

# Induction of Bacterial Antigen-Specific Colitis by a Simplified Human Microbiota Consortium in Gnotobiotic Interleukin-10<sup>-/-</sup> Mice

Chang Soo Eun,<sup>a,b</sup> Yoshiyuki Mishima,<sup>b,c</sup> Steffen Wöhlgemuth,<sup>b,d</sup> Bo Liu,<sup>b,e</sup> Maureen Bower,<sup>b,f</sup> Ian M. Carroll,<sup>b</sup> R. Balfour Sartor<sup>b</sup>

Department of Internal Medicine, Hanyang University Guri Hospital, Guri, South Korea<sup>a</sup>; Center for Gastrointestinal Biology and Disease, Division of Gastroenterology and Hepatology, Departments of Medicine, Microbiology, and Immunology, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA<sup>b</sup>; Department of Internal Medicine II, Shimane University School of Medicine, Izumo, Shimane, Japan<sup>c</sup>; Institute for Applied Microbiology, Research Centre for BioSystems, Land Use and Nutrition, Justus-Liebig University Giessen, Giessen, Germany<sup>d</sup>; Key Laboratory of Zoonosis Research, Ministry of Education, Institute of Zoonoses, College of Animal Science and Veterinary Medicine, Jilin University, Changchun, China<sup>e</sup>; Department of Laboratory Animal Medicine, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA<sup>f</sup>

**We evaluated whether a simplified human microbiota consortium (SIHUMI) induces colitis in germfree (GF) 129S6/SvEv (129) and C57BL/6 (B6) interleukin-10-deficient (IL-10<sup>-/-</sup>) mice, determined mouse strain effects on colitis and the microbiota, examined the effects of inflammation on relative bacterial composition, and identified immunodominant bacterial species in “humanized” IL-10<sup>-/-</sup> mice. GF wild-type (WT) and IL-10<sup>-/-</sup> 129 and B6 mice were colonized with 7 human-derived inflammatory bowel disease (IBD)-related intestinal bacteria and maintained under gnotobiotic conditions. Quantification of bacteria in feces, ileal and colonic contents, and tissues was performed using 16S rRNA gene selective quantitative PCR. Colonic segments were scored histologically, and gamma interferon (IFN- $\gamma$ ), IL-12p40, and IL-17 levels were measured in supernatants of unstimulated colonic tissue explants and of mesenteric lymph node (MLN) cells stimulated by lysates of individual or aggregate bacterial strains. Relative bacterial species abundances changed over time and differed between 129 and B6 mice, WT and IL-10<sup>-/-</sup> mice, luminal and mucosal samples, and ileal and colonic or fecal samples. SIHUMI induced colitis in all IL-10<sup>-/-</sup> mice, with more aggressive colitis and MLN cell activation in 129 mice. *Escherichia coli* LF82 and *Ruminococcus gnavus* lysates induced dominant effector *ex vivo* MLN TH1 and TH17 responses, although the bacterial mucosal concentrations were low. In summary, this study shows that a simplified human bacterial consortium induces colitis in ex-GF 129 and B6 IL-10<sup>-/-</sup> mice. Relative concentrations of individual SIHUMI species are determined by host genotype, the presence of inflammation, and anatomical location. A subset of IBD-relevant human enteric bacterial species preferentially stimulates bacterial antigen-specific TH1 and TH17 immune responses in this model, independent of luminal and mucosal bacterial concentrations.**

Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis, appear to result from overly aggressive immune responses to a subset of commensal enteric bacteria in genetically susceptible hosts (1, 2). Commensal enteric bacteria have an essential role in driving immune-mediated experimental inflammation in the distal intestine (1, 3–6). Most germfree (GF) genetically susceptible rodents exhibit absent intestinal inflammation or immune activation under GF conditions but rapidly develop chronic colitis and pathogenic immune responses to commensal bacteria after colonization with specific-pathogen-free (SPF) enteric bacteria (5–8).

An altered intestinal microbial composition (dysbiosis) is associated with intestinal inflammation in human IBD and with cases of acute and chronic rodent experimental enterocolitis harboring complex enteric microbiotas, which are characterized by decreased bacterial diversity and an altered ratio of dominant bacterial species (9, 10). In addition, rodent models monoassociated with bacteria relevant to the dysbiosis of IBD, such as certain *Escherichia coli*, *Enterococcus faecalis*, *Bacteroides vulgatus*, and *Bacteroides thetaiotaomicron* strains, demonstrated that single intestinal bacteria can selectively induce host inflammation and also provided insights into underlying disease mechanisms (6, 11–13).

Nevertheless, the study of specific bacterium-host interactions by use of conventional and monoassociated rodent models has some limitations. The microbiotas of conventional animal models are too complex and variable to easily define and manipulate the functionally dominant component species and strain. In addition,

monoassociated mice do not reflect the actual situation in the distal intestine, where different bacterial groups reciprocally interact with each other and the host in multiple ways. To resolve these shortcomings, new animal models with defined, interacting intestinal microbiotas need to be developed.

Recently, several studies described the development of alternative rodent models of a simplified human microbiota, showing stable microbial and metabolomic characterization (14, 15). To study IBD-related microbiota-host interactions at a more complex yet defined level, in preliminary studies we established a simplified human microbiota consortium (SIHUMI), composed of 7 well-characterized and sequenced human-derived and IBD-related enteric bacteria, in wild-type (WT) mouse strains and demonstrated that it forms a stable community in the mouse intestine that is maintained under gnotobiotic conditions (16).

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Address correspondence to R. Balfour Sartor, rbs@med.unc.edu.

