Submucosal Crohn's Lymphoid Aggregates and endothelial proliferation as disease drivers in Fibrostenosing Crohn's Disease

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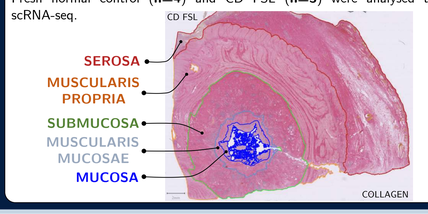
STUDY DESIGN AND SUMMARY

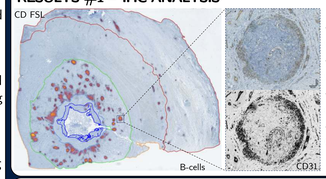
Crohn's Disease fibrostenotic lesions (CD FSL) are still not well characterised for their major cellular components.

Approach #1:
Archival CD FSL and normal control samples (**n=30, each**) were stained with immunohistochemistry (IHC) for major cell types and analysed using QuPath to quantify the changes in each ileal layer.

Approach #2:
Fresh normal control (**n=4**) and CD FSL (**n=3**) were analysed using scRNA-seq.



RESULTS #1 – IHC ANALYSIS

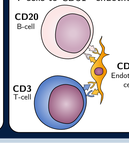


- Largest collagen increase in submucosa.
- Highest increase in CLAs in submucosa.
- Proliferated and clustered CD31$^+$ endothelial cells around CLAs.
- Submucosa as nucleation layer with CLAs and endothelium driving fibrosis.

RESULTS #2 – scRNA-seq ANALYSIS

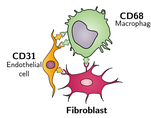
Multiple ligand-receptor (L-R) signalling pathways:

L-R signals going from CD20$^+$ B-cells and CD3$^+$ T-cells to CD31$^+$ endothelial cells:



↑ CD99, SELL, CYPA, VISFATIN

L-R signals going from CD31$^+$ cells to macrophages and to fibroblasts:



↑ COLLAGEN, LAMININ, NOTCH, GALECTIN, CD45, VISFATIN, CYPA, MHC-I and II, MPZ, IGF

Acknowledgments, sponsorship and links

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Link to raw images used in the analysis

Introduction

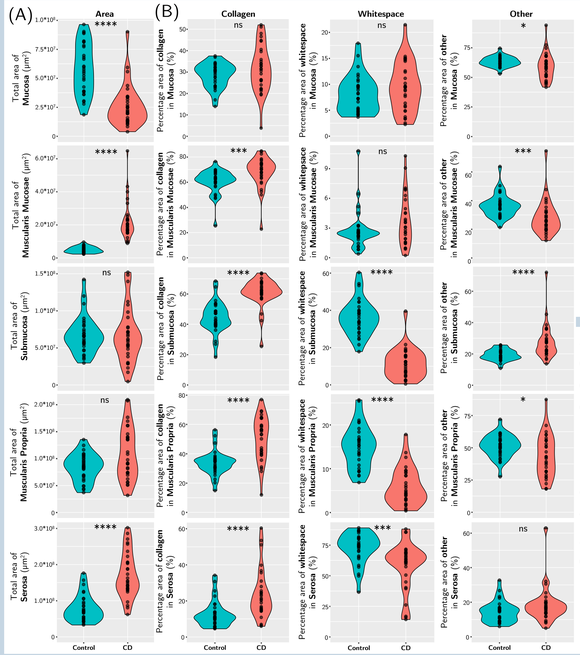
Crohn's Disease (CD) affects up to 1% of the European population over the next 5 years and is increasing in incidence. Although well-established single nucleotide polymorphisms (SNPs) influence risk, the effects of Crohn's Disease are mostly due to recurrent acute and chronic inflammation of the intestinal wall, in some cases leading to progressive fibrosis, resulting in partial luminal obstruction. Although immune cells present in these fibrostenosing lesions (FSL) are qualitatively well described, accurate quantification of such immune cell populations within each intestinal wall layer has not yet been reported.

