Abstract DOP22

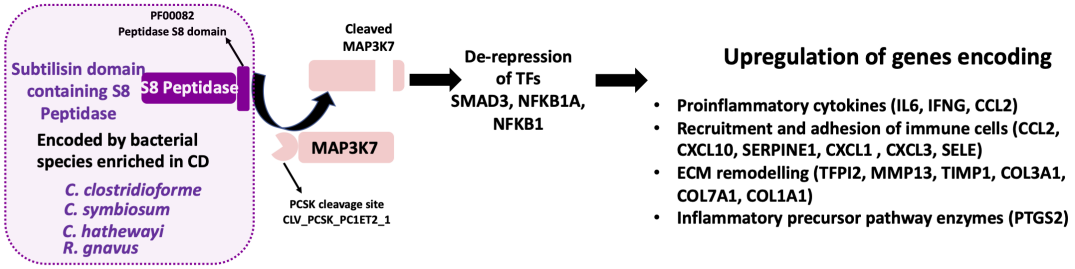

Figure 1. Inferred molecular mode of action of the subtilisin-domain containing type S8 peptidase (encoded by four of the eight species with enhanced abundance/acitivity in CD) on ileal gene expression in CD patients.

validation cohort (R2 = 0.907). Bacterial proteins post-translationally modifying host receptors resulted in the up-regulation of several pro-inflammatory cytokines via critical hub proteins such as NFkB (Figure 1). We observed different levels of locational specificity (from 35 to 61%) for the top regulators such as SPI1, STAT1 and NFKB1in terms of genes regulated by them in ileum and rectum. 24 proteins including ITGA4 and JAK1 from the ileal and rectal signaling networks are existing targets of CD drugs such as vedolizumab and tofacitinib, filgotinib and upadacitinib respectively.

Conclusion: Our findings outline the potential mechanisms of microbiome-induced host responses and provide insights into designing microbiome-mediated therapies to prevent and/or treat CD.

DOP23

Single-cell RNA sequencing identifies an important role for class I histone-deacetylase enzymes in intestinal myofibroblasts from patients with Crohn's Disease strictures

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Background: Histone-deacetylase (HDAC) enzymes are a broad class of ubiquitously expressed enzymes that modulate histone acetylation, chromatin accessibility and gene expression. In models of Inflammatory bowel disease (IBD), HDAC inhibitors, such as Valproic acid (VPA) are proven anti-inflammatory agents and evidence suggests that they also inhibit fibrosis in non-intestinal organs. However, the role of HDAC enzymes in stricturing Crohn's disease (CD) has not been characterised; this is key to understanding the molecular mechanism and developing novel therapies.

Methods: To evaluate HDAC expression in the intestine of SCD patients, we performed unbiased single-cell RNA sequencing (sc-RNA-seq) of over 10,000 cells isolated from full-thickness surgical resection specimens of non-SCD (NSCD; n=2) and SCD intestine (n=3). Approximately, 1000 fibroblasts were identified for further analysis, including a distinct cluster of myofibroblasts. Changes in gene expression were compared between myofibroblasts and other resident intestinal fibroblasts using the sc-RNA-seq analysis pipeline in Partek. Changes in HDAC expression and markers of HDAC activity (H3K27ac) were confirmed by immunohistochemistry in FFPE tissue from patient matched NSCD and SCD intestine (n=14 pairs). The function of HDACs in intestinal fibroblasts in the CCD-18co cell line and primary CD myofibroblast cultures (n=16 cultures) was assessed using VPA, a class I HDAC inhibitor. Cells were analysed using a variety of molecular techniques including ATAC-seq, gene expression arrays, qPCR, western blot and immunofluorescent protein analysis.

Results: Class I HDAC (HDAC1, p= 2.11E-11; HDAC2, p= 4.28E-11; HDAC3, p= 1.60E-07; and HDAC8, p= 2.67E-03) expression was increased in myofibroblasts compared to other intestinal fibroblasts subtypes. IHC also showed an increase in the percentage of stromal HDAC2 positive cells, coupled with a decrease in the percentage of H3K27ac positive cells, in the mucosa overlying SCD intestine relative to matched NSCD areas. In the CCD-18co cell line and primary myofibroblast cultures, VPA reduced chromatin accessibility at Collagen-I gene promoters and suppressed their transcription. VPA also inhibited TGFB-induced up-regulation of Collagen-I, in part by inhibiting TGFB1|1/SMAD4 signalling. TGFB1|1 was identified as a mesenchymal specific target of VPA and siRNA knockdown of TGFB1|1 was sufficient suppress TGFB-induced up-regulation of Collagen-I.

Conclusion: In SCD patients, class I HDAC expression is increased in myofibroblasts. Class I HDACs inhibitors impair TGFB-signalling and inhibit Collagen-I expression. Selective targeting of TGFB1|1 offers the opportunity to increase treatment specificity by selectively targeting meschenymal cells.