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In this study, we colonized interleukin-10-deficient (IL-10<sup>-/-</sup>) and WT mice on two different genetic backgrounds (129S6/SvEv [129] and C57BL/6 [B6]) with SIHUMI to address the following aims: (i) to evaluate whether SIHUMI induces colitis in GF IL-10<sup>-/-</sup> mice on both strain backgrounds, (ii) to determine murine genetic background strain effects on colitis and the microbiota, and (iii) to examine the effects of intestinal inflammation on relative bacterial compositions of colonized 129 and B6 WT and IL-10<sup>-/-</sup> mice. Our results show that a well-characterized defined human bacterial consortium, SIHUMI, induced colitis in ex-GF IL-10<sup>-/-</sup> mice, with more aggressive colitis in 129 than in B6 mice; that enteric concentrations of individual SIHUMI species are determined by host genetic background, anatomical distribution within the distal intestine, and the presence of colonic inflammation; and that human enteric bacterial strains differentially stimulate murine immune responses, with no direct correlation between luminal bacterial species concentration and the ability to induce mucosal effector immune responses in IL-10<sup>-/-</sup> mice. Humanized gnotobiotic mice with SIHUMI or other defined human bacterial consortia provide a valuable tool to study the inflammatory and protective roles and mechanisms of action of individual bacterial strains from IBD patients.

## MATERIALS AND METHODS

**Mice and bacterial species.** GF IL-10<sup>-/-</sup> and WT control mice (on both the inbred 129 and B6 backgrounds) were derived and maintained in the National Gnotobiotic Rodent Resource Center (NGRRC), University of North Carolina (UNC) at Chapel Hill. Mice were colonized at 8 to 12 weeks of age with 7 human-derived and IBD-related intestinal bacterial strains (SIHUMI), namely, *E. coli* LF82 (isolated from the ileum of a CD patient by Arlette Darfeuille-Michaud) (17), *E. faecalis* OG1RF (18), *Ruminococcus gnavus* ATCC 29149, *Bacteroides vulgatus* ATCC 8482, *Faecalibacterium prausnitzii* A2-165, *Lactobacillus plantarum* WCFS1, and *Bifidobacterium longum* subsp. *longum* ATCC 15707, by oral gavage on day 0 and day 3. Bacterial species of the SIHUMI were selected based on the following criteria: reported to be altered in IBD patients or to affect experimental colitis, human origin, availability of genomic sequence, and able to form a stable community in rodents. Animal use protocols were approved by UNC Institutional Animal Care and Use Committees.

**Histological scoring and colonic tissue fragment and mesenteric lymph node (MLN) cell cultures.** Mice were killed 6 and 12 weeks after colonization with SIHUMI. At necropsy, sections of the cecum, proximal colon, and distal colon were fixed in 10% neutral buffered formalin, embedded in paraffin, and stained with hematoxylin and eosin (H&E). Histologic analysis was performed in a blinded fashion. The evaluation of inflammation in the entire large intestine was performed by summation of the scores of the different regions, with each region being graded from 0 to 4, based on the degree of lamina propria and submucosal mononuclear cellular infiltration, crypt hyperplasia, goblet cell depletion, and architectural distortion. The total histology score represents the summation of the scores for the cecum, proximal colon, and distal colon, with a potential maximum score of 12.

Colonic tissue fragment and mesenteric lymph node cell cultures were prepared as previously described (7), and details are provided in the supplemental material.

**Bacterial lysates.** Lysates of each bacterial strain and the seven-bacterium combination were prepared from individual colonies of *E. coli*, *E. faecalis*, *R. gnavus*, *B. vulgatus*, *F. prausnitzii*, *L. plantarum*, and *B. longum*, as previously described (11). Details are provided in the supplemental material.

**Cytokine measurements.** The concentrations of gamma interferon (IFN- $\gamma$ ), IL-12/23p40, and IL-17 in supernatants were measured in triplicate by using enzyme-linked immunosorbent assays (ELISAs) from R&D Systems (Minneapolis, MN). Details are provided in the supplemental material.

**Bacterial DNA extraction.** Bacterial DNA was extracted at different time points (1, 2, 4, 8, and 12 weeks postcolonization) from the feces, luminal contents, and tissues by using a phenol-chloroform extraction method combined with physical disruption of bacterial cells and a DNA cleanup kit (Qiagen DNeasy Blood and Tissue extraction kit [Qiagen, Valencia, CA]), as previously described (19). Details are provided in the supplemental material.

**Quantitative real-time PCR.** For quantitative PCR, species-specific oligonucleotide primers were used to target bacterial species-specific hypervariable regions of the 16S rRNA gene (see Table S1 in the supplemental material). Standard curves were generated for each bacterial species and used to enumerate copy numbers in individual samples. Relative bacterial abundances from mean copy numbers for each bacterium were compared among all four groups of colonized mice. Bacterial concentrations were calculated as percentages relative to the total bacterial 16S rRNA gene copy number, normalized to the 129 WT mouse group, and expressed as mean fold changes  $\pm$  standard errors of the means (SEM). Details are provided in the supplemental material.

**Statistical analysis.** Statistical analyses were performed using SPSS 12.0 for Windows (SPSS Inc., Chicago, IL). Parametric data were analyzed by the independent Student *t* test. Nonparametric data (histologic scores) were analyzed by the Mann-Whitney test. Statistical significance was defined as having a *P* value of  $<0.05$  for the comparisons indicated.

