Ecobiotherapy Rich in Firmicutes Decreases Susceptibility to Colitis in a Humanized Gnotobiotic Mouse Model

Jane M. Natividad, PhD, Maria I. Pinto-Sanchez, MD,* Heather J. Galipeau, PhD,* Jennifer Jury, MSc,* Manel Jordana, MD, PhD,† Walter Reinisch, MD,* Stephen M. Collins, MD,* Premsyl Bercik, MD,* Michael G. Surette, PhD,* Emma Allen-Vercoe, PhD,‡ and Elena F. Verdu, MD, PhD**

Background: Alterations in the intestinal microbiota, characterized by depletion of anti-inflammatory bacteria, such as Firmicutes, in patients with ulcerative colitis (UC) have prompted interest in microbiota-modulating strategies for this condition. The aim of this study was to evaluate the role of fecal and synthetic human microbial ecosystems, low or enriched in Firmicutes, on colitis susceptibility and host immune responses.

Methods: The microbiota of selected healthy and UC human donors was characterized by culture method and 16S rRNA-based sequencing. Germ-free mice were colonized with fecal or a synthetic ecosystem enriched (healthy donors) or low (UC donors) in Firmicutes. Experimental colitis was induced using dextran sodium sulfate. Colon transcriptome and colon lamina propria cells were evaluated in mice postcolonization by RNA-seq and flow cytometry, respectively, and T helper (T_H) 17 differentiation was assessed in vitro.

Results: Mice colonized with microbiota from patients with UC low in Firmicutes had increased sensitivity to colitis compared with mice colonized with fecal or synthetic ecosystems rich in Firmicutes. Microbiota low in Firmicutes increased expression of T_H17-related genes and expansion of interleukin-17A–expressing $CD4^+$ cells in vivo. Supplementation with bacterial isolates belonging to the Firmicutes phylum abrogated the heightened T_H17 responses in vitro.

Conclusions: A microbiota rich in Firmicutes derived from fecal samples of a healthy human donor, or assembled synthetically, downregulated colonic inflammation and T_H17 pathways in mice. The results support the use of ecobiotherapy strategies, enriched in Firmicutes, for the prevention or treatment of UC.

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Key Words: ulcerative colitis, microbiota, T_H17, Firmicutes

Over the past decade, the intestinal microbiota has become one of the most studied areas in biomedical research. Gnotobiotic studies have provided key evidence on how specific bacterial strains influence the development and maturation of host physiology and immunity, including secondary lymphoid development, antimicrobial peptide maturation, and induction of T helper (T_H) cells, such as T regulatory (T_{REG}) and proinflammatory T_H17 cells.[1–3] Components of the intestinal microbiota have differential capacity to influence host responses.[4] For instance, experimentally defined communities, such as the altered Schaedler flora (ASF), Clostridium species, or the polysaccharide A–expressing *Bacteroides fragilis* predominantly induce T_{REG} in the colon, whereas pathobionts *Bilophila wadsworthia* and segmented filamentous bacteria are potent inducers of T_H1 and T_H17 cells, respectively.[5–8] Others have shown that colonization with an enterotoxigenic *B. fragilis* worsens experimental colitis in mice compared with a nontoxigenic strain.[9] Altogether, this suggests that the host-microbiota interaction sits in a continuum between mutualism and pathogenicity, and the composition of the microbiota is one factor that could potentially upset this delicate balance.

Disturbed microbiota, termed dysbiosis, which is a state in which the homeostatic balance between the microbiota and the host is shifted toward a proinflammatory state, has been described in a number of autoimmune and intestinal inflammatory conditions.[10,11] It is currently accepted that ulcerative colitis (UC), one of the 2 forms of inflammatory bowel disease (IBD), results from the development of abnormal immune responses to the colonic microbiota.[12] Although therapies for UC currently aim at modulating the immune response, there has been increasing interest in microbiota-modulating therapies,

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From the *Farncombe Family Digestive Health Research Institute, McMaster University, Hamilton, ON, Canada; †Department of Pathology, and Molecular Medicine, McMaster Immunology Research Centre, McMaster University, Hamilton, ON, Canada; and ‡Department of Molecular and Cellular Biology, University of Guelph, Guelph, ON, Canada.

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Reprints: Elena F. Verdu, MD, PhD, Room HSC 3N4A, 1200 Main Street West, Hamilton, ON L8N 3Z5, Canada (e-mail: verdue@mcmaster.ca).

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including fecal microbiota transplantation, probiotics, prebiotics, and antibiotics. However, questions remain regarding the mechanism of action, efficacy, and safety of some of these therapies.

In this article, we studied whether microbiota low or rich in Firmicutes, derived from patients with active UC or healthy volunteers, respectively, influenced colitis susceptibility and host immune responses in naive germ-free (GF) mice. Colonization with microbiota low in Firmicutes led to increased sensitivity to experimental colitis, compared with mice colonized with fecal or synthetic microbial ecosystems, rich in Firmicutes. Examination of immune responses before colitis induction did not reveal overt inflammation; however, microbiota low in Firmicutes influenced the proinflammatory T_H17-related gene network and expansion of interleukin (IL)-17A–expressing $CD4^+$ cells both in vitro and in vivo. The T_H17 response was reversed after supplementing the dysbiotic microbiota with bacterial isolates from healthy donors belonging to Lachnospiraceae and Ruminococcaceae families. The data indicate that a microbiota low in Firmicutes increases the risk of intestinal inflammation, whereas members of the Lachnospiraceae and Ruminococcaceae families have anti-inflammatory activity.

MATERIALS AND METHODS

Healthy Volunteer and UC Donors

Stool was collected from a healthy donor ($Healthy_{stool}$), which was previously used in a clinical fecal transplantation study in patients with UC.[13,14] From a second healthy donor, 33 bacterial culture isolates were obtained ($Healthy_{cultured}$); this synthetic ecosystem was successfully used to treat recurrent *Clostridium difficile* infection.[15] Fecal samples from 2 patients with active UC ($UC1_{stool}$, $UC2_{stool}$) were also collected. The 2 patients had severe pancolitis and were hospitalized at the time of fecal collection (Table 1 for patients' characteristics).

TABLE 1. Demographic Information of Patients with UC

	UC1	UC2
Gender	Female	Male
Race	First nation	White
Diagnosis	UC, severe pancolitis	UC, severe pancolitis
Disease state	Active, total colectomy 4 d after stool collection	Active
Stool collection	During hospitalization	During hospitalization
Age	48	39
Current medication	Methylprednisolone, adalimumab	Prednisone

Microbiota Preparations for Mouse Colonizations

Healthy ($Healthy_{stool}$) and UC ($UC1_{stool}$, $UC2_{stool}$) microbiota was prepared by diluting 1 g of frozen human fecal samples in 10 mL of prereduced sterile 0.9% normal saline under anaerobic conditions and then suspended by vortexing. The synthetic healthy human ($Healthy_{cultured}$) microbiota was formulated and prepared as previously described (see Fig, Supplemental Digital Content 7, http://links.lww.com/IBD/A869).[15]

Bacterial Lysates for In Vitro Studies

UC fecal samples were cultured using various media types including brain heart infusion agar, Wilkins–Chalgren agar, reinforced clostridial agar, and deMan, Rogosa, and Sharpe agar under strict anaerobic conditions. Twenty-four different bacterial isolates were recovered from $UC1_{stool}$ and identified by 16S ribosomal ribonucleic acid (rRNA) gene sequencing. Bacterial suspensions of the 33 isolates from the synthetic healthy human microbiota ($Healthy_{cultured}$) or the 24 isolates from $UC1_{stool}$ were formulated and then sonicated to obtain bacterial lysates. $UC1_{cultured}$ + Lac + Rum bacterial suspension was formulated by adding bacterial isolates belonging to Lachnospiraceae and Ruminococcaceae families isolated from $Healthy1_{cultured}$ into the $UC1_{cultured}$ bacterial suspension (see Table, Supplemental Digital Content 1, http://links.lww.com/IBD/A863, for list of bacterial isolates).

