THE JOURNAL OF IMMUNOLOGY

IL-22 Deficiency Alters Colonic Microbiota To Be Transmissible and Colitogenic

Lauren A. Zenewicz, Xiaochen Yin, Guoyang Wang, Eran Elinav, Liming Hao, Liping Zhao and Richard A. Flavell

J Immunol 2013; 190:5306-5312; Prepublished online 12 April 2013;
doi: 10.4049/jimmunol.1300016
http://www.jimmunol.org/content/190/10/5306

The Journal of Immunology is published twice each month by
The American Association of Immunologists, Inc.,
9650 Rockville Pike, Bethesda, MD 20814-3994.

Print ISSN: 0022-1767 Online ISSN: 1550-6606.

IL-22 Deficiency Alters Colonic Microbiota To Be Transmissible and Colitogenic

Lauren A. Zenewicz,*,[1] Xiaochen Yin,[†] Guoyang Wang,[†] Eran Elinav,*,[2] Liming Hao,[‡] Liping Zhao,[†,§] and Richard A. Flavell*,[¶]

IL-22 is a good candidate to play a critical role in regulating gut microbiota because it is an important inducer of antimicrobial peptides and mucins in the gut. However, whether IL-22 participates in immune homeostasis by way of modulating gut microbiota remains to be elucidated. In this study, we find, through 16S rRNA gene-pyrosequencing analysis, that healthy IL-22–deficient mice had altered colonic microbiota, notably with decreased abundance of some genera, including *Lactobacillus*, and increased levels of others. Mice harboring this altered microbiota exhibited more severe disease during experimentally induced colitis. Interestingly, this altered gut microbiota can be transmitted to cohoused wild-type animals along with the increased susceptibility to this colitis, indicating an important role for IL-22 in shaping the homeostatic balance between immunity and colonic microbiota for host health. ***The Journal of Immunology*****, 2013, 190: 5306–5312.**

The cytokine IL-22 is an important modulator of tissue responses during inflammation in many tissues, including the skin, lung, and gastrointestinal (GI) tract (1). We and other investigators showed that IL-22 is an important cytokine that protects the colon during inflammation (2, 3). IL-22 is highly upregulated in the sera of patients with Crohn's disease or ulcerative colitis (4). In dextran sodium sulfate (DSS)-mediated colitis, IL-22–deficient mice develop more severe disease than do wild-type controls and have a longer recovery period (2, 3). IL-22 was also shown to be protective in a T cell–mediated colitis model (2) and infectious colitis, such as that caused by the pathogenic bacterium *Citrobacter rodentium* (5).

Barrier integrity is essential to maintain immune homeostasis within the colon. IL-22 is important to preserving the integrity of the single-layer GI epithelium during inflammation (1). Recognition of IL-22 by epithelial cells leads to activation of Stat3-signaling pathways, which, in turn, leads to both proliferative and antiapoptotic pathways (6). IL-22 is a potent inducer of antimicrobial molecules, including β-defensin, lipocalin-2, and RegIIIγ, as well as mucins (3, 5, 7, 8). RegIIIγ, which binds to lipoteichoic acid and, therefore, is effective against Gram-positive bacteria, is highly regulated by IL-22; in the absence of IL-22 there is little induction of this antimicrobial (9). Mucins are highly inducible by IL-22 and constitute the thick mucosal layer that coats the epithelium and that is impenetrable to many commensal bacteria, thereby limiting their potential to cause inflammation (3). In the absence of IL-22 signaling, levels of these molecules can be greatly diminished (5, 9), and we hypothesize that this could affect the composition of the microbiome.

IL-22 is upregulated in many types of lymphocytes during inflammation; however, during immune homeostasis, one lymphocyte subset primarily expresses IL-22: the RORγ*t*-dependent innate lymphocyte (1, 10, 11). These cells are found in the small intestine in Peyer's patches and in the colon in similar structures referred to as "cryptopatches," as well as in the lamina propria (12–16). As with other aspects of the immune system, commensal bacteria play a critical role in the development and differentiation of these cells (14, 15).

Previous studies from our laboratory (17), as well as those from other laboratories (18, 19), showed that mice lacking specific components of the immune system, such as TLR5 or the inflammasome, have altered microbiota. Furthermore, we showed that mice deficient in a component of the inflammasome have altered microbiota, as well as that this different microbiota can be transferred to wild-type mice and is colitogenic (17). We hypothesized that because IL-22 is important in the expression of antimicrobial peptides and mucin, its absence may modify the colonic niche, altering the flora composition of the GI tract. This led us to investigate whether the absence of IL-22 could lead to altered microbiota and whether this had a role in colitis.

To examine the effect of IL-22 on the structure of the microbiota of the colon, we performed experiments in which microbiota from IL-22–deficient mice were naturally transmitted to wild-type mice. We found, using pyrosequencing analysis of 16S rRNA gene V3 region, that wild-type mice cohoused with IL-22–deficient mice had a microbiome more similar to IL-22–deficient

*Department of Immunobiology, Yale University School of Medicine, New Haven, CT 06520; [†]State Key Laboratory of Microbial Metabolism, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China; [‡]Department of Pathology, Yale University School of Medicine, New Haven, CT 06520; [§]Ministry of Education Key Laboratory of Systems Biomedicine, Shanghai Center for Systems Biomedicine, Shanghai 200240, China; and [¶]Howard Hughes Medical Institute, Yale University School of Medicine, New Haven, CT 06520

[1]Current address: Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City, OK.

[2]Current address: Immunology Department, Weizmann Institute of Science, Rehovot, Israel.

Received for publication January 4, 2013. Accepted for publication March 12, 2013.

L.A.Z. was supported by an American Cancer Society fellowship. E.E. was supported by the Cancer Research Institute (2010–2012) and the Israel–US Educational Foundation (2009) and was the recipient of the Claire and Emmanuel G. Rosenblatt Award from the American Physicians for Medicine in Israel Foundation. R.A.F. is an Investigator at the Howard Hughes Research Institute.

The sequences presented in this article have been submitted to the GenBank Sequence Read Archive database (http://www.ncbi.nlm.nih.gov/sra) under accession number SRA054081.

Address correspondence and reprint requests to Dr. Richard A. Flavell, Howard Hughes Medical Institute, Yale University School of Medicine, 300 Cedar Street, The Anlyan Center S-569, New Haven, CT 06520. E-mail address: richard.flavell@yale.edu

The online version of this article contains supplemental material.

