Intergenerational transfer of antibiotic-perturbed microbiota enhances colitis in susceptible mice

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Antibiotic exposure in children has been associated with the risk of inflammatory bowel disease (IBD). Antibiotic use in children or in their pregnant mother can affect how the intestinal microbiome develops, so we asked whether the transfer of an antibiotic-perturbed microbiota from mothers to their children could affect their risk of developing IBD. Here we demonstrate that germ-free adult pregnant mice inoculated with a gut microbial community shaped by antibiotic exposure transmitted their perturbed microbiota to their offspring with high fidelity. Without any direct or continued exposure to antibiotics, this dysbiotic microbiota in the offspring remained distinct from controls for at least 21 weeks. By using both IL-10-deficient and wild-type mothers, we showed that both inoculum and genotype shape microbiota populations in the offspring. Because IL10−/− mice are genetically susceptible to colitis, we could assess the risk due to maternal transmission of an antibiotic-perturbed microbiota. We found that the IL10−/− offspring that had received the perturbed gut microbiota developed markedly increased colitis. Taken together, our findings indicate that antibiotic exposure shaping the maternal gut microbiota has effects that extend to the offspring, with both ecological and long-term disease consequences.
three donors for each inoculum (Fig. 1a,ii). Pooling allowed us to create one inoculum for each treatment group and to generate a large study cohort, although it may have limited the variability probably seen in humans. To maintain dietary consistency with the donor mice and thus reduce stress on the microbial community, all mice were maintained on a 45% high fat diet (HFD) for the entirety of the experiment.

The STAT inoculum had fewer operational taxonomic units (OTUs) and a remodelled community composition and structure (Supplementary Fig. 1a,b), with relative taxa abundance differences including decreased Erysipelotrichi and increased Verrucomicrobiae, consistent with previous observations (Supplementary Fig. 1c). The microbial communities at one day post-gavage in the dams became similar (Fig. 1b,i), but by four days post-gavage, each group was instability in the dam microbiota for the first days post-gavage (Fig. 1b,ii and Supplementary Table 1). Although the STAT and control inocula were similarly diverse, α-diversity dropped markedly in all groups, with partial recovery within four days and with stabilization by seven days (Supplementary Fig. 3b–d and Supplementary Table 3). Although the STAT and control inocula were similarly diverse, α-diversity became lower in dams receiving the STAT than the control inoculum, continuing throughout the experiment (Supplementary Fig. 3b–d and Supplementary Table 3). These trends were due to rapid community succession, as a few opportunists (Lactococcus, Turicibacter and Staphylococcus) bloomed immediately after transfer, then were outcompeted by the usual resident microbiota (Supplementary Fig. 1c and Supplementary Discussion).

Next, we assessed the effects of the transfers on community stability. We found that OTU loss was greater in the intergenerational transfer from the dams that had received STAT microbiota than in those that received control microbiota, and the response varied by genotype (Fig. 2a, Supplementary Fig. 4a and Supplementary Table 7). Mouse genotype played a role; transfer was more complete in IL10−/− mice (Fig. 2a, Supplementary Figs. 4a and 5, Supplementary Tables 7 and 10 and Supplementary Notes). We hypothesize that this more complete transfer may be due to there being more extensive niches for colonization in a host with a defective immune system, although ultimately it is not clear why IL10−/− mice had richer microbial colonization from the inoculum. By Jaccard analysis of pairs of samples, there was instability in the dam microbiota for the first days post-gavage (highest Jaccard indices), mostly stabilizing by four days post-gavage (Fig. 2b). By four weeks post-gavage, both dam and pup microbiota stabilized, although the STAT pups had greater variation over time than Controls (Fig. 2b,c and Supplementary Table 4). Thus, after immediate short-term community instability.

Effects of mouse passage on community dynamics. In pups, the first faecal samples (age three weeks) showed close resemblance to samples from their dams (Fig. 1b,iiii), indicating that gavage of pregnant germ-free dams is effective for introducing inoculum to pups at birth. The four groups determined by inoculum and host genotype were distinct, remaining consistently so to week 21 (Fig. 1b,iii–v and Supplementary Table 1).