DOP24

Crohn's Disease fistula show skewed lymphoid/myeloid balance, altered myeloid cell profiles and high TNF-α expression

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Background: A fistula is an abnormal tract connecting two epithelialized surfaces, for example the intestine and the skin. Perianal fistula are a common complication of patients suffering Crohn's Disease (CD), but also occur in non-IBD patients in the form of cryptoglandular fistula. Around one third of all CD patients develop fistula at some point during their disease course. Fistula are often refractory to therapy, due to poor wound healing responses. In contrast, cryptoglandular fistula often respond to standard therapy. The biological background of this difference is unknown, and comparative studies between the two groups are lacking. The aim of this study was to characterize the cellular composition in fistula tracts of CD and cryptoglandular patients.
Methods: Curettage material of perianal fistula tracts was obtained during surgical intervention from patients with CD (n=15) and cryptoglandular fistulas (n=5). Single-cell suspensions were stained with a 35-antibody panel, focusing on myeloid and T-cell markers and were analyzed using mass cytometry (CyTOF). To visualize macrophages in the fistula tract we performed in situ hybridization with CD68 and TNF-α.
Results: The main cellular component of both fistula tracts consisted of CD66a+ granulocytes (64 +/- 24%). However, the remaining mononuclear compartment differed significantly between Crohn and cryptoglandular fistula. In CD, the majority was of lymphoid nature (CD3+ T cells 57 +/-21%, CD19+ B cells 14 +/-15%), while in cryptoglandular tracts, the majority consisted of myeloid origin (61+/- 15%). Within the T cell compartment, the majority of cells was CD45RO+, indicating activation. Presence of a seton increased the proportion of CD45RO+ T cells, in particular in CD4+ cells. In the myeloid compartment, CD14high/HLA-int monocytes, CD14int/HLA-high inflammatory macrophages and CD14high/CD163+ resident macrophages were identified. Interestingly, CD patient samples contained less monocyte-like cells, and substantially more resident macrophages compared to cryptoglandular samples. This feature tended to be even more enhanced in the presence of a seton, although this did not reach statistical significance. In situ hybridization showed a high production of TNF-α in epithelial-like cells in fistula tract of Crohn's disease patients, but not in macrophages.
Conclusion: Despite granulocytes being the main contributor to the cellular composition of fistula tracts, striking differences were found between Crohns and cryptoglandular fistula, both in lymphoid/myeloid balance, and in the presence of resident macrophages. We also showed that epithelial-like cells in Crohns's disease fistula tracts produce high amounts of TNF-α. These differences may contribute to the lack of response to therapy in CD.

DOP25

Association of Enterobacteriaceae with Crohn's Disease subtypes during remission

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Background: Members of the *Enterobacteriaceae* have been associated with active Crohn's Disease (CD), possibly as a result of intestinal inflammation via production of a lipopolysaccharide that can trigger TLR4 signalling. This study aims to assess whether this association persists in remission of CD patients and whether correlation with disease phenotype is present.
Methods: Stool samples of 32 CD patients in remission and 97 healthy controls were analyzed by 16S rRNA sequencing. High quality Amplicon sequence variants (ASV) were derived and classified via DADA2.
Results: ASV 6-*Escherichia/Shigella uncl*. was found to be more abundant in CD (padj=0.0003) while ASV 24, another member of the *Escherichia/Shigella* cluster was identified as being an indicator species for CD (padj=0.09). Differential abundance analysis according to phenotype as per Montreal classification revealed that, compared to patients with the B1 phenotype, patients with the B2 and/or B3 have a higher abundance of *Escherichia/Shigella uncl*. (ASVs 13, 31, 282 and 422), *Klebsiella uncl*. (ASVs 75 and 101) and *Enterobacter uncl*. (ASV 219) (Figure 1). Furthermore, patients with L3 involvement had higher abundances of *Klebsiella uncl*. (ASVs 75 and 101) and *Parasutturella uncl*. (ASVs 22, 53, 120, 199, 249 and 510), the latter being a *Proteobacteria*, compared to patients with L1 and/or L2 involvement. No significant association with "Age of Onset" was identified. In addition, network analyses revealed a strongly correlated group of *Enterobacteriaceae* ASVs (*Klebsiella*, *Escherichia/Shigella*, *Enterobacter*, *Citrobacter*) which appear to collectively associate to CD.
Conclusion: *Enterobacteriaceae* persist in the faecal microbiota in significantly higher levels than controls despite remission and furthermore are associated with the more severe phenotypes of stricturing and penetrating disease. Further studies might indicate whether microbiota assessment on diagnosis might predict CD subtypes and therefore influence therapeutic choices.

DOP26

The relationship between vedolizumab therapeutic drug monitoring, biomarkers of inflammation, and clinical outcomes in Inflammatory Bowel Disease in the real-world setting

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Background: Despite widespread use of therapeutic drug monitoring to guide anti-TNF biologic prescribing in IBD, its role for other biologic classes remains unclear. The present study aimed to assess the relationship between early vedolizumab trough concentrations (VTC) and real-world outcomes in inflammatory bowel disease (IBD).
Methods: Individuals with IBD enrolled in the Takeda Canada Patient Support Program were assessed at regular intervals from 2018–2020. VTC, albumin, faecal calprotectin (FC), C-reactive protein (CRP), and disease scores were collected from Crohn's disease (CD; Harvey-Bradshaw Index, HBI) and ulcerative colitis (UC; Partial Mayo scores, PMS) patients. The relationship between