NLRP12 attenuates colon inflammation by maintaining colonic microbial diversity and promoting protective commensal bacterial growth

Liang Chen1,2,11, Justin E Wilson2,3,11, Mark J Koenigsknecht4, Wei-Chun Chou3, Stephanie A Montgomery2,5, Agnieszka D Truax2,3, W June Brickey1,2, Christopher D Packey6, Nitsan Maharshak7, Glenn K Matsushima1,8, Scott E Plevy9, Vincent B Young4, R Balfour Sartor10 & Jenny P-Y Ting1–3

Inflammatory bowel diseases involve the dynamic interaction of host genetics, the microbiome and inflammatory responses. Here we found lower expression of NLRP12 (which encodes a negative regulator of innate immunity) in human ulcerative colitis, by comparing monozygotic twins and other patient cohorts. In parallel, Nlrp12 deficiency in mice caused increased basal colonic inflammation, which led to a less-diverse microbiome and loss of protective gut commensal strains (of the family Lachnospiraceae) and a greater abundance of colitogenic strains (of the family Erysipelotrichaceae). Dysbiosis and susceptibility to colitis associated with Nlrp12 deficiency were reversed equally by treatment with antibodies targeting inflammatory cytokines and by the administration of beneficial commensal Lachnospiraceae isolates. Fecal transplants from mice reared in specific-pathogen-free conditions into germ-free Nlrp12-deficient mice showed that NLRP12 and the microbiome each contributed to immunological signaling that culminated in colon inflammation. These findings reveal a feed-forward loop in which NLRP12 promotes specific commensals that can reverse gut inflammation, while cytokine blockade during NLRP12 deficiency can reverse dysbiosis.

The healthy intestine is inhabited by trillions of bacteria and has evolved a fine-tuned balance between pathogen recognition and commensal tolerance. Sixty percent of individuals with inflammatory bowel disease (IBD) develops colitis, which is associated with dysbiosis, is associated with inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn’s disease, yet the treatment of IBD is limited to immunotherapy. Hence, understanding the host gene–microbiota interactions that contribute to colitis holds promise for the identification of novel microbiome-based therapeutic options.

A hallmark of IBD is the dysregulated activation of inflammatory cytokines and components of signaling pathways, such as members of the NF-κB, MAPK and STAT families. Several innate immunological receptors and sensors of the NLR family (‘nucleotide-binding-domain, leucine-rich-repeat’ or ‘NOD-like receptor’) regulate these pathways. NLRs are intracellular innate immunological sensors that exert either pro-inflammatory functions or anti-inflammatory functions. The NLR family member NOD2 activates NF-κB, and mutations in NOD2 represent the first genetic association with susceptibility to Crohn’s disease. Nod2-deficient mice display gut dysbiosis, which might contribute to exacerbated colitis. Similarly, loss of the NLRP6 inflammasome is associated with increased pathogenic colitis-associated microbes. Although the NLRP12 inflammasome has been linked to the recognition of specific infections, NLRP12 serves as a negative regulator of inflammatory signaling in experimental colitis in mice, its effect on the colonic bacterial ecology and the translational relevance to humans are unknown. Here we identified an additional function of NLRP12 in restricting intestinal inflammation by promoting beneficial strains of Lachnospiraceae.

RESULTS
Exacerbated colitis in Nlrp12−/− mice depends on microbiota
NLRP12 performs an anti-inflammatory function in experimental colitis. To determine if NLRP12 is clinically relevant to human colitis, we performed a meta-analysis of a paired comparison of gene-profiling studies of ten pairs of monozygotic twins (one with UC and...
one healthy) and seven additional UC patient cohorts and found that NLRP12 was significantly downregulated in cohorts with active UC relative to its expression in healthy (control) subjects or cohorts with inactive UC (Fig. 1a and Supplementary Fig. 1). In animal studies, conventionally raised Nlrp12−/− mice displayed more severe colitis than that of wild-type mice following oral administration of 3% dextran sodium sulfate (DSS), which induces experimental colitis. These differences include greater weight loss, mortality, histology scores and signs of clinical disease (measured as the disease-associated index (DAI)) in Nlrp12−/− mice than in wild-type mice; Nlrp12−/− mice also exhibited shorter colons than those of wild-type mice (indicative of increased colonic inflammation)12–13 (Fig. 1b,c and Supplementary Fig. 2a–d). Additionally, the loss of Nlrp12 resulted in heightened activation of NF-kB, the kinase ERK and STAT3 (Supplementary Fig. 2e–g). These findings established a link between reduced expression of NLRP12 and human IBD and consolidated NLRP12’s protective role in limiting colon inflammation via the suppression excessive immunological signaling.

In addition to the association between genetic susceptibility and colon inflammation, mounting evidence supports the proposal of a role for the microbiota in the pathogenesis of IBD14–16. To assess the effect of the microbiota on the enhanced susceptibility of Nlrp12−/− mice to colitis, we treated germ-free (GF) wild-type and Nlrp12−/− mice with a low dose of 1.5% DSS in drinking water (we used a low dose due to the reported enhanced sensitivity of GF mice to DSS)17. In contrast to conventionally raised mice, GF wild-type mice and GF Nlrp12−/− mice showed indistinguishable weight loss, mortality, DAI scores, colon length and histology scores following treatment with DSS (Fig. 1d–h). Colons from GF wild-type and GF Nlrp12−/− mice showed similar levels of activated NF-kB, ERK and STAT3 (Fig. 1i–k). These results indicated a role for the microbiota in the severe colitis found in Nlrp12−/− mice.

The presence of NLRP12 curtails the activation of inflammatory cell-signaling pathways induced by bacteria-derived ligands11–13,18 which suggests that Nlrp12−/− mice might have greater basal colonic inflammation in the presence of resident bacteria at steady state than that of wild-type mice. Indeed, colons from naive specific-pathogen-free (SPF) Nlrp12−/− mice displayed more activation of the NF-kB subunits p65 and p52 than that of wild-type mice raised in SPF conditions (control mice), as assessed by immunoblot analysis (Fig. 1l). Of note, this basal activation was low relative to that of mice treated with DSS, as these blots (Fig. 1l) required a longer exposure time for visualization of the proteins than did the blots above (Fig. 1l–k). To determine the role of NLRP12 in regulating basal colon inflammation in the presence of microbiota, we housed GF wild-type mice and GF Nlrp12−/− mice in a SPF vivarium for 4 weeks (Fig. 1m). GF Nlrp12−/− mice colonized for 4 weeks displayed more colonic activation of NF-kB than that of their wild-type counterparts or that of GF wild-type and GF Nlrp12−/− mice maintained in sterile isolators at steady state (Fig. 1n). These results demonstrated that basally activated NF-kB immunological signaling pathways required Nlrp12 deficiency and the microbiota of mice housed in conventional conditions. The C-type lectin and antimicrobial peptide REG3y and the cathelicidin-related antimicrobial peptide CRAMP were greater in abundance in the GF Nlrp12−/− mice housed in a SPF vivarium for 4 weeks than in their wild-type counterparts or GF wild-type and GF Nlrp12−/− mice maintained in sterile isolators (Fig. 1o,p), in agreement with published work showing that inflammation promotes the expression of antimicrobial peptides, which results in a dysbiotic intestinal microbiome19. These findings indicated that the intestinal microbiota exacerbated the colitis of Nlrp12−/− mice.

NLRP12 deficiency promotes a dysbiotic microbiome
To determine if NLRP12 alters the microbiome, we performed high-throughput gene-sequencing analysis of 16S rRNA in fecal bacterial DNA isolated from untreated wild-type and Nlrp12−/− mice originally generated from the same heterozygous Nlrp12−/+ parents and raised in our facilities (at the University of North Carolina) for more than nine generations. We used rarefaction analysis to compare bacterial diversity within individual mice of a group20, Nlrp12−/− mice harbored a microbiota with significantly less diversity (Fig. 2a) and a different community composition (Fig. 2b) relative to that of wild-type mice. Comparison of within- and between-group dissimilarity indicated that the microbiome difference between wild-type mice and Nlrp12−/− mice was significantly greater than the difference between mice within each genotype (Fig. 2c, calculated from Fig. 2b). Housing and diet are key factors that can influence the intestinal microbiota21, thus, we repeated the microbiome analysis 3 years later with mice housed in a second vivarium with different formula chow and obtained similar results (Supplementary Fig. 3a–c). These results demonstrated that Nlrp12−/− mice retained this altered microbiome in different housing conditions.

