

Differences in expression of PPAR γ in small intestine vs colon impact the effect of 5-aminosalicylates in Inflammatory Bowel Disease

Ingunn Bakke^{4,5}, Torunn Bruland^{2,4}, Kaja S.Eriksen⁴, Helene K. Skovdahl^{2,3,4}, Shreya Gopalakrishnan⁴, Silje Thorsvik^{1,2,4}, Atle vB. Granlund^{3,4}, Arne K. Sandvik^{1,2,3,4}, Ann E. Østvik^{1,2,4}

¹Department of Gastroenterology and Hepatology, St Olav's University Hospital, Trondheim, Norway.

²Clinic of Medicine, St Olav's University Hospital, Trondheim, Norway.

³Centre of Molecular Inflammation Research, Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, NTNU - Norwegian University of Science and Technology, Trondheim, Norway.

⁴Department of Clinical and Molecular Medicine, Faculty of Medicine and Health Sciences, NTNU - Norwegian University of Science and Technology, Trondheim, Norway.

⁵Clinic of Laboratory Medicine, St. Olav's University Hospital, Trondheim, Norway.

Background

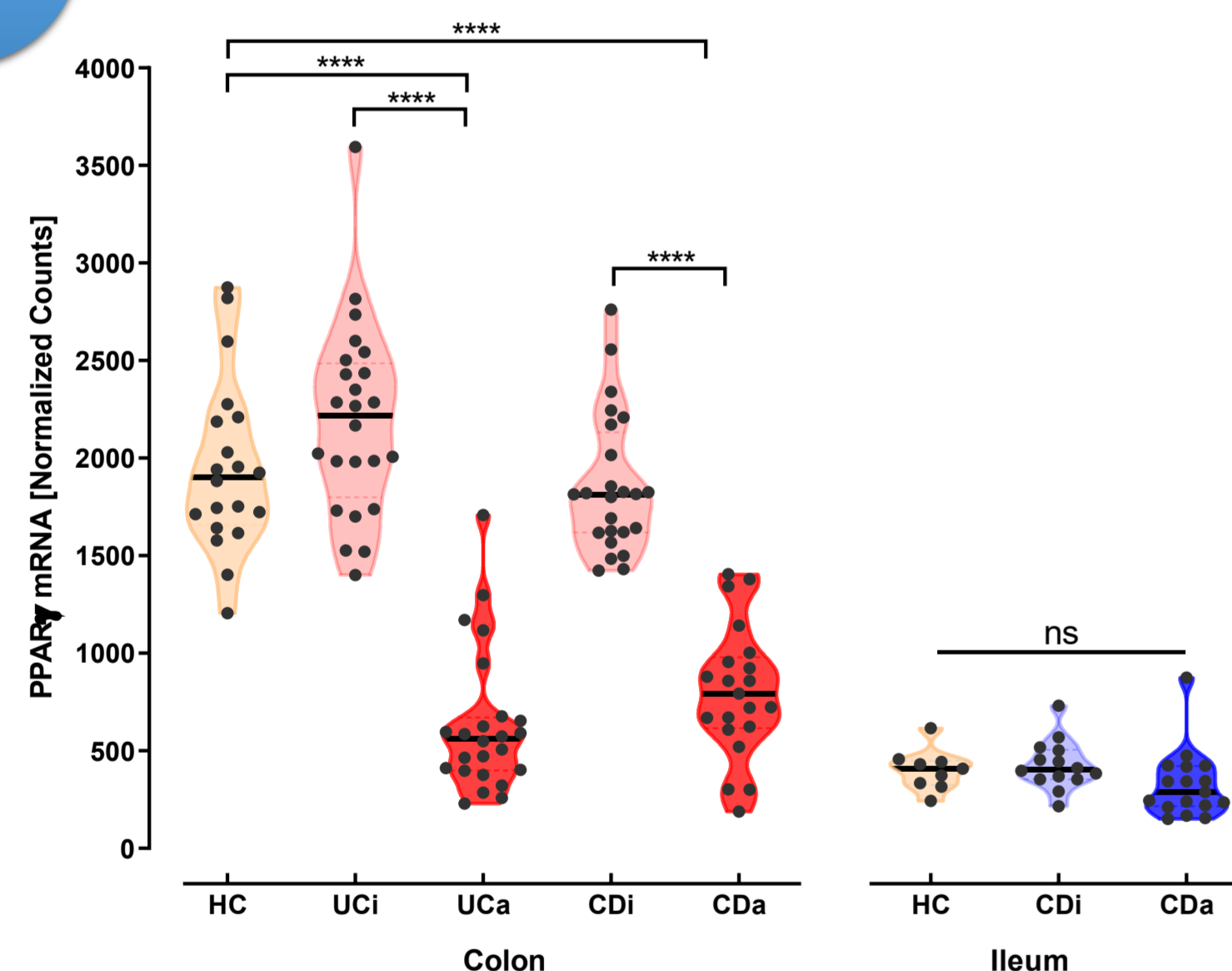
The nuclear receptor Peroxisome Proliferation-Activated Receptor gamma (PPAR γ) harbours anti-inflammatory effects. There is evidence that PPAR γ mediates the effect of 5-aminosalicylic acid (5-ASA). 5-ASA is the first line drug in ulcerative colitis (UC), while its use in Crohn's disease (CD) is debated and not recommended according to guidelines. We hypothesize that the inconsistent therapeutic effect of 5-ASA in CD is caused by differences in expression of PPAR γ in the large and small intestine. This work examines cellular source of PPAR γ gene and protein expression in colon and ileum in inflammatory bowel disease (IBD) and the effect of 5-ASA in colonic epithelial cells.