## RESULTS

**SIHUMI induces colitis in ex-GF IL-10<sup>-/-</sup> mice, with greater aggressiveness of colitis in 129 than in B6 mice.** We investigated whether SIHUMI induced colitis in selectively colonized gnotobiotic IL-10<sup>-/-</sup> and WT mice on both the 129 and B6 backgrounds and the relative aggressiveness of colitis in the two mouse strains. Histologic colitis scores in the cecum and the proximal and distal colon regions were evaluated 6 and 12 weeks after colonization with SIHUMI. SIHUMI induced colitis in IL-10<sup>-/-</sup> mice on both the 129 and B6 backgrounds by 12 weeks of colonization (Fig. 1A). IL-10<sup>-/-</sup> 129 mice developed moderate colitis that was most active in the distal colon (see Fig. S1 in the supplemental material). Meanwhile, IL-10<sup>-/-</sup> B6 mice developed mild colitis that was most evident in the cecum. The degree of colitis was significantly increased in 129 IL-10<sup>-/-</sup> mice versus B6 IL-10<sup>-/-</sup> mice (total score,  $6.2 \pm 0.6$  versus  $3.3 \pm 0.3$ ;  $P = 0.002$ ), although there was no significant difference in cecal inflammation between the two groups. WT mice on both the 129 and B6 backgrounds had no histological evidence of colitis at 12 weeks; therefore, colitis scores were significantly increased in IL-10<sup>-/-</sup> mice relative to WT mice on both strain backgrounds. After 6 weeks of colonization, SIHUMI induced mild pancolitis in IL-10<sup>-/-</sup> 129 mice. In contrast, IL-10<sup>-/-</sup> B6 mice had no histological evidence of colitis at 6 weeks (Fig. 1A; see Fig. S1 in the supplemental material).

We next addressed whether mucosal immune responses accompanied histological inflammation. IFN- $\gamma$ , IL-12p40, and IL-17 levels in the supernatants of unstimulated colonic tissue explants at 6 and 12 weeks were measured by ELISA. After 12 weeks of SIHUMI colonization, spontaneous colonic secretion of IFN- $\gamma$ , IL-12p40, and IL-17 was significantly increased in IL-10<sup>-/-</sup> mice compared to WT 129 and B6 mice (Fig. 1B to D). Significant increases in IFN- $\gamma$ , IL-12p40, and IL-17 secretion measured from colonic explant cultures were evident in 129 IL-10<sup>-/-</sup> mice relative to B6 IL-10<sup>-/-</sup> mice, paralleling the degree of histological inflammation. Six weeks after SIHUMI colonization, spontaneous *ex vivo* colonic secretion of IFN- $\gamma$ , IL-12p40, and IL-17 was significantly increased in 129 IL-10<sup>-/-</sup> mice relative to B6 IL-10<sup>-/-</sup> mice, whose cytokine levels were minimally elevated