Abbreviations used in this article: DSS, dextran sodium sulfate; GI, gastrointestinal; IBD, inflammatory bowel disease; IVF, in vitro fertilization; OTU, operational taxonomic unit; PCA, principal component analysis.

mice than to that of wild-type mice not exposed to IL-22–deficient mice, showing that the altered flora found in IL-22–deficient mice is transmissible. This altered microbiota was shown to be responsible for at least some of the increased disease observed during colitis in IL-22–deficient mice.

Materials and Methods

Mice

Il22$^{-/-}$ mice were described previously (2). Age- and sex-matched C57BL/6 mice were purchased from the National Cancer Institute (Frederick, MD). To rederive our IL-22–deficient mice, in vitro fertilization (IVF) was performed with sperm isolated from an *Il22*$^{-/-}$ male mouse and C57BL/6 ova, and the resulting embryos were implanted into a CD-1 female. The resulting heterozygous offspring were housed in a specific pathogen–free room that excluded *Helicobacter* and were separate from our original *Il22*$^{-/-}$ colony. The heterozygous mice were intercrossed to generate *Il22*$^{-/-}$ mice. All mice were cared for in accordance with Institutional Animal Care and Use Committee–approved protocols at the Yale University animal facility. For cohousing, three mice of each strain were housed together for 4–6 wk prior to DSS treatment. Mice were 8–12 wk old at the initiation of DSS treatment.

DSS-induced colitis

Mice were given 2% (w/v) DSS (molecular mass = 36,000–50,000 Da; MP Biomedicals) ad libitum in their drinking water for 7 d, which was then replaced with normal water.

Histology

Colons were excised, rinsed with PBS, fixed overnight in 10% formalin, and then embedded in paraffin, sectioned, and stained with H&E. Slides were prepared at the Yale University Program for Critical Technologies in Molecular Medicine, Department of Pathology. Sections were blindly analyzed by a trained gastroenteropathologist. Each segment was given a score of 0–4. For chronicity (the degree of chronic inflammation): grade 0, no significant changes; grade 1, mildly increased inflammatory cells in the lamina propria; grade 2, moderately increased inflammation in the lamina propria (multiple foci); grade 3, high level of inflammation with evidence of wall thickening by inflammation; and grade 4, maximal severity of inflammation with transmural lymphocytic infiltration and/or architectural distortion. For activity (the degree of epithelial injury): grade 0, no significant changes; grade 1, occasional epithelial injury with focal and superficial or rare cryptitis; grade 2, foci of cryptitis, including rare crypt abscess; grade 3, multiple crypt abscesses and/or focal ulceration; grade 4, grade three plus extensive ulceration.

Colonoscopies

Colonoscopy was performed using a high-resolution mouse video endoscopic system (Coloview; Karl Storz, Tuttlingen, Germany). The severity of colitis was blindly scored using the Murine Endoscopic Index of Colitis Severity, which is based on five parameters: granularity of the mucosal surface, vascular pattern, translucency of the colon mucosa, visible fibrin, and stool consistency (20).

16S rRNA analyses

Aliquots of frozen fecal samples were processed for DNA isolation using a previously validated protocol (21). Forward 5′-NNNNNNNNAT-TACCGCGGCTGCT-3′ and reverse 5′-NNNNNNNNCCTACGGGAGG-CAGCAG-3′ primers were used to amplify the 16S rRNA gene V3 region. The NNNNNNNN was the sample-unique eight-base barcode used for sorting of PCR amplicons. PCR reactions, pyrosequencing, and sequence quality controls were performed as described previously (22). High-quality sequences were uploaded into QIIME and processed as described earlier (23), using Pynast (24) to align the sequences and RDP classifier (25) for the classification (cutoff 50%). Operational taxonomic unit (OTU) data were generated with 97% identity. Principal component analysis (PCA) and cluster analysis were performed in the MATLAB 7.11.0 (R2010b) environment (The MathWorks). The sequences have been deposited in GenBank Sequence Read Archive database (http://www.ncbi.nlm.nih.gov/sra) under accession number SRA054081.

Real-time RT-PCR

RNA from colons was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA). RNA was subjected to reverse transcriptase with Superscript II (Invitrogen) and oligo deoxythymidine primer. cDNA was semiquantitated with commercially available primer and probe sets (Applied Biosystems, Foster City, CA) and the ΔΔCT method. Hypoxanthine guanine phosphoribosyltransferase was included as an internal control.

Statistical analyses

For data with normal distribution, one-way ANOVA was performed; otherwise, Mann–Whitney analysis was used. The p values <0.05 were considered significant.

Results

Specific pathogen–free mice deficient in IL-22 exhibit no abnormal pathology

Previously, in the absence of inflammation, we did not detect any differences in IL-22–deficient mice compared with wild-type mice (2). Because low levels of IL-22 are expressed in the colon during immune homeostasis (26), we hypothesized that a lack of the cytokine may have much longer-term consequences on colonic health. Therefore, we examined the colonic architecture of 1-y-old wild-type or IL-22–deficient mice. We found normal colonic architecture in both groups of mice, with minimally increased inflammation in some mice but no significant colitis in any animals (Fig. 1). Thus, a long-term deficiency in IL-22 does not affect the colon morphology.

