

# Therapeutic target PDE8A is located at the site of adhesion between leukocytes and blood vessels during Ulcerative Colitis

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## Research Objective

The objective of this study was to investigate the protein expression of PDE8A in the submucosa of intestinal resections from patients with active Ulcerative Colitis (UC). Specifically, we aimed to determine the subcellular localization of PDE8A in leukocytes and blood vessels since recruitment is a critical step in establishing UC and other inflammatory diseases.

## Background

Phosphodiesterase (PDE) inhibitors block termination of intracellular cAMP and have anti-inflammatory effects on leukocytes [1]. However, so far no PDE inhibitors have been successful in clinical trials for UC due to dose-limiting side effects [2]. Recently identified PDE8A has been demonstrated to be involved in the control of leukocyte adhesion to endothelial cells *in vitro* [3]. PDE8A is the highest affinity enzyme of all known PDEs and a small increase in PDE8A expression may be sufficient to make a therapeutic difference [4]. Therefore, PDE8A inhibitors may have an effect at a lower dosage and be a promising therapy where other PDE drugs have failed. No *in situ* data exists concerning the cell specific localization in humans of PDE8A protein during UC.

## Hypotheses

- 1) As cAMP and PDEs work in microdomains [5], we hypothesize that PDE8A protein expression will be compartmentalized within the single cell.
- 2) Based on PDE8A gene expression data from other studies [4,6], we hypothesize that PDE8A protein will be expressed in various leukocytes and endothelial cells.

## Methods

Hematoxylin and Eosin stained tissue resections for potential inclusion in this project were identified during the course of routine histological diagnosis of UC following colonoscopy by the pathologist at The National Hospital of The Faroe Islands. Following inclusion, resection pairs (macroscopically uninvolved region and UC region) from two patients were included in the cohort. Additional uninvolved resections from three patients were included. Resections were graded using a detailed UC grading scale created for research based histology. Immunohistochemistry was performed on 5 µm FFPE tissue sections using an anti-PDE8A antibody. ImageJ software was used to segment PDE8A staining by using the saturation parameter (Figure 1 and 2, red).

