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AN AGGREGATION GENE PROFILE IN BLOOD MONOCYTES AND RISK OF PERIANAL FISTULA: UTILITY OF SELECTIN P AND CD226 BIOMARKERS

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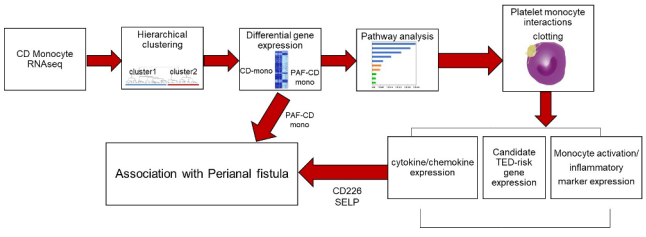
Background: In Crohn's disease (CD) extravasation of pro-inflammatory peripheral monocytes plays a fundamental role in innate immunity, gut homeostasis and modulating intestinal disease. However, the molecular mechanisms mediated by circulating monocytes contributing to chronic inflammation and disease progression remains largely unknown. **Aim:** Identify molecular pathways underlying peripheral monocytes pathophysiology in severe treatment-resistant CD patients. **Methods:** $CD14^+$ monocytes were purified from 73 CD patients requiring surgery. Expression profiles were generated by RNAseq and pathway analysis using ENRICHR. The analysis strategy is presented in Figure 1. **Results:** Unsupervised clustering of $CD14^+$ gene expression stratified CD-mono into 2 transcriptomic signature subtypes, one of which designated PAF-CD-mono, was clinically associated with perianal fistula (p=3.7E-04). $CD14^+$ transcriptomic stratification and PAF-CD-mono association with perianal fistula was confirmed in a separate patient cohort. CD monocyte subtype signatures were not associated with gender, age, disease location/behavior or therapeutic treatment nor with WBC, platelet and monocyte count or CRP lab values at time of surgery (Table 1). Nearly all (81%) differentially expressed genes (DEG) (830, p<0.001, FC 1.5) were down-regulated in the PAF-CD-mono vs CD-mono subtype. DEG were significantly enriched in GWAS-associated IBD variant loci (47/241 loci). The top identified DEG enrichment analysis pathways were strongly associated with megakaryocyte progenitor cell markers, monocyte-platelet aggregation/activation/signaling and clotting cascade (p=E-29 to -07). These pathways provided the focus to further define perianal-fistula biomarkers utilizing DEG implicated in platelet-monocyte complex formation (SELPL, SELP), monocyte inflammatory cytokine/chemokine expression (TNF, CCL4, CXCL3,CCL3), IBD candidate thromboembolic disease (TED)-risk genes (PROS1, PROC, MTR) and monocyte activation/inflammatory markers (CD1D, CD226). Low expression of CD226 (p=0.01) and SELP (p=0.002), both flanking GWAS-identified IBD-risk loci, were associated with perianal-fistula. Initial experiments demonstrated that CD226 mRNA expression is reflected in parallel by the level of circulating CD226 in plasma at time of surgery and low levels of circulating CD226 protein are associated with perianal fistula. **Conclusion:** Severe CD can be stratified based on peripheral monocyte gene expression into 2 functionally diverse profiles associated with IBD GWAS loci, monocyte-platelet activation and clotting molecular pathways and clinically with perianal fistula. Diminished expression of SELP and CD226 mRNA and soluble circulating CD226 protein levels may serve as valuable diagnostic parameter for monitoring perianal disease progression and aid in the design of novel CD subtype-specific therapeutics.



Analysis Strategy

Table 1: Patient demographics	CD-mono	PAF-CD-mono
Gender Female (%)	46	35
Age at diagnosis (median and IQR), yr.	25 (16-31)	19 (16-30)
Disease duration (median and IQR), yrs.	8 (2-17)	9 (4-18)
Age at surgery (median and IQR), yr.	39 (28-53)	33 (24-38)
elevated CRP at surgery (%)	58	67
Steroids (%)	73	85
5-ASA (%)	78	67
anti-TNF (%)	57	63
resected bowel length (median and IQR), cm.	31(20-51)	33(11-60)
Hematology lab values		
WBC (10^9/L)	8 (6-12)	10 (7-15)
MCV fl	86 (78-90)	87 (82-91)
MCH pg	29 (26-31)	30 (27-32)
Platelet count (10^9/L)	303 (214-381)	262 (207-397)
Mean platelet volume fl	9.4 (8.5-10.2)	9.9 (8.6-11.2)
ABS Monocytes (10^9/L)	0.55 (0.33-0.7)	0.6 (0.43-0.87)

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A SINGLE-CELL THERAPEUTIC ATLAS OF ANTI-TUMOUR NECROSIS FACTOR THERAPY IN INFLAMMATORY BOWEL DISEASE