Reduced microbial diversity is associated with IBD1,2 and other illnesses, so we examined the effects of inoculum, host genotype and mouse generation on community richness (α-diversity). After gavage, α-diversity dropped markedly in all groups, with partial recovery within four days and with stabilization by seven days (Supplementary Fig. 3b–d and Supplementary Table 3). Although the STAT and control inocula were similarly diverse, α-diversity became lower in dams receiving the STAT than the control inoculum, continuing throughout the experiment (Supplementary Fig. 3b–d and Supplementary Table 3). These trends were due to rapid community succession, as a few opportunists (Lactococcus, Turicibacter and Staphylococcus) bloomed immediately after transfer, then were outcompeted by the usual resident microbiota (Supplementary Fig. 1c and Supplementary Discussion).

Fig. 1 | Microbiome transfer to pregnant germ-free mice colonized 23 dams and 112 pups. a, (i) STAT and control gut microbiota inocula were taken from a previous experiment. (ii) Germ-free (GF) mice were mated to yield pregnant GF dams. This procedure was done in C57BL/6 mice of wild-type (WT) or IL10-deficient (IL10−/−) genotype. (iii) Approximately one week before delivery, the pregnant GF dams were gavaged with either the STAT or the Control inoculum, to conventionalize them. Pups were weaned at 4 weeks of age and killed at 21 weeks of age. For the IL10−/− mice, they were also killed at age 6 and 14 weeks (black stars). b, Principal coordinate plots of unweighted UniFrac distances. The inocula used to colonize the dams before delivery are shown together with dam and pup faecal samples from the indicated time points. n = 4 replicate aliquots for each inoculum. All numbers of mice/groups are provided in Supplementary Table 1, as well as P values from Adonis testing.
in the dams, the dams and pups were colonized with a mostly stable microbiota, highly resembling the inoculum. We show that the inheritance of a perturbed microbiota leads to increased instability of community composition in a later generation, with loss of richness.

Specific taxa were represented nearly identically in the control WT pups and their dams (Supplementary Fig. 4b); however, for both inocula, Akkermansia was more abundant in dams than their pups (Supplementary Fig. 4c and Supplementary Table 8). Populations in control pups were more similar to their inoculum than were STAT pups, respectively, by both Jaccard Index (Supplementary Fig. 4d and Supplementary Table 9) and as a percent of shared OTUs (Supplementary Fig. 4c and Supplementary Table 8). Populations in control pups were more similar to their inoculum than were STAT pups, respectively, by both Jaccard Index (Supplementary Fig. 4d and Supplementary Table 9) and as a percent of shared OTUs (Supplementary Fig. 4c and Supplementary Table 8), indicative of higher transfer fidelity. In total, mouse genotype influences community structure after inoculation, with specific taxa differing in propensity for intergenerational transfer.
Effects of treatment and host genotype on the metagenome. To assess the differential selection associated with founding inoculum and host genotype, faecal samples from three-week-old pups underwent shotgun sequencing to identify metagenomic effects. Analysis of Bray–Curtis distances of the metagenomic data showed significantly separated groups, except among the WT mice (Supplementary Fig. 6a). Metagenomic analysis also permitted identification and fine-scale resolution of specific taxa that were differentially present in the control and STAT pups (Supplementary Fig. 6b). There were many parallel changes in KEGG pathways in the IL10−/− compared to WT mice for recipients of the STAT and control microbiota (Supplementary Fig. 6c). This conservation suggests genotype-specific selection for function. In particular, the pathways over-represented in WT STAT mice include ones involved with branched-chain amino acids (BCAAs), similar to an earlier observation, whereas in the IL10−/− mice, many differences were identified that we have not seen reported elsewhere (Supplementary Fig. 6d,e). This is potentially significant because BCAAs have been associated with differential risk for auto-immune and metabolic diseases including type 1 diabetes, as well as type 2 diabetes and obesity. The abundance of antibiotic-resistance genes was similar in the inocula from the control and STAT mice (Supplementary Fig. 6f). However, in the pups, the abundance of resistance genes was lower in recipients of the STAT microbiota than the control microbiota, in both mouse backgrounds, and significant (P < 0.01) in the WT mice. These findings suggest that without further antibiotic pressure, selection in STAT mice favoured the growth of transferred organisms without resistance determinants (that is, greater fitness in the absence of selection). Although unexpected, there are several possible explanations for this finding (Supplementary Discussion). The metagenomic analysis also revealed a significant enrichment of genes involved in the Sulfate Reduction I pathway in IL10−/− STAT pups, which has been associated with both Akkermansia muciniphila and colonic dysfunction (Supplementary Fig. 7 and Supplementary Notes). In total, both the maternal inocula and the host genotype led to differential metagenomic gene and pathway effects.