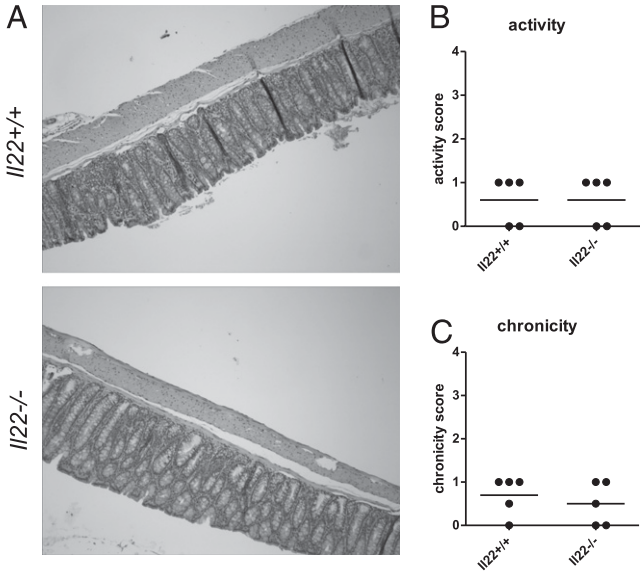

FIGURE 1. IL-22–deficient mice have normal colonic architecture. One-year-old specific pathogen–free IL-22–deficient (*Il22*$^{-/-}$) or wild-type (*Il22*$^{+/+}$) control mice were euthanized. Colons were excised, fixed, sectioned. and stained with H&E. (**A**) Representative micrographs (original magnification ×100) of five mice/group. Activity (the degree of epithelial injury) (**B**) and chronicity scores (the degree of chronic inflammation) (**C**) for the colon sections. Each segment was given a score of 0–4. For chronicity (the degree of chronic inflammation); grade 0, no significant changes; grade 1, mildly increased inflammatory cells in the lamina propria; grade 2, moderately increased inflammation in the lamina propria (multiple foci); grade 3, high level of inflammation with evidence of wall thickening by inflammation; grade 4, maximal severity of inflammation with transmural lymphocytic infiltration and/or architectural distortion. For activity (the degree of epithelial injury); grade 0, no significant changes; grade 1, occasional epithelial injury with focal and superficial or rare cryptitis; grade 2, foci of cryptitis, including rare crypt abscess; grade 3, multiple crypt abscesses and/or focal ulceration; grade 4, grade three plus extensive ulceration. Each symbol represents one mouse; horizontal line indicates the mean.

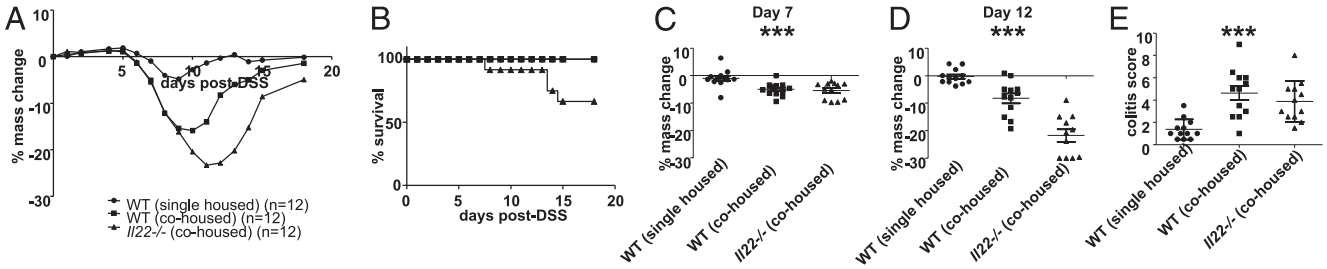

FIGURE 2. Cohousing wild-type mice with IL-22–deficient mice increases susceptibility to DSS-induced colitis. C57BL/6 mice were either housed by themselves or cohoused with *Il22*$^{-/-}$ mice for 4 wk. Mice were then given 2% DSS in their drinking water for 7 d. (**A**) Mice were weighed daily. Graph shows mean change in mass for each group over time. (**B**) Survival of mice at different days post-DSS. Mass loss at day 7 post-DSS (**C**) and day 12 post-DSS (**D**). Each symbol represents one mouse; the horizontal line indicates the mean. (**E**) At day 7 post-DSS, colonoscopies were performed and blindly scored, as described in *Materials and Methods*. Each symbol represents one mouse; the horizontal line indicates the mean. Experiment is representative of three independent experiments. ***$p < 0.001$, one-way ANOVA.

Cohousing wild-type mice with IL-22–deficient mice increases the severity of induced colitis

Previous studies from our laboratory showed that mice deficient in immune system components have altered microbiota that is transmissible to wild-type mice and can increase inflammation (17). Because we showed previously that IL-22–deficient mice are more susceptible to DSS-mediated colitis (2), we questioned whether the microbiota of these mice could contribute to this phenotype. Thus, we designed an experiment in which wild-type C57BL/6 mice were cohoused with IL-22–deficient mice or remained housed alone. After 4 wk of cohousing [a time period previously established by our laboratory to be sufficient for the transmission of microbiota between mice (17)], we added 2% DSS to the drinking water for 7 d and examined the resulting wasting and inflammatory diseases. We found that C57BL/6 mice cohoused with IL-22–deficient mice lost significantly more mass than did C57BL/6 mice that were housed alone (Fig. 2A, 2C, 2D). To examine inflammation, we performed colonoscopies at day 7 post-DSS treatment and found increased severity in the mice that were cohoused with the IL-22–deficient mice (Fig. 2E). We also found that the IL-22–deficient mice lost more mass than did the wild-type mice with which they were cohoused (Fig. 2A, 2D), and

