#0206

ALTERED GUT IMMUNITY IN IMMUNOLOGICAL NON-RESPONDERS IS PARTLY RESTORED BY PROBIOTICS

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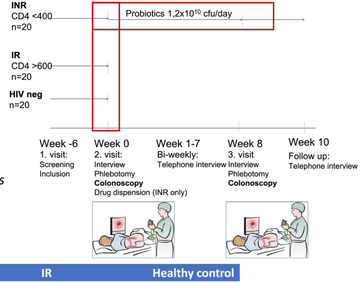
Introduction

- Immunological non-responders (INR) have increased non-AIDS morbidity.
- A proposed mechanism for INR's inferior prognosis is microbial translocation across gut mucosa, which promotes chronic immune activation.
- Our objectives were to study in-depth immune function in gut mucosa of INR and the impact of a probiotic intervention.

Methods and patient characteristics

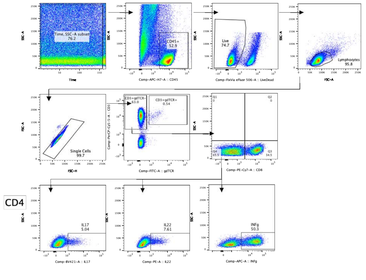
- Study completed, preliminary results.
- Cross-sectional study.
- Caucasian age-matched men:
  - 20 INR (ART >4 years with HIV RNA <50 copies/ml and CD4 count <400 cells/µL for >3.5 years);
  - 20 immunological responders (IR) (ART >4 years with HIV RNA <50 copies/mL and CD4 count >600 cells/µL for >3.5 years) matched on nadir CD4 count
  - 20 HIV-negative controls (HIV neg)
- Probiotic intervention for eight weeks:
  - Idoform Travel *Lactobacillus rhamnosus* (LGG®), *Lactobacillus acidophilus* LA-5®, *Bifidobacterium* (BB-12®), *Lactobacillus bulgaricus* LBY-27®, *Streptococcus thermophilus* STY-31®)



- Main characteristics of study subjects:

PARAMETER median (IQR)	INR	IR	Healthy control
Age (years)	49.6 (43.9-58.9)	52.5 (48.2-59.3)	54.8 (50.7-59.2)
Nadir CD4+ T-cell count, cells/µL	90 (22-157)	101 (31-178)	-
CD4+ T-cell count by inclusion, cells/µL	327 (269-374)	777 (690-867)	-
CD4/CD8 ratio inclusion	0.48 (0.34-0.74)	1.0 (0.74-1.21)	-
CD4+ T-cell count after probiotics, cells/µL	334 (257-407)	-	-
CD4/CD8 ratio after probiotics	0.47 (0.34-0.64)	-	-
Time since first positive test (years)	10.2 (7.3-21.8)	18.2 (11.8-24.9)	-
Risk group	16 MSM; 2 MSW; 1 unknown	18MSM; 2 unknown	Unknown

- Flow cytometry of isolated lamina propria mononuclear cells after mitogenic stimulation with PMA (final concentration 5ng/ml) and Ionomycin 1µg/ml for 12 hours. CD4+ T cells were characterized as CD45+live+CD3+gdTCR-CD8-. Frequencies of Th17 (CD4+IL-17+), Th22 (CD4+IL-22+) and Th1 (CD4+IFNγ+) were assessed, see gating chart.
- ELISA: Soluble (s)CD14, IL-6, sCD163, CRP, Zonulin, IL-18, intestinal fatty acid binding protein (I-FABP), LBP, LPS and sCD25.
- Microbiome characterized by 16S rRNA gene sequencing (V3-V4).



Results

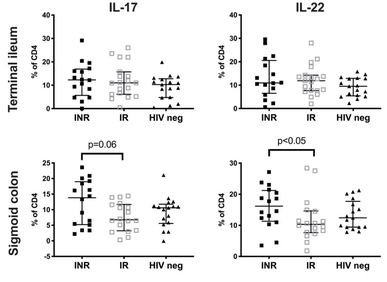


Figure 1: Mucosal CD4+ T cells from sigmoid colon in INR secrete more IL-22 after PMA stimulation compared to IR. Bar representing median and IQR. Statistical analysis by Mann-Whitney test.