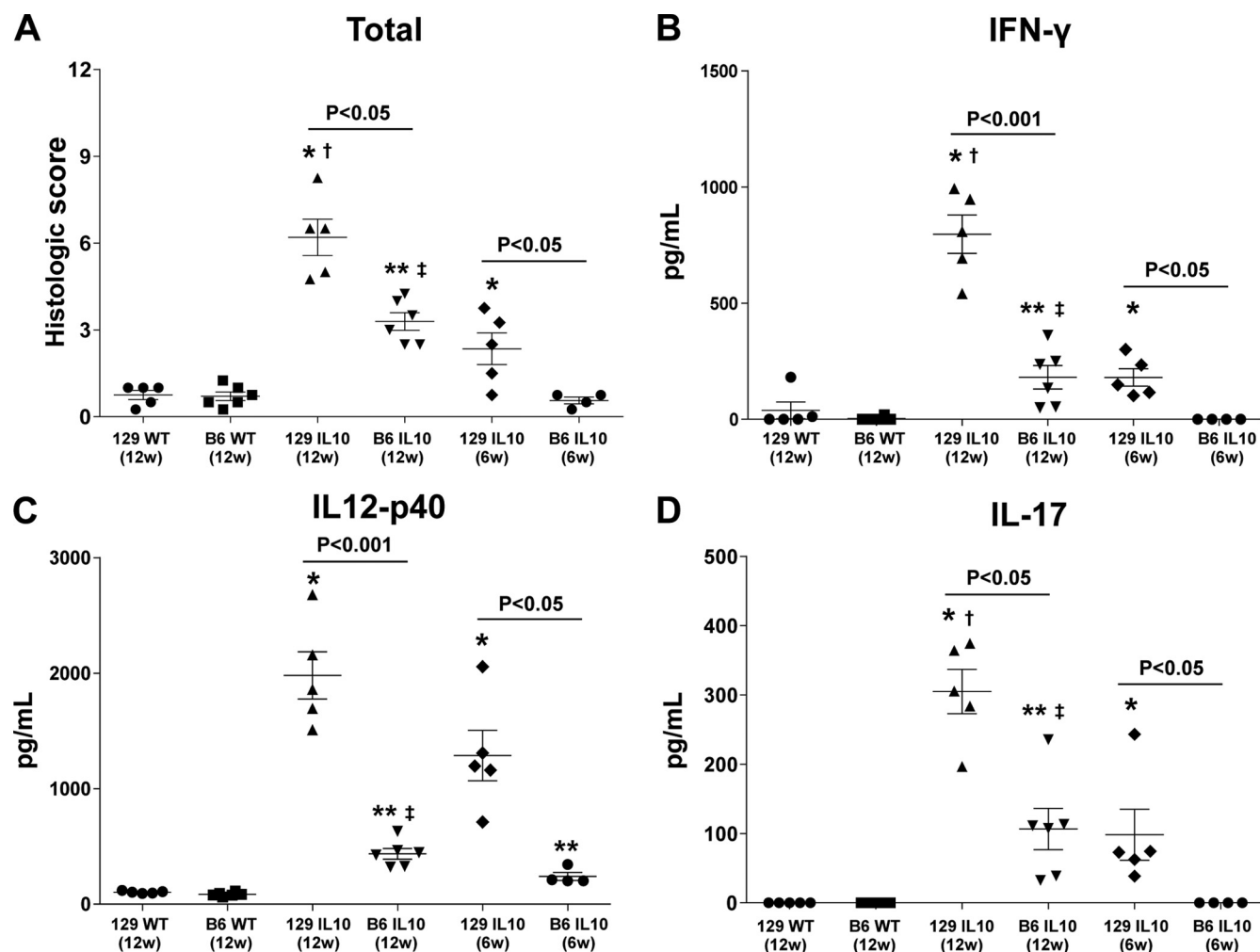


FIG 1 Blinded total histologic scores (A) and spontaneous secretion of IFN- $\gamma$  (B), IL-12p40 (C), and IL-17 (D) by colonic tissue explants from WT and IL-10<sup>-/-</sup> mice on the 129 and B6 backgrounds ( $n = 4$  to 6/group) after 6 and 12 weeks of SIHUMI colonization. The total histologic score represents the summation of the scores for the cecum, proximal colon, and distal colon (see Fig. S1 in the supplemental material), with a potential maximum score of 12. Results show means  $\pm$  standard errors of the means. \*,  $P < 0.05$  versus 129 WT (12w) mice; \*\*,  $P < 0.05$  versus B6 WT (12w) mice; †,  $P < 0.05$  versus 129 IL-10 (6w) mice; ‡,  $P < 0.05$  versus B6 IL-10 (6w) mice. 6w or 12w, mice at 6 or 12 weeks of colonization; IL-10, IL-10<sup>-/-</sup> mice.

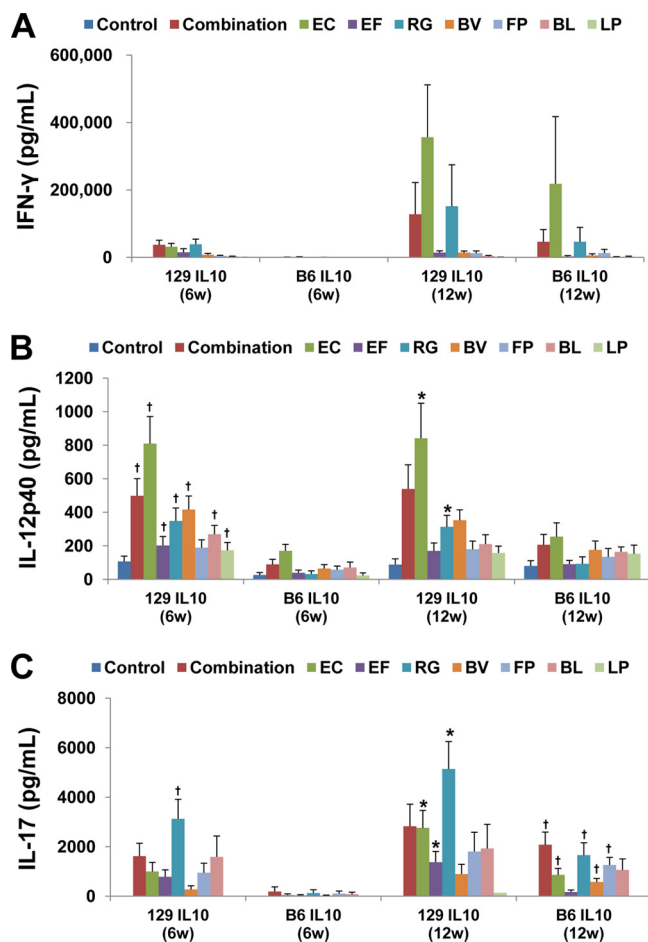
over those of WT mice. Levels of these proinflammatory cytokines secreted from IL-10<sup>-/-</sup> mice at 12 weeks were significantly increased compared to levels from IL-10<sup>-/-</sup> mice after 6 weeks of colonization, except for an insignificant difference of IL-12p40 levels from 129 IL-10<sup>-/-</sup> mice between 12 weeks and 6 weeks of colonization (Fig. 1B to D).

**Human enteric bacterial strains differentially stimulate murine immune responses, with *E. coli* and *R. gnavus* preferentially inducing effector immune responses in IL-10<sup>-/-</sup> mice.** To address whether SIHUMI-colonized mice exhibited bacterial antigen-specific immune responses, we cultured MLN cells (isolated from IL-10<sup>-/-</sup> mice at 6 and 12 weeks of SIHUMI colonization) with lysates of each of the seven bacterial species or a combination lysate of all strains. After 12 weeks of SIHUMI colonization, unfractionated MLN cells from 129 or B6 IL-10<sup>-/-</sup> mice produced the highest IFN- $\gamma$  levels when stimulated with *E. coli* lysate and moderate levels of IFN- $\gamma$  when stimulated with either the combination lysate or *R. gnavus* lysate (Fig. 2A). The levels of IFN- $\gamma$  secretion from stimulated MLN cells were consistently, but not

significantly, higher in 129 IL-10<sup>-/-</sup> mice than in B6 IL-10<sup>-/-</sup> mice. After 6 weeks of colonization, MLN cells from 129 IL-10<sup>-/-</sup> mice predominantly produced IFN- $\gamma$  when stimulated with the *R. gnavus*, combination, or *E. coli* lysate. The levels of IFN- $\gamma$  secretion from stimulated MLN cells were lower in 129 IL-10<sup>-/-</sup> mice at 6 weeks than in 129 IL-10<sup>-/-</sup> mice at 12 weeks, although there was no statistically significant difference. IFN- $\gamma$  secretion by MLN cells in response to specific bacterial lysate stimulation was quite low in B6 IL-10<sup>-/-</sup> mice at 6 weeks of colonization (Fig. 2A).

