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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

BRIEF REPORT

Metagenomic Characterization of Microbial Communities *In Situ* Within the Deeper Layers of the Ileum in Crohn's Disease

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SUMMARY

Our study shows that the *in situ* microbial community of the diseased bowel in patients with Crohn's disease is distinct from inflammatory bowel disease–free individuals. In Crohn's disease patients, the microbial composition at the phyla level did not differ markedly between healthy and diseased areas, but at the species level an enrichment of potentially pathogenic organisms was observed in the diseased ileum.

BACKGROUND & AIMS: Microbial dysbiosis and aberrant host–microbe interactions in the gut are believed to contribute to the development and progression of Crohn's disease (CD). Microbiome studies in CD typically have focused on microbiota in feces or superficial mucosal layers of the colon because accessing DNA from deeper layers of the bowel is challenging. In this study, we analyzed the deep tissue microbiome in patients who underwent surgical resection of the small intestine.

METHODS: Paraffin blocks were obtained from 12 CD patients undergoing ileocecal resection, and healthy ileum samples (inflammatory bowel disease–free controls) were obtained from 12 patients undergoing surgery for right-sided colon cancer. Diseased and healthy-appearing ileum was identified using microscopy, and paraffin blocks were macrodissected using a core needle to specifically isolate DNA. Illumina Whole Genome Sequencing was used for microbial sequence identification and subsequent taxonomic classification using the *PathSeq* tool.

RESULTS: We observed significant differences between the microbiome of CD samples vs inflammatory bowel disease–free controls, including depletion of *Bacteroidetes* and *Clostridia*. Notably, microbial composition at the phyla level did not differ markedly between healthy and diseased areas of CD patients. However, we observed enrichment of potentially pathogenic organisms at the species level.

CONCLUSIONS: Our study showed dysbiosis within deeper layers of the ileum of CD patients, specifically enrichment of enterotoxigenic *Staphylococcus aureus* and an environmental *Mycobacterium* species not described previously. Future studies with larger cohort sizes are warranted to confirm these findings. Studies would benefit from effective microbial DNA extraction methods from paraffin sections and host nucleic acid depletion approaches to increase microbial read coverage. *(Cell Mol Gastroenterol Hepatol 2016;2:563–566; http://dx.doi.org/10.1016/j.jcmgh.2016.05.011)*

Keywords: Crohn's Disease; Deeper Mucosal Layers; Microbial Dysbiosis.

Microbial communities in the gut lumen of patients with Crohn's disease (CD) are distinct from those observed in healthy controls and include depletion of commensal phyla such as *Firmicutes* and *Bacteroidetes*.[1,2] Although the etiology of this dysbiosis is unclear, it is thought to trigger intestinal inflammation.[3] Microbiome studies in CD typically have focused on the microbiota in feces or in the superficial mucosal layers of the colon because accessing tissue from the deeper layers of the bowel is challenging.

To identify potential pathogens we took an *in situ* approach to determine invasive tissue microbiota. We analyzed the deep tissue microbiome in patients who underwent surgical resection of the small intestine. Inflamed (diseased or involved) and uninflamed (healthy or uninvolved) areas of the resection specimens were selected from the same patient for analysis (Supplementary Table 1). Specifically, samples were obtained from 12 patients who underwent ileocecal resections at Massachusetts General Hospital (Boston, MA) for treatment of Crohn's disease and who were part of the Prospective Registry in Inflammatory Bowel Disease (IBD) study at Massachusetts General

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Abbreviations used in this paper: CD, Crohn's disease; FDR, false-discovery rate; IBD, inflammatory bowel disease; LDA, linear discriminant analysis.

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Hospital. We selected patients who had surgery early in the course of the disease (<5 years after diagnosis). Healthy ileum samples were obtained from patients undergoing a similar surgery for right-sided colon cancer and were designated as IBD-free controls. Deeper sections of the small bowel were macrodissected from these surgical specimens. The submucosal lymphoid areas, granulomas, and lymphoid reactions around fissures were identified by histologic examination of the tissue by a pathologist and co-investigator (A.K.B.). The diseased (involved) area was identified and cored on paraffin blocks (Supplementary Figure 1*B* and *C*) using a 1.5-mm dermal punch as described previously.[4] Punch biopsies were performed using sterilized, disposable needles and samples were placed directly into sterile Eppendorf tubes for further processing. Healthy-appearing (uninvolved) ileum from these CD patients and IBD-free ileum from patients undergoing surgery for right-sided colon cancer were cored similarly. DNA extraction and preparation of bar-coded DNA sequencing libraries were performed as previously described.[4] Microbial sequence identification and subsequent taxonomic classification was performed using the *PathSeq* tool.[5]