Mice

Six- to 12-week-old female and male C57BL/6 mice were used in experiments. GF C57BL/6 mice were rederived by axenic 2-cell embryo transfer technique and maintained in flexible film isolators at the McMaster University Axenic Gnotobiotic Unit.[16] GF status was evaluated weekly by a combination of culture and nonculture techniques. Periodic serologic testing was also performed for viruses, parasites, and known pathogens (Charles Rivers Laboratories, Wilmington, MA). Some experiments used mice colonized with a benign murine microbiota (the ASF)[17] as controls. These mice are gnotobiotically housed in the McMaster's Axenic Gnotobiotic Unit. All mice had unlimited access to autoclaved mouse breeder's diet (Harlan, Indianapolis, IN) and water. All experiments were carried out in accordance with the McMaster University animal utilization protocols.

Human-mouse Colonizations

Each human donor was used to gavage 5 to 14 mice, using 0.2 mL of the fecal or cultured bacteria suspensions. Colonized mice were then housed under gnotobiotic conditions. All colonized mice were maintained with the same sterilized diet and water as before colonization.

Intestinal Injury and Inflammation Analysis

Intestinal injury was induced using 3% dextran sulfate sodium (DSS). Degree of injury and inflammation were evaluated using a variety of histologic and immunologic methods as previously described.[16,18]

Microbiota Analysis

Genomic bacterial DNA was extracted,[19] V3 region of 16S rRNA gene was amplified and sequenced using MiSeq Illumina platform. Data were processed using an in-house bioinformatics pipeline.[19] α-diversity and β-diversity metrics were carried out using QIIME.[20]

RNA-seq Analysis

Colonic mRNA library was prepared using NEB Next Ultra Directional RNA kit. RNA-seq was run using HiSeq Illumina platform and then analyzed using Tuxedo protocol.[21]

Flow Cytometry

Colonic lamina propria cells were isolated by collagenase method and then surface stained with appropriate antibodies. Cells were intracellularly stained after 4 hours of stimulation with phorbol 12-myristate 12-acetate (Sigma, Ontario, Canada) and ionomycin (Invitrogen, Eugene, OR) with Brefeldin A (Sigma).[22]

In Vitro T-cell Differentiation

For T-cell differentiation, $CD4^+CD25^-$ T cells were stimulated with plate-bound anti-CD3 antibodies and bacteria-pulsed $CD11c^+$ cells with or without recombinant cytokines. For differentiation using $CD11c^+$ cells supernatant, anti-CD28 antibodies were additionally added to the culture. Cytokines were analyzed by cytometric bead array or enzyme-linked immunosorbent assay or reverse transcription quantitative polymerase chain reaction.

Statistics

Data were presented as either dot plots or bar graph (mean ± SD). Statistical analysis was performed using the 1-way analysis of variance followed by the Bonferroni test or the 2-tailed Student's *t* test, when applicable. Except for RNA-seq data, all statistical testing was performed using GraphPad Prism 6 (GraphPad Software Inc., La Jolla, CA), and the *P* value of 0.05 was considered as significant. For RNA-seq, Cuffdiff[23] was used to test for statistical significance, and the *P* value of 0.01 was considered as significant.

RESULTS

Characterization of Human Microbiota Communities for Mouse Colonizations

Using 16S rRNA-based sequencing, we characterized the human microbiota communities ($UC1_{stool}$ and $UC2_{stool}$) and compared them with those acquired from the healthy subject ($Healthy_{stool}$). We also characterized a synthetic microbiota community derived from the feces of the second healthy subject ($Healthy_{cultured}$).[15] As expected from the previous studies,[24] the taxonomic distribution of reads in the human stool communities was unique to each individual. However, the 2 UC microbiota were enriched in bacteria from the phylum Bacteroidetes and had significantly lower abundance of Firmicutes, including Lachnospiraceae and Ruminococcaceae families (Fig. 1; see Table, Supplemental Digital Content 2, http://links.lww.com/IBD/A864).

Mice Colonized with Both Fecal and Synthetic Microbiota Rich in Firmicutes Had Lower Sensitivity to Colitis and Reduced Inflammatory Parameters

To investigate functional and pathologic outcomes in recipient mice after microbiota transfer, experimental colitis was induced using DSS 3 weeks after colonization. Colon sections were examined histologically for signs of architectural distortion and damage (Fig. 2A). Colonic myeloperoxidase (MPO) activity was

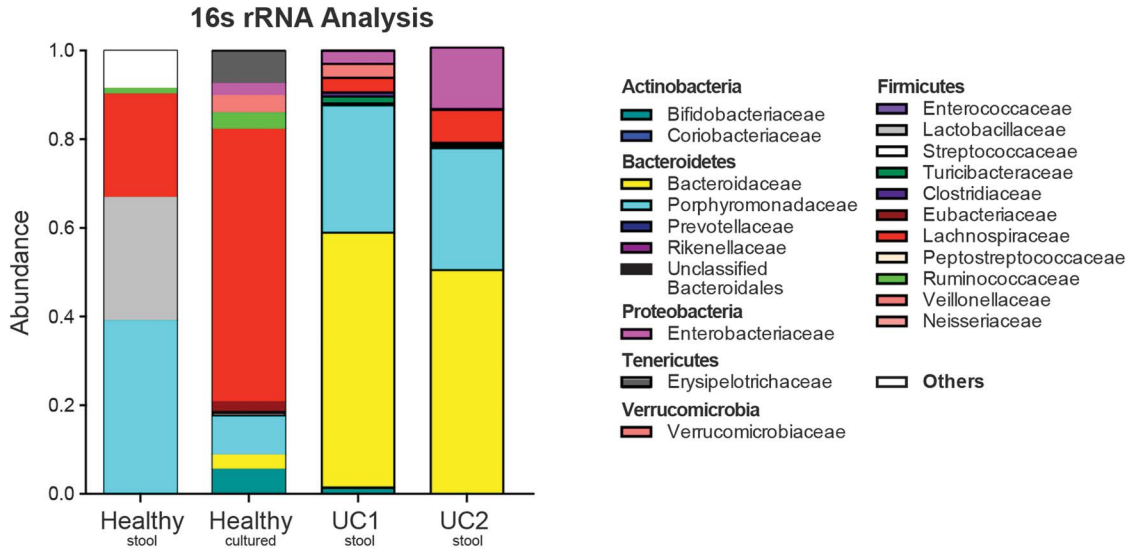

FIGURE 1. Taxonomic composition of different donor ecosystems. Genomic DNA from $Healthy_{cultured}$ (33 selected human strains), $Healthy_{stool}$ (fecal samples from healthy individual), $UC1_{stool}$, and $UC2_{stool}$ (fecal samples from 2 patients with UC) microbiota was isolated and then assessed by 16S rRNA-based sequencing method. Result was expressed as bar plot of relative abundance at the family level.