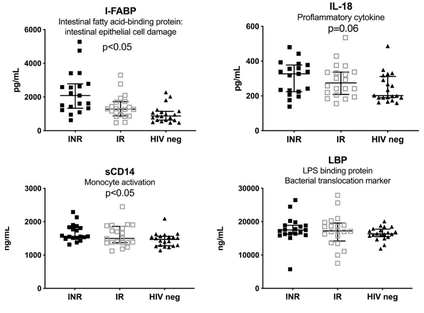


Figure 2: INR have increased markers of intestinal cell damage, inflammation and monocyte activation. Bar representing median and IQR. Statistical analysis by Kruskal-Wallis test.

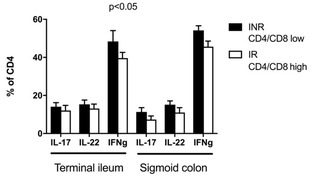


Figure 3: INR with low CD4/CD8 ratio have higher cytokine production than IR with high CD4/CD8 ratio. Statistical analysis by two-way ANOVA.

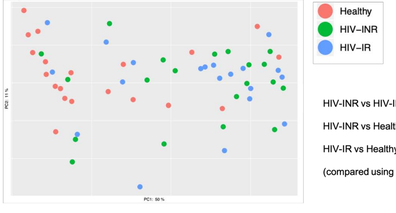


Figure 4: Beta diversity in fecal samples by weighted UniFrac. Significant differences between HIV positive and HIV negative study subjects, but not between INR and IR. Statistical analysis by PERMANOVA.

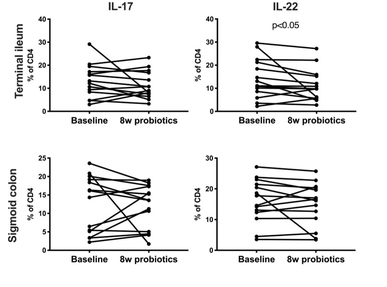


Figure 5: Reduced proportion of IL-22 producing mucosal CD4+ T cells after 8 weeks of probiotics. Statistical analysis by Wilcoxon matched-pairs signed rank test.

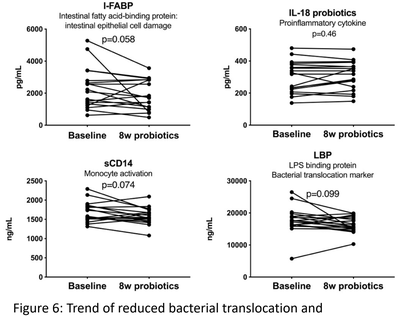


Figure 6: Trend of reduced bacterial translocation and monocyte activation after probiotic intervention. Statistical analysis were performed using Wilcoxon matched-pairs signed rank test.

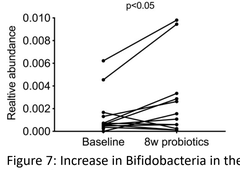


Figure 7: Increase in Bifidobacteria in the terminal ileum after intervention. Statistical analysis by Wilcoxon matched-pairs signed rank test.

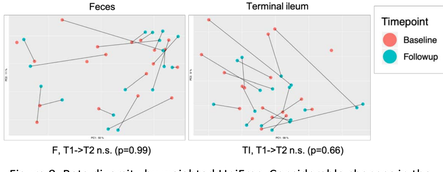


Figure 8: Beta diversity by weighted UniFrac. Considerable changes in the individual microbiota after intervention, but not significant on the group level. Statistical analysis by PERMANOVA.

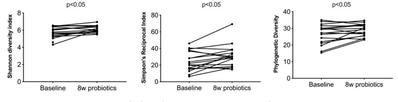


Figure 9: Increasing alpha diversity in mucosal microbiota in the terminal ileum after intervention. Statistical analysis by paired T test.

Conclusions

- INR had increased I-FABP as a marker of impaired mucosal barrier function.
- INR with low blood CD4/CD8 T cell ratio had elevated frequencies of cytokine producing mucosal CD4 subsets, compared to IR with high CD4/CD8 ration. This indicates a more pro-inflammatory tissue environment.
- There was an increase in the mucosa-adherent bacterial alpha diversity after probiotic intervention.
- The immunologic alterations were partially reversed by probiotics, providing a rationale for further trials of gut targeted treatment in INR.

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