Methods

- ❖ IBD-patients and healthy controls were included at the Gastrointestinal Endoscopy Unit, St. Olav's University Hospital.
- ❖ Biopsies from I) colon of active/inactive UC (n=24/24), active/inactive CD (n=24/21) and healthy controls (n=20); and from II) ileum of active/inactive CD (n= 14/17) and healthy controls (n=9) were stored in liquid nitrogen or fixed in 10% formalin.
- ❖ For RNASeq of biopsies and epithelial colonoids, libraries were generated using SENSE total RNASeq library prep kit (with RiboCop rRNA depletion) on an Illumina Next Seq500 instrument.
- ❖ Immunohistochemistry (IHC) and *in situ* hybridisation (ISH) were done to localize PPAR γ positive cells, and Western blot to quantify PPAR γ , in biopsies of colonic and ileal mucosa.
- ❖ Human 3D colonoid cultures were established from pinch biopsies, using an optimized protocol (Jung et al, Nat Med 2011, Mahe et al, J Vis Exp 2015)
- ❖ The intestinal cell line HT-29 and colonoids were stimulated for 24h with TNF or poly(I:C) during 5-ASA exposure, in HT-29 also with and without the PPAR γ -antagonist GW9662.
- ❖ Colonoids from IBD-patients and controls were stimulated with TNF, TNF+IL17 and TNF+poly(I:C) and the mRNA sequenced.
- ❖ Cytokine levels were analysed in supernatants from HT-29 and colonoid stimulating experiments using ELISA.