Because the wild-type and Nlrp12−/− mice we analyzed had been bred separately for multiple generations, it was possible that familial transmission instead of NLRP12 deficiency was responsible for the altered microbiome22. Therefore, we analyzed feces from wild-type and Nlrp12−/− littermates birthed from the same Nlrp12−/+ parents and housed separately by genotype after weaning (Supplementary Fig. 3d). Nlrp12−/− mice from this heterozygous mating harbored a microbiota with significantly less diversity (Fig. 2d) and greater dissimilarity (Supplementary Fig. 3e) than that of their wild-type littermates.

To establish rigor of the microbiota results, we again used high-throughput gene sequencing of 16S rRNA to characterize the microbiome of naive wild-type and Nlrp12−/− mice in three mouse cohorts (vivarium 1, vivarium 2 and littermates) (Supplementary Tables 1–3) and observed a consistent lower abundance of the order Bacteroidales and Clostridiales and family Lachnospiraceae and a greater abundance of the family Erysipelotrichaceae in Nlrp12−/− mice than in wild-type mice (Fig. 2e). This was a notable finding, as patients with IBD have been reported to display a microbiome profile similar to that14,16,23 (Fig. 2f and Supplementary Table 4). Thus, we focused on these bacterial groups in further studies.

Attenuation of disease in Nlrp12−/− mice by fecal transplantation
To determine if the altered microbiome observed in Nlrp12−/− mice was responsible for their exacerbated colon inflammation, we conducted microbiota-transfer studies by cohousing mice, which leads to exchange of the microbiota through coprophagia24,25. Age- and sex-matched wild-type and Nlrp12−/− mice were either housed singly (SiHo mice) or cohoused (CoHo mice) for 6 weeks before treatment with 3% DSS (Fig. 3a). SiHo wild-type mice and SiHo Nlrp12−/− mice (controls) showed significant differences in disease severity (Fig. 3b–f). After receiving DSS, Nlrp12−/− mice that were cohoused with wild-type mice (CoHo Nlrp12−/− mice) displayed less disease and immunological signaling than that of their SiHo Nlrp12−/− littermates (Fig. 3b–i and Supplementary Fig. 4a). Moreover, CoHo Nlrp12−/− and their wild-type cage-mates were similar in all measurements (Fig. 3b–i). As a control, we found that cohousing of wild-type mice with inflammasome-deficient Asc−/− mice failed to ameliorate the colitis of DSS-treated Asc−/− mice but instead resulted in increased death, DAI scores and histopathology in wild-type mice relative to that of SiHo wild-type mice, as reported8 (Supplementary Fig. 4b–f).
Figure 1 The microbiota distinguishes the colitis severity in wild-type mice from that in Nlrp12−/− mice. (a) NLRP12 expression by monozygotic twins (10 pair), healthy and with active UC (lines connect members of each set of twins) in one of eight studies of UC by NCBI GEO (left; other seven studies, Supplementary Fig. 1), and composite of those eight studies (right); results are presented relative to those of healthy control subjects. (b,c) Body weight (b) and survival (c) of conventionally raised wild-type (WT) and Nlrp12−/− mice treated with 3% DSS (above horizontal axes); results are presented relative to initial values, set as 100% (throughout). (d–g) Body weight (d), survival (e), DAI score (f) and colon length (g) of GF wild-type and Nlrp12−/− mice treated with 1.5% DSS. (h) Histopathology scores of colons from mice as in d–g, i–k Immunoblot analysis (left) and densitometry (right) of phosphorylated (p-) p65 and total actin (loading control throughout), p52 and histone H3 (l) and phosphorylated and total ERK (m) or STAT3 (n) in the distal colon of DSS-treated GF mice. AU, arbitrary units. (l) Immunoblot analysis and densitometry of phosphorylated and total p65, p52, and histone H3 in cytosolic (Cyto) and nuclear (Nuc) fractions of distal colon from untreated ('Steady state') SPF mice in three different cages (below lanes). (m) Generation of GF and 'conventionalized' (exGF) mice. (n–p) Immunoblot analysis (left) and densitometry (right) of proteins as in l (n), REG3γ (o) and CRAMP (p) in the distal colon of untreated GF and exGF mice. Each symbol (f–h, i–l (right), n–p (right)) or lane (i–l (left), n–p (left)) represents an individual mouse. NS, not significant (P > 0.05); *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (two-tailed paired t-test (a, left), unpaired t-test (a, right), b,d,f–l,n–p) or log-rank (Mantel Cox) test (c,e). Data are from one experiment representative of eight (a, left) or are pooled from eight experiments (a, right), four independent experiments with n = 41 mice (WT) or n = 42 mice (Nlrp12−/−) (b,c; error bars, s.e.m.) or two independent experiments with n = 18 mice (WT) or n = 19 mice (Nlrp12−/−) (d–g; error bars, s.e.m.) or n = 6 mice per group (h) or are from one experiment with n = 9 mice per group (i) or two independent experiments with n = 7 mice per group (i–k; error bars, s.e.m.) or n = 4 (n,p) or n = 3 (o) mice per group (n–p).
This was in contrast to the improved survival and diminished colitis of CoHo Nlrp12−/− mice and made Nlrp12 deficiency the first case, to our knowledge, in which enhanced susceptibility to colitis resulting from an NLR deficiency was reversed by the transfer of normal microbiota.

To investigate in detail the changes in the microbiome after cohousing of wild-type mice with Nlrp12−/− mice, we performed gene-microbiome-sequencing analysis of 16S rRNA in feces from wild-type and Nlrp12−/− mice in a cohousing setting. (Fig. 2a, b) High-throughput sequencing of 16S rRNA in the wild-type mice and Nlrp12−/− mice was performed (Table S2), and the microbiota composition (Fig. 2c, d) was determined using unweighted UniFrac principal-coordinate analysis (PCoA) of the microbiota composition (b) in wild-type and Nlrp12−/− mice in vivarium 1 (second independent experiment in vivarium 2, Supplementary Fig. 3). Each symbol (b) represents an individual mouse. PC1 and PC2, principal components 1 and 2, respectively. (c) Quantification of UniFrac distance in a, presented as dissimilarity values (first (box bottom) and third (box top) quartiles and the median (line inside box) and 1.5 interquartile range (line ends)). (d) High-throughput sequencing of 16S rRNA in the wild-type mice and Nlrp12−/− littermates. (e) High-throughput sequencing of 16S rRNA in the wild-type mice and Nlrp12−/− mice as in a-d (and Supplementary Fig. 3), results are presented as operational taxonomic units (OTUs). Each symbol represents an individual mouse (n values below plots); small horizontal lines indicate the mean (± s.e.m.). (f) Microbiota differences between Nlrp12−/− mice and wild-type mice in vivarium 1 (second independent experiment in vivarium 2, Supplementary Table 4). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (two-tailed unpaired t-test (a.d,e) or analysis of similarities (ANOSIM) test (c)). Data are from one experiment with n = 9 mice (WT) and n = 8 mice (Nlrp12−/−) (a-c; error bars (a), s.e.m.), are pooled two independent experiments with n = 10 mice (WT) and n = 14 mice (Nlrp12−/−) (d) or are from three independent experiments (e,f).

