα IgG (rat clone; MPG-XT3) was kindly provided by T. Moore (University of Michigan). 4-6-week-old littermate (both male and female) Tac-DKO mice were injected with

500 μg of TNFα antibody or isotype-matched control rat IgG (Leinco Technologies) intraperitoneally twice (every 5 days) and sacrificed 9 days following the first injection. To monitor the presence of intestinal inflammation, fecal samples were collected before the injection and then every other day up to 9 days and used to perform Lcn-2 ELISA analysis. Mice were sacrificed 9 days following the first injection. Small intestine, cecum and colon were flushed with PBS and fixed in 10% formalin and then processed for H&E staining and histological evaluation.

Mucispirillum* and *B. uniformis infections

Mucispirillum isolated from the mouse cecum was a gift from Jani O'Rourke (The University of New South Wales, Sydney) (*38*). For inoculations, bacteria were grown anaerobically on 5% horse blood agar plates. A ratio of one plate/mouse (every other day) was used for *in vivo* experiments. 3 week-old (both male and female) Jax mice were co-housed, right after weaning, for 2 weeks and then administrated with vancomycin (250 mg/L) in drinking water for 7 days before *Mucispirillum* infection and then switched to regular water. Mice were infected by oral gavage with 0.2 ml of BHI broth containing approximately 1 x 10^8 CFU of *Mucispirillum* every other day up to 7 days. *B. uniformis* (strain dnlKV2) (*72*) was grown anaerobically at 37°C overnight in custom chopped meat broth (*73*) and then 0.2 mL of the bacterial suspension was orally gavaged to mice every other day up to 7 days. Controls mice received oral gavage of 0.2 ml of BHI broth every other day up to 7 days. Mouse survival was monitored for 7 days after the first oral administration. To determine bacterial loads and to monitor the development of intestinal inflammation, fecal samples were collected before and every other day up to 7 days, following the first bacterial administration. The presence of *Mucispirillum* and *B. uniformis* was

quantified by qPCR and the level of intestinal inflammation was determined using Lcn-2 ELISA. Mice were sacrificed 7 days after the first infection. Small intestine, cecum and colon were flushed with PBS and fixed in 10% formalin and then processed for H&E staining and histological evaluation.

B-cell depletion

$CD20^+$ B cells were depleted using a mouse anti-mouse CD20 monoclonal antibody (clone 5D2, murine IgG2a; Genentech). 3 week-old Jax mice were co-housed for 2 weeks and then given vancomycin (250 mg/L) in the drinking water for 7 days. During vancomycin treatment, mice were given intraperitoneal injections with 0.1 mg of anti-CD20 antibody or isotype-matched control IgG (Genentech) (at day -2). Efficiency of $CD20^+$ cell depletion was assessed by flow cytometry one day after treatment with anti-CD20 antibody. Upon confirmation of $CD20^+$ cell depletion, mice were infected by oral gavage with 0.2 ml of BHI broth containing approximately 1×10^8 CFU of *Mucispirillum* every other day, for up to 10 days. A second dose of anti-CD20 or control protein was given at day +3 (during infection). CD20 depletion efficiency was again assessed by flow cytometry during the infection (6 days after the initial bacterial administration). To determine bacterial loads and to monitor the development of intestinal inflammation, fecal samples were collected before and every other day for up to 10 days following the first bacterial administration. The presence of *Mucispirillum* was quantified by qPCR and the levels of intestinal inflammation were determined using Lcn-2 ELISA. Fecal samples were also collected before (day 0) and on day 7 after infection and the presence of *Mucispirillum*-specific IgA and IgG was determined by ELISA.

Statistics

Statistical analyses were performed using GraphPad Prism software version 7 (GraphPad Software Inc.). Sample sizes were chosen to reach statistical significance ($P < 0.05$) for a pre-determined effect based on the sample variation observed in previous studies. Sample sizes for microbiota analysis were chosen based on sample sizes published in previous studies. Differences between two groups were evaluated using Two-tailed Unpaired t-test or Mann-Whitney U test. Differences between paired groups were evaluated using Paired t-test. Comparison of more than two groups was performed with one-way ANOVA or two-way repeated measures ANOVA followed by Tukey's, Sidak's or Dunn's multiple comparisons test. Survival between groups of mice was compared using Log-rank (Mantel-Cox) test. Correlation analyses were performed using Spearman rank correlation. Differences at $P<0.05$ were considered significant.