Similar to the case for IFN- $\gamma$  secretion, MLN cells from IL-10<sup>-/-</sup> mice on both the 129 and B6 backgrounds produced the highest level of IL-12p40 when stimulated with *E. coli* lysate, and the next highest levels were seen with combination, *B. vulgatus*, and *R. gnavus* lysates (Fig. 2B). The levels of IL-12p40 secretion from stimulated MLN cells were higher in 129 IL-10<sup>-/-</sup> mice than in B6 IL-10<sup>-/-</sup> mice at 6 and 12 weeks of SIHUMI colonization, but secretion levels of IL-12p40 in 129 IL-10<sup>-/-</sup> mice colonized for 6 and 12 weeks were similar. On the other hand, MLN cells from IL-10<sup>-/-</sup> mice on both strain backgrounds produced the





**FIG 2** Secretion of IFN- $\gamma$  (A), IL-12p40 (B), and IL-17 (C) by MLN cells stimulated with individual lysates of seven bacterial species or a combination lysate of pooled bacterial species. Cells were obtained from 129 and B6 IL-10 $^{-/-}$  mice ( $n = 4$  to 6/group) after 6 or 12 weeks of SIHUMI colonization. Results show means  $\pm$  standard errors of the means. \*,  $P < 0.05$  versus B6 (12w) mice with the same lysate stimulation; †,  $P < 0.05$  versus B6 (6w) mice with the same lysate stimulation. Combination, combination lysate of 7 SIHUMI bacteria; EC, *E. coli*; EF, *Enterococcus faecalis*; RG, *Ruminococcus gnavus*; BV, *Bacteroides vulgatus*; FP, *Faecalibacterium prausnitzii*; BL, *Bifidobacterium longum*; LP, *Lactobacillus plantarum*.

highest levels of IL-17 when stimulated with the *R. gnavus* lysate or combination lysate for 6 and 12 weeks. The levels of IL-17 secretion from stimulated MLN cells were higher in 129 IL-10 $^{-/-}$  mice than in B6 IL-10 $^{-/-}$  mice, especially at 6 weeks of SIHUMI colonization (Fig. 2C).

**Concentrations of individual SIHUMI species are determined by host genetic background and are related to colonic inflammation.** Next, we investigated whether intestinal bacterial compositions are influenced by host genetic background, anatomical distribution, and the presence of colonic inflammation. Fecal bacterial concentrations were quantified by 16S rRNA gene-based quantitative PCR at different times (1, 2, 4, 8, and 12 weeks), and luminal contents and tissues of the cecum, colon, and distal ileum were analyzed 12 weeks after SIHUMI colonization.

Quantification of bacterial concentrations in pooled feces contained in two cages for each group showed that the seven human bacterial species formed a stable community under gnotobiotic

conditions and that relative bacterial abundances changed over time in the intestines of WT and IL-10 $^{-/-}$  mice on both the 129 and B6 backgrounds (Fig. 3; see Fig. S2 in the supplemental material). During the first and second weeks of SIHUMI colonization, the proportion of *B. vulgatus* was highest in all four groups. Over time, the proportion of *R. gnavus* increased in the 129 WT, 129 IL-10 $^{-/-}$ , and B6 IL-10 $^{-/-}$  mouse groups, to become a dominant species with *B. vulgatus*. By 12 weeks of colonization, the relative proportions of *R. gnavus* in 129 WT and IL-10 $^{-/-}$  mice were higher than those of *B. vulgatus*, while the proportions of *R. gnavus* in B6 WT and IL-10 $^{-/-}$  mice were lower than those of *B. vulgatus*. In contrast, *E. coli* concentrations decreased slightly over time and remained relatively low in all groups.

To determine relative differences in bacterial composition related to anatomical distribution, we compared relative bacterial species abundances among ileal, cecal, and colonic luminal contents and feces at 12 weeks of colonization. Relative bacterial species abundances from cecal and colonic contents in all mouse groups were similar to those in feces at 12 weeks, with *R. gnavus* and *B. vulgatus* being the most predominant species, except in WT B6 mice, where *E. faecalis* was more prevalent than *R. gnavus* (Fig. 4). In contrast, relative bacterial concentrations from ileal contents in all four groups were considerably different from those in feces at 12 weeks. The relative proportion of *E. faecalis* was highest in the ileal contents of all groups, with contraction of *R. gnavus* and *B. vulgatus* compared with colonic concentrations.

We next investigated differences in relative bacterial abundances between luminal contents and mucosal tissues. In contrast to the predominant proportions of *R. gnavus* and *B. vulgatus* in cecal and colonic contents and feces, the proportions of *B. longum* and *L. plantarum* in mucosal tissues were increased, with concomitant markedly decreased *R. gnavus* proportions, compared to the case in luminal contents and feces (Fig. 5). *B. vulgatus* and *E. faecalis* were other major components at all mucosal sites. These differential patterns of relative bacterial species abundance were consistent in ileal tissue, cecal tissue, and colonic tissue from all four groups, in contrast to obvious differences in ileal versus colonic luminal contents (Fig. 4) and in fecal bacterial profiles between 129 and B6 mice (Fig. 3 and 4).