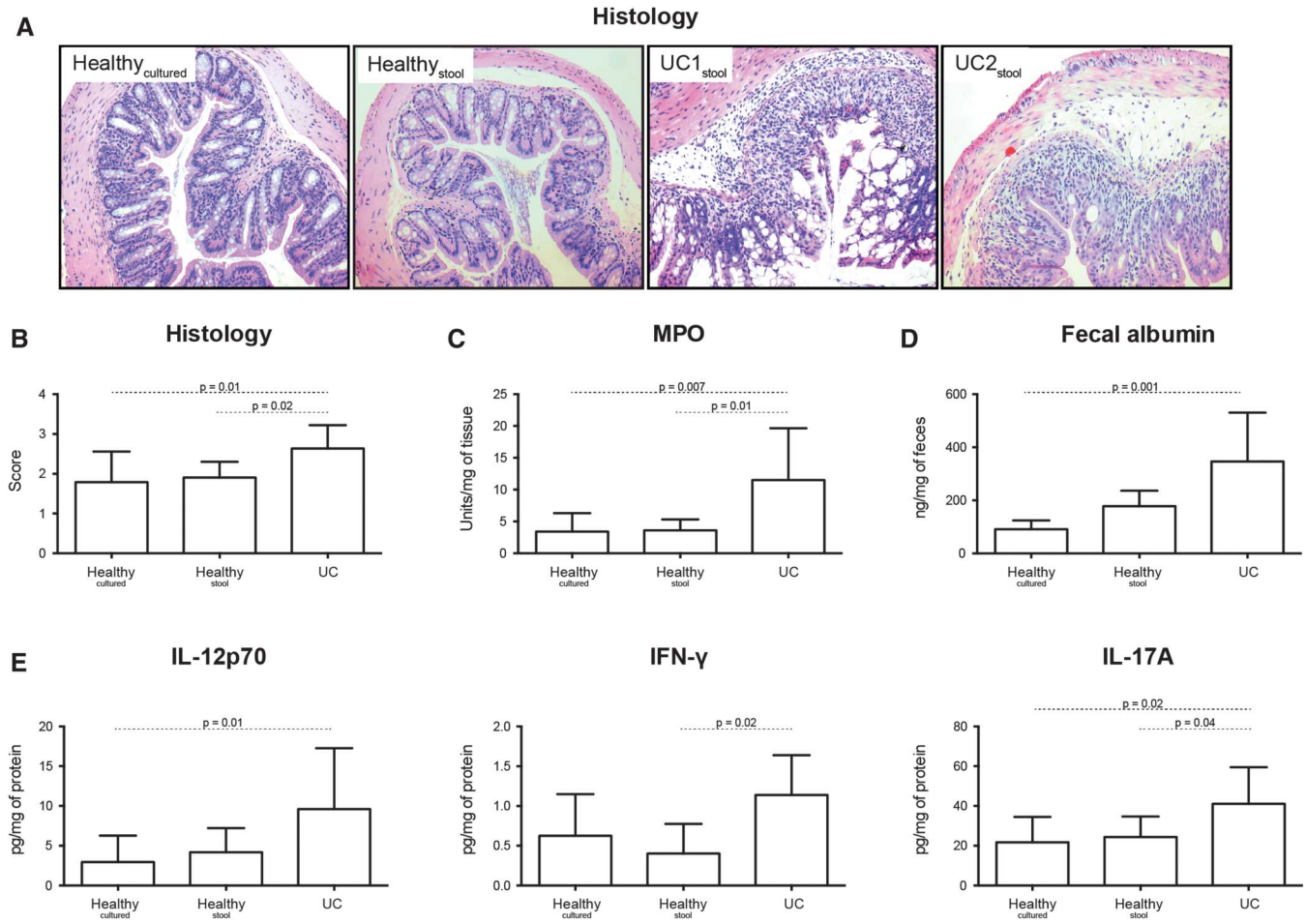

FIGURE 2. Mice colonized with UC microbiota had higher sensitivity to experimental colitis. Three weeks after colonization, intestinal injury was induced in C57BL/6 mice colonized with healthy human derived ($Healthy_{cultured}$), healthy stool ($Healthy_{stool}$), or ($UC1_{stool}$, $UC2_{stool}$) microbiota using DSS. (A), Representative hematoxylin and eosin staining of proximal colon. (B), Blinded histologic evaluation for signs of architectural distortion and damage. (C), Colonic MPO activity as a marker of colonic inflammatory activity. (D), Presence of albumin in the feces determined by enzyme-linked immunosorbent assay as a marker of enteropathy. (E), Colonic cytokine expression. Data were pooled from 2 to 3 independent experiments, 5 to 14 mice per group and shown in bar graph (mean ± SD). *P* values were calculated using analysis of variance followed by Bonferroni post hoc test.

measured as an index of granulocyte infiltration (Fig. 2B) and the presence of albumin in the feces as a marker of enteropathy (Fig. 2C). Mice colonized with microbiota low in Firmicutes (UC_{stool}) exhibited higher histologic scores, MPO activity, and increased expression of colonic IFN-γ and IL-17A after colitis induction compared with mice colonized with microbiota rich in Firmicutes ($Healthy_{stool}$). Similarly, mice colonized with $Healthy_{culured}$ exhibited lower colitis severity. No differences in histologic damage and activity of colitis (MPO) were detected between $Healthy_{stool}$ or $Healthy_{cultured}$ colonized mice. There were some efficacy differences between $Healthy_{stool}$ and $Healthy_{cultured}$, with $Healthy_{cultured}$ colonized mice displaying significantly lower fecal albumin and IL-12 levels than UC_{stool} colonized mice. However, $Healthy_{stool}$ colonized mice had lower levels of IFN-γ compared with UC_{stool}. Overall, the results suggest that colonization with defined microbial ecosystems rich in bacterial families associated with gut health can ameliorate subsequent development of colitis. The results also indicate the effects are beneficial whether the transferred microbiota originates from a fecal donor ($Healthy_{stool}$) or a synthetic ecosystem ($Healthy_{cultured}$).

Naive GF Mice Colonized with Microbiota Low in Firmicutes Had Increased Colonic T_H17 Gene–related Signatures

Both $Healthy_{stool}$ and $Healthy_{cultured}$ ameliorated colitis susceptibility and reduced inflammatory parameters. We then investigated the type of immune responses induced in GF mice, in the absence of colitis, by colonization with low Firmicutes. Given the current safety concerns regarding the use of uncharacterized fecal slurs in human transplantation trials, we chose to focus on the

synthetic microbiota (rich in Firmicutes) as control. Henceforth, we colonized additional groups of GF mice with UC_{stool} or $Healthy_{cultured}$ and investigated the host responses 3 weeks after colonization.

