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ALTERED CELL CYCLING AND APOPTOSIS IN COLONIC MUCOSA OF IMMUNOLOGICAL NONRESPONDERS

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BACKGROUND

- **Immunological nonresponders (INR)** have increased risk of non-AIDS-related morbidity and mortality
- We have recently shown that compared to immunological responders (IR) and HIV negative healthy controls (HC), **INR** have signs of **increased enterocyte damage and gut mucosal immune dysfunction restricted to colon** (Meyer-Myklestad et al., 2020)
- **We hypothesize that INR have a differentially expressed gut transcriptome and proteome compared with IR and HC**

METHODS

Human clinical samples (n=5/group):

- INR: ART>4 years with HIV RNA <50 copies/ml ; CD4 count <400 cells/µL for >3.5 years
- IR: ART>4 years with HIV RNA <50 copies/mL and CD4 count >600 cells/µL for >3.5 years, age-, sex-, and CD4nadir- matched with INR
- HIV-negative healthy controls, age- and sex-matched with INR and IR

mRNA sequencing and global proteome analyses (mass spectrometry: MS) on gut mucosal endoscopic pinch biopsies from Sigmoid colon and Terminal ileum

- *INR* display highly *differentiated* mucosal transcriptome and proteome in the *sigmoid colon*, but not in the terminal ileum.
- *INR* have *altered expression* of proteins involved in *survival*, *cell cycle regulation* and *apoptosis*.
- *Differentially regulated factors* identified may be *targets* for future *adjuvant therapy* to improve prognosis for INR.

RESULTS

Transcriptome analysis by RNA-seq:

- Sigmoid colon: ~ 3300 mRNA transcripts significantly differentially expressed in INR compared to IR (Figure 1)
- Terminal ileum : no differential expression was observed between INR and IR (Figure 1)

Proteome analysis by MS:

- 3700 proteins evaluated for differential expression
- Higher level of differential regulation between INR and IR in colon than in terminal ileum (Figure 2)
- Sigmoid colon: Lower level of periostin (survival) and Musashi RNA binding protein 2 (MSI2) (cell cycle regulation) in INR compared to IR. Higher level of of CASP3 (apoptosis) in INR compared to IR . No differential regulation of these proteins in the terminal ileum (Figure 3)

CONCLUSIONS

- Sigmoid colon, as opposed to terminal ileum, is implicated as an anatomic site linked to mechanisms causing complete immune recovery in PLHIV
- Differentially regulated genes and proteins identified in sigmoid colon may contribute to the INR phenotype through reduced cell survival, cell cycle regulation and increased apoptosis
- These factors may be candidates for adjuvant therapy to improve the prognosis and quality of life for PLHIV

ADDITIONAL KEY INFORMATION

- *Author Contact Information*: Mari Kaarboe mari.kaarbo@rr-research.no
- *Acknowledgements:* All of the study participants and Hanne Guldsten (OUH) and PROMEC (NTNU) for technical assistance
- *Reference:* Meyer-Myklestad, M. H., et al "HIV-infected immunological non-responders have colon-restricted gut mucosal immune dysfunction." J Infect Dis. (2020).

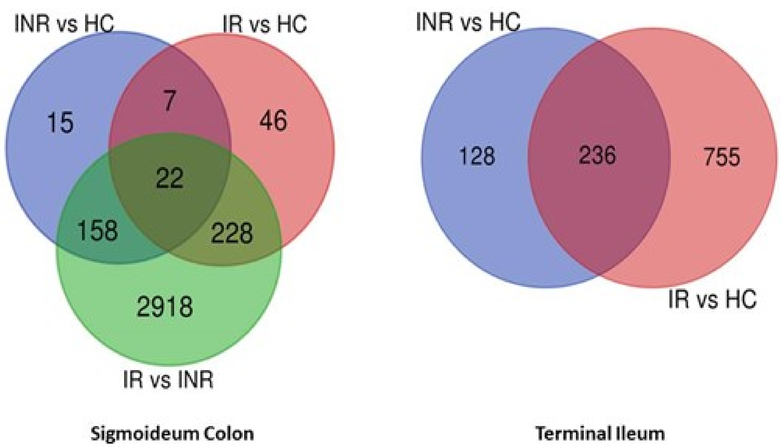

Figure 1. INR show more transcriptional regulation in sigmoideum colon compared to terminal ileum. Total RNA was isolated from gut mucosal endoscopic pinch biopsies and expression analyses were performed by RNA-seq. The Venn diagrams show overlap in sets of significant differentially regulated mRNAs in INR, IR and HC in sigmoideum colon and terminal ileum, respectively. A modified T-test (Wald test) was used to identify differentially expressed mRNAs.

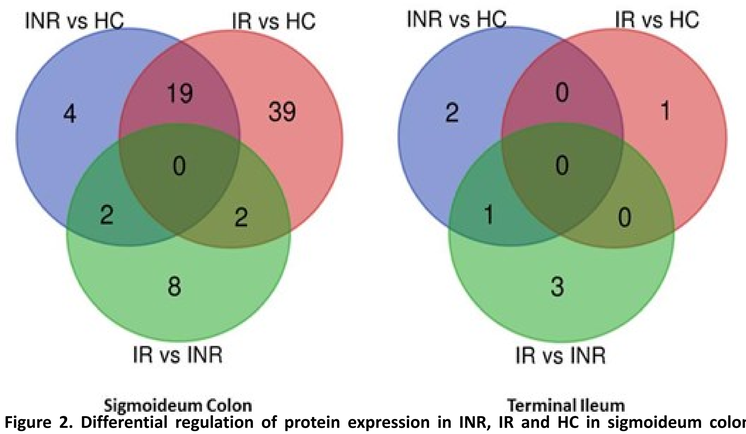

Figure 2. Differential regulation of protein expression in INR, IR and HC in sigmoideum colon and terminal ileum. Differential protein expression in gut mucosal samples was analyzed by MS based global proteomic analyses. The Venn diagrams illustrate significant differentially expressed proteins in INRs IR and HC in sigmoideum colon and terminal ileum, respectively. Differential expression analyses were performed using Empirical Bayes statistics test.

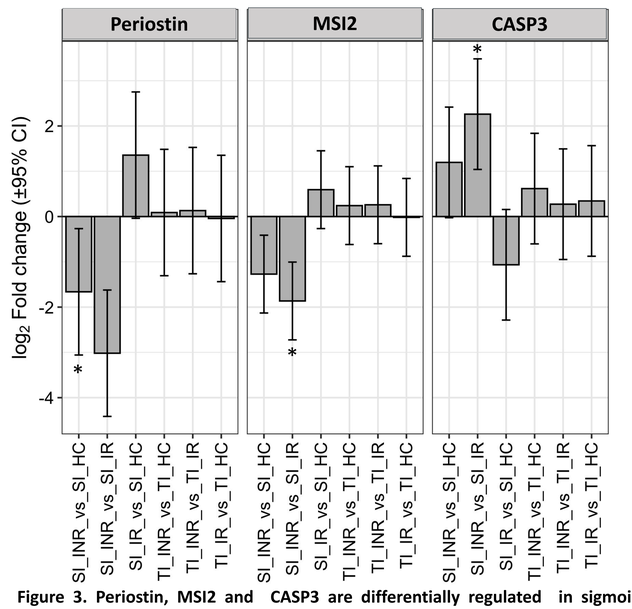

Figure 3. Periostin, MSI2 and CASP3 are differentially regulated in sigmoid colon but not in terminal ileum. The graph ilustrates expression profiling (log 2 fold change) of factors in gut mucosal samples. Empirical Bayes statistics test (*: $p < 0.05$)