Reference

1 O'Brien CL, Pavli P, Gordon DM, et al. Detection of bacterial DNA in lymph nodes of Crohn's disease patients using high throughput sequencing. *Gut* 2014; **63**: 1596–606.

An antibiotic cocktail that results in a dysbiotic microbiome improves spontaneous colitis in the *Winnie* mouse

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Introduction: There is evidence from germ-free animal models and in inflammatory bowel diseases, from disease location and fecal diversion, that the microbiota drive ulcerative colitis. However, while antibiotics can treat pouchitis, there is scant evidence to show that antibiotics are useful in ulcerative colitis. We investigated the effect of antibiotic treatment in *Winnie* C57Bl/6 mice (chronic spontaneous colitis mouse model) and also the role of microbial dysbiosis in transmitting colitis.
Methods: Four-week-old *Winnie* mice and C57Bl/6 (wild type [WT]) controls were intra-gastrically gavaged daily with either vehicle or 5-antibiotic cocktail (AB5), at a dose of 10 μL/g for 10 days. Disease activity was scored daily, and at sacrifice, colonic histology, cytokine message, and cecal mucosal microbiomes were analyzed. Cecal contents donated from untreated or antibiotic-treated *Winnie* or WT mice were gavaged daily for 7 days into 1% dextran sulfate sodium (DSS)-treated WT mice.
Results: AB5 treatment improved disease activity scores in *Winnie* mice. Blinded histological colitis scores of colonic segments improved more than twofold ($P < 0.01$). Pro-inflammatory cytokine markers of endoplasmic reticulum stress improved ($P < 0.05$). Remarkably, there was no difference in the total bacterial load between untreated and antibiotic treatment in both mice strains. However, there were significant shifts at phyla and genus level towards what is normally considered a dysbiotic profile. Gavage of donor untreated *Winnie* into WT DSS recipients dramatically exacerbated colitis, with 3.5-fold increased disease activity from Day 4 of DSS ($P < 0.0001$).
Conclusion: The data show an unambiguous beneficial effect of the AB5 cocktail in this colitis model. The effect of AB5 on the microbiome was similar in WT and *Winnie*. Spontaneous colitis was improved despite the microbiome profile becoming more "dysbiotic." The functional outputs appear to be more important in affecting colitis than the shifts in microbiome.

Gene expression differences between Crohn's disease aphthous ulcers and healthy Peyer's patches highlight novel therapeutic targets

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Background and Aim: The earliest macroscopic lesion in Crohn's disease (CD) is the aphthous ulcer, which overlies Peyer's patches and lymphoid follicles. Our aim was to characterize differences in gene expression and the virome of aphthous ulcers and Peyer's patches.
Methods: Biopsies ($n = 24$) were obtained from the terminal ileum of 12 patients (six with CD and six healthy controls). Aphthous ulcers and adjacent unaffected mucosa were obtained from patients with CD, and Peyer's patches and adjacent mucosa from the controls. All patients, except one, were medication free. RNA was extracted using Qiagen kits. NextSeq 500 libraries were constructed using NextSeq 500/550 High output kits (Illumina) in a 150 bp paired-end format. Whole genome transcripts were assessed for quality using FASTQC, trimmed using Trimmomatic, and aligned to the human reference genome using subread mapper. Fragment counts were obtained using featureCount, and expression values normalized using the trimmed mean of M-values normalization method (TMM). Differential gene expression analyses were performed using generalized linear models in edgeR. Cell-specific gene expression was determined using ImSig. Reads that did not map to the human genome were used to mine for virus sequences using the VirusSeeker pipeline.
Results: We obtained 36 million tags per sample, 87% were retained for downstream processing, and 93% of these mapped to the human genome. A total of 920 genes were significantly differentially expressed between aphthous ulcers and Peyer's patches ($P = <0.001$); all were upregulated in aphthous ulcers. Differential gene expression analysis revealed 34 pathways that were upregulated in aphthous ulcers relative to Peyer's patches. Pathways that were over-expressed included those involved in responding to bacteria, leukocyte chemotaxis, inflammatory response, and creation of C2 and C4 activators. Receptors for the constant region of IgG were represented in 13 pathways. The cytokine OSM and its receptor (OSMR) were also over-expressed in aphthous ulcers. ImSig, which is capable of using transcriptome data to indicate cell types and their activation state, revealed that core marker genes for plasma cells were overrepresented in aphthous ulcers relative to Peyer's patches and unaffected or normal mucosa. There was no virus common to all aphthous ulcers. We detected human herpes virus 4 in one aphthous ulcer, human herpes virus 1 in mucosa from a patient with CD, and a novel *Totivirus* in mucosa of one control patient.
Conclusions: A number of gene clusters and immune pathways are over-expressed in aphthous ulcers compared with Peyer's patches. These biologically relevant gene lists highlight a number of new therapeutic targets and potential biomarkers for early stage disease. Viruses are an unlikely initiator of CD.