The fecal microbiota of recipient and donor mice were compared 3 weeks after colonization using 16S rRNA-based sequence profiling and β diversity metrics. Mouse recipients showed similar microbiota profiles as their respective human donors and were distinct from those harvested from other donor microbiota (Fig. 3; see Table, Supplemental Digital Content 2, http://links.lww.com/IBD/A864, and Figs., Supplemental Digital Content 3 and 4, http://links.lww.com/IBD/A865 and http://links.lww.com/IBD/A866), indicating successful engraftment of the microbiota in the new host. The presence of spontaneous inflammation after colonization was evaluated histologically (Fig. 4A) and by measuring colonic MPO activity (Fig. 4B) and the presence of albumin in the feces (Fig. 4C). No differences were found between groups in any of these parameters, indicating that colonizations per se did not induce colitis in recipient mice. Furthermore, we found no differences in mice colonized with either human ($Healthy1_{cultured}$ or UC_{stool}) or a well-defined murine microbiota (ASF),[22] indicating that colonization with human microbiota did not induce intestinal pathology in mice.

We then evaluated the colon transcriptome using RNA-seq (Fig. 4D; see Table, Supplemental Digital Content 5, http://links.lww.com/IBD/A867). Regardless of the type of microbiota transferred, 944 genes were modulated ($P \leq 0.01$) after colonization. These included genes involved in nutrient absorption, xenobiotic metabolism, antimicrobial peptides, and regulatory genes.[4,6,22] We performed hierarchical clustering to determine the pattern of genes differentially expressed between mice colonized with microbiota low or enriched with Firmicutes. Immune system pathways, including innate and T-cell immune responses, were among the programs most notably changed microbiota low in Firmicutes, including genes associated with inflammatory immune responses (Fig. 4E). Furthermore, genes (IL-6, IL-1β, IL-23r, IL-17rc, Ccl6, Cxcl3, Gzmb, Pou2af1) that positively affect T_H17 differentiation and expansion[25] were expressed at a higher level, whereas expression of the T_H17 negative regulator (Tsc22dd3) was lower in mice colonized with microbiota low in Firmicutes.

Naive Mice Colonized with Microbiota Low in Firmicutes Had Increased Proportion of Colonic T_H17 Cells

We did not find differences neither in $CD3^+$ cells nor in the proportion of colon lamina propria $CD4^+$ and $CD8^+$ cells in mice colonized with microbiota either low (UC_{stool}) or enriched in Firmicutes ($Healthy_{cultured}$) (see Fig., Supplemental Digital Content 6, http://links.lww.com/IBD/A868). However, in agreement with the previous studies,[22] we observed an increase in the proportion of Foxp3-expressing $CD4^+CD25^+$ cells, regardless of the type of colonization (Fig. 5A). We performed intracellular staining for IL-17A and IFN-γ as markers of T_H17 and T_H1 cells, respectively. Although we detected an induction of T_H17 cells in all colonized mice, significantly higher induction was observed in mice colonized with UC microbiota (Fig. 5B). No significant difference was observed in the proportion of IFN-γ expressing T cells among colonized mice. The increased T_H17 induction was observed only when cells were stimulated with phorbol 12-myristate 13-acetate and ionomycin, indicating that a certain degree of activation is necessary to detect a difference in colonic T_H17 levels (see Fig., Supplemental Digital Content 7, http://links.lww.com/IBD/A869). Finally, the increased proportion of T_H17 was only evident in the colonic lamina propria and not in other mucosal and systemic immune compartments (see Fig., Supplemental Digital Content 8, http://links.lww.com/IBD/A870). Thus, colonization with microbiota low in Firmicutes from patients with UC resulted in activation of a colonic T_H17 network in mice.

T_H17-cell Expansion Induced by Microbiota Low in Firmicutes Was Abrogated by Lachnospiraceae and Ruminococcaceae Families

Based on our RNA-seq data, $UC1_{stool}$ upregulated a greater number of T_H17-related genes compared with $UC2_{stool}$. Thus, we further investigated the ability of $UC1_{stool}$ microbiota to directly influence the T_H17 immune response using in vitro culture assays. We characterized the $UC1_{stool}$ by culture method and recovered 24 bacterial isolates (see Table, Supplemental Digital Content 1, http://links.lww.com/IBD/A863) and then tested whether $UC1_{cultured}$ bacterial lysates induced the expression of proinflammatory cytokines in $CD11c^+$ cells pooled from spleen and mesenteric lymph nodes as well as whole splenic and mesenteric lymph nodes cells. In all cases, we observed increased levels of IL-6, a critical positive regulator of T_H17 differentiation,[26] in cultures stimulated with $UC1_{cultured}$ bacterial lysates but not with $Healthy_{cultured}$ lysates (Fig. 6). No differences in the level of IL-12p70, IFN-γ, TNF-α, IL-10, MIP-2, and IL-17A were observed (data not shown).

To further test the ability of $UC1_{cultured}$ to induce T_H17 cells, we tested whether coculturing $CD4^+CD25^-$ T cells with $CD11c^+$ bacteria-pulsed cells, or their supernatants, induced greater T_H17 cell differentiation. Recombinant IL-6 and TGF-β were included into all culture conditions as these cytokines are required for T cells to commit to the $CD4^+$ T_H17 lineage in vitro.[27] T cells challenged with $UC1_{cultured}$ bacterial lysates, or their supernatant, produced higher levels of IL-17A compared with T cells incubated with $Healthy_{cultured}$ bacterial lysates (Fig. 7A–B, E–F). In the absence of recombinant cytokines, there was no significant difference between T cells exposed to $Healthy_{cultured}$ and $UC1_{cultured}$ bacteria, indicating that cytokine induction by UC1 bacteria is insufficient to promote a greater T_H17 differentiation (see Fig., Supplemental Digital Content 9, http://links.lww.com/IBD/A871). Furthermore, T cells challenged with $UC1_{cultured}$ showed elevated transcripts of IL-21 and IL-17F, which have been associated with pathogenic T_H17 cells (Fig. 7c-d, g-h).[28,29] Thus, UC microbiota promoted an overall in vitro expansion of T_H17 cells.