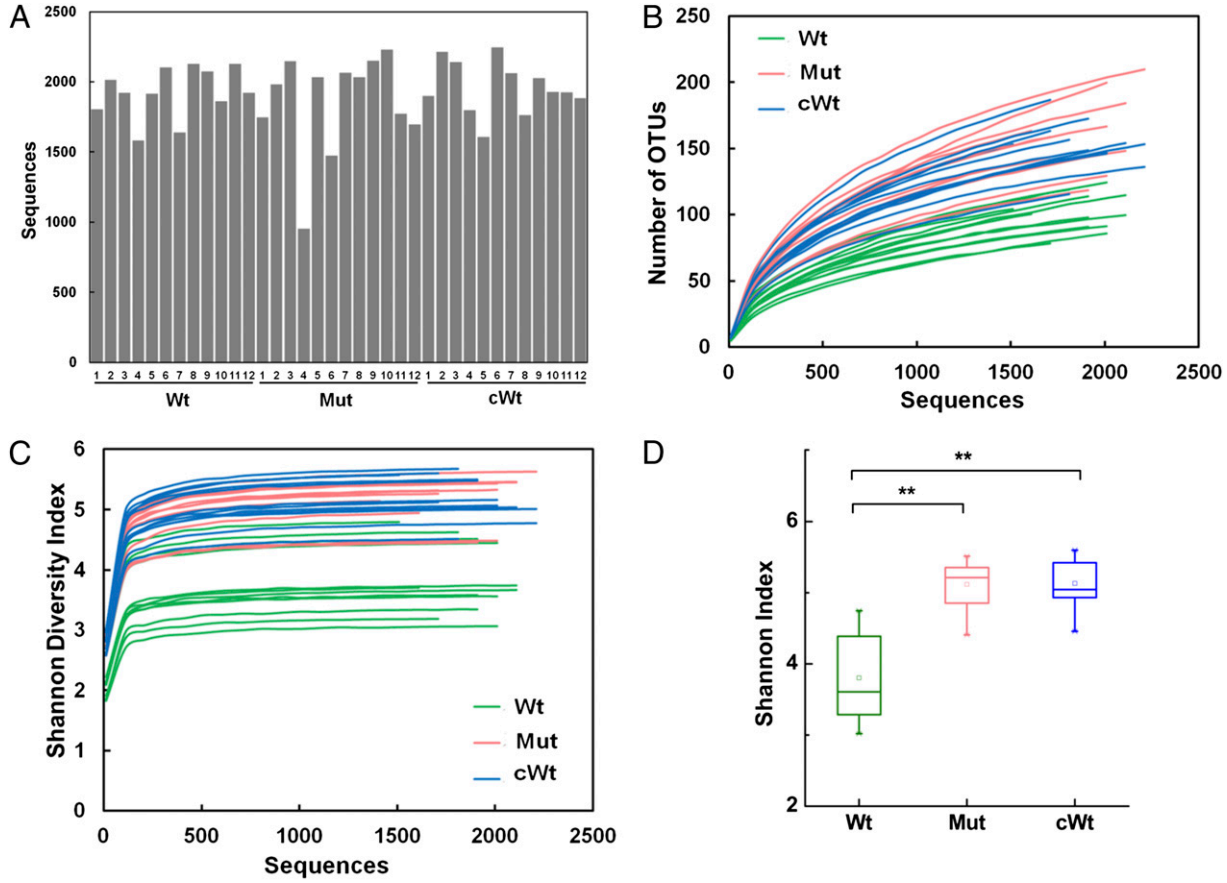

FIGURE 3. Diversity estimation of gut microbiota. Prior to DSS treatment, fecal samples were obtained from the mice described in Fig. 1. DNA was prepared and subjected to pyrosequencing. A total of 68,822 sequences was obtained from the 36 samples (n = 12/group). (**A**) Average sequences per sample. Each bar represents one mouse. (**B**) OTU rarefaction curves. Each line represents one mouse. (**C**) Shannon–Wiener Diversity Index curves. Each line represents one mouse. (**D**) Difference in Shannon-Wiener Diversity among the three groups (at the depth of 910 sequences). **$p < 0.01$, Mann–Whitney test.

IL-22–deficient mice were the only mice to have mortality associated with disease (Fig. 2B). These data suggest that the increase in severity of DSS-mediated colitis in IL-22–deficient mice is dependent on their microbiota, as well as a biological role for IL-22 independent of the microbiota.

To independently confirm our findings, we performed the same experiment with a distinct colony of IL-22–deficient mice obtained through IVF. Sperm collected from an IL-22–deficient male mouse was used to fertilize wild-type C57BL/6 oocytes, and the resulting heterozygous embryos were implanted into CD1 mothers. After weaning, the newly derived heterozygous mice were housed under *Helicobacter*-excluded conditions and intercrossed to generate homozygous IL-22–deficient mice. We then performed a similar cohousing experiment in which wild-type mice were either cohoused with IL-22–deficient mice or not and then subjected to DSS-mediated colitis. We found a similar phenotype in the rederived colony as in our original colony; wild-type mice cohoused with IL-22–deficient mice had significantly greater wasting disease than did wild-type mice not exposed to IL-22–deficient mice (Supplemental Fig. 1). However, in this particular experiment we did not observe a significant difference in mass loss between wild-type mice cohoused with IL-22–deficient mice and the IL-22–deficient mice, perhaps due to transfer of some protective flora from the wild-type mice to the IL-22–deficient mice. Thus, passage of our IL-22–deficient mouse line through a CD1 parent and subsequent heterozygous ($Il22^{+/-}$) mice shows that IL-22–deficient mice harbor transmissible colitic microbiota that is specific to the genotype and not the specific colony.

IL-22–deficient mice have altered commensal microbiota

Our data support the hypothesis that IL-22–deficient mice have altered microbiota that is transmissible to wild-type mice. Thus, we undertook bacterial 16S rRNA gene pyrosequencing to examine the microbiome in three groups of mice: wild-type mice and wild-type mice cohoused with IL-22–deficient mice. Prior to DSS treatment we collected fecal samples from each mouse, prepared fecal DNA, and used barcoded pyrosequencing of the 16S rRNA gene V3 region to explore the change in the microbiome.

We obtained 68,822 high-quality sequences with an average ~2000 reads/sample (Fig. 3A). A total of 657 OTUs was obtained, and Shannon–Wiener Diversity Index was calculated for each sample (Fig. 3B, 3C). These curves reached stable values, showing that the depth of sequencing would include most species found in the samples. Wild-type mice housed alone had much less diversity than did either IL-22–deficient mice or wild-type mice housed with IL-22–deficient mice (Fig. 3D).

Multivariable analysis of the colonic microbiome structure was then performed. By PCA, IL-22–deficient mice had a significantly different bacterial composition in their gut microbiota compared with wild-type mice housed alone (Fig. 4A).

To assess whether the altered microbiota of IL-22–deficient mice were transmissible to wild-type mice, we further compared the microbiota from cohoused wild-type mice with that of IL-22–deficient mice and wild-type mice housed alone. This revealed that the microbiome of cohoused wild-type mice was indistinguishable from that of IL-22–deficient mice (Fig. 4B) but was unrelated to the wild-type mice housed alone, indicating that the altered microbiota of the mice deficient in IL-22 was transmissible to wild-type mice. A multivariate ANOVA test also indicated that the gut microbiota of cohoused wild-type mice was much more similar to that of IL-22–deficient mice (Fig. 4C).