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Background: Single cell RNA sequencing (scRNAseq) offers unparalleled resolution into the cellular drivers of inflammatory bowel disease (IBD). Understanding the cellular culprits of disease in context of therapy is necessary not only to actualise precision medicine and aid drug development, but also to shape clinical strategies of drug sequencing in patients. **Methods:** To assess the impact of anti-TNF therapy, we generated a longitudinal scRNA-seq therapeutic atlas of IBD. Site- and patient-matched biopsies of 38 biologic-naïve patients with Crohn's disease (CD) or ulcerative colitis (UC) from 5 gut regions (terminal ileum, ascending colon, descending colon, sigmoid and the rectum) before and after treatment with adalimumab and biopsies from 3 healthy controls were obtained. Levels of adalimumab confirmed adequate dosing and excluded patients with anti-drug antibody-mediated loss of response. **Results:** 987,743 high-quality single-cell transcriptomes from 216 gut samples were obtained from 16 responders and 22 non-responders. Cellular characteristics associated with site (ileum, colon), and disease (CD, UC) were identified. Transcriptomic variance across healthy ileum and colon was restricted to epithelial cells indicating specialised functions in vitamin B12, iron, and fat-soluble vitamin absorption. Epithelial and lymphocyte compartments had disease specific features. The impact of TNF modulation in-vivo in patients with IBD was explored. Activated CD4+ (CD: 29%, UC: 29.6%) and CD8+ T memory cells (CD: 20.2%, UC: 18.2%) represent the largest sources of TNF mRNA transcripts in the inflamed gut. The propensity of cell types to respond to TNF signalling pre-treatment negatively correlated with decreases in TNF signalling following treatment in responders; CD: R=-0.43, p=0.0031 and UC: R=-0.45, p=0.0049. Genes enriched in inflammatory monocytes (*S100A9*, *FTH1*, *IL1RN*) increased despite treatment in non-responders with CD. In non-responders with UC, persistent NF-KB activation amongst other pathways occurred across cell compartments including the stroma, CD4+ T cells, and epithelium. To characterise covarying cell states, a graph-based approach identified 'hubs' of inflammation across CD and UC. Gene expression programs (GEPs) in hubs were associated with histological features. After projection to large scale bulk RNA sequencing data, constituent GEPs were associated with anti-TNF therapy non-response at baseline. **Conclusion:** We have generated a single cell atlas of IBD in context of anti-TNF therapy. By systematically deconstructing IBD in context of tissue site and disease, the cellular drivers of CD and UC have been distinguished. Changes induced by anti-TNF at single cell resolution have been revealed by leveraging paired sampling within patients, highlighting pathways that remain persistently active in the context of tissue architecture.

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SINGLE-CELL AND SPATIAL MULTI-OMICS IDENTIFY INNATE AND STROMAL MODULES TARGETED BY ANTI-INTEGRIN THERAPY IN ULCERATIVE COLITIS

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Background: Ulcerative colitis (UC) is a chronic inflammatory disorder of the intestine driven by mucosal immune and stromal subsets, culminating in intestinal epithelial injury. Vedolizumab (VDZ) is an anti-integrin monoclonal antibody that interferes with intestinal leukocyte trafficking and is effective for treating UC. VDZ is thought to primarily inhibit lymphocyte trafficking to the intestine, but its effect on other cell subsets is poorly understood. **Methods:** To better understand the inflammatory cells that contribute to colitis and respond to VDZ, we performed a comprehensive single-cell transcriptomic and proteomic analysis (scRNA-seq, CITE-seq, and CyTOF) of peripheral blood and colonic biopsies in healthy controls (HC, n=4), patients with UC on aminosalicylates (n=4), and patients with UC on VDZ (n=4). On matching tissues, spatial transcriptomic and proteomic assays on formalin-fixed paraffin-embedded (FFPE) biopsies were performed using different technologies, including multiplex ion beam imaging (MIBI), co-detection by indexing (CODEX), and two platforms for multiplexed RNA-ISH with subcellular resolution (12-plex and 960-plex). Gene set enrichment analysis (GSEA) of identified gene signatures was validated using a longitudinal transcriptomic dataset (n=11 VDZ responders and n=9 VDZ non-responders). Both unsupervised and supervised methods were used to infer intestinal cell subset variation by disease and treatment status. **Results:** VDZ showed small effects in peripheral blood compared to colonic tissues. We identified tissue trafficking of mononuclear phagocytes (MNPs) as a primary mechanism of action for VDZ, with comparatively modest effects on lymphocytes. Spatial proteomics and transcriptomics demonstrated increased density and proximity of inflammatory MNP and fibroblast subsets in UC compared to HC, with inhibition by VDZ. GSEA of longitudinal transcriptomic data confirmed the reduction of inflammatory MNP and stromal signatures, with epithelial healing in VDZ responders. VDZ non-responders could be differentiated from responders pre-treatment by enrichment of endothelial, activated fibroblast, and macrophage signatures. **Conclusion:** This study combines multiple technologies to enable comprehensive single-cell and spatial tissue multi-omics, revealing a significant effect of VDZ on inhibiting MNP intestinal trafficking, reducing stromal cell activation, and healing the mucosa. This spatial transcriptomic and proteomic cell atlas is compatible with FFPE tissue and will aid in future studies evaluating signatures associated with disease and treatment response.

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THE SINGLE-CELL LANDSCAPE OF GENETIC MODULES BASED ON INFLAMMATORY BOWEL DISEASE SEVERITY

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For inflammatory bowel diseases (IBD), such as Crohn's Disease (CD) and Ulcerative Colitis (UC), the identification of cellular and molecular markers can potentially provide both mechanistic insights as well as predictors of disease severity and clinical outcomes. Previous GWAS studies identified SNPs within higher-risk loci in IBD patients; however, they fell short of determining who would actually develop the disease or the expected clinical outcomes. In this study, we used the inflammatory state (as reported by endoscopy) to dissect the cell type-specific expression signatures of putative IBD risk genes that contribute to CD. Biopsy samples of the ascending colon and terminal ileum from 13 healthy individuals and 26 CD