Effect of inoculum on characteristics of the pups. Finally, we examined the effects on host properties of receiving the transferred microbiota. Confirming previous reports, bone mineral content and density were decreased in IL10−/− compared to WT mice (Supplementary Fig. 8a,b and Supplementary Table 11). However, body composition was not affected by inoculum, differing from previous studies, which might be related to dietary differences between the studies.

To determine the role of the antibiotic-altered microbiota on IBD development, we collected intestinal samples before the onset of severe weight loss, rectal prolapse or bloody stool, which often appear by week 26 (ref. 14). The highest incidence of UC and CD occurs in 15- to 30-year-old young adults, but can occur at any age; 26-week-old mice are adult. IL10−/− mice in the C57BL/6J background develop less aggressive IB in than in other genetic backgrounds, consistent with the low IBD histology activity index (HAI) we observed for the IL10−/− control pups. However, even without apparent illness, the STAT mice had IBD lesions (colonic inflammation with lamina propria mononuclear cell infiltration, mucosal hyperplasia, dysplasia) at week 6 of life, with progressively increasing severity; as expected, the WT mice had no lesions (Fig. 3a). In the 21-week-old IL10−/− mice, the mean IBD lesion was 55-fold higher (P < 0.0001) in pups inheriting the STAT versus control microbiota (Fig. 3b and Supplementary Table 5). Across all ages, based on HAI, the antibiotic-altered microbiota led to more severe inflammatory disease than the control microbiota (odds ratio (OR) 20.5, confidence interval (CI) 6.5–64.1) in IL10−/− pups) (Fig. 3b, Supplementary Fig. 9a and Supplementary Table 5). Using calprotectin and lipocalin-2, biomarkers of colonic inflammation in both human and murine IBD, temporal patterns of colitis development paralleled the observed histopathology (Fig. 3c,d and Supplementary Table 5), with differences between the recipients of the two inocula most pronounced at week 21 (Fig. 3a, inset). We show that inheritance of an antibiotic-perturbed microbiota is not only faithfully recapitulated in the next generation, but that it confers added disease risk.

In analyses using a panel of 547 mouse genes related to immunity and inflammation, we found that the IL10−/− mice showed increased colonic expression of Granzyne A, MHC Class II, Tnf, Ccl2 and Cxcl10 and decreased CD36, CD163 and IL15 compared to WT mice (Supplementary Fig. 9b and Supplementary Table 12), consistent with previous studies. Among the IL10−/− mice, the control and STAT groups showed differential intestinal gene expression; 10 of the 12 significantly differential immune genes (Fig. 3e and Supplementary Table 5), a hallmark of IL10−/− colitis. These histologic, immunologic and gene expression findings indicate that conventionalization of the IL10−/− mothers with the antibiotic-perturbed microbiota led to enhanced colitis in their offspring during adulthood.

Relationship between taxa and host phenotypes. We next used LEfSe and Random Forest modelling to determine which taxa could be important in the phenotypes we have characterized. Although these bioinformatic tools are insufficient to determine the significance of taxa without further experimental validation, they can identify candidates for further study. By LEfSe analyses, only two genera (Bacteroides and Odoribacter) more abundant in the STAT than the control inoculum (Supplementary Fig. 10a,i) were also more abundant in the pre-inflammation pup samples. Five taxa (Supplementary Fig. 10a,ii) including Blautia producta, Staphylococcus and Lachnospiraceae were more abundant in the pre-colitis IL10−/− STAT pups and might be considered disease-promoting candidates.