Microbial DNA was identified in the deeper bowel layers in all 3 experimental groups: involved (n = 12 samples) and uninvolved (n = 11 samples; 1 surgical resection did not contain an uninvolved segment) regions from ileal segments of 12 CD patients, and IBD-free control (n = 12 samples) ileal segments from 12 right-sided colon cancer patients. The microbiomes of all 3 groups were dominated by 4 phyla (*Actinobacteria*, *Firmicutes*, *Bacteroidetes*, and *Proteobacteria*). The relative abundance of these phyla, however, varied between the groups (Figure 1*A*). Reads corresponding to viral, archaeal and fungal sequences were virtually absent (Supplementary Tables 2 and 3). At the phylum level, the microbial community was similar in involved and uninvolved regions but differed markedly from the microbiomes of IBD-free patients (Figure 1*A*). In particular, we observed depletion of commensal bacterial phyla such as *Bacteroidetes* in the involved and uninvolved regions compared with IBD-free ileum. The depletion in *Bacteroidetes* was especially significant ($P = .0002$; involved vs IBD-free ileum), with nearly 10-fold greater relative abundance in IBD-free ileum (10.22% mean relative abundance, compared with 1.15% and 0.82% mean relative abundance in involved and uninvolved regions, respectively). The relative abundance of *Firmicutes* conversely was higher in the involved regions vs IBD-free ileum ($P = .0204$), whereas abundance of the 2 other dominant phyla, *Actinobacteria* ($P = .2189$) and *Proteobacteria* ($P = .1005$), was not significantly different in involved vs IBD-free ileal specimens (Figure 1*A*). Here, it is important to note that the CD patients in this study were prescribed metronidazole and levofloxacin during the course of their treatment. Metronidazole specifically targets anaerobic and microaerophilic bacteria, including *Bacteroidetes* and members of the *Firmicutes* phylum containing the class *Clostridia*. We therefore cannot rule out the possibility that antibiotic treatment may have contributed to the depletion of members of these phyla in patients with Crohn's disease. Although all patients were treated with antibiotics, the duration of treatment varied. We decided to compare longer-term (>2 wk) to short-term (≤2 wk) treatment in the involved (antibiotic treatment > 2 wk, n = 7 samples; antibiotic treatment ≤ 2 wk, n = 5 samples) and uninvolved (antibiotic treatment >2 wk, n = 7 samples; antibiotic treatment ≤ 2 wk, n = 4 samples) regions. We observed neither a significant difference in the presence of *Bacteroidetes* or *Clostridiales* nor a clear enrichment of aerobic organisms and depletion of anaerobic or microaerophilic organisms in the longer-term treated group compared with short-term treatment (data not shown). Because we did not have an untreated cohort we could not determine reliably the consequences of antibiotic treatment on the microbiome in this study. However, Gevers et al[6] previously investigated the influence of antibiotic treatment for mucosal samples from CD

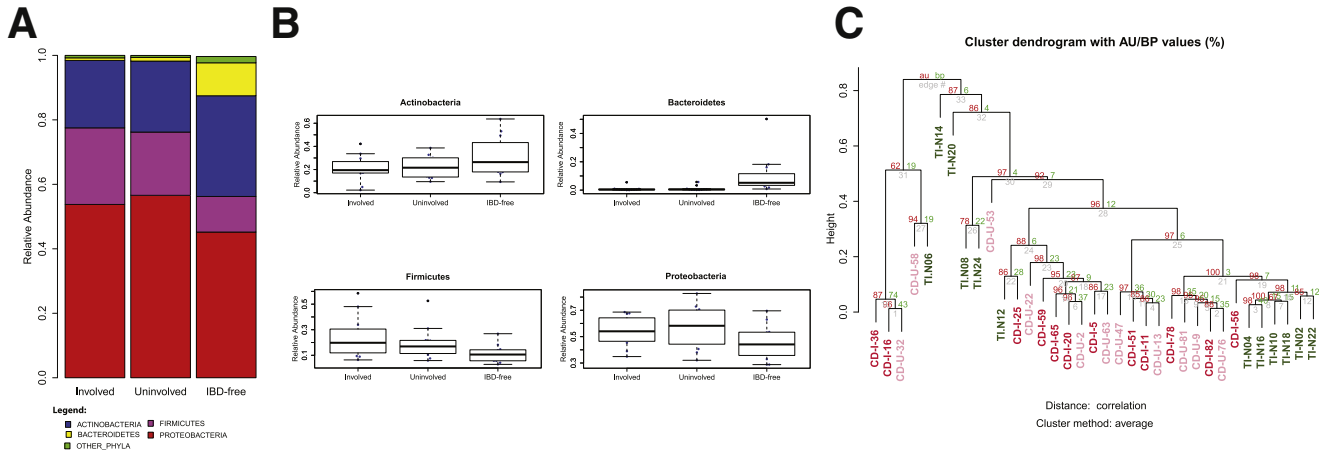

Figure 1. (*A*) Relative abundance of bacterial phyla in involved and uninvolved Crohn's and IBD-free ileum segments. (*B*) Box plots showing relative abundance of *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Proteobacteria* phyla. (*C*) Unsupervised hierarchical clustering of bacterial genus level relative abundance from 3 sample groups (*red*, involved; *pink*, uninvolved; *green*, IBD-free). AU, approximately unbiased; BP, bootstrap probability.