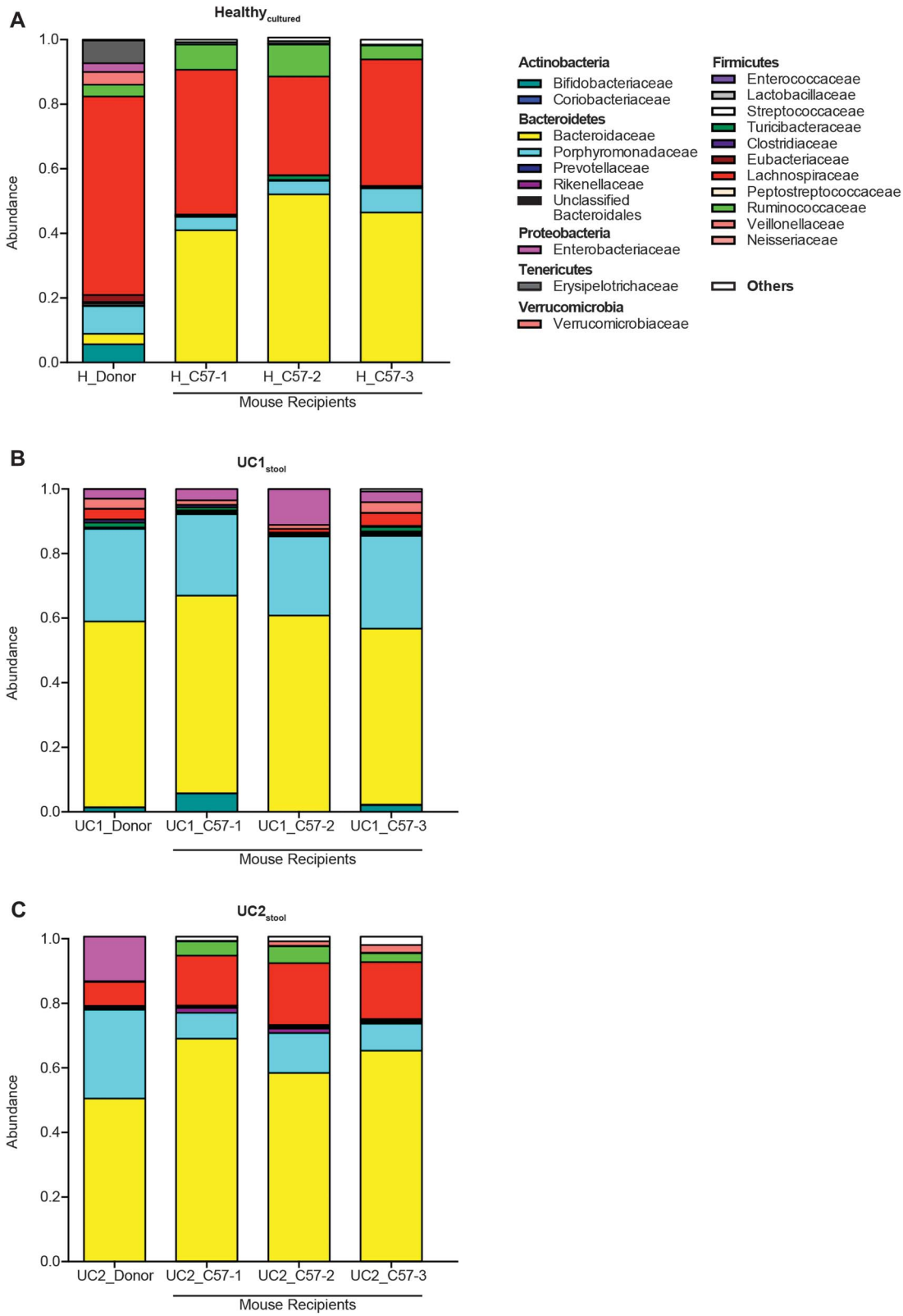

FIGURE 3. Mouse recipients had similar microbiota profiles as their respective human donors. Three weeks after colonization, fecal pellets were collected from C57BL/6 mice colonized with healthy human ($Healthy_{cultured}$) or ($UC1_{stool}$, $UC2_{stool}$) microbiota. Bacterial DNA was extracted and analyzed using 16S rRNA-based sequencing technology. (A–C), Relative abundance at family level of donor and their respective recipient microbiota was plotted as bar graphs.