Figure 2 Nlrp12 deficiency results in a dysbiotic colonic microbiome. (a, b) High-throughput sequencing of 16S rRNA in fecal bacterial DNA (observed species mean; indicative of bacterial diversity) (a) and unweighted UniFrac principal-coordinate analysis (PCoA) of the microbiota composition (b) in wild-type and Nlrp12−/− mice in vivarium 1 (second independent experiment in vivarium 2, Supplementary Fig. 3). Each symbol (b) represents an individual mouse. PC1 and PC2, principal components 1 and 2, respectively. (c) Quantification of UniFrac distance in b, presented as dissimilarity values (first (box bottom) and third (box top) quartiles and the median (line inside box) and 1.5 interquartile range (line ends)). (d) High-throughput sequencing of 16S rRNA in the microbiome of wild-type and Nlrp12−/− littermates. (e) High-throughput sequencing of 16S rRNA in the microbiome of wild-type and Nlrp12−/− mice as in a-d (and Supplementary Fig. 3), results are presented as operational taxonomic units (OTUs). Each symbol represents an individual mouse (n values below plots); small horizontal lines indicate the mean (± s.e.m.). (f) Microbiota differences between Nlrp12−/− mice and wild-type mice in vivariums 1 and 2 (top) and between patients with IBD and healthy subjects (bottom), showing strains altered in Nlrp12−/− mice and patients with IBD, and the overlap in those groups (middle) (details, Supplementary Table 4). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (two-tailed unpaired t-test (a,d,e) or analysis of similarities (ANOSIM) test (c)). Data are from one experiment with n = 9 mice (WT) and n = 8 mice (Nlrp12−/−) (a-c; error bars (a), s.e.m.), are pooled two independent experiments with n = 10 mice (WT) and n = 14 mice (Nlrp12−/−) (d) or are from three independent experiments (e,f).
donor into a GF wild-type host mouse (FM(WT)→GF WT) resulted in significantly less DSS-induced colitis, survival and DAI scores but greater colon length than did the transfer of FM from an SPF Nlrp12-/- donor into GF Nlrp12-/- mice (FM(Nlrp12-/-)→GF Nlrp12-/-) (Fig. 4a–d). However, FM(WT)→GF Nlrp12-/- mice and FM(Nlrp12-/-)→GF WT mice exhibited similar weight loss, survival, disease index and colon length, all of which were less severe than that of FM(Nlrp12-/-)→GF Nlrp12-/- mice (controls) (Fig. 4a–d). This indicated that the microbiome shaped by the Nlrp12-/- genotype and genetic deficiency in Nlrp12 were both needed to produce the fulminating colitis of Nlrp12-/- mice.

Next we assessed the contribution of FM versus that of host genetics on immunological signaling pathways. As expected, colon samples from FM(WT)→GF WT mice (controls) exhibited minimal activation of NF-kB and STAT3, whereas samples from FM(Nlrp12-/-)→GF Nlrp12-/- mice showed a greater abundance of phosphorylated STAT3 and nuclear p52 than those of FM(Nlrp12-/-)→GF WT mice and a greater abundance of phosphorylated p65 than those of FM(WT)→GF

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Figure 3 Nlrp12-/- mice co-housed with wild-type mice display attenuated colitis. (a) CoHo–SiHo strategy, fecal microbiome sequencing and DSS treatment. (b–e) Body weight, (b) survival, (c) DAI score (d) and colon length (e) of littermates as in a. (f) Histopathology score of colons from littermates as in a. (g) PCoA analysis (as in Fig. 3a) of fecal microbial composition of littermates as in a after single housing or co-housing. (k) Quantification of UniFrac distance between the mice in j (presented as in Fig. 3c). (l) High-throughput sequencing of 16S rRNA in the microbiome of littermates as in a. Each symbol (d–f, g–i, right), l or lane (g–i, left) represents an individual mouse; small horizontal lines (g, l) indicate the mean (± s.e.m.). ∗P < 0.05, †††P < 0.001 and ††††P < 0.0001 (t, SiHo WT versus SiHo Nlrp12-/-; *; SiHo Nlrp12-/- versus CoHo Nlrp12-/- (b) (two-tailed unpaired t test (b), log-rank (Mantel Cox) test (c), one-way ANOVA with Fisher’s least-significant difference (LSD) test (d–i), ANOSIM test (k) or two-way ANOVA (l)). Data are pooled from three independent experiments with n = 16 mice (SiHo WT), n = 18 mice (SiHo Nlrp12-/-), n = 12 mice (CoHo WT) or CoHo Nlrp12-/-) (b–e, error bars, s.e.m.) or three independent experiments with n = 10 mice per group (f; error bars, s.e.m.) or from three independent experiments (g–i; error bars, s.e.m.) or one experiment with n = 9 mice per group (j–l).
**Figure 4** Transferring microbiota from *Nlrp12*^−/−^ mice induces colonic inflammation. (a–d) Body weight (a), survival (b), DAI score (c) and colon length (d) of GF wild-type and *Nlrp12*^−/−^ mice reconstituted with FM from SPF wild-type or *Nlrp12*^−/−^ mice (key (a,b) or below plot (c,d)) and treated with DSS. (e) Immunoblot analysis (left) and densitometry (right) of total p52 and histone H3 and phosphorylated and total p65 and STAT3 in the distal colon of the mice in a–d. Each symbol (c,d,e (right)) or lane (e (left)) represents an individual mouse. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (two-tailed unpaired t-test (a,e), log-rank (Mantel Cox) test (b) or one-way ANOVA with Fisher’s LSD test (c,d)). Data are pooled from two independent experiments with n = 11 mice (FM(WT)→GF WT), n = 10 mice (FM(Nlrp12^−/−^)→GF WT) and n = 9 mice (FM(WT)→GF Nlrp12^−/−^ and FM(Nlrp12^−/−^)→GF Nlrp12^−/−^) (a–d; error bars, s.e.m.) or one experiment (e; error bars, s.e.m.).

*Nlrp12*^−/−^ mice (Fig. 4e). Colons from FM(Nlrp12^−/−^)→GF WT and FM(WT)→GF Nlrp12^−/−^ mice showed intermediate, but distinct activation patterns (Fig. 4e). Colons from FM(Nlrp12^−/−^)→GF WT mice had a greater abundance of phosphorylated p65 than that of colons from FM(WT)→GF WT mice, similar to colons from FM(Nlrp12^−/−^)→GF Nlrp12^−/−^ mice, but had less translocation of p52 to the nucleus and activation of STAT3 than that of colons from FM(Nlrp12^−/−^)→GF Nlrp12^−/−^ mice (Fig. 4e). In contrast, FM(WT)→GF Nlrp12^−/−^ colons showed elevated phosphorylated STAT3 and nuclear p52, but modest phosphorylated p65 (Fig. 4e). These results indicated that both the microbiome of *Nlrp12*^−/−^ mice and deficiency in Nlrp12 were needed to cause the full activation of p52, p65 and STAT3 and fulminant colitis.

**Suppression of colitis in Nlrp12^−/−^ mice by Lachnospiraceae**

We had determined that the Clostridiales order and Lachnospiraceae family were significantly lower in abundance and Erysipelotrichaceae was greater in abundance in *Nlrp12*^−/−^ mice than in wild-type mice (Fig. 3). Lachnospiraceae strains are decreased in abundance in Crohn’s disease14,16,23, and Lachnospiraceae limits experimental colitis induced by *Clostridium difficile*26. To determine if bacterial groups that are lacking in *Nlrp12*^−/−^ mice can restrict colitis, we inoculated wild-type and *Nlrp12*^−/−^ mice with 23 strains of Lachnospiraceae (simultaneously) via oral gavage for 21 d, followed by 1 week of rest before treatment with DSS (Fig. 5a). Mice given Lachnospiraceae and control mice given only the brain–heart–infusion (BHI) vehicle (used for growing Lachnospiraceae) had similar weight before treatment with DSS (Fig. 5a). Strikingly, DSS-treated *Nlrp12*^−/−^ mice inoculated with Lachnospiraceae displayed less colitis, including less weight loss, lower DAI scores, less colon histopathology and greater colon lengths, than that of DSS-treated *Nlrp12*^−/−^ mice inoculated with BHI vehicle (Fig. 5a–d and Supplementary Fig. 5). Proinflammatory cytokines (Fig. 5e) and the abundance of phosphorylated p65, ERK and STAT3 in addition to nuclear p52 showed similar a reduction in DSS-treated *Nlrp12*^−/−^ mice inoculated with Lachnospiraceae relative to that in DSS-treated *Nlrp12*^−/−^ mice inoculated with BHI vehicle (Fig. 5f–h).

To determine whether Lachnospiraceae modulated the severity of inflammation by altering the composition of the microbiota of *Nlrp12*^−/−^ mice, we sequenced the microbiome of the mice inoculated with BHI vehicle and *Nlrp12*^−/−^ mice, but had less translocation of p52 to the nucleus and activation of STAT3 than that of colons from FM(Nlrp12^−/−^)→GF Nlrp12^−/−^ mice (Fig. 4e). In contrast, FM(WT)→GF Nlrp12^−/−^ colons showed elevated phosphorylated STAT3 and nuclear p52, but modest phosphorylated p65 (Fig. 4e). These results indicated that both the microbiome of *Nlrp12*^−/−^ mice and deficiency in Nlrp12 were needed to cause the full activation of p52, p65 and STAT3 and fulminant colitis.