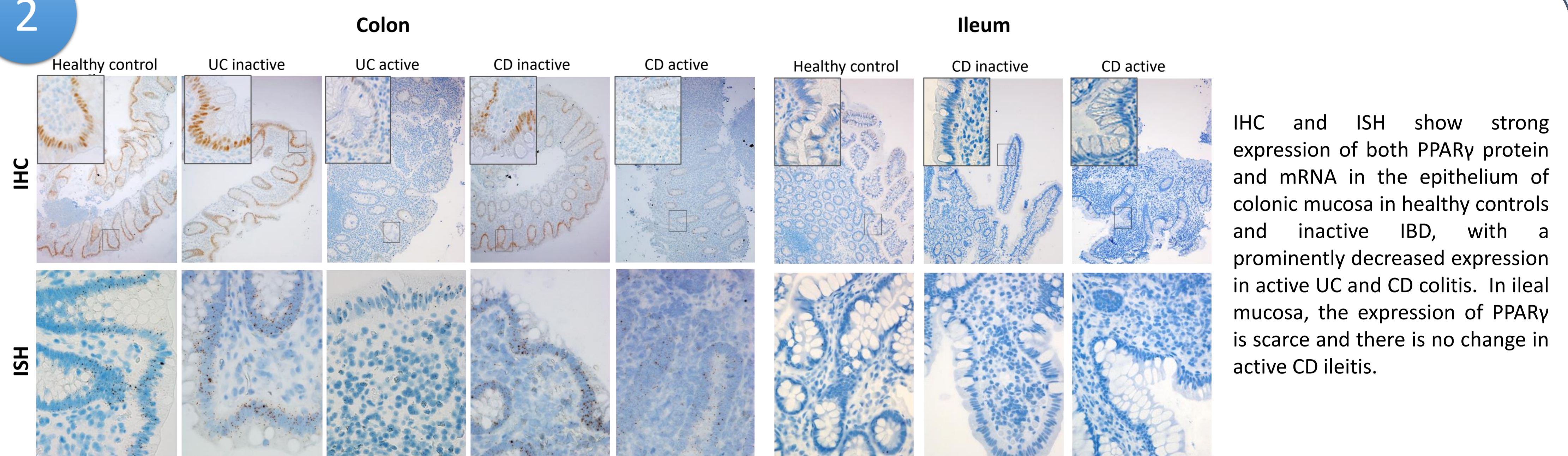
Results

1

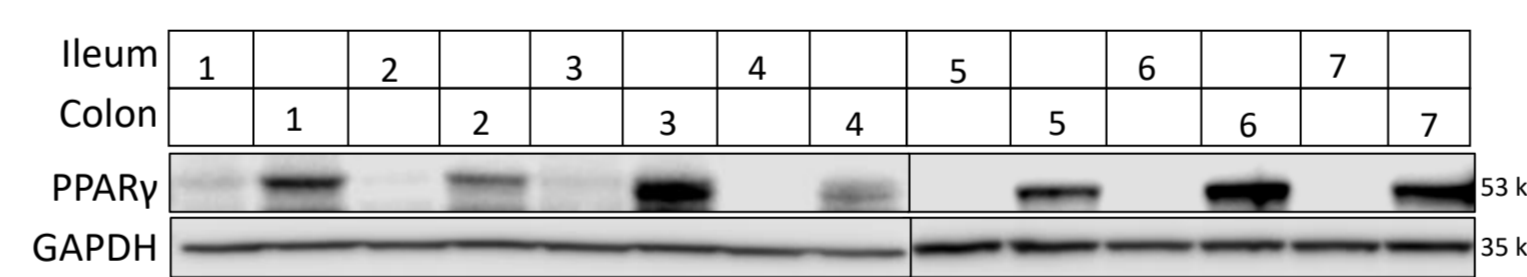


PPAR γ gene expression is significantly downregulated in colonic mucosa of active UC and CD compared to HC (UCa log₂ = -1.65, CDa log₂ = -1.29) and to inactive disease (UCa log₂ = -1.83, CDa log₂ = -1.23). ****p<0.0001. PPAR γ is not regulated in active CD ileitis. Individual values are plotted and median expression (black line) shown. a-active, i-inactive.