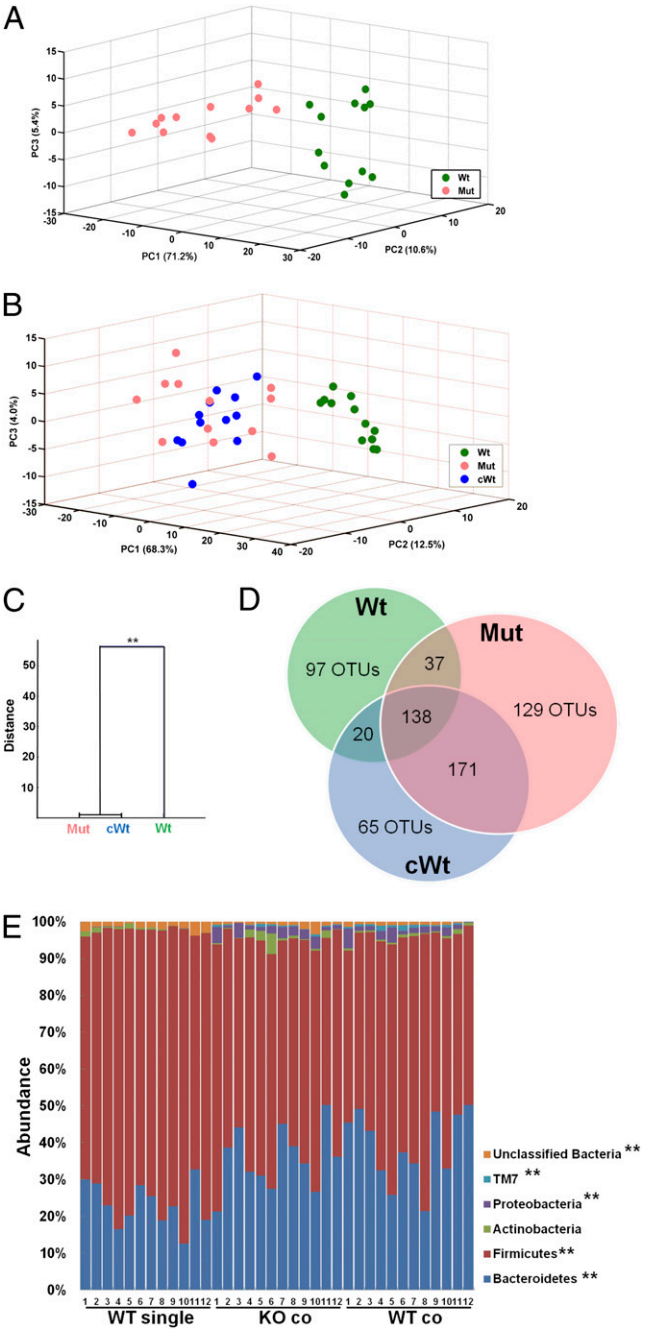

FIGURE 4. IL-22–deficient and cohoused wild-type mice have altered microbiota compared with wild-type mice by 16S rRNA analysis of fecal-derived bacteria. Prior to DSS treatment, fecal samples were obtained from the mice described in Fig. 2. DNA was prepared and subjected to pyrosequencing. A total of 68,822 sequences was obtained from the 36 samples (n = 12/group). (**A**) Score plot of PCA of the microbiomes of wild-type (Wt) and IL-22–deficient (Mut) mice based on the first three principal components. Each symbol represents one mouse. (**B**) Score plot of PCA based on the first three principal components of wild-type mice (Wt), IL-22–deficient mice (Mut), and wild-type mice housed with IL-22–deficient mice (cWt). Each symbol represents one mouse. (**C**) Clustering of bacteria based on distances between different groups calculated with multivariate ANOVA test of the first seven principal components of UniFrac PCoA analysis data. The Mahalanobis distances between group means are shown. $^{**}p < 0.01$. (**D**) Venn diagram showing distribution of the shared OTUs. (**E**) Taxon-based analysis at phylum level among the groups. Phyla with >1% abundance are shown. $^{*}p < 0.05$, $^{**}p < 0.01$, Kruskal–Wallis test.

Table I. Taxon-based analysis at the family level among the groups

Family	*p* Value (Kruskal–Wallis Test)	Wt		Mut		cWt	
		Median	Range	Median	Range	Median	Range
Lactobacillaceae	0.0055224	43.45	24.4–52.4	25.44	5.3–49.7	22.38	8.9–43.9
Bacteroidaceae	0.0000083	13.48	7.8–30.6	1.09	0.6–2.2	1.18	0.5–3.6
Clostridiaceae	0.0000821	0.98	0.4–2.5	0.06	0–1.5	0.05	0–0.8
Peptococcaceae	0.0000001	0.91	0.4–2.3	0.00	0–0	0.00	0–0
Porphyromonadaceae	0.0001488	5.22	0.7–17.9	20.94	11.4–34.8	21.60	12.0–29.6
Prevotellaceae	0.0000029	0.00	0–1.3	8.06	4.0–14.8	10.66	4.5–20.9
Rikenellaceae	0.0000031	0.00	0–0.1	1.12	0.3–2.9	2.16	0.4–3.0
Coriobacteriaceae	0.0193184	0.26	0–0.6	0.32	0.2–1.4	0.47	0.2–1.1
Helicobacteraceae	0.0000111	0.00	0–0	0.33	0.1–1.5	0.41	0–0.6
Desulfovibrionaceae	0.0000230	0.00	0–0	0.11	0–0.7	0.25	0.1–1.2

The data represent the abundance (%) of each family.
Wt, C57BL/6 mice housed alone; Mut, IL-22–deficient mice housed with C57BL/6 mice; cWt, C57BL/6 mice housed with IL-22–deficient mice.

We next examined the OTU distribution to estimate shared species among the three groups of animals. Results showed that wild-type mice cohoused with IL-22–deficient mice had 65 unique OTUs and shared 309 OTUs with the IL-22–deficient mice; however, they only shared 158 OTUs with the wild-type mice housed alone (Fig. 4D, Supplemental Tables I–VII). Taxon-based analysis revealed that the microbiome of each group was mainly composed of *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *TM7*, and *Actinobacteria* (Fig. 4E). Ten families (Table I) and 14 genera (Table II) showed significant differences among the three groups. In all, compared with wild-type mice housed alone, seven genera were reduced in the IL-22–deficient and the cohoused wild-type mice (*Lactobacillus*, *Bacteroides*, *Ruminococcus*, *Turicibacter*, *Anaerobacter*, *Parabacteroides*, and *Hespellia*) and seven genera were increased (*Coprococcus*, *Allobaculum*, *Barnesiella*, *Alistipes*, *Xylanibacter*, *Butyricimonas*, and *Helicobacter*).

Wild-type mice cohoused with IL-22–deficient mice have altered colonic antimicrobial peptide expression

Because IL-22 is an important regulator of certain antimicrobial peptides, we hypothesized that deficiency in IL-22 would decrease their expression. We examined expression of the genes encoding RegIIIβ and RegIIIγ in the colons of IL-22–deficient mice, wild-type mice cohoused with IL-22–deficient mice, or control wild-type mice. As previously observed by us (9) and other investigators (5), expression of both RegIIIβ and RegIIIγ was decreased in IL-22–deficient mice compared with wild-type mice housed alone (Fig. 5). In contrast to the levels that we observed in these wild-type mice, we noticed significantly reduced expression of RegIIIβ and RegIIIγ in wild-type mice cohoused with IL-22–deficient mice. Thus, cohousing of wild-type mice with IL-22–deficient mice reduced their expression of IL-22–regulated antimicrobial proteins.