Nine taxa were more abundant in the control than the STAT inoculum (Supplementary Fig. 10a,iii), for seven (including Lactobacillus reuteri and Allobaculum), differences persisted in the pups. Nine other taxa had consistently increased abundance in the IL10−/− control pups (including Bilophila, Roseburia and Ruminococcus) (Supplementary Fig. 10a,iv). These 16 taxa, more abundant in Controls, might have disease-protecting roles.

Similarly, we used supervised learning to identify taxa predictive of high-level HAI in the IL10−/− mice at week 21 (Supplementary Fig. 10b). From samples obtained more than five weeks postgavage, Random Forest modelling identified five OTUs (within Bilophila, Lachnospiraceae, Lactobacillus reuteri, Ruminococcus and Bacteroidales S24-7) that highly predicted HAI scores. Four other OTUs (Rikenellaceae, Lactobacillus, Allobaculum and Roseburia) were predictive mostly before weaning. Thus, while LEfSe and Random Forest modelling use different approaches, both analyses indicated that Bilophila, Lactobacillus reuteri, Roseburia, Ruminococcus and Allobaculum were associated with protection against IBD onset, with the opposite for Odoribacter, Bacteroides, Lachnospiraceae and Rikenellaceae.

Discussion

These results show that exposing pregnant germ-free mice to specified inocula allows high-fidelity transmission of the taxa to the dams over a period of weeks and to their pups over months. Fidelity was maintained despite housing the mice in a conventional facility in which mouse-adapted strains are circulating and which could invade the test mice. Nevertheless, it is clear that the control inoculum was better maintained in the recipients than was the STAT.
Fig. 3 | STAT microbiota in IL10−/− mouse increases colonic inflammation. a, Representative H&E-stained colon sections from WT (top row of panels, week 21) and IL10−/− mice (bottom three rows of panels, weeks 6, 14 and 21) colonized with Control microbiota (left) or STAT microbiota (right). Inset (bottom right): higher-magnification image of inflammation in IL10−/− STAT mice at week 21. Images are representative, from a single experiment. Scale bars, 200 μm. b, Mean HAI ± s.d. for WT and IL10−/− mice colonized with Control (C) or STAT (S) microbiota. ****P < 0.0001, Kruskal–Wallis test with Dunn’s post-test. In a and b, WT: n = 10 per group; IL10−/− Control n = 13, 14, 10 and STAT n = 11, 13, 10 at weeks 6, 14 and 21, respectively. c, d, ELISA results of calprotectin and lipocalin-2 normalized to total protein levels from faecal supernatants. WT: n = 9 per group; IL10−/− Control n = 10, 11, 9 and STAT n = 9, 10, 9 at weeks 7, 14 and 19, respectively. Mean ± s.e.m., *P < 0.05, **P < 0.01, ****P < 0.0001, one-way ANOVA with Sidak’s multiple comparison test. e, Expression of colonic genes that were significantly altered by inoculum in IL10−/− pups (n = 3 each) at 21 weeks, measured by the Nanostring nCounter Mouse Immunology panel. Genes shown in bold have FDR-adjusted P values < 0.05, by t-test. Genes shown with normal font have FDR-adjusted P values between 0.05 and 0.10. f, Relative expression of TNF-α and IFN-γ in colonic tissue from week 21 IL10−/− pups, measured by reverse transcription quantitative-polymerase chain reaction (RT-qPCR) (STAT TNF-α: n = 12; all other groups: n = 8), mean ± s.e.m. *P < 0.05 t-test. For a–f, Supplementary Tables 5 and 6 provide detailed statistics.
inoculum, which showed both loss of taxa and lower stability in Jaccard analyses. We also show that host genotype matters, with the IL10−/− (immunodeficient) mice more permissive for transmission of the original inocula. A very recently published study, using a different study design in the IL10−/− model in which broad-spectrum antibiotics were directly given to pregnant dams, provides evidence similar to our findings and the results of these studies together can be used to benchmark future studies of transfers to pregnant germ-free recipients.