2

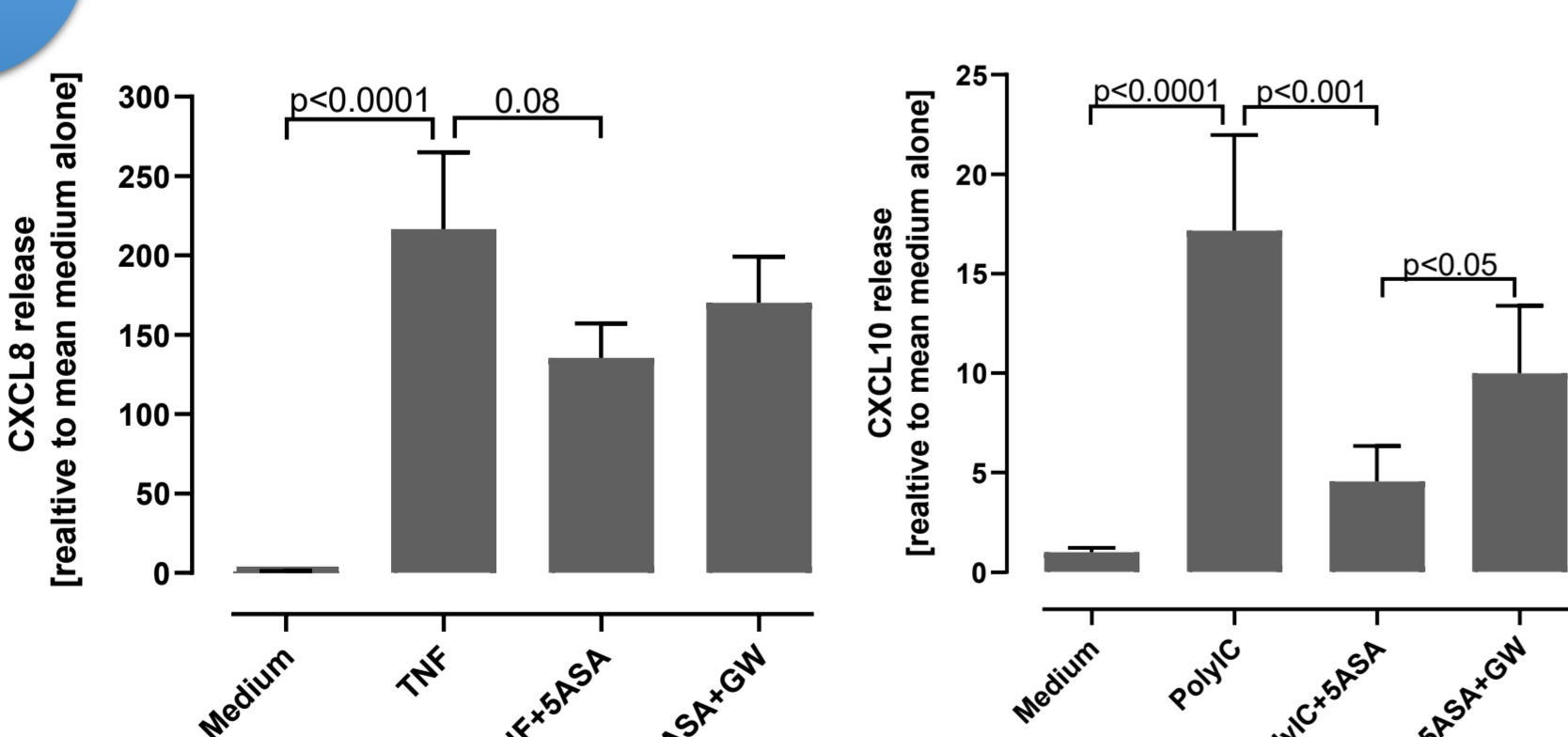


IHC and ISH show strong expression of both PPAR γ protein and mRNA in the epithelium of colonic mucosa in healthy controls and inactive IBD, with a prominently decreased expression in active UC and CD colitis. In ileal mucosa, the expression of PPAR γ is scarce and there is no change in active CD ileitis.



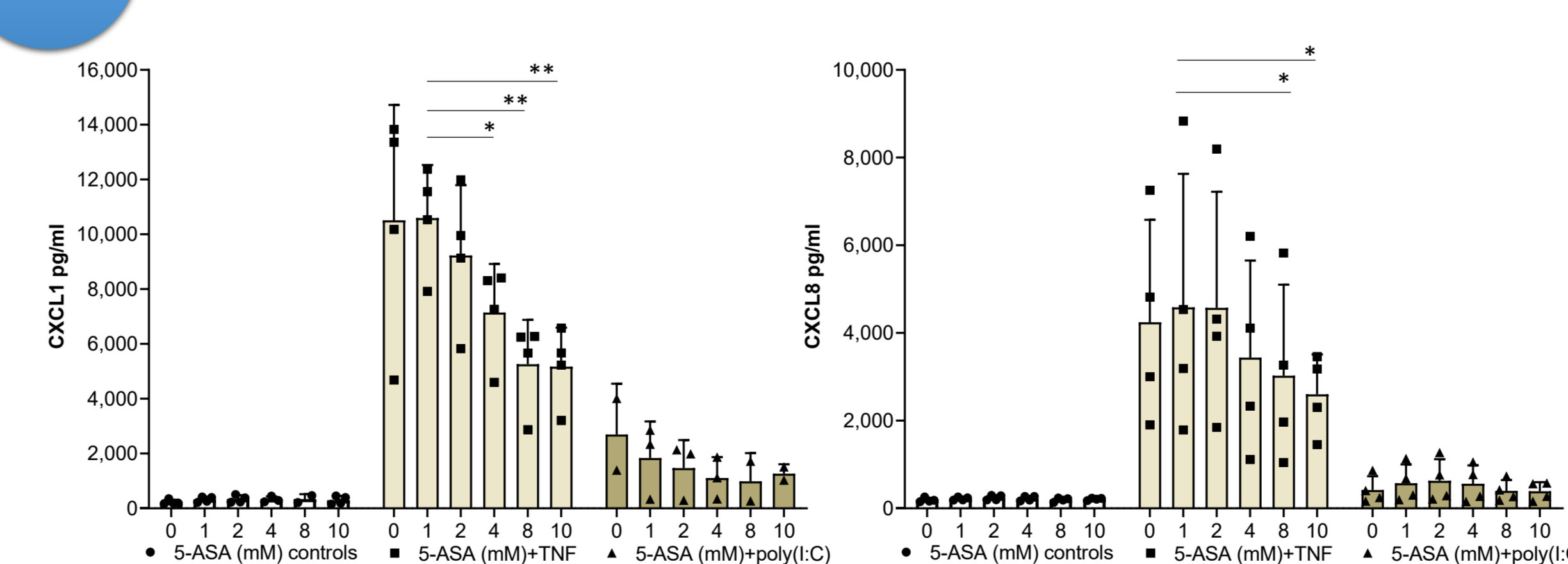
Western blots of mucosal homogenates show a clear difference in PPAR γ expression in paired samples of ileal and colonic biopsies from 7 healthy controls.