Discussion

In this study, we showed that the cytokine IL-22 contributes to the composition of the colonic microbiota. In the absence of IL-22, the commensal flora homeostasis could be tipped in favor of different species or one particular keystone species that can stabilize the altered flora (27). IL-22 is a potent inducer of several antimicrobial peptides and mucins, which help to protect the epithelial barrier. RegIIIγ is active against Gram-positive bacteria because of its binding to the peptidoglycan surface (28), and it is important for microbiota localization in the small intestine but not the colon (29). Changes that we observed in the colonic microbiome in IL-22–deficient and cohoused wild-type mice could be explained by the observed reduced levels of RegIIIβ and RegIIIγ. In the absence of IL-22, commensals that are normally suppressed by RegIIIβ and/or RegIIIγ may now expand. Upon cohousing, these altered flora are transmitted to wild-type mice, but their persistence is unknown. In turn, these mice have suppression of the genes whose encoded proteins may have generated the altered flora in the first place. The mechanism by which this occurs is unknown, but we speculate that the altered flora may be inhibitory

Table II. Taxon-based analysis at the genus level among the groups

Genus	*p* Value (Kruskal–Wallis Test)	Wt		Mut		cWt	
		Median	Range	Median	Range	Median	Range
Lactobacillus	0.0055224	43.45	24.40–52.32	25.44	5.34–49.62	22.38	8.92–43.88
Bacteroides	0.0000083	13.48	7.77–30.61	1.09	0.57–2.24	1.18	0.52–3.64
Ruminococcus	0.0000004	1.68	0.84–2.83	0.00	0–0	0.00	0–0
Turicibacter	0.0001339	1.10	0.43–3.73	0.19	0–1.75	0.00	0–2.8
Anaerobacter	0.0000220	0.93	0.43–2.44	0.00	0–1.49	0.00	0–0.63
Parabacteroides	0.0053751	0.85	0.52–1.81	0.52	0.25–1.16	0.51	0.19–1.8
Hespellia	0.0108047	0.88	0.3–1.88	0.34	0.1–1.15	0.37	0.16–1.00
Coprococcus	0.0456690	3.09	1.49–8.34	8.93	1.98–25.04	6.80	2.27–28.38
Allobaculum	0.0005015	0.00	0-0	2.94	0–14.39	0.65	0–12.27
Barnesiella	0.0000046	0.00	0–0.14	2.77	1.72–5.31	3.29	1.59–7.59
Alistipes	0.0000031	0.00	0–0.1	0.94	0.3–2.69	1.99	0.1–2.74
Xylanibacter	0.0000583	0.00	0–0	0.39	0–1.48	0.25	0–1.28
Butyricimonas	0.0000386	0.00	0–0	0.34	0–0.83	0.25	0.09–1.43
Helicobacter	0.0000111	0.00	0–0	0.33	0.1–1.49	0.41	0–0.62

The data represent the abundance (%) of each family.
Wt, C57BL/6 mice housed alone; Mut, IL-22–deficient mice housed with C57BL/6 mice; cWt, C57BL/6 mice housed with IL-22–deficient mice.

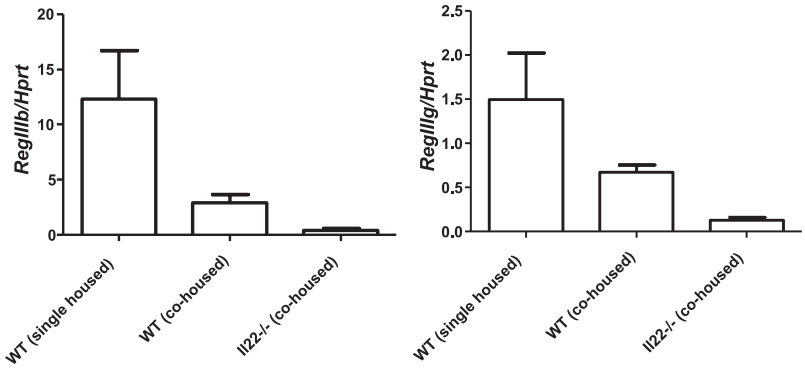

FIGURE 5. Wild-type mice cohoused with IL-22–deficient mice have reduced levels of RegIIIβ and RegIIIγ. C57BL/6 mice were either housed by themselves or cohoused with *Il22*$^{-/-}$ mice for 4 wk. Levels of RegIIIβ and RegIIIγ mRNA in the colon tissue were semiquantitated by real-time RT-PCR. Bars represent mean ± SD (n = 5). Experiment was performed twice with similar results.

to IL-22 or upstream IL-23 signaling or, alternatively, MyD88-mediated signaling pathways (30).

We found many differences in the altered microbiota generated by the absence of IL-22 compared with the microbiota in immune-intact mice. The bacteria that were decreased in the absence of IL-22, and, therefore, appear to require IL-22 to maintain their niche in the microbiota, included the family Lactobacillacae, a Gram-positive family of lactic acid–producing bacteria that is generally thought to be part of the healthy flora (31). A reduced population of beneficial microbiota may have allowed the increase in colitis severity of the IL-22–deficient and cohoused wild-type mice. However, the more notable trend was the presence of low levels of bacterial phyla in IL-22–deficient mice and cohoused wild-type mice that were generally absent or extremely rare in wild-type mice. This included TM7 and Proteobacteria, and the genus *Barnesiella.* TM7 is an unculturable phylum that was identified by 16S rRNA sequencing in environmental soil samples and has been since found in the feces of mice (17) and the human oral cavity (32). *Barnesiella* is one of the most abundant of the taxa that were newly identified by metagenomic sequence analysis of human stool samples, and it still lacks a known role in the GI tract (33). The phylum Proteobacteria are Gram-negative bacteria and include such notable genera as *Escherichia*, *Salmonella*, *Vibrio*, and *Helicobacter.* These findings suggest that the absence of IL-22 allows for the colonization or outgrowth of these organisms.