By transferring microbes rather than treating mothers with antibiotics, we have shown in this model system that the antibiotic-perturbed microbiota, not the antibiotic itself, was responsible for the accelerated disease onset seen in our mice. Of course, an important caveat of human IBD development of disease is the loss of protective organisms, such as Faecalibacterium prausnitzii and Rikenellaceae species. These taxa have been identified in previous studies in models of IBD development and in human subjects. The metagenomic studies also point to a potentially critical injurious role of one taxon Akkermansia muciniphila, and an important metabolic pathway leading to H2S production. However, it may be unlikely that any single taxon will be considered to be especially strong candidates for playing causal roles. As noted in the results, these include Odoribacter, Bacteroides, Lachnospiraceae, Blautia and Rikenellaceae species. These taxa have been identified in previous studies in models of IBD development and in human subjects. The metagenomic studies also point to a potentially critical injurious role of one taxon Akkermansia muciniphila, and an important metabolic pathway leading to H2S production. However, it may be unlikely that any single taxon will be considered to be especially strong candidates for playing causal roles. As noted in the results, these include Odoribacter, Bacteroides, Lachnospiraceae, Blautia and Rikenellaceae species. These taxa have been identified in previous studies in models of IBD development and in human subjects.

Methods

Animals

All animal experiments were conducted according to protocols approved by the New York University School of Medicine Institutional Animal Care and Use Committee (IACUC). After mice were received from the National Gnotobiotic Animal Resource Center (NGRRC), University of North Carolina, Chapel Hill, they were maintained on irradiated high fat diet (HFD, Rodent Diet D12451, Research Diets, 45% kcal%).

Microbiota inoculum preparation. WT C57BL/6 mice were raised to 18 weeks of age with sub-therapeutic levels of penicillin in their drinking water (ST) or not (control). Caecal contents were collected from three control and three ST mice. Contents were homogenized in an anaerobic environment, and tissue was removed and pooled according to experimental group (total 3 ml) and diluted with 5 ml of pre-reduced anaerobically sterilized saline (Anaero Systems). This inoculum was stored in 1 ml aliquots at −80°C until use.

A pool was used rather than individual mice because we could only realistically perform one transfer, as the experiment involved four groups of mice (two genotypes, two inocula) representing 16 litters and 135 mice studied in all (and 1,137 samples). Also, importantly, the intestinal microbiota showed individual variation, even in genetically identical mice housed in the same cage. To generate a representative microbiota from our control and antibiotic-treated mice, we selected the three mice with weights closest to the median.

Microbiota transfer into pregnant germ-free mice. Pregnant germ-free adult mice ≥8 weeks of age (WT C57BL/6) and IL10−/− C57BL/6J, received from the NGRRC—14 days after mating plugs were detected, were acclimated to the NYU animal facility overnight in their sterilized shipping containers. Control and STAT murine inocula, which had been frozen at −80°C (ref. ), were allowed to thaw, and the mice were briefly restrained while 250 μl of control or STAT inoculum was placed into their stomach by oral gavage. Recipient mice were randomly and inoculations were alternated between control and STAT with glove changes between each. Immediately after gavage, a faecal sample was collected from each mouse. There was no amplification of the 16S rRNA gene in any of these samples, confirming their germ-free status. Mice were then housed individually in autoclaved cages. We aimed to have five litters per group to produce at least 15 mice per group for statistical significance. All mice were maintained on a 45% HFD (Research Diets D12451) as the donor mice were receiving this diet before they were killed to create the caecal inocula. Dams gave birth 6.8±2.3 days after gavage. Five IL10−/− dams were gavaged with each inoculum and all 10 litters survived. Of the 12 WT dams gavaged, in six cases the mothers ate their pups shortly after birth (3/7 given the control inoculum and 3/5 receiving the STAT inoculum). Faecal pellets and scale weights were collected daily from the dams for the first week post-gavage.

Maintenance of pups.