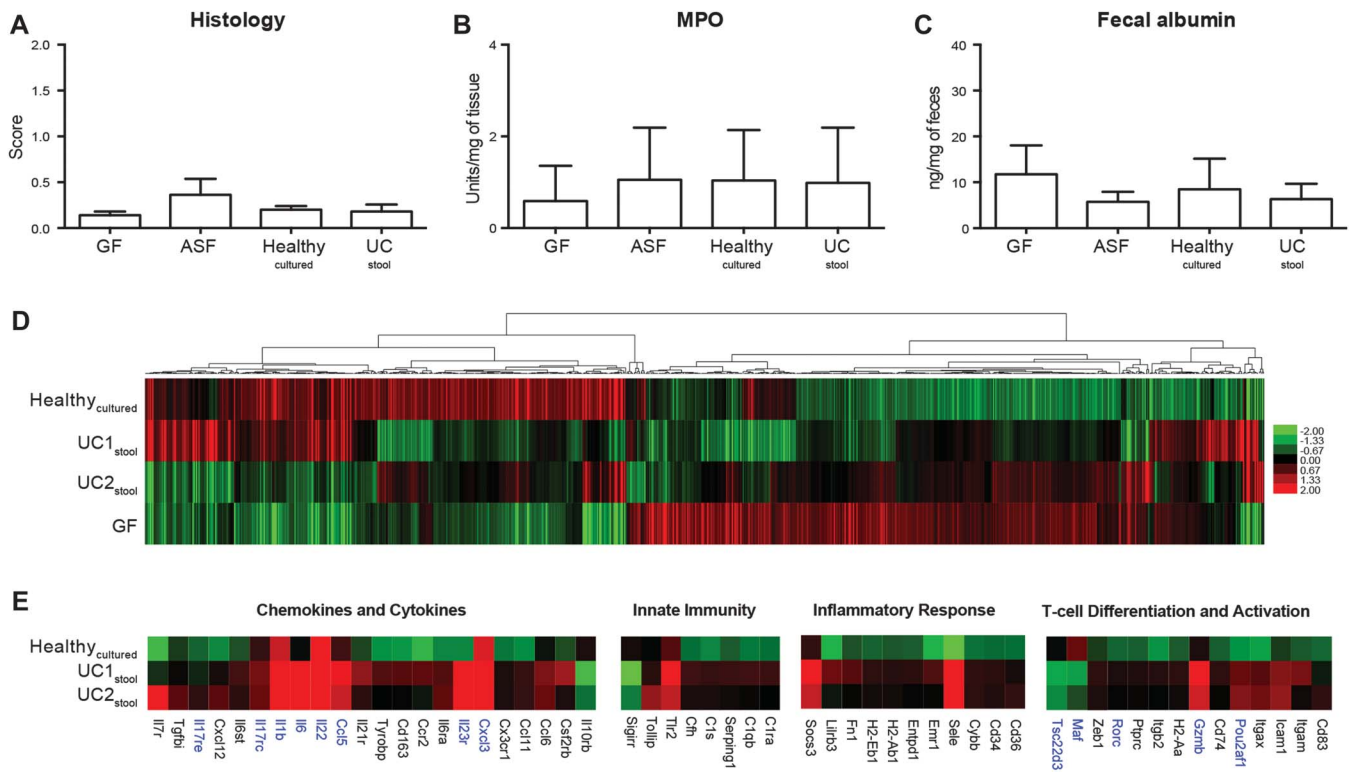

FIGURE 4. UC microbiota upregulated colonic TH17 gene–related signatures in the absence of overt colonic inflammation. Three weeks after colonization, colon health was assessed in C57BL/6 mice colonized with a benign murine ASF, healthy human ($Healthy_{cultured}$), or ($UC1_{stool}$, $UC2_{stool}$) microbiota. (A), Blinded histologic evaluation for signs of architectural distortion and damage. (B), Colonic MPO activity as a marker of colonic inflammatory activity. (C), Presence of albumin in the feces determined by enzyme-linked immunosorbent assay as a marker of enteropathy. Data were pooled from 2 to 3 independent experiments, 5 to 12 mice per group and shown in bar graph (mean ± SD) (A–C). (D), Heatmap of differentially expressed genes after colonization assessed by RNA-seq. Only genes with *P* values ≤0.01 versus GF mice were presented. (E), Heatmap showing immune genes that were significantly modulated in UC_{stool} compared with $Healthy_{cultured}$ colonized mice. Heatmap was plotted as $\log_2$ fold change versus GF mice. T_H17-associated molecular signature was highlighted in blue.

Specific subsets of bacteria have been shown to differentially induce T_H17 cells.[6,30] None of the 4 bacterial isolates (*Kluyvera cryocrescens, Klebsiella oxytoca, Veillonella atypica,* and *B. fragilis*) present in $UC1_{cultured}$, but not in $Healthy_{cultured}$, individually enhanced T_H17 differentiation (see Fig. A, Supplemental Digital Content 10, http://links.lww.com/IBD/A872). However, when cocktails of bacterial lysates with varying proportion of $Healthy_{cultured}$ and $UC1_{cultured}$ bacteria were used to stimulate T cells, we observed an enhanced T_H17 response when the ratio of $Healthy_{cultured}$ was lower than $UC1_{cultured}$ (see Fig. B and C, Supplemental Digital Content 10, http://links.lww.com/IBD/A872). It is plausible that certain groups present in $Healthy_{cultured}$ dampen the $UC1_{cultured}$-induced T_H17 response. Indeed, the $UC1_{cultured}$ microbiota has underrepresented bacteria belonging to Lachnospiraceae and Ruminococcaceae families; thus, we added representative isolates from $Healthy_{cultured}$ belonging to these families. Stimulation of naive T cells with either $CD11c^+$ cells pulsed with the Lachnospiraceae and Ruminococcaceae supplemented cocktail (UC1 + Lac + Rum), or their supernatants, decreased the proportion of T_H17. Finally, culture supernatants from $CD11c^+$ cells exposed to the supplemented cocktail had lower IL-6 levels (Fig. 6).

DISCUSSION

Host-intestinal microbiota interactions are primarily mutualistic; however, a breakdown of this equilibrium may contribute to disease. Studies in animal models have implicated the microbiota in colitis, and the clinical studies have revealed relevant information regarding the characteristics of dysbiosis in IBD, including UC.[11,31] However, there is still little mechanistic information underlying the nature of this association and its relevance to disease susceptibility. This understanding is key to optimize new therapeutic options for IBD, such as the recently proposed fecal bacteriotherapy.[32,33]

We found that transplantation of fecal, or cultured, communities into GF mice led to different colitis susceptibility and host responses in naive mice depending of the relative abundance of Lachnospiraceae and Ruminococcaceae in the transplanted material. The experimental microbiota used for

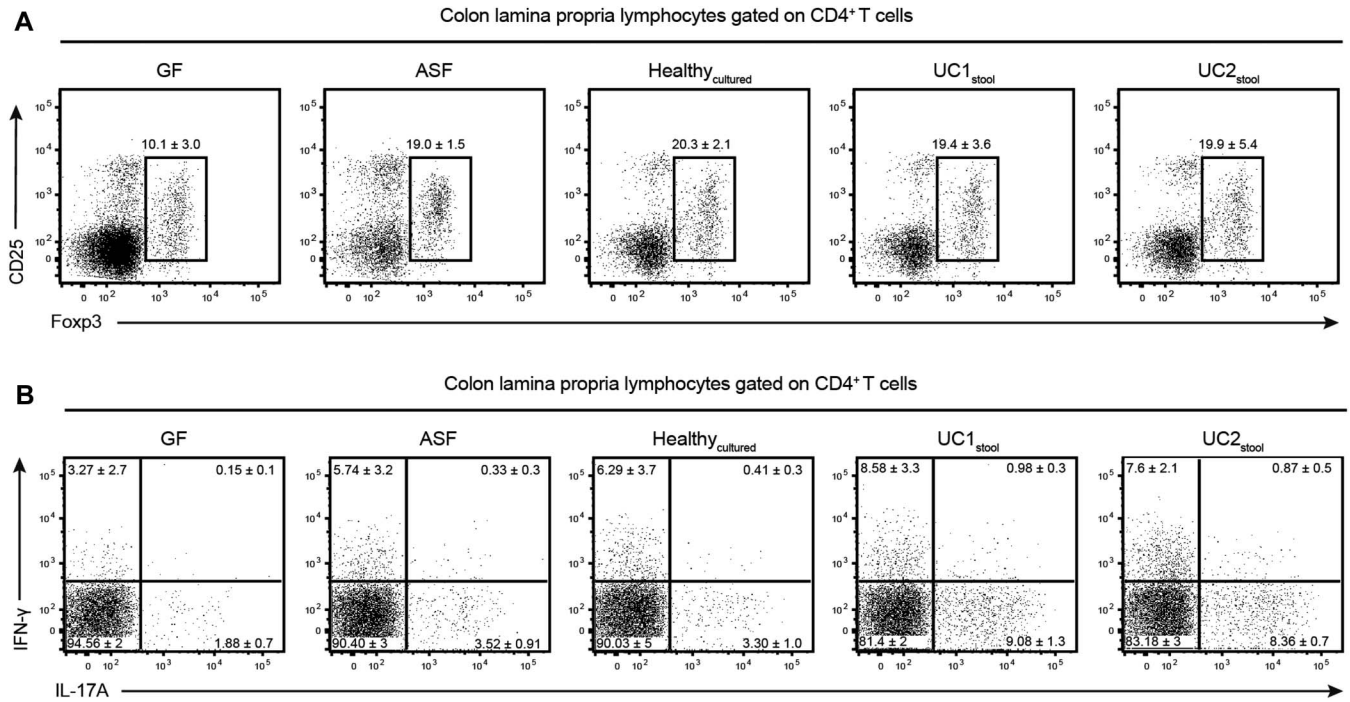

FIGURE 5. UC microbiota increased the proportion TH17 cells in the colon. Three weeks after colonization, colon health was assessed in C57BL/6 mice colonized with a benign murine ASF, healthy human ($Healthy_{cultured}$), or ($UC1_{stool}$, $UC2_{stool}$) microbiota. (A), Representative flow cytometry plots after intracellular staining for Foxp3 colon lamina propria $CD4^+$ cells. (B), Representative flow cytometry plots after intracellular staining for IL-17A and IFN-γ of colon lamina propria $CD4^+$ cells after phorbol myristate acetate and ionomycin stimulation in the presence of Brefeldin A. Flow data represent one of 4 to 5 independent experiments, 3 mice pooled per group, and the average proportion of Foxp3 or cytokine-producing $CD4^+$ T cells for all independent experiments was indicated in the figure ± SD.