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the distance between the $Nlrp12^{−/−}$ mice inoculated with BHI vehicle and $Nlrp12^{−/−}$ mice inoculated with Lachnospiraceae (Fig. 5j,k), which suggested that $Nlrp12^{−/−}$ mice inoculated with Lachnospiraceae develop a microbiome more reminiscent of that of wild-type mice. Among all the microbiota strains sequenced (Supplementary Table 5), Clostridiales underwent growth promoted by the administration.

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**Figure 5** Administration of Lachnospiraceae suppresses colitis in $Nlrp12^{−/−}$ mice. (a) Body weight of wild-type and $Nlrp12^{−/−}$ mice inoculated (downward arrowheads) with Lachnospiraceae (Lachno) or BHI vehicle (BHI), assessed before (left) and after (right) treatment with DSS (protocol, above plots). (b,c) DAI score (b) and colon length (c) of the mice in a, assessed after DSS treatment. (d) Histopathology score of mice as in a, assessed after DSS treatment. (e) Quantification of cytokines in colon explant cultures of mice as in a, assessed after DSS treatment; results are presented per mg colon tissue. (f-h) Immunoblot analysis (left) and densitometry (right) of phosphorylated IκBα, phosphorylated and total p65, and total p52 and histone H3 (f) and phosphorylated and total ERK (g) or STAT3 (h) in the distal colon (cytosolic and nuclear fractions in f) of mice as in a, assessed after DSS treatment. (i,j) High-throughput sequencing of 16S rRNA in fecal bacterial DNA (i) and unweighted UniFrac PCoA (j) of the microbiome composition of mice as in a, assessed before DSS treatment (presented as in Fig. 2a (i) or Fig. 2b (j)). (k) Quantification of UniFrac distance of mice as in a, assessed before DSS treatment (presented as in Fig. 2c). (l) High-throughput sequencing of 16S rRNA in the microbiome of mice as in a, assessed before DSS treatment (presented as in Fig. 2e). Each symbol (b-e, f-h (right), I) or lane (f-h (left)) represents an individual mouse; small horizontal lines indicate the mean (s.e.m.). *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001 (unpaired t-test (a,b), one-way ANOVA with Fisher’s LSD test (b-h), ANOSIM test (k) or two-way ANOVA (l)). Data are pooled from three independent experiments with $n = 24$ mice (WT BHI), $n = 33$ mice (WT Lachno), $n = 22$ mice ($Nlrp12^{−/−}$ BHI or $Nlrp12^{−/−}$ Lachno) (a-c; error bars, s.e.m.) or one experiment with $n = 4$ mice (WT BHI, WT Lachno and $Nlrp12^{−/−}$ Lachno) or $n = 5$ mice ($Nlrp12^{−/−}$ BHI; d; error bars, s.e.m.) or are from three independent experiments with $n = 9$ mice (WT BHI and WT Lachno), $n = 14$ mice ($Nlrp12^{−/−}$ BHI) or $n = 8$ mice ($Nlrp12^{−/−}$ Lachno) (e) or $n = 7$ mice per group (f-h) (error bars, s.e.m.), one experiment with $n = 7$ mice (WT BHI and $Nlrp12^{−/−}$ BHI) or $n = 8$ mice (WT Lachno and $Nlrp12^{−/−}$ Lachno) (I-J; error bars (I-J), s.e.m.).
of Lachnospiraceae in Nlrp12<sup>−/−</sup> mice, but Erysipelotrichaceae had growth that was significantly reduced (Fig. 5i). As the abundance of Erysipelotrichaceae was greater in the Nlrp12<sup>−/−</sup> mice than in wild-type mice, and this positively correlated with disease severity (Fig. 3b–f), the administration of Lachnospiraceae might have limited colitis in part by suppressing the expansion of Erysipelotrichaceae.

**Prevention of dysbiosis by hematopoietic NLRP12 expression**

The enhanced intestinal inflammation in Nlrp12<sup>−/−</sup> mice is driven by both hematopoietic components and non-hematopoietic components<sup>12,13</sup>, but the cellular compartment expressing NLRP12 that shapes the intestinal commensals is unknown. To address this, we generated chimeras by lethally irradiating wild-type and Nlrp12<sup>−/−</sup>

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**Figure 6** NLRP12 expression by hematopoietic cells prevents intestinal dysbiosis. (a) Bone-marrow transplantation (BMT) and collection of fecal DNA for analysis of the microbiome. (b–d) High-throughput sequencing of 16S rRNA in fecal bacterial DNA (b), unweighted UniFrac PCoA (c) and quantification of UniFrac distance in (d) of mice treated as in a (presented as in Fig. 2a (b) or Fig. 2b (c) or Fig. 2c (d)). (e) Flow cytometry (concatenated plots) of cLP macrophage (Mac) and DC subpopulations in SPF wild-type and Nlrp12<sup>−/−</sup> mice (left margin). Numbers in outlined areas indicate percent cells in each gated area. (f) Quantification of results in e. (g) RT-qPCR of genes encoding proinflammatory cytokines in colon-resident macrophages and DCs sorted (as in e; below plots) from wild-type and Nlrp12<sup>−/−</sup> mice (key) and left untreated (UT) or stimulated for 3 h with cecal contents from wild-type mice (CC); results are presented relative to those of wild-type CD11b<sup>+</sup>CD11c<sup>+</sup>F4/80<sup>+</sup>CD103<sup>+</sup> macrophages. Each symbol (f,g) represents cells pooled from two to three colonics; small horizontal lines indicate the mean (± s.e.m.). *P < 0.05; **P < 0.01; ***P < 0.001 and ****P < 0.0001 (two-tailed unpaired t-test (b,f,g) or ANOSIM test (d)). Data are pooled from two independent experiments with n = 8 mice per group (b–d; error bars (b), s.e.m.) or two independent experiments with n = 9 (macrophage) or n = 5 (DC) replicates per group (g) or are from four independent experiments with cells pooled from two to three mice (e,f).
and then transferring bone marrow from wild-type donors or Nlrp12−/− donors to generate wild-type mice with myeloid cells deficient in Nlrp12 (Nlrp12−/−→WT) and Nlrp12−/− mice expressing Nlrp12 only in myeloid cells (WT→Nlrp12−/−), as well as control mice (Nlrp12−/−→Nlrp12−/− or WT→WT) (Fig. 6a). We then assessed the microbiome after a 10-week-reconstitution period (Fig. 6a). As expected, WT→WT mice retained greater microbiota diversity than did Nlrp12−/−→Nlrp12−/− mice (Fig. 6b). Nlrp12−/−→WT mice displayed less bacterial diversity than that of WT→WT mice (Fig. 6b). Additionally, WT→Nlrp12−/− mice had greater bacterial diversity than that of Nlrp12−/−→Nlrp12−/− mice (Fig. 6b). These results indicated that NLRP12 expression in the hematopoietic compartment dominantly shaped the diversity of the intestinal bacteria. Analysis of bacterial composition confirmed these findings (Fig. 6c,d).

We next characterized the hematopoietic cells in the colon lamina propria (cLP) that had contributed to the enhanced basal inflammation in Nlrp12−/− mice. Although NLRP12 suppresses inflammatory pathways in bone-marrow-derived macrophages and dendritic cells (DCs)12, there is no evidence to support the proposal of a role for NLRP12 in colonic macrophages and DCs, which are phenotypically different from their bone-marrow-derived counterparts27. Within the cLP, colon-resident macrophages are identified as two subsets that express the fractalkine receptor (CX3CR1), macrophage marker F4/80 and the integrin CD11c and the integrin CD103 (αEβ7) and were considered CD11b+CD11c+ cells (R2, ref. 28) (Fig. 6e). To analyze the effect of NLRP12 on these macrophage and DC populations at steady state, we isolated cLP cells from naive wild type and Nlrp12−/− mice and gated on CD45+I-A^b+ cells with differential expression of F4/80, CD11b, CD11c and CD103, by flow cytometry. In accordance with the enhanced colonic inflammation of SPF Nlrp12−/− mice, these mice displayed a significantly greater frequency of CD11c+CD11b+ cells (R3, Fig. 6e) and a modestly greater frequency of CD11c+CD11b+ cells (R1, Fig. 6e) and CD11c+CD11b+ cells (R2, Fig. 6e) than that of SPF wild-type mice. These R1–R3 fractions were further stained for F4/80 and CD103. In wild type and Nlrp12−/− mice, the R1 and R2 populations were found to be mainly macrophages, and R3 cells were DCs (Fig. 6e). The composite data of multiple mice showed a greater frequency of CD11b+CD11c+F4/80+CD103+ DCs in the cLP of Nlrp12−/− mice than in that of wild-type mice (Fig. 6f). The frequency of CD11b+CD11c+F4/80+CD103− and CD11c+CD11b+F4/80+CD103− populations, which were characterized as macrophages, was also greater in Nlrp12−/− mice than in wild-type mice, but this difference was not significant (Fig. 6f). These results suggested that Nlrp12 deficiency increased the frequency of CD11b+CD11c+F4/80+CD103+ colonic DCs.