The dams were left undisturbed after giving birth until their pups were 21 days old, except for the IL10−/− dams, which were weighed, and faecal pellets were obtained on days 4 and 11 post-partum. After the pups reached 21 days, dams and pups were weighed and faecal pellets were obtained once to five times per week. After weaning, pups were housed four per cage with other pups from the same inoculum and genotype group (WT control with WT control, WT STAT with WT STAT, and so on). Both sexes were studied. Cage assignments were random within each inoculum and genotype group to minimize litter/cage differences on the microbiome. Dams were killed two to three weeks after the pups were weaned. All 35 WT pups were killed at 21 weeks of age. Subgroups of IL10−/− pups were killed at 6 (n = 24), 14 (n = 27) and 21 (n = 26) weeks of age.

Histopathology.

At death, tissues were collected as described in ref. , fixed with 10% neutral-buffered formalin, routinely processed, and paraffin-embedded. Colon samples were sectioned and stained with haematoxylin and eosin (H&E), coded and scored by a board-certified veterinary pathologist (A.R.R.) who was blinded to experimental status. These samples were scored according to a previously established histopathologic grading scheme for experimental colitis.
with scores of 0–4 given for each of three categories (inflammation, hyperplasia and dysplasia). A total HAI was calculated by combining these three separate values. Sections from 10–14 mice were scored in each group, means were calculated and tested for statistical significance using Kruskal–Wallis test with Dunn’s post-test.

Odds ratio calculation. The severity scores of the disease status of the guts of the mice were scored on a scale of 0–6 in 11 response categories (Supplementary Fig. 9A). A proportional odds model was used to fit a common-slopes cumulative model, which is a parallel lines regression model based on the cumulative probabilities of the response categories rather than on their individual probabilities. The cumulative model has the form: \( g(\text{Pr}(Y \leq y_i)) = \alpha + \beta X_i + \epsilon \), where \( g \) is the logit link function, \( a, \ldots, a_n \) are intercept parameters and \( \beta \) is the vector of slope parameters. Based on this model, treatment effects can be represented as the odds ratio (OR) of going to the next level in the disease score of treatment versus control.

Microbial community analysis. DNA was extracted from frozen faecal, caecal and ileal samples using the MO BIO PowerSoil DNA Isolation Kit in a 96-well format. The 16S rRNA gene was amplified with barcoded fusion primers targeting the V4 region, as described in ref. 56. Amplicon pools were sequenced on the Illumina MiSeq platform with 150 bp paired-end reads. The QIIME pipeline was used for downstream analysis.

Bioinformatic analysis. Paired-end reads were joined using.fastq-join from EA-utils with a minimum overlap of 30 base pairs. Only reads that were perfectly matched in this overlapping region were kept. Sequences were demultiplexed and quality-filtered with QIIME. If a read had more than three consecutive low-quality bases (phred q score ≥ 20) it would be truncated, and any read that ended up being <75% of its original length was discarded. Sequences were assigned taxonomy with the open-reference method in QIIME using UCLUST50 and the Green Genes 2013 May database release as a reference.50 Samples with fewer than 5,000 reads were removed from the data set. This data set was then used to generate relative abundance plots, calculate alpha diversity and measure UniFrac distances to generate PCoA plots. Linear discriminant analysis effect size (LDA)52 was used to detect significantly differential taxa among various pairings of groups. To reduce the number of features the data set was filtered to exclude any taxa that accounted for 0.1% or less of the total observation count. The average sequencing depth for this study was 15,564. As such, 0.01% abundance represents only about one to two reads per sample. Although there are low-abundance taxa that could be relevant, they were considered to be below the detection threshold of the current study, as single reads may represent sequencing artefacts. Significance thresholds were retained as the default LEfSe settings.

Whole-genome sequencing. Faecal samples from four female pups from each group at three weeks of age were chosen for metagenomic study, along with each inoculum. Extracted genomic DNA (2 ng) from each sample was used for library preparation and subsequent whole-genome sequencing using the Illumina HiSeq 2500 platform. Samples were sequenced over four flow cell lanes as 100 bp paired-end reads. The metagenomes were pre-processed for quality metrics by removing nucleotides with a Phred score <20 and reads shorter than 60 bp using Trimmomatic.58 Sequences were then aligned to the mouse (mm10) genome using the bowtie2 program with standard settings. Only reads that were perfectly aligned and with maximum overlap of 30 base pairs were retained as the default LEfSe settings.