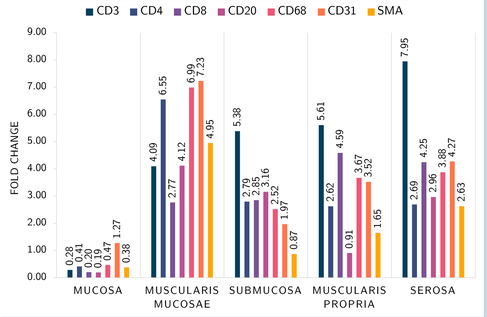
Methods

Archival formalin-fixed, paraffin-embedded ileal resections from 30 normal control and 30 Crohn's fibrostenosing cases were stained using immunohistochemistry (IHC) to identify specific cell types: CD3$^+$, CD4$^+$ and CD8$^+$ T-Cells, CD20$^+$ B-cells, CD68$^+$ macrophages, CD31$^+$ endothelium and Smooth Muscle Actin-positive cells. Collagen deposits were visualised using Picro-Sirius Red (PSR) staining. The sections were captured digitally and analysed with QuPath to quantify the fibrosis and cell populations within each ileal wall layer, using pre-trained machine-learning classifiers, while the DAB-positive IHC-detected cells were counted using the built-in positive-cell detection function. Lymphoid aggregates were detected using the cluster heatmap function for CD3$^+$ and CD20$^+$ cells in QuPath. For scRNA-seq, 4 normal control and 3 CD FSL terminal ileal fresh whole wall thickness blocks were sequenced and analysed for cell-cell ligand-receptor interactions using CellChat.

Results

CD FSL shows expansion and increased collagen and immune cells throughout ileal wall layers



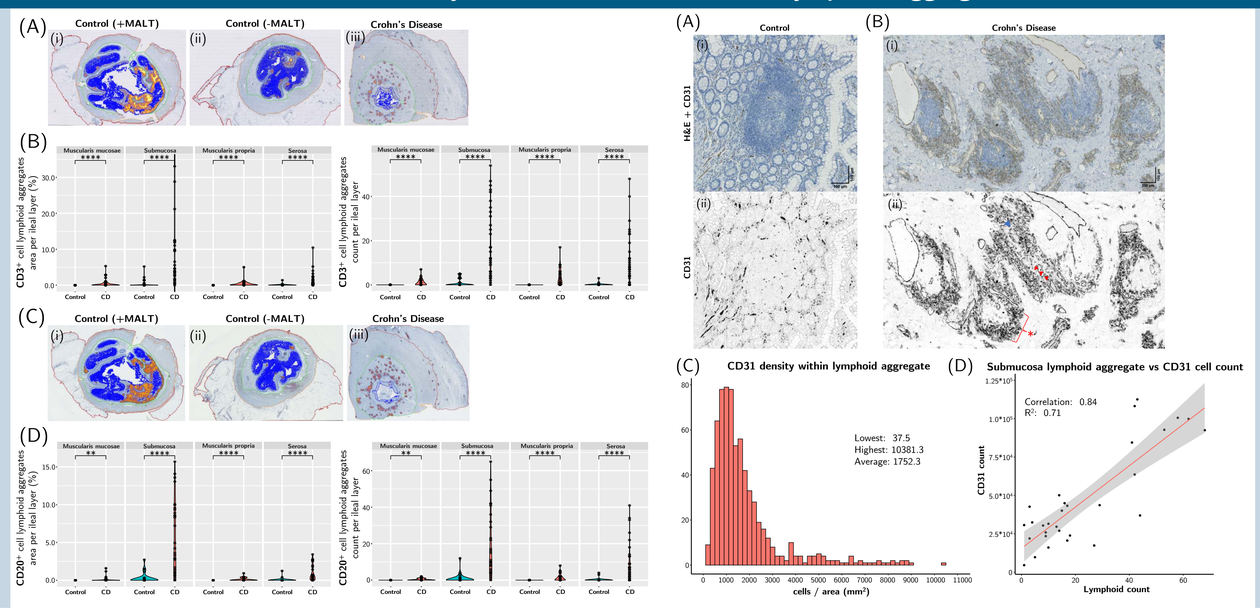


Above: Mean fold change (CD FSL / normal) of T- and B-cells, macrophages, endothelial cells and smooth muscle actin cells within all terminal ileal layers. The results show expansion of all cell types within all layers except ulcerated mucosa.

Left: Violin plots showing the changes in area, collagen, whitespace (fat cells, vessel lumens) and other (immune cells, vasculature, etc.) between control (blue) and CD FSL (red). A significant expansion of muscularis mucosae and serosa was observed. Collagen expansion was found in all layers, except mucosa, and was most marked in submucosa.