To maintain colon homeostasis, macrophages and DCs in the cLP acquire anti-inflammatory phenotypes28 and become anergic to bacterial stimulation. To determine if NLRP12 has a role in maintaining the quiescence of colonic macrophages and DCs, we exposed cLP macrophages and DCs from naïve wild-type and Nlrp12−/− mice to cecal contents from wild-type mice to mimic the cell-resident bacterial interactions in vivo. Consistent with the concept that cLP macrophages and DCs are anergic to bacterial stimulation27, exposure to cecal contents did not increase the expression of genes encoding inflammatory cytokines in wild-type macrophages and DCs, with the exception of a slight increase in the expression of Il23a (which encodes the cytokine subunit p19) by wild-type DCs (Fig. 6g). In contrast, cecal contents significantly increased the expression of Il6 (which encodes the cytokine IL-6), Tnf (which encodes the cytokine TNF), Il12b (which encodes the cytokine subunit p40) and Il23a in Nlrp12−/− CD11b+CD11c+ macrophages and CD11b+CD11c+ DCs.
relative to their expression in wild-type cells treated with cecal contents (Fig. 6g). Nlrp12−/− CD11b+CD11c+ macrophages displayed only higher Tnf expression than that of wild-type CD11b+CD11c+ macrophages, after exposure to cecal contents (Fig. 6g). These results suggested that NLRP12 maintained homeostasis in colonic macrophages and DCs in the presence of cecal material.

**TNF and IL-6 cause microbial dysbiosis in Nlrp12−/− mice**

The findings reported above indicate that Nlrp12 deficiency and the microbiome derived from Nlrp12−/− mice resulted in elevated inflammation (Fig. 4). We next directly investigated if increased inflammation could drive microbial dysbiosis. Antibody to TNF (anti-TNF) is a common treatment for patients with IBD30, while antibody to the cytokine receptor IL-6R (anti-IL-6R) has been discussed as a therapeutic strategy for colitis31,32. We targeted the inflammatory cytokines TNF and IL-6 in vivo by giving mice injection of anti-TNF and anti-IL-6R before and during exposure to DSS (Supplementary Fig. 6a,b). Blocking TNF and IL-6R ameliorated the DSS-induced colitis of Nlrp12−/− mice, as indicated by their significantly improved weight gain, survival and clinical scores compared with those of PBS-treated Nlrp12−/− mice, while the effect of anti-TNF and anti-IL-6R was less pronounced on wild-type mice than on their Nlrp12−/− counterparts (Fig. 7a–c).

To determine if antibody treatment reversed the dysbiosis of Nlrp12−/− mice, we performed microbiome analysis before and after antibody treatment. Prior to antibody treatment, naive Nlrp12−/− mice displayed a microbiome that was significantly altered compared with that of wild-type mice (Supplementary Fig. 6c–e). However, Nlrp12−/− mice treated with anti-TNF and anti-IL-6R displayed significantly greater intestinal bacterial diversity than that of Nlrp12−/− mice treated with PBS (Fig. 7d) and were more reminiscent of antibody-treated wild-type mice and PBS-treated wild-type mice, in their microbiome composition (Fig. 7e,f), with a greater abundance of Bacteroidales and Clostridiales taxa than that of PBS-treated Nlrp12−/− mice (Fig. 7g). In contrast, PBS-treated Nlrp12−/− mice maintained a significantly lower bacterial diversity and abundance of Bacteroidales and Clostridiales than that of antibody-treated wild-type mice and PBS-treated wild-type mice (Fig. 7e–g). These results indicated that blocking excessive inflammatory cytokines reversed the altered microbiome and diminished colitis in Nlrp12−/− mice. In conclusion, our results indicated that instead of proceeding along a one-way linear trajectory, the interaction between aberrant immunological signaling and dysbiotic microbiota in Nlrp12−/− mice proceeded in a feed-forward cycle in which increased inflammation driven by loss of NLRP12 expression produced a proinflammatory microbiota that further escalated inflammation (Supplementary Fig. 7).

**DISCUSSION**

NLRP12 serves a protective role in intestinal inflammation by suppressing canonical and non-canonical NF-κB12,13. In this study, we identified an additional critical role for NLRP12: regulating gut microbial communities. IBD-profiling studies revealed that NLRP12 expression was negatively correlated with active UC. In agreement with those findings, we found that fecal microbiota from Nlrp12−/− mice enriched for Erysipelotrichaceae induced more activation of NF-κB and STAT3 than did fecal microbiota from wild-type mice with a low abundance of Erysipelotrichaceae. Lachnospiraceae might provide a protective function in part by suppressing overgrowth of intestinal Erysipelotrichaceae. Accompanied by the outcome of human gene profiling, in which mucosal NLRP12 expression is reduced in active colitis, our results suggest that Lachnospiraceae might be therapeutic when applied to patients with UC who have reduced expression of NLRP12. In sum, our reciprocal fecal transplantation experiments, cohousing and bacteria-transfer severe susceptibility of Nlrp12−/− mice to disease. Intestinal microbial diversity and human disease have a complex reciprocal cause-and-effect relationship. A reduction in the richness of the gut microbiome is a biomarker for human metabolic and inflammatory disorders33, including IBD24. Loss of commensal diversity due to genetic alterations34 or an unhealthy diet35 correlates with exacerbated colitis. Our results indicate that NLRP12 acts as central component of this relationship by curtailing excessive inflammatory cytokine production to limit intestinal inflammation and maintain commensal diversity and protective microbiota. Neutralization of TNF is a current IBD therapy, and targeting IL-6R is being assessed as a similar therapeutic approach. Our findings indicate that in addition to suppressing inflammatory signaling, targeting these cytokines might also alleviate colitis by reversing dysbiosis.

Many protective intestinal bacterial groups are necessary for the maintenance of gut homeostasis. We observed that similar to the microbiota found in patients with Crohn’s disease14, there was a lower abundance of Bacteroidales and Clostridiales (in particular Lachnospiraceae) strains in untreated Nlrp12−/− mice, which correlated with a greater sensitivity to DSS-induced colitis. Deficiency in Nod2 or Nlrp6 results in a colitogenic microbiota that can exacerbate colitis5-8. In contrast, we observed that dysbiosis and colitis severity caused by loss of an NLR was reversed by transfer of the microbiota from wild-type mice, in further support of our conclusion that NLRP12 has a predominant role in maintaining protective commensal bacterial groups and limiting colitogenic strains. In line with that, Nlrp12−/− mice cohoused with wild-type mice exhibited elevated intestinal Clostridiales and Lachnospiraceae, decreased Erysipelotrichaceae and reduced DSS-induced colitis relative to that of singly housed Nlrp12−/− mice. Although the abundance of Lachnospiraceae is diminished in patients with IBD14,16,23,36,37 and C. difficile-associated colitis38, little is known about how these protective strains affect the host immune system. Lachnospiraceae show greater enrichment in the mucosal folds than in the central lumen49. The spatial location of Lachnospiraceae favors their interaction with lamina-propria-resident immune cells, which suggests that Lachnospiraceae might function as an immunological regulator to prevent enteric pathogen adhesion and/or colonization. Lachnospiraceae are also major producers of the short-chain fatty acid propionate in the human gut60, which promotes the generation of peripheral regulatory T cells41,42. The loss of Lachnospiraceae species in the Nlrp12−/− mice might have resulted in reduced production of short-chain fatty acids, which could have contributed to the elevated inflammation in Nlrp12−/− mice. Moreover, there is a negative correlation between intestinal Lachnospiraceae and Erysipelotrichaceae. Erysipelotrichaceae have been linked to elevated levels of TNF and chronic intestinal inflammation in animals infected with simian immunodeficiency virus and in patients infected with human immunodeficiency virus who are receiving antiretroviral therapy43,44, which suggests that these organisms seem to be highly inflammatory.
experiments collectively indicated that the interaction between aberrant immune signaling and dysbiotic microbiota in Nlpr12−/− mice did not proceed along a one-way linear trajectory but instead proceeded in a vicious feed-forward cycle in which increased inflammation driven by loss of NLRP12 expression produced a proinflammatory microbiota that further escalated inflammation.