## Results

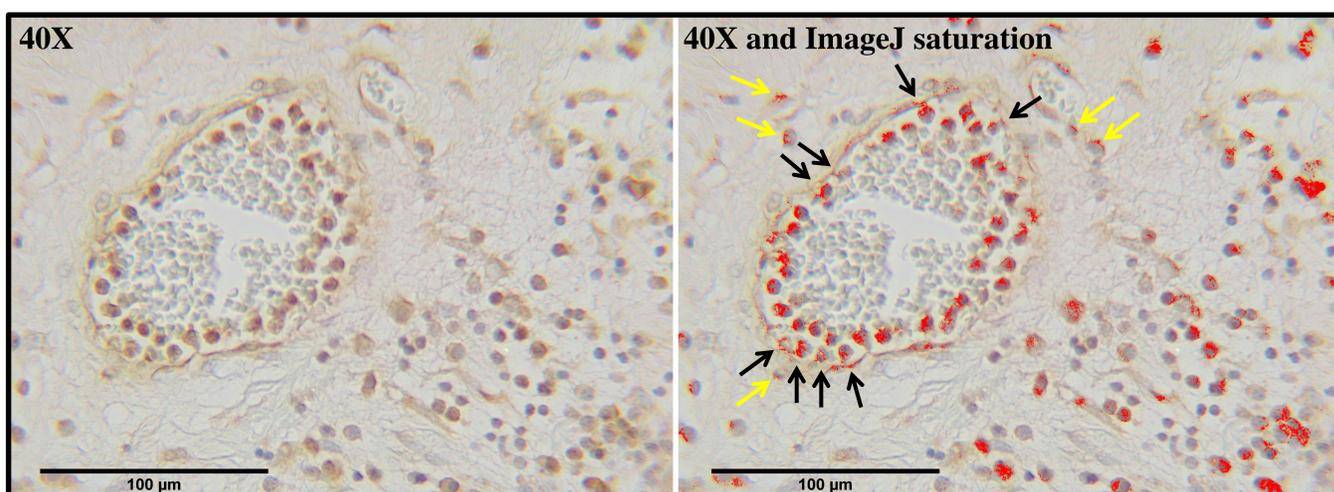
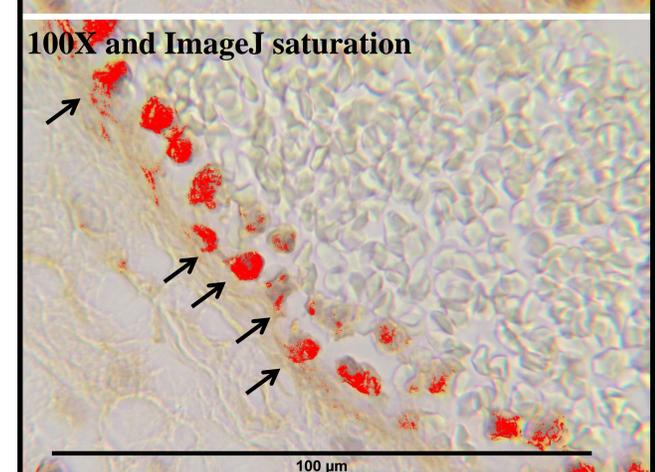
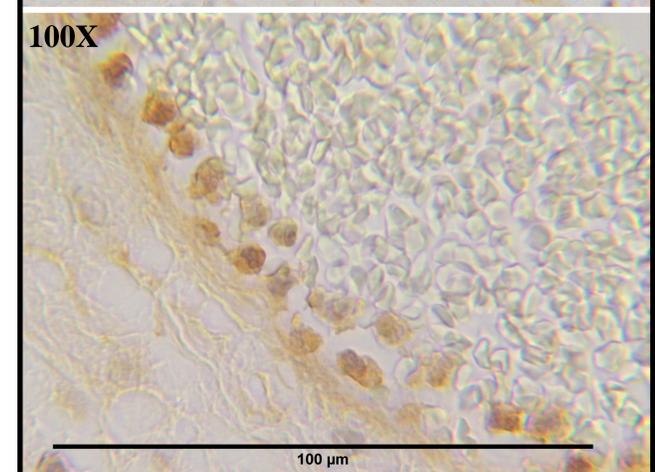
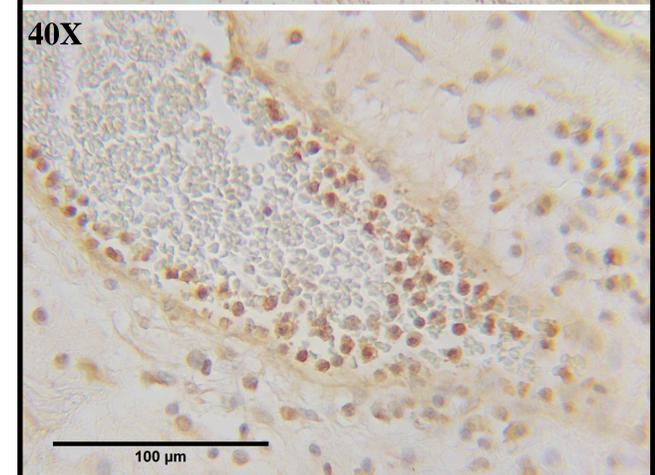
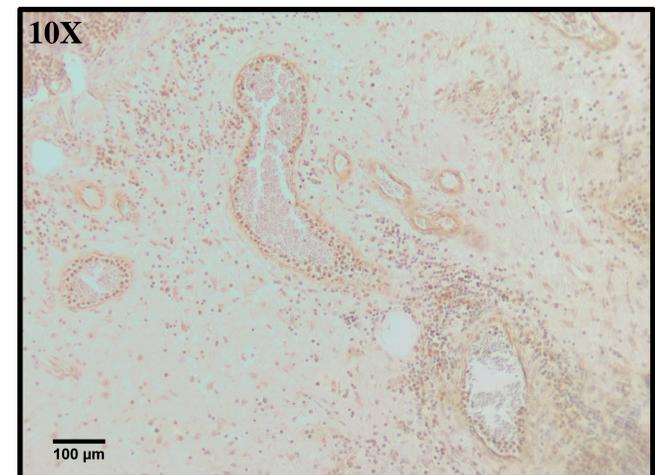
Robust PDE8A staining was detected in leukocytes and endothelial cells participating in adhesion (black arrows) and in recently recruited perivascular leukocytes (yellow arrows) (Figure 1 and 2). PDE8A was compartmentalized in subcellular microdomains associated the plasma membrane where leukocytes were in close proximity or contact with endothelial cells.

## Conclusion

Localizing PDE8A in inflammatory disease is a unique contribution and relevant as *in situ* data of PDE8A can link published PDE8A *in vitro* and *in vivo* data to clinical disease in humans. This study is the first to demonstrate PDE8A compartmentalization in leukocytes and endothelial cells as they participate in adhesion and transmigration in human UC tissue. PDE8A microdomain signalling domains in leukocytes and intestinal endothelial cells are a potential therapeutic target for UC.

## Future directions

Examining the immunophenotype of activated leukocytes following pharmacological disruption of PDE8A functional microdomains.



40X and ImageJ saturation

Figure 1

Figure 2

## References

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