Our study examines the microbiota composition, but we did not address whether IL-22 alters the localization of commensal bacteria within the lumen. In the absence of IL-22 and the accompanying decrease in the expression of antimicrobial proteins and mucin, commensal bacteria may encroach closer to the epithelium, where they are normally excluded. This may have further affected the course of DSS-mediated colitis in the IL-22–deficient and cohoused wild-type mice.

Colonic flora is easily transmitted between mice living in the same cage because of their copraphagia. Why does the altered bacteria transmit from the IL-22–deficient mice to the wild-type mice but not the other way around? Our data suggest that once the altered flora has been established, it might be more persistent and/or stable than the normal microbiota. In addition, because we found that the cohoused mice had lower expression levels of IL-22–regulated genes than did the noncohoused mice, it appears that the altered flora may create a niche in which it is favored.

We know from our previous studies that the role of IL-22 in colitis can be independent of commensal microbiota. Our experiments with a CD4 T cell–mediated model of disease used cohorts of the same *Rag1*$^{-/-}$ mice and only differed in the genotype of T cells adoptively transferred into these hosts (2). We also observed a similar protective role for IL-22 in DSS-mediated colitis that was independent of adaptive immunity (2). DSS is thought to disrupt the colonic epithelium, allowing commensal bacteria to come into contact with, and possibly translocate, the epithelial barrier (34). We now show in this innate colitis model that the microbiota play at least a partial role in the severity of disease in the absence of IL-22. This agrees with findings that mice treated with neutralizing IL-22 Ab during colitis, which, therefore, have not developed an altered homeostatic flora, have a less pronounced phenotype than do *Il22* gene–deficient mice; however, these studies were not performed contemporaneously (2, 3).

Our findings in experimental colitis models are relevant to human disease. Patients with inflammatory bowel disease (IBD) are known to have altered gut microbiota, although it is unclear whether this is a cause or an outcome of disease (35). As we showed for the absence of IL-22 in mice, an increase in IL-22 may play a similar role in altering the composition of the microbiome. The upregulation of IL-22 that accompanies disease may be responsible for altering the patient's microbiota as a result of changes in levels of antimicrobial molecules or through other mechanisms. This shows that altered microbiota of patients with IBD may result from the changes in cytokine expression that accompany disease. If this proves to be true, therapies that try to return cytokine levels in patients with IBD to those of healthy people may be beneficial from an immune system viewpoint, as well as from the perspective of altering the microbiota.

This study is a striking example of how the absence of one specific cytokine can have effects on the commensal microbiota and, in turn, the course of inflammation. Although IL-22 is well known to be involved during inflammation, it clearly can also play a role in immune homeostasis by altering the commensal microbiota. The transmission of the altered gut microbiota from IL-22–deficient mice to cohoused wild-type mice, along with increased susceptibility to DSS-induced colitis, indicates that the altered gut microbiota may work as a contributing factor for, rather than a consequence of, the disease. Future studies will investigate whether particular species of bacteria are responsible for mediating these effects.

Acknowledgments

We thank Cindy Hughes for rederivation of our IL-22–deficient mouse colony.

Disclosures

The authors have no financial conflicts of interest.

References

1. Sonnenberg, G. F., L. A. Fouser, and D. Artis. 2011. Border patrol: regulation of immunity, inflammation and tissue homeostasis at barrier surfaces by IL-22. *Nat. Immunol.* 12: 383–390.
2. Zenewicz, L. A., G. D. Yancopoulos, D. M. Valenzuela, A. J. Murphy, S. Stevens, and R. A. Flavell. 2008. Innate and adaptive interleukin-22 protects mice from inflammatory bowel disease. *Immunity* 29: 947–957.