METHODS

Methods, including statements of data availability and any associated accession codes and references, are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.E.W. and J.P.-Y.T. designed the experiments and wrote the manuscript, with critical input from V.B.Y. and R.B.S.; M.I.K. and V.B.Y. generated the purified Lachnospiraceae strains; W.-C.C. contributed to the immunoblot analysis, cytokine measurement and flow cytometry; S.A.M. performed the histopathological scoring; A.D.T., W.J.B and G.K.M. generated the radiation-bone-marrow chimeras; C.D.P., N.M.S., S.E.P. and R.B.S. contributed to the isolation of fecal DNA and 16S rRNA gene-sequencing experiments; and R.B.S. generated the GF mice.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Mice. All animal procedures were approved by the University of North Carolina Chapel Hill (UNC) Institutional Animal Care and Use Committee (IACUC) according to US National Institutes of Health guide for the Care and Use of Laboratory Animals. Animal numbers were empirically determined to optimize numbers necessary for statistical significance based on our previous reports using these disease models (minimum of four to seven animals per group). Animals were excluded if they exhibited signs of disease not associated with the condition of the colitis (for example, fight wounds and malocclusion). No randomization method was used due to the single-housing and cohousing strategy required for the microbiome studies, *ad libitum* administration of DSS in drinking water and use of littermate controls.

Wild-type C57BL/6J mice were obtained from Jackson Laboratory and house-raised at UNC for at least nine generations. Nlrp12−/− and Asc−/− mice are on C57BL/6J background after at least nine generations of backcross. Conventionally raised mice were bred and housed in specific-pathogen-free (SPF) conditions, and germ-free (GF) animals were generated and housed in the National Gnotobiotic Rodent Resource Center of North Carolina at Chapel Hill or in the Center for Gastrointestinal Biology and Disease, Gnotobiotic Core at North Carolina State University. For cohabitation experiments, 4-week-old mice originating from the same breeders were divided to be either housed singly (SiiH0) or cohoused with age- and sex-matched mice (CoHo) for 6 weeks. CoHo mice were compared with their SiiH0 littermates as controls. For the ‘conventionalization’ study, GF animals were transferred into SPF conditions and were housed for 4 weeks. The ‘conventionalized’ (exGF) mice were compared with mice kept in the GF condition as controls. For the reciprocal fecal-transplantation study, GF wild-type or Nlrp12−/− mice were treated by oral gavage once a week for 3 weeks with a PBS suspension of feces derived from SPF wild-type or Nlrp12−/− mice, following the previously described protocol24. The mice were given DSS 1 week after the final gavage. For adoptive bone-marrow-transplantation study, wild-type and Nlrp12−/− chimeric mice were generated as previously described23. No antibiotics were used during the reconstitution phase, and fecal DNA was collected 10 weeks after the transplantation for microbiome analysis.

Experimental colitis. Experimental colitis was initiated by treatment of mice with 1.5–3% dextran sulfate sodium (DSS) (36,000–50,000 M.Wt, MP Biomedicals) in autoclaved drinking water for 5 d. DSS was then replaced by normal autoclaved water for 6–7 d. Body weight was monitored daily, and the disease-associated index (DAI) was determined by an investigator blinded to experimental conditions on day 8 after DSS treatment, according to the following parameters: weight loss (0 points = 0% weight loss from baseline; 1 point = 1–5% weight loss; 2 points = 5–10% weight loss; 3 points = 10–20% weight loss; 4 points = more than 20% weight loss); Rectal bleeding (0 points = negative; 2 points = positive hemoccult test; and 4 points = gross bleeding); and stool consistency (0 points = normal; 2 points = semiformed stool; and 4 points = liquid that adheres to the anus). The sum of these three parameters results in the total DAI score ranging from 0 (healthy) to 12 (severe disease).

Histopathology. Colonies were Swiss rolled, fixed in 10% neutral-buffered formalin and paraffin embedded and processed for histological analysis. 5-μm-thick colon sections were stained with hematoxylin and eosin (H&E) and semiquantitatively scored for histopathology by a board-certified veterinary pathologist in a blinded manner. Histology scores represent the sum of each histological alteration outlined below. This system assesses inflammation, epithelial defects, area of inflammation, area of epithelial defect, crypt atrophy and dysplasia-neoplasia by giving each parameter a separate score (0–4) for severity and extent as previously described12.

Immunoblot analysis. Colonies were excised, opened longitudinally and washed with cold PBS. The distal-most 3-cm section of each colon was collected and mechanically homogenized in NE-PER Nuclear and Cytoplasmic Extraction Reagent (Thermo Scientific) containing Complete Protease Inhibitor and PhosSTOP (Roche) per the manufacturer’s protocol. Cytosolic and nuclear lysates were subjected to SDS–PAGE and immunoblot analysis. The following primary antibodies were used for immunoblot analysis, at a dilution of 1:1,000:

- anti-IL1β (Ser32) (14D4) (cat. no. 2859), anti-1-β-KK/β (Ser176/180) (16A6) (cat. no. 2697), anti-p-65 (Ser56) (93H11) (cat. no. 3033), anti-NIK (cat. no. 4994), anti-p-ERK1/2 (Thr202/Tyr204) (D13.14.4E) (cat. no. 4370), anti-p-65 (D1E12) (cat. no. 8242), anti-p-STAT3 (Tyr705) (cat. no. 9131) and anti-STAT3 (79D7) (cat. no. 4904) (Cell Signaling Technology); anti-p-p52 (C-5) (cat. no. sc-736), anti-ERK1 (G-16) (cat. no. sc-93), anti-ERK2/G-14 (cat. no. sc-154); anti-CRAMP (G-1) (cat. no. sc-166055) and anti-actin-HRP (C-11) (cat. no. sc-16151) (Santa Cruz Biotechnology); and anti-REG3γ (cat. no. ab198216) (Abcam) and anti-Histone H3 (cat. no. 07-690) (Millipore). Goat anti-rabbit-HRP (cat. no. 111-035-144) and goat anti-mouse-HRP (cat. no. 115-035-146) (Jackson Laboratories) were used as secondary antibodies. Protein densitometry was quantified by ImageJ software.

Colon explant cultures. Colonies were excised, washed several times in cold PBS containing 2x penicillin-streptomycin (Sigma-Aldrich) and cultured for 15 h in RPMI media ( Gibco) containing 2x penicillin-streptomycin at 37°C. Supernatants were centrifuged to clear debris and assessed for cytokines by Luminox Bio-Plex System per the manufacturer’s instructions.

Fecal DNA extraction. Fecal samples were collected from live mice, snap-frozen and stored at −80°C. DNA was isolated by incubating fecal material at 65°C for 30 min in Lysis Matrix E tubes (MP Biomedicals) containing 200 mM NaCl, 100 mM Tris, 20 mM EDTA (pH 8.0), SDS and proteinase K (Qiagen). Phenol:Chloroform:Isoamyl alcohol (Invitrogen) was added, and the samples were homogenized at 4°C for 3 min using a bead beater homogenizer. The samples were centrifuged at 8,000 r.p.m. for 3 min at 4°C, and the supernatant was incubated with Phenol:Chloroform (Invitrogen) for 10 min at room temperature. The samples were centrifuged at 13,000 r.p.m. for 5 min at 4°C, and the aqueous phase was incubated with isopropanol and 3M sodium acetate, pH 5.2, at −20°C for 15 h to precipitate DNA. The precipitated DNA was collected by centrifugation at 13,000 r.p.m. at 4°C for 20 min, washed twice with 100% cold ethanol and resuspended in TE buffer. The DNA was further purified using a DNAeasy Blood and Tissue Kit (Qiagen) according to the manufacturer’s protocol.