Intergenerational transfer heatmap. Relative abundances of filtered OTUs were plotted in a heatmap grouped by mouse genotype and treatment over selected timepoints. Heatmap was generated using custom R scripts and the packages biom, phyloseq and qiime.56,57

Jaccard Index analyses. Pairwise Jaccard distances were generated between each pair of samples for each pup, dam and inoculum sample, using the vegan R-package and the parameter binary = T. Plots were created using the levelplot function found within the lattice R-package. Summarized heatmaps were generated using the median distance across all sample groups for each group. Boxplots were generated using the median pairwise conspecific Jaccard distance, by comparing each pup sample to its previous sample, over time. For each mouse, the median Jaccard values were calculated by comparing between dam, pup and inoculum groups. The distribution of values was assessed by the pairwise Wilcoxon test with Holm correction for statistical significance.

Supervised classification of histopathology results. Random forest classification models were built to predict high (1+) versus low (<1) HAI histology scores (class) as a function of the microbial community OTU relative abundances (features), for each examined week of pup life, as described in ref. 31. Models were built by growing 1,000 trees per forest, and model error was calculated using a tenfold cross-validation approach. All samples were randomly subsampled at 5,000 OTUs per sample before analysis to avoid bias from uneven sampling efforts. Subsampling and analysis were performed in ten independent trials, which were then used to calculate mean model error and OTU importance. At all time points, the model had substantially better than random (error ratio >2) predictive power. The same strategy was used to predict inoculum (class) as a function of microbial community (features).

Quantification of faecal lipocalin-2 and calprotectin by enzyme-linked immunosorbent assay. Frozen faecal samples were vortexed with PBS to obtain a homogeneous suspension. Samples were then centrifuged for 10 min at 9,500g, at 4°C. Supernatants were collected and stored at −80°C until analysis. Lipocalin-2 levels were estimated in the supernatants using a Duoset murine lipocalin-2 enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems). Calprotectin levels were estimated in the supernatants using the S100A9/calprotectin mouse ELISA kit (Hyccult Biotech). Both were standardized to total protein, as determined using the Pierce BCA Protein Assay Kit (Thermo Fisher Scientific).

Gene expression studies. Colon, caecum and small intestine tissues were obtained at sacrifice, rinsed and later stored in RNA for 24 h at −1°C. Tissue was then placed in a new tube and stored at −80°C until RNA could be extracted using the RNeasy Mini Kit (Qiagen).

Nanostring. Colonic expression of a panel of immune genes was measured in 21-week-old WT and IL10−/− mice whose mothers had received either the STAT or control inoculum (four groups, n = 3 per group). Assays were performed using the NanoString Gene Expression Mouse Immunology Kit (NanoString Technologies).

qPCR. RNA was reverse-transcribed to cDNA with the Verso cDNA Synthesis kit (Thermo Fisher Scientific). qPCR was performed on a LightCycler 480 ii (Roche) using LightCycler 480 SYBR Green I Master. Specific mouse oligonucleotides were used to measure IFN-γ (5′-CGCGACAGTCTGATCAAAGCC-3′ and 5′-TGCTACACCTCTTTGGCCAGT-3′) and TNF-α (5′-GTCCTGCAAGGGAGATGTTG-3′ and 5′-GACCTTCAAGGAGATGGCT-3′). Relative expression of these genes was calibrated to the 18S rRNA endogenous control gene (5′-CATCATTACGGTGATAGG-3′ and 5′-GAACTCCCTGGTACCCCATT-3′) and analysed with the comparative C7 (ΔΔCt) method to calculate the fold-differences in expression between control and STAT IL10−/− pups.

Body composition measurements. Body composition was measured at approximately four-week intervals beginning when the pups were weaned at four weeks of age. Body composition was measured by dual energy X-ray absorptiometry (DEXA) with a Lunar PIXimus II mouse densitometer (GE Medical Systems) with anaesthesia by isofluorane inhalation.