3



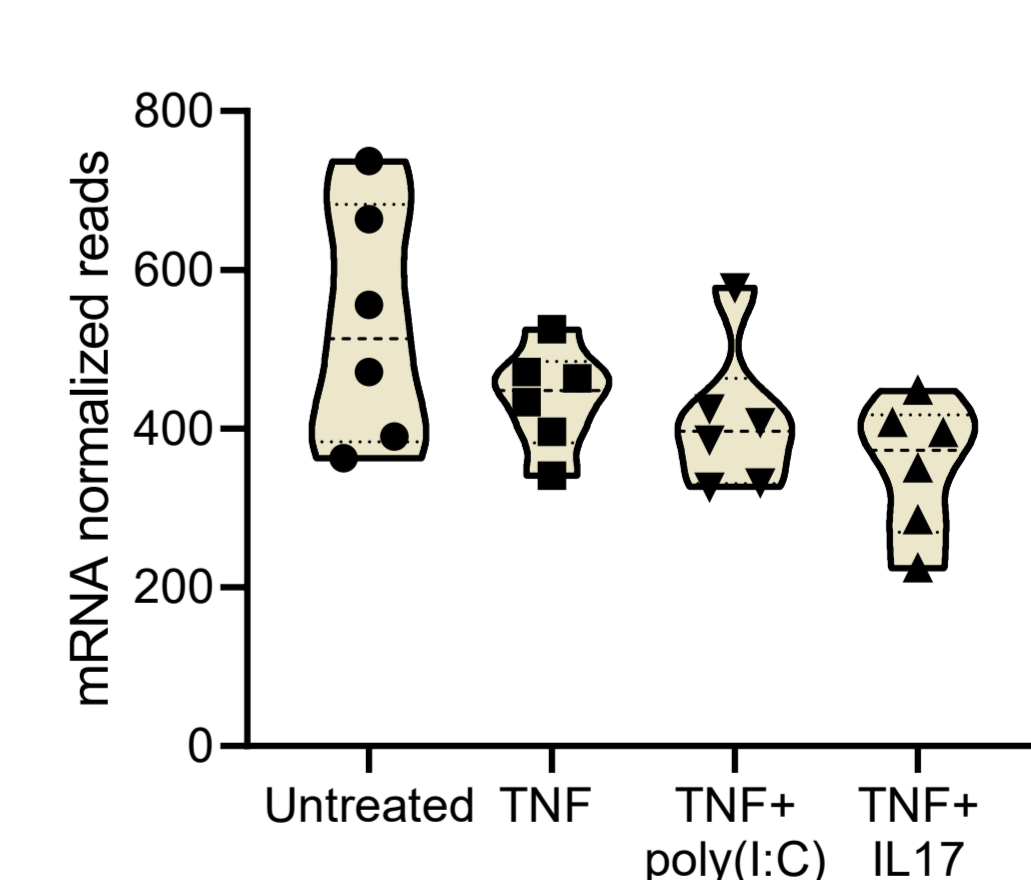
5-ASA (8mM) has an inhibitory effect on TNF (0,1 μ g/ml) or poly(I:C) (5 μ g/ml) induced release of CXCL8 and CXCL10 from HT-29 cells. The PPAR γ -antagonist GW9662 (10 μ M) tended to reverse this effect. Mean and 95% CI are shown (n=2-5 experiments).

4



Dose-response inhibition of 5-ASA (mM) on the release of CXCL1 and CXCL8 from colonoids (n=4 experiments) treated with TNF (0.2 μ g/ml) and poly(I:C) (25 μ g/ml). **p<0.01, *p<0.05. Mean and SD are shown.

5



Reduced gene expression of PPAR γ in epithelial colonoids from 3 healthy controls and 3 UC after stimulation with pro-inflammatory mediators. TNF+IL17 vs. untreated: log₂=-0.578. adjusted p=0.193

Conclusion

- ❖ The scarce expression of PPAR γ mRNA and protein in ileum contrasts the strong expression in uninflamed colonic epithelium and the marked downregulation in active IBD-colitis.
- ❖ 5-ASA harbours anti-inflammatory effects on colonic epithelial cells that could be mediated by PPAR γ .
- ❖ Pro-inflammatory stimulation reduces PPAR γ mRNA in human 3D colonoids.

We suggest that the previously observed lack of effect of 5-ASA in CD is related to differences in PPAR γ expression in small intestine versus colon. These results suggest that patients with Crohn's colitis may benefit from 5-ASA similarly to UC patients and challenge the current view on use of 5-ASA in CD.