16S rRNA gene sequencing and data analysis. Fecal DNA samples were amplified by PCR using barcoded primer pairs targeting the V1–V2 region of the 16S gene. PCR amplicons were sequenced at the V1–V2 region using Roche 454 for the vivarium #1 study27, and the same V1–V2 region was sequenced using an Mi-Seq Illumina sequencer for all other experiments described. The resulting bacterial sequence fragments were clustered into Operational Taxonomic Units and aligned to microbial genes with 97% sequence similarity from Greengenes Database using UCLUST method in QIME. Bacterial taxa summarization and rarefaction analyses of microbial diversity or compositional differences (dissimilarity value indicated by Unweighted UniFrac Distance) were calculated in QIME (1.8.0) as previously described using QIIME scripts (including pick_open_reference_otus.py, summarize_taxa.py, alpha_rarefaction.py, jackknifed_beta_diversity.py and make_distance_box_plots.py). PCoA plots indicating compositional difference were generated by QIME script, make_2d_plots.py. Each point represents one mouse, and the ellipses represent the interquartile range (IQR) during the rarefaction analyses (scripts details, http://www.wernerylab.org/teaching/qiime/overview). All 16S rRNA microbiome sequences have been deposited in the European Nucleotide Archive (http://www.ebi.ac.uk/ena) and QIITA (https://qiita.ucsd.edu). The accession codes are listed in the ‘Data Availability’ section below.

Isolation and stimulation of mouse cLP macrophages and DCs. Mouse colonies were opened longitudinally and washed with cold PBS to remove the fecal contents. Pooled colonies from two mice were further cut into 1.5-cm pieces and washed twice with 30 ml of HBSS containing 10% FBS and 2 mM EDTA on an orbital shaker at 250 r.p.m. for 30 min at 37°C. After washing, the colonies were finely minced and digested with 20 ml of HBSS containing 10% FBS, 1.5 mg/ml Type VIII Collagenase (C2139; Sigma-Aldrich) and 40 μg/ml DNase I (4527; Sigma-Aldrich) at 200 r.p.m. for 20 min at 37°C. After the digestion, the digested cLP cells were filtered through a 100-μm strainer, centrifuged at 1,500 r.p.m. for 5 min at 4°C and resuspended in 2 ml MACS buffer for flow cytometry or fluorescence-activated cell sorting16.
For flow cytometry, cLP cells were incubated with mouse FcγRI/III receptor blocker (553141; BD Pharmingen), used at a dilution of 1:20 for 15 min on ice and were then stained with the following labeled antibodies (at a dilution of 1:200) for 30 min on ice: Brilliant Violet 421-conjugated anti-CD45 (103133; BioLegend), PE-conjugated anti-I-A<sup>B</sup> (553552; BD Pharmingen), PE-Cy5-conjugated anti-CD11c (117324; BioLegend), PE-Cy7-conjugated anti-CD11b (101216; BioLegend), APC-conjugated anti-CD103 (17-1031-80; eBioscience), and Alexa Fluor 488-conjugated anti-F4/80 (123120; BioLegend). Samples were washed twice in MACS buffer and were analyzed on a BD LSR II flow cytometry at the UNC Flow Cytometry Core Facility. Dead cells and doublets were excluded from the cLP mononuclear cell population on the basis of appropriate forward- and side-scatter plots. Antigen-presenting cells were defined as CD45<sup>I-Ab</sup>-<sup>B</sup>. Macrophage and DC populations were first categorized by the expression of CD11b and CD11c and then were further characterized by CD103 and F4/80 expression as previously described. For separation of cLP macrophages and DCs by fluorescence-activated cell sorting and <i>ex vivo</i> stimulation, CD11b<sup>+</sup> and CD11c<sup>+</sup> microbeads (Miltenyi Biotec) were used for the enrichment of CD11b<sup>+</sup> or CD11c<sup>+</sup> cells. The enriched cells were stained with labeled antibodies aforementioned. Stained cells were sorted to purify the indicated population at the UNC Flow Cytometry Core Facility. Sorted macrophages and DCs were seeded in a 96-well plates (0.5 × 10<sup>3</sup> to 1 × 10<sup>5</sup> cells per well) with 200 µl RPMI medium containing 10% FBS. For cell content stimulation, cecal contents were generated from wild-type mice as described elsewhere and were added to the cell culture medium at a 1:200 dilution for 3 h. Total RNA was then isolated by TRIzol according to the manufacturer's instructions (Thermo Fisher Scientific) for qRT-PCR analysis.

### Design of Lachnospiraceae-specific primers

Lachnospiraceae strains were identified using an adapted version of the plate wash PCR technique. This allowed rapid screening by PCR using phytoplasmic specific primers of the 16S rRNA gene to screen selective media plates in order to identify conditions that enrich for specific bacteria. To generate Lachnospiraceae specific primers, the nearly full-length 16S rRNA gene sequences from 34 bacterial strains were used to generate <i>CLUSTALW</i> multiple-sequence alignments. The 16S rRNA gene sequences used were the following: nine of the most represented bacterial sequences from the mouse large intestine (Akkermansia muciniphila, Alastipes fontidooi, Bacteroides acidifaciens, Enterococcus fecalis, Lactobacillus murinus, Oscillo bacter <i>lostridium</i> <i>citroniae</i>, <i>Clostridium</i> <i>indolit</i>, <i>Clostridium</i> <i>propionici</i>um and <i>Clostridium</i> <i>syl</i>osus), six Lachnospiraceae (<i>C</i>lostridium <i>al</i>denese, <i>C</i>lostridium <i>bolett</i>ae, <i>C</i>lostridium <i>c</i>tori<nine>, <i>C</i>lostridium <i>i</i>dol<i>it</i>, <i>C</i>lostridium <i>p</i>ropionici<i>um</i> and <i>C</i>lostridium <i>syl</i>osus), three Lachnospiraceae strains isolated before and after stimulation, cecal tissue homogenization and stool was serial diluted in anaerobic PBS and were placed into a sterile container for tissue homogenization (50 μm pore size) using a Medimachine tissue homogenizer (BD Biosciences). The tissue was grinded for 15 s and homogenized tissue was used for plating. The cecal contents, cecal tissue homogenization and stool was serial diluted in anaerobic PBS and plated in duplicate onto brain heart infusion agar (BD Biosciences) with 0.01% cysteine (BHI). A variety of media conditions were used to enrich for Lachnospiraceae isolates. Bacterial growth from the first agar plate was collected and genomic bacterial DNA was isolated using an Easy-DNA kit (Invitrogen). Using the Lachnospiraceae specific primers and PCR conditions described above, we identified several media conditions that showed enrichment for Lachnospiraceae strains. Once a media condition was identified with enrichment for Lachnospiraceae strains, single colonies from the duplicate plate were used to inoculate 1 ml of BHI plus 5% FBS into a sterile 96-well plate. These cultures were grown anaerobically for 3 d at 37 °C, and then 1 ml of the liquid culture was used as a template for the PCR reaction described above. Lachnospiraceae-specific primers (<i>Supplementary Table 6</i>) were used to identify potential Lachnospiraceae isolates. If any of the primers used yielded a successful PCR reaction, 50 µl of the corresponding culture was plated for single colonies anaerobically on BHI plus 5% FBS for 1–3 d at 37 °C. A single colony was used to inoculate a 5 ml BHI plus 5% FBS culture that grew anaerobically for 1–3 d at 37 °C. This culture was used to create 20% final concentration glycerol stocks of all the isolates that were stored at −80 °C. The following media conditions were used to isolate Lachnospiraceae strains in this study: BHI and 5% FBS; BHI, 1 μg/ml astreomam, 10 μg/ml colistin and 2 μg/ml gentamycin; and BHI, 0.5 μg/ml ampicillin, 2 μg/ml erythromycin and 0.25 μg/ml vancomycin. Single colonies from the duplicate plate were used to inoculate 1 ml of BHI and 5% FBS. Specific media conditions used to isolate each Lachnospiraceae strains are defined (<i>Supplementary Table 7</i>).