To evaluate differences in specific bacterial profiles between WT and IL-10 $^{-/-}$  mice and between 129 and B6 mice, we compared specific bacterial levels, calculated as percentages relative to the total bacterial 16S rRNA gene copy number, in the luminal contents and mucosal tissues from the four murine groups (see Fig. S3 and S4 in the supplemental material). Colonic and cecal luminal concentrations of *R. gnavus* and *F. prausnitzii* were significantly increased in 129 WT and IL-10 $^{-/-}$  mice relative to those in B6 WT and IL-10 $^{-/-}$  mice. Changes in other bacterial populations were somewhat variable.

## DISCUSSION

This study shows that a defined, simplified consortium of well-characterized human enteric bacteria (SIHUMI), whose components were chosen for their relevance to IBD, induces colitis in selectively colonized ex-GF IL-10 $^{-/-}$  mice. We used this unique model to explore host-microbe interactions, demonstrating that the aggressiveness of colitis is greater in 129 than in B6 mouse strains and that concentrations of individual SIHUMI species are determined by host genetic background, anatomical distribution, and the presence of colonic inflammation. In addition, human

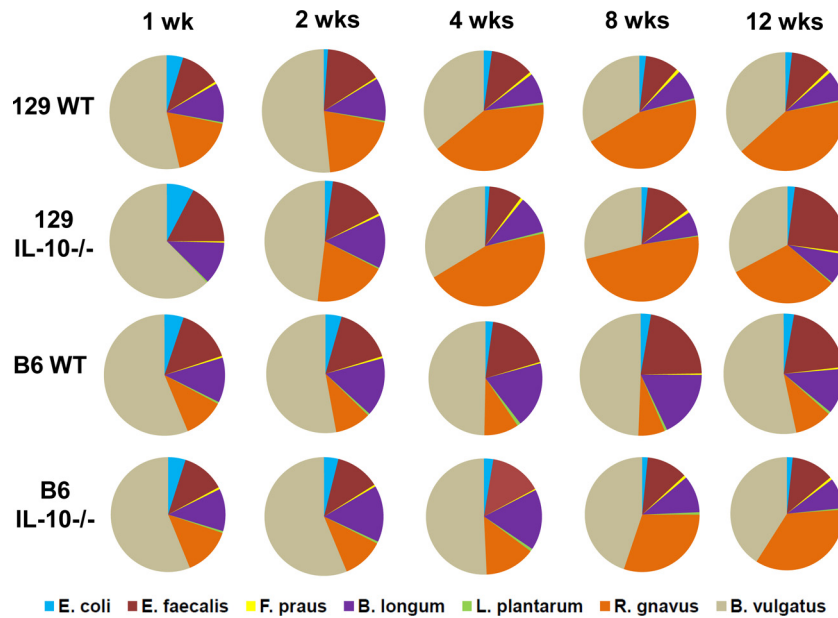


FIG 3 Relative abundances of seven SIHUMI bacterial species in feces from WT and IL-10<sup>-/-</sup> mice on the 129 and B6 backgrounds ( $n = 5$  or  $6$ /group) after 1, 2, 4, 8, and 12 weeks of SIHUMI colonization. F. praus, *F. prausnitzii*.

enteric bacterial strains differentially stimulate murine immune responses, with *E. coli* and *R. gnavus* preferentially inducing TH1 and TH17 effector immune responses in IL-10<sup>-/-</sup> mice. Importantly, the ability to induce TH1 and TH17 cell responses by bacterial lysates is not necessarily correlated with concentrations of individual bacterial species, especially in mucosal tissues. Finally, we demonstrated that luminal and mucosal bacterial profiles differ and are not reflected by fecal sampling; in particular, fecal microbiota analysis does not effectively identify the ileal bacterial community structure.

To investigate IBD-related microbiota-host interactions at a fully defined but more complex interactive level than that with

monoassociated mouse models, we established a simplified human microbiota consortium, composed of 7 human-derived and IBD-relevant gut bacteria, in WT and IL-10<sup>-/-</sup> mice on 2 genetic backgrounds. We demonstrated that this human microbiota forms a stable community in the mouse intestine that is maintained under gnotobiotic conditions. Although several studies have described simplified gnotobiotic rodent models containing 7 to 10 human bacteria or interactions between a commensal and enteric pathogens, those studies primarily showed successful microbial colonization and metabolomic characterization in WT rodents (14, 15, 20). In addition, “humanized” ex-GF mice colonized with a complex human fecal microbiota have been used to