Statistical significance: p>0.05 ns, p≤0.05 *, p≤0.01 **, p<0.001 ***, p<0.0001 ****

CD31$^+$ endothelial cells freely accumulate around Crohn's lymphoid aggregates in CD FSL

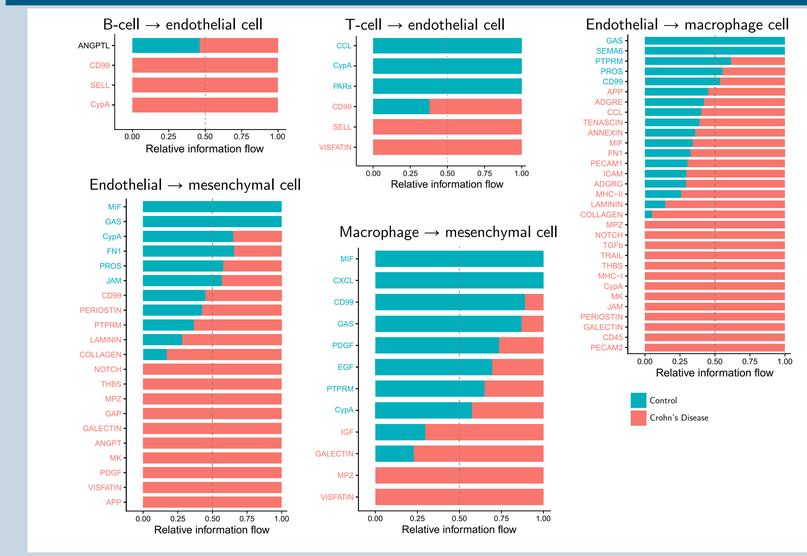


Left: Analysis of Crohn's lymphoid aggregates (CLAs) counts (CD3 (A, B) and CD20 (C, D) positive lymphocytes) within muscularis mucosae, submucosa, muscularis propria and serosa in CD FSL (red) relative to normal controls (blue). The most significant increase in CLAs in CD FSL was present in the submucosa.

Right: Observation and quantification of CD31$^+$ endothelial cells around Crohn's Lymphoid Aggregates (CLAs) as observed in CD FSL. (A) and (B) show the differences between control normal mucosal MALT and Crohn's lymphoid aggregates in CD FSL (red arrows show CD31+ cells mostly in clusters and free endothelial cells, with few vessels). (C) Density distribution of endothelial cells around lymphoid aggregates; and (D) correlation between the numbers of lymphoid aggregates and CD31$^+$ cells. In summary, a marked accumulation of endothelial cells was observed around CLAs.

Statistical significance: p>0.05 ns, p≤0.05 *, p≤0.01 **, p<0.001 ***, p<0.0001 ****

Ligand-receptor interaction analysis reveals numerous pathways involved between endothelium and lymphocytes within lymphoid aggregates and the macrophage-fibroblast axis



Left: Analysis of cell-cell ligand-receptor (L-R) pairs using CellChat between B-, T-lymphocytes, endothelium, macrophages and mesenchymal fibroblast cells with involvement of multiple and common pathways:CD99, SELL, CYPA, VISFATIN, COLLAGEN, LAMININ, NOTCH, GALECTIN, CD45, CYPA, MHC-I and II, MPZ, IGF.

Hypothesis and Conclusions

Immune cell infiltration in Crohn's Disease leads to formation of transmural Crohn's Lymphoid Aggregates (with intermingled T- and B-lymphocytes), mostly in submucosa where most collagen is seen. Around these CLAs is a marked increase in endothelial cells mostly in clusters with some free individual cells. We hypothesise that CLA lymphocytes can signal to endothelium to stimulate their recruitment and proliferation around CLAs, presumably via release of cytokines. Further, we hypothesise that the interaction between endothelium and CLAs continues to promote CLA accumulation and signals to mesenchymal fibroblasts to stimulate collagen synthesis and fibrosis, either directly or via signalling to macrophages. This signalling involves multiple cell-cell ligand-receptor signalling pathways including some of the most common Crohn's Disease associated pathways, such as TGF-β pathway, as well as the collagen pathway amongst others. Signalling via TGF-β leads to activation of mesenchymal fibroblasts, stimulating the release of collagen, leading to formation of fibrotic scar tissue mostly in the submucosa of CD FSLs.