mouse colonizations in this study harbored dysbiotic microbial ecosystems previously reported to be associated with IBD.[34] As controls, we identified fecal communities from healthy subjects and a cultured "synthetic" community, which were previously used for fecal transplantation in UC and *C. difficile* infection, respectively.[13–15] Consistent with previous work,[35] we found that, at the family level the microbiota from the 2 patients with UC had a similar profile characterized by lower representation of bacterial groups belonging to Lachnospiraceae and Ruminococcaceae families, from the Firmicutes phylum. This is important, since anti-inflammatory bacteria, such as *Faecalibacterium prausnitzii* belonging to the Ruminococcaceae family, have been reported to be low in patients with IBD.[34] We investigated the functionality of these specific microbiota transplants in vivo by inducing colitis with DSS in colonized mice. UC microbiota recipient mice showed higher sensitivity to colitis compared with mice colonized with healthy microbiota. The decreased sensitivity to colitis was observed after administration of both fecal and cultured healthy

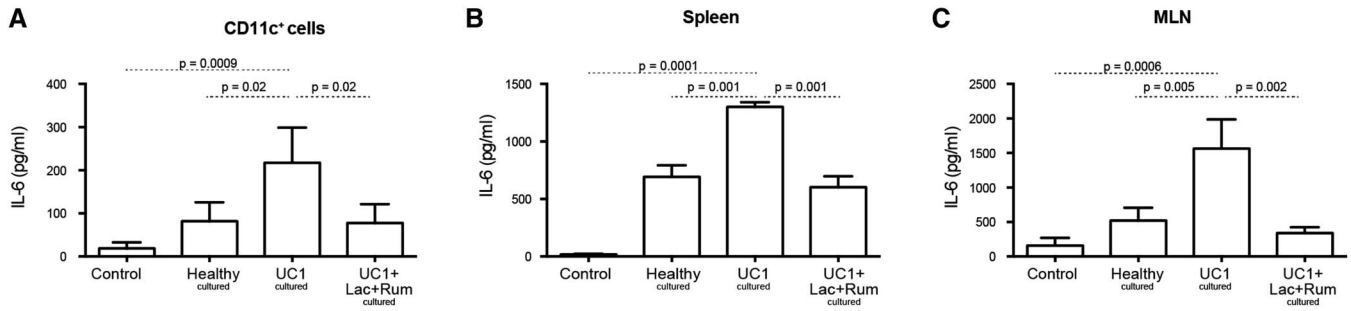

FIGURE 6. IL-6 production induced by UC microbiota in vitro was abrogated by Lachnospiraceae (Lac) and Ruminococcaceae (Rum) families. Bacterial lysates were prepared from healthy human ($Healthy_{cultured}$) and ($UC1_{cultured}$; consists of 24 bacterial strains isolated from $UC1_{stool}$) microbiota. IL-6 production of (A) $CD11c^+$, (B) splenic, and (C) mesenteric lymph node cells stimulated with bacterial lysates measured by cytometric bead array. Data were pooled from 2 independent experiments, 3 to 6 mice per group and shown in bar graph (mean ± SD). *P* values were calculated using analysis of variance followed by Bonferroni post hoc test.