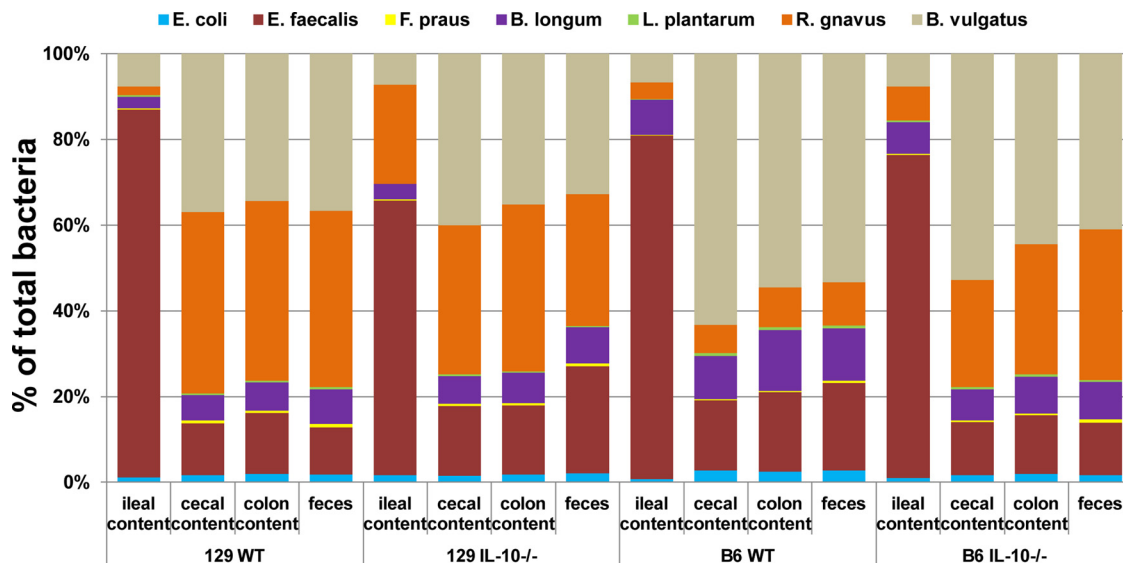


FIG 4 Relative abundances of seven SIHUMI bacteria among ileal contents, cecal contents, colonic contents, and feces from WT and IL-10<sup>-/-</sup> mice on the 129 and B6 backgrounds ( $n = 5$  or  $6$ /group) after 12 weeks of SIHUMI colonization.

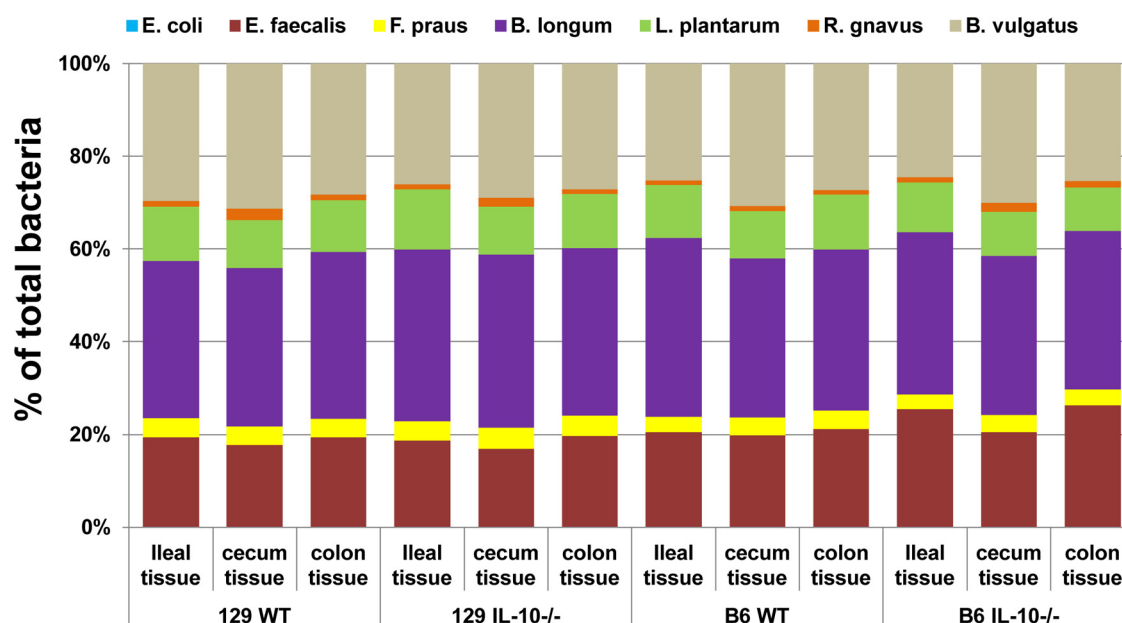


FIG 5 Relative abundances of seven SIHUMI bacteria among ileal tissues, cecal tissues, and colonic tissues from WT and IL-10<sup>-/-</sup> mice on the 129 and B6 backgrounds ( $n = 5$  or 6/group) after 12 weeks of SIHUMI colonization.

explore dietary effects on enteric bacterial composition and function (21). However, our study demonstrates for the first time that a defined human bacterial consortium can induce colonic inflammation and bacterium-driven TH1 and TH17 mucosal immune responses in genetically susceptible gnotobiotic IL-10<sup>-/-</sup> mice. Previous studies showed that humanized mice (GF mice colonized with human fecal samples) may more closely resemble GF mice in many immunological traits than conventional mice and that colonizing mice with a foreign microbiota cannot completely restore immune defects seen in GF mice (22, 23). Our current studies differ in that we induced immune-mediated inflammation in genetically susceptible mice with a defined group of human enteric bacterial strains relevant to IBD. Further studies using genetically susceptible mice colonized with human fecal samples will be needed to confirm whether the complex human intestinal microbiota can induce colonic inflammation and immune activation.

In the present study, we assessed the influence of host genetic background on the severity of SIHUMI-induced colitis in susceptible mice, using two widely used inbred mouse strains: 129 and B6. After 6 and 12 weeks of SIHUMI colonization, the histological aggressiveness of colitis, spontaneous colonic secretion of IFN- $\gamma$ , IL-12p40, and IL-17, and MLN cell immune activation by bacterial lysates were significantly increased in 129 IL-10<sup>-/-</sup> mice compared to B6 IL-10<sup>-/-</sup> mice. These results agree with previous reports demonstrating that B6 mice are relatively resistant to colitis in multiple models of acute and chronic intestinal inflammation, whereas 129 mice are highly colitogenic (24–26). Although the exact genetic loci responsible for colitis susceptibility have not been identified completely in murine models (27, 28), our data confirm that the host genetic background has a strong influence on the aggressiveness of colitis and demonstrate differential genetic susceptibility to chronic bacterium-induced, immune-mediated colitis.