3. Sugimoto, K., A. Ogawa, E. Mizoguchi, Y. Shimomura, A. Andoh, A. K. Bhan, R. S. Blumberg, R. J. Xavier, and A. Mizoguchi. 2008. IL-22 ameliorates intestinal inflammation in a mouse model of ulcerative colitis. *J. Clin. Invest.* 118: 534–544.
4. Andoh, A., Z. Zhang, O. Inatomi, S. Fujino, Y. Deguchi, Y. Araki, T. Tsujikawa, K. Kitoh, S. Kim-Mitsuyama, A. Takayanagi, et al. 2005. Interleukin-22, a member of the IL-10 subfamily, induces inflammatory responses in colonic subepithelial myofibroblasts. *Gastroenterology* 129: 969–984.
5. Zheng, Y., P. A. Valdez, D. M. Danilenko, Y. Hu, S. M. Sa, Q. Gong, A. R. Abbas, Z. Modrusan, N. Ghilardi, F. J. de Sauvage, and W. Ouyang. 2008. Interleukin-22 mediates early host defense against attaching and effacing bacterial pathogens. *Nat. Med.* 14: 282–289.
6. Pickert, G., C. Neufert, M. Leppkes, Y. Zheng, N. Wittkopf, M. Warntjen, H. A. Lehr, S. Hirth, B. Weigmann, S. Wirtz, et al. 2009. STAT3 links IL-22 signaling in intestinal epithelial cells to mucosal wound healing. *J. Exp. Med.* 206: 1465–1472.
7. Wolk, K., S. Kunz, E. Witte, M. Friedrich, K. Asadullah, and R. Sabat. 2004. IL-22 increases the innate immunity of tissues. *Immunity* 21: 241–254.
8. Raffatellu, M., M. D. George, Y. Akiyama, M. J. Hornsby, S. P. Nuccio, T. A. Paixao, B. P. Butler, H. Chu, R. L. Santos, T. Berger, et al. 2009. Lipocalin-2 resistance confers an advantage to *Salmonella enterica* serotype Typhimurium for growth and survival in the inflamed intestine. *Cell Host Microbe* 5: 476–486.
9. Kinnebrew, M. A., C. Ubeda, L. A. Zenewicz, N. Smith, R. A. Flavell, and E. G. Pamer. 2010. Bacterial flagellin stimulates Toll-like receptor 5-dependent defense against vancomycin-resistant *Enterococcus* infection. *J. Infect. Dis.* 201: 534–543.
10. Colonna, M. 2009. Interleukin-22-producing natural killer cells and lymphoid tissue inducer-like cells in mucosal immunity. *Immunity* 31: 15–23.
11. Spits, H., and J. P. Di Santo. 2011. The expanding family of innate lymphoid cells: regulators and effectors of immunity and tissue remodeling. *Nat. Immunol.* 12: 21–27.
12. Cella, M., A. Fuchs, W. Vermi, F. Facchetti, K. Otero, J. K. Lennerz, J. M. Doherty, J. C. Mills, and M. Colonna. 2009. A human natural killer cell subset provides an innate source of IL-22 for mucosal immunity. *Nature* 457: 722–725.
13. Luci, C., A. Reynders, I. I. Ivanov, C. Cognet, L. Chiche, L. Chasson, J. Hardwigsen, E. Anguiano, J. Banchereau, D. Chaussabel, et al. 2009. Influence of the transcription factor RORgammat on the development of NKp46+ cell populations in gut and skin. *Nat. Immunol.* 10: 75–82.
14. Sanos, S. L., V. L. Bui, A. Mortha, K. Oberle, C. Heners, C. Johner, and A. Diefenbach. 2009. RORgammat and commensal microflora are required for the differentiation of mucosal interleukin 22-producing NKp46+ cells. *Nat. Immunol.* 10: 83–91.
15. Satoh-Takayama, N., C. A. Vosshenrich, S. Lesjean-Pottier, S. Sawa, M. Lochner, F. Rattis, J. J. Mention, K. Thiam, N. Cerf-Bensussan, O. Mandelboim, et al. 2008. Microbial flora drives interleukin 22 production in intestinal NKp46+ cells that provide innate mucosal immune defense. *Immunity* 29: 958–970.
16. Satoh-Takayama, N., S. Lesjean-Pottier, S. Sawa, C. A. Vosshenrich, G. Eberl, and J. P. Di Santo. 2011. Lymphotoxin-β receptor-independent development of intestinal IL-22-producing NKp46+ innate lymphoid cells. *Eur. J. Immunol.* 41: 780–786.
17. Elinav, E., T. Strowig, A. L. Kau, J. Henao-Mejia, C. A. Thaiss, C. J. Booth, D. R. Peaper, J. Bertin, S. C. Eisenbarth, J. I. Gordon, and R. A. Flavell. 2011. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell* 145: 745–757.
18. Vijay-Kumar, M., J. D. Aitken, F. A. Carvalho, T. C. Cullender, S. Mwangi, S. Srinivasan, S. V. Sitaraman, R. Knight, R. E. Ley, and A. T. Gewirtz. 2010. Metabolic syndrome and altered gut microbiota in mice lacking Toll-like receptor 5. *Science* 328: 228–231.
19. Salzman, N. H., K. Hung, D. Haribhai, H. Chu, J. Karlsson-Sjöberg, E. Amir, P. Teggatz, M. Barman, M. Hayward, D. Eastwood, et al. 2010. Enteric defensins are essential regulators of intestinal microbial ecology. *Nat. Immunol.* 11: 76–83.
20. Becker, C., M. C. Fantini, and M. F. Neurath. 2006. High resolution colonoscopy in live mice. *Nat. Protoc.* 1: 2900–2904.
21. Turnbaugh, P. J., M. Hamady, T. Yatsunenko, B. L. Cantarel, A. Duncan, R. E. Ley, M. L. Sogin, W. J. Jones, B. A. Roe, J. P. Affourtit, et al. 2009. A core gut microbiome in obese and lean twins. *Nature* 457: 480–484.
22. Zhang, C., M. Zhang, S. Wang, R. Han, Y. Cao, W. Hua, Y. Mao, X. Zhang, X. Pang, C. Wei, et al. 2010. Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME J.* 4: 232–241.
23. Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K. Costello, N. Fierer, A. G. Peña, J. K. Goodrich, J. I. Gordon, et al. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nat. Methods* 7: 335–336.
24. Caporaso, J. G., K. Bittinger, F. D. Bushman, T. Z. DeSantis, G. L. Andersen, and R. Knight. 2010. PyNAST: a flexible tool for aligning sequences to a template alignment. *Bioinformatics* 26: 266–267.
25. Wang, Q., G. M. Garrity, J. M. Tiedje, and J. R. Cole. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* 73: 5261–5267.
26. Sonnenberg, G. F., L. A. Monticelli, M. M. Elloso, L. A. Fouser, and D. Artis. 2011. CD4(+) lymphoid tissue-inducer cells promote innate immunity in the gut. *Immunity* 34: 122–134.
27. Hajishengallis, G., R. P. Darveau, and M. A. Curtis. 2012. The keystone-pathogen hypothesis. *Nat. Rev. Microbiol.* 10: 717–725.
28. Cash, H. L., C. V. Whitham, and L. V. Hooper. 2006. Refolding, purification, and characterization of human and murine RegIII proteins expressed in *Escherichia coli*. *Protein Expr. Purif.* 48: 151–159.
29. Vaishnava, S., M. Yamamoto, K. M. Severson, K. A. Ruhn, X. Yu, O. Koren, R. Ley, E. K. Wakeland, and L. V. Hooper. 2011. The antibacterial lectin RegIIIgamma promotes the spatial segregation of microbiota and host in the intestine. *Science* 334: 255–258.
30. Brandl, K., G. Plitas, B. Schnabl, R. P. DeMatteo, and E. G. Pamer. 2007. MyD88-mediated signals induce the bactericidal lectin RegIII γ and protect mice against intestinal *Listeria monocytogenes* infection. *J. Exp. Med.* 204: 1891–1900.
31. Sanders, M. E. 2011. Impact of probiotics on colonizing microbiota of the gut. *J. Clin. Gastroenterol.* 45(Suppl.): S115–S119.
32. Liu, B., L. L. Faller, N. Klitgord, V. Mazumdar, M. Ghodsi, D. D. Sommer, T. R. Gibbons, T. J. Treangen, Y. C. Chang, S. Li, et al. 2012. Deep sequencing of the oral microbiome reveals signatures of periodontal disease. *PLoS ONE* 7: e37919.
33. Wylie, K. M., R. M. Truty, T. J. Sharpton, K. A. Mihindukulasuriya, Y. Zhou, H. Gao, E. Sodergren, G. M. Weinstock, and K. S. Pollard. 2012. Novel bacterial taxa in the human microbiome. *PLoS ONE* 7: e35294.
34. Pizarro, T. T., K. O. Arseneau, G. Bamias, and F. Cominelli. 2003. Mouse models for the study of Crohn's disease. *Trends Mol. Med.* 9: 218–222.
35. Frank, D. N., A. L. St Amand, R. A. Feldman, E. C. Boedeker, N. Harpaz, and N. R. Pace. 2007. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. *Proc. Natl. Acad. Sci. USA* 104: 13780–13785.