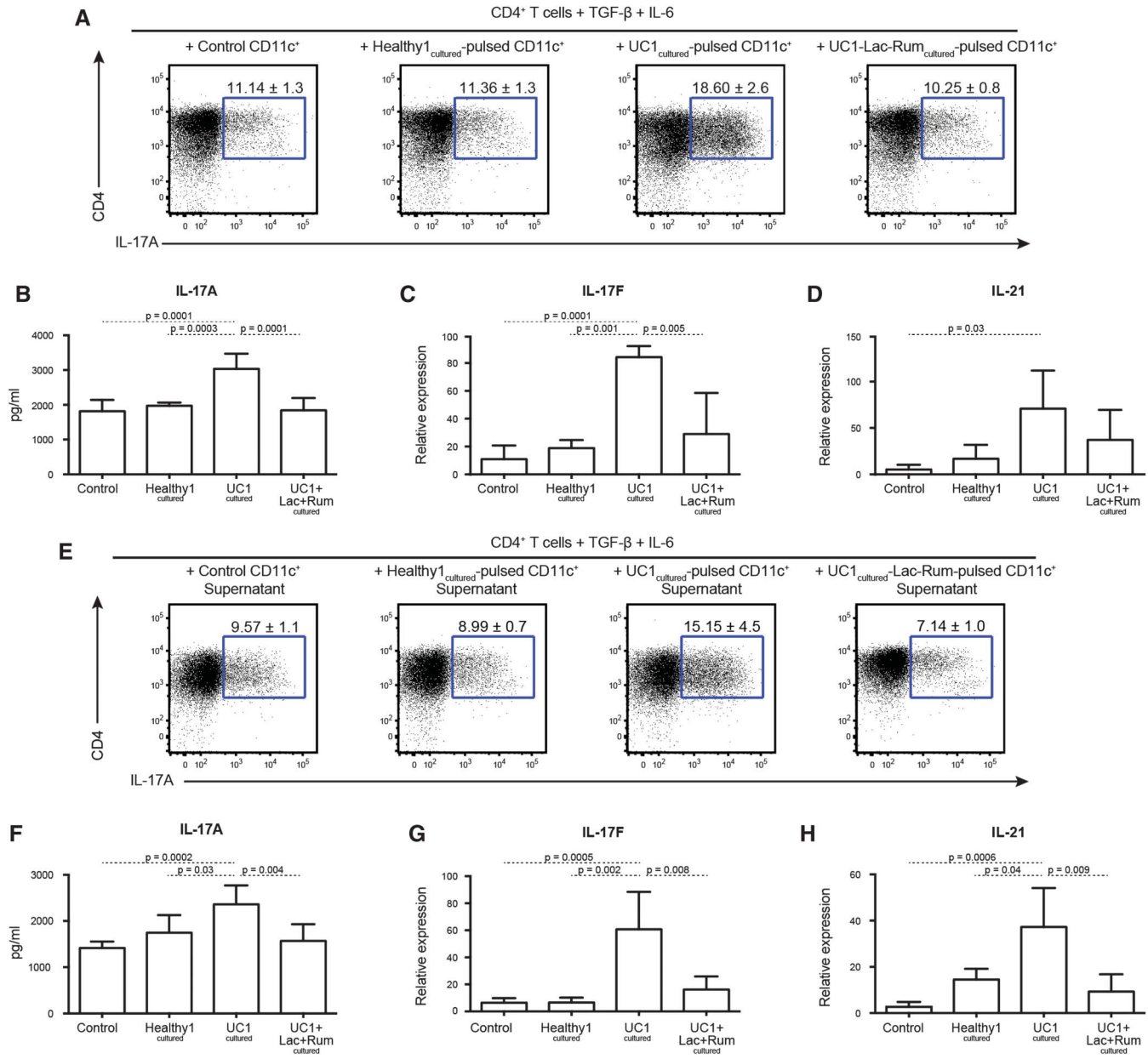

FIGURE 7. Lachnospiraceae (Lac) and Ruminococcaceae (Rum) families abrogated the UC microbiota-induced in vitro T_H17 cells expansion. Bacterial lysates were prepared from healthy human ($Healthy_{cultured}$) and ($UC1_{cultured}$; consists of 24 bacterial strains isolated from $UC1_{stool}$) microbiota. $CD4^+CD25^-$ T cells were cocultured with bacterial lysates-pulsed $CD11c^+$ cells or their supernatants in the presence of IL-6 and TGF-β. (A), Representative flow cytometry plots of T cells stimulated with bacterial lysates-pulsed $CD11c^+$ cells. (B), IL-17A production of T cells stimulated with bacterial lysates-pulsed $CD11c^+$ cells measured by enzyme-linked immunosorbent assay. (C–D), Normalized IL-17F and IL-21 expression (to *gapdh*) of T cells stimulated with bacterial lysate-pulsed $CD11c^+$ cells measured by reverse transcription quantitative polymerase chain reaction. (E), Representative flow cytometry plots of T cells stimulated with supernatants from bacterial lysate-challenged $CD11c^+$ cells. (F), IL-17A production of T cells stimulated with supernatants from bacterial lysate-challenged $CD11c^+$ cells. (G–H), Normalized IL-17F and IL-21 expression (to *gapdh*) of T cells stimulated with supernatants from bacterial lysate-challenged $CD11c^+$ cells. Flow data (A and E) represents 2 to 3 independent experiments, 6 to 6 mice per group, and the average proportion of cytokine-producing cells after phorbol myristate acetate and ionomycin stimulation with Brefeldin A for all independent experiments was indicated in the figure ± SD. Data (B–D, F–H) were pooled from 2 independent experiments, 3 to 6 mice per group and shown in bar graph (mean ± SD). *P* values were calculated using analysis of variance followed by Bonferroni post hoc test.

microbiota. This suggests that although $Healthy_{cultured}$ microbiota is a synthetic community, it recapitulates similar host responses to those induced with fecal slurs. Our results suggest that this, or other similar well-defined communities,[36] may be useful in fecal ecobiotherapy for UC and help mitigate some of the concerns regarding the use of uncharacterized fecal samples in strategies such as fecal transplant.

It has been suggested that patients with UC have altered T-cell profiles including aberrant expression of circulating T_H17 cells.[37,38] Furthermore, colonic biopsies from these patients have increased levels of T_H17-related cytokines.[37,38] In agreement with previous reports,[30,39] we found that GF mice had considerably lower T_H17 cells compared with mice colonized with murine microbiota. We further showed that colonization with a healthy human microbiota, or with mouse ASF, induced T_H17 responses of similar degree in naive GF mice. However, greater induction was observed in mice colonized with UC microbiota low in Lachnospiraceae and Ruminococcaceae. In parallel, colon transcriptomic analysis revealed that UC microbiota modulated the T_H17 gene-related network. T_H17 function is highly plastic, such that these cells can alter their differentiation program depending on the environment.[40] Specifically, several molecular signatures have been identified that discriminate between protective and proinflammatory T_H17 cells.[41] On this point, most genes that were upregulated in mice colonized with UC microbiota are those thought to be associated with proinflammatory T_H17 cells. It remains to be determined whether the increased T_H17 response is a mere host response to compensate for the different microbiota profile in UC. These results support the conclusion that colonization with a microbiota low in Firmicutes activates colonic T_H17 network in mice, suggesting that dysbiosis and depletion in Lachnospiraceae and Ruminococcaceae, is 1 possible driver of abnormal T_H17 responses in IBD.

T_{REG} cells were induced on colonization with UC to a similar degree than in mice colonized with healthy microbiota. It is possible that this regulatory induction is sufficient to control the heightened T_H17 immune response in UC colonized mice under naive conditions and may explain the lack of clinical signs of inflammation. However, in the presence of an additional trigger such as DSS, the regulatory compensation may fail to control the inflammatory response and lead to the higher colitis sensitivity observed. This is consistent with the hypothesis of multifactorial interactions forming a "perfect storm" critical to the development of dysregulated inflammation.[42]

As T_H17 cells have been implicated in the course of intestinal inflammation,[43] we further explored the capacity of the microbiota to influence the T_H17 immune response. T cells challenged with UC microbiota increased the induction of T_H17 cells, which in turn showed elevated transcripts of IL-21 and IL-17F. It is important to point out that whereas IL-17A has been shown to play a protective role against chemically induced intestinal injury in mice,[44] other T_H17-related cytokines such as IL-21 and IL-17F have been associated with pathogenic T_H17 phenotype.[28,29] TGF-β is required for $CD4^+$ cells to commit to T_{REG} lineage; however, the additional presence of IL-6 inhibits T_{REG} development and induces T_H17 differentiation instead.[27] We showed that UC microbiota increased the production of IL-6 by both mesenteric lymph nodes and splenic cells. Furthermore, we confirmed that UC microbiota promoted the production of IL-6 by $CD11c^+$ cells, which are key producers of IL-6. Interestingly, increased IL-6 production in vitro was consistent with in vivo RNA-seq data showing that mice colonized with $UC1_{stool}$ had higher IL-6 gene expression compared with mice colonized with $Healthy_{cultured}$ colonized mice. We did not identify specific bacterial isolates from UC microbiota, which preferentially induce T_H17 differentiation. However, since the most evident difference between the UC and healthy ecosystems resided in the depletion of Lachnospiraceae and Ruminococcaceae in the former, it is possible that members of these families are key in controlling inflammatory responses. We tested this hypothesis by adding bacterial isolates belonging to Lachnospiraceae and Ruminococcaceae families into UC microbiota, and we found that the heightened T_H17 responses were abrogated. Overall, the results support that increased inflammatory process in a subset of patients with UC may be the result of a lower capacity to dampen the proinflammatory immune responses because of the low abundance of certain bacteria from Lachnospiraceae and Ruminococcaceae families.

In conclusion, we showed that GF mice colonized with microbiota from patients with UC low in Firmicutes was associated with more severe risk to experimental colitis than mice colonized with either fecal or synthetic microbiota rich in this phylum. Examination of colonized mice before colitis induction revealed that a microbiota low in Lachnospiraceae and Ruminococcaceae did not induce overt inflammation; however, it promoted a heightened T_H17 immune response. In vitro data showed that supplementing the UC microbiota with bacterial isolates belonging to Lachnospiraceae and Ruminococcaceae families from microbiota derived from healthy donors abrogated the heightened T_H17 responses. Our data thus suggest a microbiota rich in members of the Lachnospiraceae and Ruminococcaceae can downregulate colonic inflammation when other inflammatory or injury triggers are present. Our results demonstrate that the efficacy of microbiota-directed therapies for UC, including fecal transplantation strategies,[32] could be improved by the specific inclusion of Lachnospiraceae and Ruminococcaceae families.

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