Several human and murine studies have shown that host factors, including genetic polymorphisms, and environmental fac-

tors, such as maternal transmission, early life exposures, diet, infections, and antibiotics, strongly influence the composition of the intestinal microbiota (9, 29). However, deep-sequencing studies of complex commensal microbiotas necessarily concentrate on family and genus community structure rather than individual species. In this study, we examined whether the intestinal bacterial species composition in SIHUMI-colonized mice differs according to host genetic background. Global microbial community structures and relative bacterial species abundances in feces, luminal contents, and mucosal tissues were distinct between 129 and B6 strains that were colonized with identical bacterial species and concentrations in the initial inocula. These data are consistent with previous studies that showed that broad luminal microbial populations are strongly affected by host-derived factors, including host genetic background (30–33), but failed to document species-specific changes. Similar to several previous reports (19, 34, 35), we also showed differences in bacterial species concentrations between intestinal contents and mucosal tissues and between colonic contents and ileal contents, indicating that host-derived factors are associated with changes in the composition and distribution of intestinal microbial communities.

Meanwhile, several studies indicate that the overall bacterial composition changes with colonic inflammation (36–39), suggesting that the inflamed mucosa and the altered inflammatory milieu selectively affect growth and adherence of different bacterial species. Our choice of bacterial species was guided in part by studies showing expansion or contraction of certain bacterial populations and experimental colitis in IL-10<sup>-/-</sup> mice colonized with complex SPF microbiotas (10, 40, 41). Our results demonstrated differences in relative abundances of specific IBD-related bacterial species between WT mice and IL-10<sup>-/-</sup> mice with experimental colitis, confirming that chronic immune-mediated intestinal inflammation is associated with compositional changes of the intestinal microbiota putatively linked to disease pathogenesis. To our surprise, luminal and mucosal *E. coli* concentrations did not ex-

pand during development of colitis in SIHUMI-colonized IL-10<sup>-/-</sup> mice, in contrast to the results of most sequencing studies, which demonstrate consistent expansion of gammaproteobacteria in IBD patients (1, 10, 40). It is important that our data were generated using quantitative PCR analysis of the 16S rRNA gene. Since the copy number of this gene varies between bacterial species, it is possible that the reported abundances of specific bacteria may differ. Since we cannot be sure that the PCR efficiencies of each 16S rRNA copy will be equal, we did not integrate this phenomenon into our calculations. Indeed, as it is impossible to determine the copy number of all 16S rRNA genes from more complex microbial communities (such as the intestinal microbiota), this is an inherent issue with modern molecular analysis of microbial ecosystems. Whether altered intestinal microbiota composition is a cause or a consequence of intestinal inflammation remains to be determined.

To examine which bacteria primarily induce immune responses in SIHUMI-colonized IL-10<sup>-/-</sup> mice, we stimulated MLN cells isolated from SIHUMI-colonized mice with lysates of each of the seven bacterial species or an aggregate lysate and measured proinflammatory cytokines in the supernatants of stimulated MLN cells. *E. coli* LF82 and *R. gnavus* predominantly induced secretion of proinflammatory cytokines from *ex vivo*-stimulated MLN cells. Interestingly, *E. coli* mainly induced secretion of IFN- $\gamma$  and IL-12p40, whereas *R. gnavus* primarily induced secretion of IL-17. The *E. coli* LF82 strain used in our study is a prototype adherent-invasive *E. coli* (AIEC) strain originally isolated from an ileal lesion of a CD patient (17) and is functionally different from commensal enteric *E. coli* strains. AIEC strains are associated with CD (42, 43), and the level of *R. gnavus* with mucolytic activities is increased in a subset of CD patients (39, 44, 45). Our results demonstrate that *E. coli* LF82 and *R. gnavus* predominantly induce effector immune responses in IL-10<sup>-/-</sup> mice, even though the proportions of those bacteria in mucosal tissues of the distal intestine are small. *R. gnavus* is one of the most abundant luminal components in the colons of IL-10<sup>-/-</sup> 129 and B6 mice, but *E. coli* concentrations are relatively low. These results suggest that immunodominant bacterial species of a numerically small fraction of the intestinal microbiota may primarily induce effector TH1 and TH17 immune responses that mediate chronic intestinal inflammation and that mucosally associated bacterial species are not necessarily more immunologically active than luminal species. Our findings suggest that future therapeutic and preventive strategies to manipulate the commensal microbiota in IBD should target bacterial species with immunodominant antigenic properties, in addition to correcting the dysbiosis in genetically susceptible individuals.

Taken together, our results suggest that AIEC and *R. gnavus* may play an important role in inducing chronic intestinal inflammation in susceptible hosts and that colitogenic bacteria can differentially induce TH1 and TH17 cell responses. Our results emphasize the need to identify tangentially relevant subsets of the commensal microbiota that activate pathogenic T cell responses.

In summary, this study provides novel data showing that a human bacterial consortium, SIHUMI, can induce bacterial antigen-specific colitis in IL-10<sup>-/-</sup> mice and that the ability to induce effector TH1 and TH17 immune responses by enteric bacterial species does not reflect the total bacterial number in the lumen or mucosa. The aggressiveness of colitis depends on the host genetic background. Our results support the important association of in-

testinal microbial composition with host genotype, anatomical distribution, and colonic inflammation. Our humanized gnotobiotic model with SIHUMI is an important resource that can be used in further clinically relevant studies to elucidate the mechanisms by which CD-associated innate immunity genes regulate the composition, spatial relationships, and function of a defined intestinal microbiota and to investigate the impact of environmental factors, such as diet, antibiotics, and nonsteroidal anti-inflammatory drugs, on host immune responses, bacterial community structure and function, and colonic inflammation in IBD.

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