Statistical analyses. Values were expressed as mean ± s.e.m. or s.d., as noted in the figure legends. Analyses were performed using GraphPad Prism (GraphPad Software). A one-way analysis of variance (ANOVA) with Sidak’s multiple comparison test or unpaired two-tailed students’ t-test was used to analyse parametric data while non-parametric data were analysed with a Mann–Whitney U-test.

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**Author contributions**
A.F.S., R.B.S. and M.J.B. designed experiments and interpreted data. A.F.S. performed experiments and participated in analysis of the data. Y.A. and V.E.R. contributed to interpretation of immunological data. Y.A. and M.H. performed protein expression assays. T.B. advised on microbiome analytical methods and performed data analyses. L.B. performed odds ratio calculations and other statistical tests. S.R., T.W. and D.K. contributed to microbiota stability analysis. A.B.R. performed histological analyses. L.M.C. and R.B.S. provided essential reagents and procedural advice. A.F.S. and M.J.B. were responsible for writing the manuscript, which was reviewed and edited by all authors.

**Competing interests**
The authors declare no competing financial interests.

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Experimental design

1. Sample size
   Describe how sample size was determined.
   [Lines 605-606] We aimed to have 5 litters per group to produce at least 15 mice per group for statistical significance.

2. Data exclusions
   Describe any data exclusions.
   No data were excluded.

3. Replication
   Describe whether the experimental findings were reliably reproduced.
   Due to the length of the experiment and availability of mice and inocula, this experiment has not been replicated.

4. Randomization
   Describe how samples/organisms/participants were allocated into experimental groups.
   [Lines 598-602] Control and STAT murine inocula, which had been frozen at -80°C, were allowed to thaw, and the mice were briefly restrained while 250 μl of Control or STAT inoculum was placed into their stomach by oral gavage. Recipient mice were chosen randomly and inoculations alternated between Control and STAT with glove changes between each.
   [Lines 621-622] Cage assignments were random within each inoculum and genotype group to minimize litter/cage differences on the microbiome.

5. Blinding
   Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   [Lines 627-629] Colon samples were sectioned and stained with hematoxylin and eosin (H&E), coded, and scored by a board-certified veterinary pathologist (A.B.R.) who was blinded to experimental status.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.
6. Statistical parameters
For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

| n/a | Confirmed |
|-----|-----------|
| ☑   | The exact sample size \( n \) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.) |
| ☑   | A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly |
| ☑   | A statement indicating how many times each experiment was replicated |
| ☑   | The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section) |
| ☑   | A description of any assumptions or corrections, such as an adjustment for multiple comparisons |
| ☑   | The test results (e.g. \( P \) values) given as exact values whenever possible and with confidence intervals noted |
| ☑   | A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range) |
| ☑   | Clearly defined error bars |

See the web collection on statistics for biologists for further resources and guidance.

7. Software
Policy information about availability of computer code

Describe the software used to analyze the data in this study.

See manuscript lines 647-684 for detailed descriptions of microbiome analysis methods. No custom code was used.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability
Policy information about availability of materials

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.

The only unique materials used in this study were the inocula which were obtained from a previously published experiment (Cox et al. 2014) and were used in their entirety in the course of these studies.

9. Antibodies
Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

[Lines 716-719] Lipocalin-2 levels were estimated in the supernatants using Duoset murine lipocalin-2 ELISA kit (R&D Systems, Minneapolis, MN). Calprotectin levels were estimated in the supernatants using the S100A9/Calprotectin mouse ELISA kit (Hycult Biotech, Plymouth Meeting, PA).

10. Eukaryotic cell lines
a. State the source of each eukaryotic cell line used.

No eukaryotic cell lines were used.

b. Describe the method of cell line authentication used.

No eukaryotic cell lines were used.

c. Report whether the cell lines were tested for mycoplasma contamination.

No eukaryotic cell lines were used.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No eukaryotic cell lines were used.
Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals
Provide details on animals and/or animal-derived materials used in the study.

[Lines 595-598] Pregnant germ-free mice (wildtype C57BL/6J and IL10-/- C57BL/6J), received from the NGRRC ~14 days after mating plugs were detected, acclimated to the NYU animal facility overnight in their sterilized shipping containers.

Policy information about studies involving human research participants

12. Description of human research participants
Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.