### Taxonomic classification of bacterial isolates

Genomic DNA was isolated using an Easy-DNA (Invitrogen) kit. PCR reaction conditions were described above and PCR product cleanup was performed using ExoSAP-IT (Affymetrix) per the manufactures protocol. Nearly full-length 16S rRNA amplicons were sequenced at the University of Michigan DNA Sequencing Core using primers 8<sup>F</sup>-AGAGTTTGTATCCTGCTCAG-3<sup>C</sup>, 515<sup>F</sup>-GTCCACAGGCGGCGGAAT-3<sup>C</sup>, and 1492R-AGAGTTTGATCCTGGCTCAG-3<sup>C</sup> in a total of 25 µl per reaction. PCR reaction was performed under the following cycling conditions: 95 °C for 2 min, 30 cycles of 95 °C for 30 s, annealing at 57 °C for 45 s, and extension at 72 °C for 90 s, 72 °C for 10 min.

### Bacterial isolation and selective growth conditions

Three of the Lachnospiraceae isolates (Lachnospiraceae D4, G11 and E7) used in this study were reported previously. The remaining twenty strains were isolated from murine cecal contents, cecal tissue and stool. Mouse stool was collected into a sterile tube directly from a restrained mouse and immediately transferred into an anaerobic chamber (Coy Industries, Grass Lake, MI). Ceca from C57BL/6 mice were collected in a sterile manner and immediately transferred into an anaerobic chamber. A sterile scalpel was used to open the cecum and separate cecal content and cecal tissue. Cecal content was added to a sterile tube and diluted into anaerobic 1× phosphate-buffered saline (PBS). Using a sterile syringe 2 ml of PBS was gently injected into the cecum to remove any additional cecal content. The cecal tissue was then added to 1 ml of anaerobic PBS and placed into a sterile container for tissue homogenization (50 μm pore size) using a Medimachine tissue homogenizer (BD Biosciences). This tissue was grinded for 15 s and homogenized tissue was used for plating. The cecal contents, cecal tissue homogenization and stool was serial diluted in anaerobic PBS and plated in duplicate onto brain heart infusion agar (BD Biosciences) with 0.01% cysteine (BHI). A variety of media conditions were used to enrich for Lachnospiraceae isolates. Bacterial growth from the first agar plate was collected and genomic bacterial DNA was isolated using an Easy-DNA kit (Invitrogen). Using the Lachnospiraceae specific primers and PCR conditions described above, we identified several media conditions that showed enrichment for Lachnospiraceae strains. Once a media condition was identified with enrichment for Lachnospiraceae strains, single colonies from the duplicate plate were used to inoculate 1 ml of BHI plus 5% FBS into a sterile 96-well plate. These cultures were grown anaerobically for 3 d at 37 °C, and then 1 ml of the liquid culture was used as a template for the PCR reaction described above. Lachnospiraceae-specific primers (<i>Supplementary Table 6</i>) were used to identify potential Lachnospiraceae isolates. If any of the primers used yielded a successful PCR reaction, 50 µl of the corresponding culture was plated for single colonies anaerobically on BHI plus 5% FBS for 1–3 d at 37 °C. A single colony was used to inoculate a 5 ml BHI plus 5% FBS culture that grew anaerobically for 1–3 d at 37 °C. This culture was used to create 20% final concentration glycerol stocks of all the isolates that were stored at −80 °C. The following media conditions were used to isolate Lachnospiraceae strains in this study: BHI and 5% FBS; BHI, 1 μg/ml astreomam, 10 μg/ml colistin and 2 μg/ml gentamycin; and BHI, 0.5 μg/ml ampicillin, 2 μg/ml erythromycin and 0.25 μg/ml vancomycin. Single colonies from the duplicate plate were used to inoculate 1 ml of BHI and 5% FBS. Specific media conditions used to isolate each Lachnospiraceae strains are defined (<i>Supplementary Table 7</i>).

### Mouse colonization with Lachnospiraceae bacteria

Mice were treated by oral gavage with a mixture containing the 23 Lachnospiraceae strains (~1 × 10<sup>8</sup> bacteria) described above, in brain-heart infusion broth (BHI) twice a week for 3 weeks. BHI broth was used as vehicle control. The mice were given DSS 1 week after the final gavage.
Mice were given 2 mg/kg body weight of anti-IL-6R (Tocilizumab, Genentech) and anti-TNF (Infliximab, Janssen Biotech) via intraperitoneal injection twice a week for 4 weeks before DSS exposure and during the 11-day DSS treatment period. Fecal DNA was collected from wild-type littermates and Nlrp12−/− littermates before and after the 4-week antibody treatment period, but before DSS treatment.

**Metadata study of human NLRP12 profiling and microbiome changes in patients with UC.** Raw data from eight studies of human UC (from NCBI GEO) were renormalized and analyzed by Genespring GX (Agilent Tech). The following data sets were used: GSE22619, GSE42911, GSE14580, GSE16879, GSE13367, GSE65114, GSE21231 and GSE57945. In the summary panels, the healthy group includes the samples from healthy participants and normal un-inflamed tissues of the patients, the active group includes the samples from the patients’ inflamed tissues, and the inactive group includes un-inflamed tissue samples of patients in remission due to the treatment (anti-TNF, steroids and others).

For microbiome changes in patients with IBD, we downloaded the published raw 16S rRNA gene sequencing file from the open-source microbiome deposition site QIITA (https://qiita.ucsd.edu/) under study ID 1939. This file includes 16S microbiome sequencing results from 28 healthy control subjects, 63 patients with colonic Crohn’s disease, 156 patients with ileal Crohn’s disease and 24 patients with UC. Raw sequencing data was reanalyzed as aforementioned. Significantly altered bacterial strains were identified by comparison of patients with IBD (colonic Crohn’s disease, ileal Crohn’s disease or UC) with healthy participants.

**Statistical analysis.** Statistical analysis was performed with GraphPad Prism 6 software. Significance between two groups was determined by unpaired, two-tailed Student’s t test, and significance between multiple groups was determined using one-way analysis of variance (ANOVA) with Fisher’s LSD test. A paired, two-tailed Student’s t test was used to compare NLRP12 mRNA between monozygotic twins in Figure 1A. A two-way ANOVA test was used to identify the significantly changed microbial groups among all strains identified by 16S microbiome sequencing between different experimental conditions. Statistical significance for survival studies was determined by the log-rank (Mantel Cox) test. For immunoblot analyses and graph plotting, one dot or lane represents one mouse. Microbiome compositional dissimilarity was displayed by PCoA plots and quantified by UniFrac Distance values, and significant separation of the microbiome composition was determined by ANOSIM test using Qiime 1.8.0. The distribution of the UniFrac Distance values were displayed in Tukey’s box plots, which display the first (bottom of the box) and third quartiles (top of the box), the median (the line inside the box) and 1.5 interquartile range of the upper or lower quartile (whiskers). For all statistical comparisons, *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001, and NS means no significance.

**Data availability.** All 16S rRNA microbiome sequences raw files that support the findings of this study have been deposited in The European Nucleotide Archive (http://www.ebi.ac.uk/ena) with primary access codes PRJEB18700, PRJEB18699 and PRJEB18678, and in the open-source microbiome sharing database QIITA (https://qiita.ucsd.edu) with the study ID 10427, 10428, 10429, 10818 and 10820. The other data are available from the corresponding author upon reasonable request.

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Erratum: NLRP12 attenuates colon inflammation by maintaining colonic microbial diversity and promoting protective commensal bacterial growth

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In the version of this article initially published, the second sentence in the first subsection of Results incorrectly described an experimental group as “…seven additional patients with UC…”. The correct description is “…seven additional UC patient cohorts….”. The error has been corrected in the HTML and PDF versions of the article.
Corrigendum: NLRP12 attenuates colon inflammation by maintaining colonic microbial diversity and promoting protective commensal bacterial growth

Liang Chen, Justin E Wilson, Mark J Koenigsknecht, Wei-Chun Chou, Stephanie A Montgomery, Agnieszka D Truax, W June Brickey, Christopher D Packey, Nitsan Maharshak, Glenn K Matsushima, Scott E Plevy, Vincent B Young, R Balfour Sartor & Jenny P-Y Ting

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In the version of this article initially published, the vertical axis of Figure 5j was incorrectly labeled 'PC1 (22.56%)'. The correct label is 'PC3 (6.47%)'. The error has been corrected in the HTML and PDF versions of this article.