Targeting Gut Dysbiosis With Prebiotics and Glutamine in HIV-Infected Subjects

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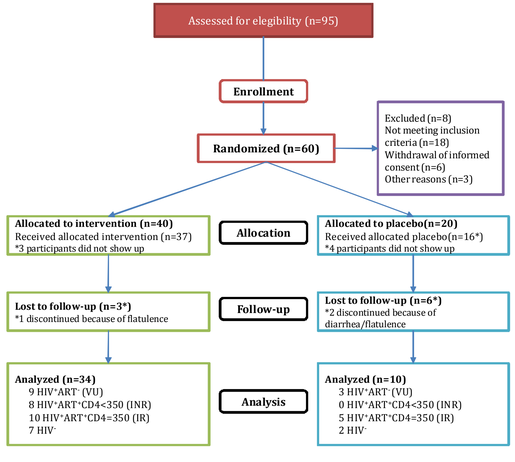
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ABSTRACT #266

INTRODUCTION

- HIV has emerged as a chronic inflammatory disease, and inflammation is probably driven by chronic immune dysfunction and contributes to excess risk of mortality
- Several recent works have shown that HIV infection elicits dysbiosis and alters the interplay between gut bacterial communities and the immune system, stimulating inflammation in the gut and systemic circulation.
- The effects of therapies aimed at restoring the gut microbial communities in humans have not been explored using deep sequencing, which would allow a deeper understanding of how these interventions could influence health.
- We hypothesized that an intervention with prebiotics and glutamine, which have shown beneficial effects on gut microbiota and the enterocyte barrier function might result in amelioration of the composition of gut bacterial and modulate markers of the innate and adaptive immunity.

METHODS

- We recruited viremic untreated (VU) HIV-infected subjects, immunological ART immunological responders and non-responders (IR and INR, ≥350 and <350 CD4+ T-cells/ml after >2 years of viral suppression), and unmatched HIV-uninfected (HIV$^-$) individuals, who were blindly randomized to receive daily either a 20g supplement of prebiotics (scGOS/lcFOS) and glutamine or placebo (34/11), during 6 weeks.



MEASUREMENTS , at baseline and week 6:
-Fecal microbiota composition using V2-V3 regions of 16S rRNA deep sequencing (7%ID OTU- level bacterial phylotypes).
-Markers of systemic T cell activation (% of T cells HLA-DR+/CD38+ and CD25+) by flow citometry.
-Markers of thymic output: levels of T-cell receptor rearrangement excision circles (using the sj/β-TREC ratio).
-Soluble markers of inflammation (Interleukin-6, high-sensitivity C reactive protein), monocyte activation (soluble CD14), endothelial dysfunction (asymmetric dimethylarginine, ADMA), coagulation (D-dimers) and bacterial translocation (bactericidal/permeability-increasing protein, BPI).

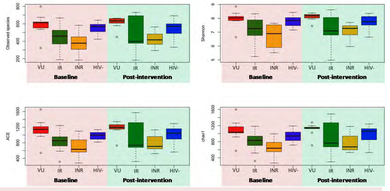
RESULTS

General characteristics of the study population

- A total of 44 individuals completed the 6-week study on-treatment, 34 received a 20g mixture of prebiotics (scGOS/lcFOS) and glutamine, and 10 received a placebo: 12 HIV+ART-, 8 INR, 15 IR, and 9 controls.
- The average compliance to the intervention in both arms was superior to 85%. HIV-infected individuals on ART were representative of a medium aged long-term treated population without comorbidities.
- Overall the intervention was well-tolerated and 7 patients dropped out of the study (3 in the active arm and 4 in the placebo arm)

HIV infection alters microbial diversity which remained altered after the intervention

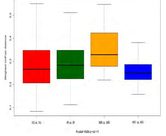
Alpha diversity (richness and evenness of bacterial taxa within a community)



VU subjects, followed by HIV$^-$ individuals, had the highest microbial richness of the cohort for each of the four different metrics (**ACE, Chao1, Shannon and the total of observed species**), while INR subjects showed the lowest diversity

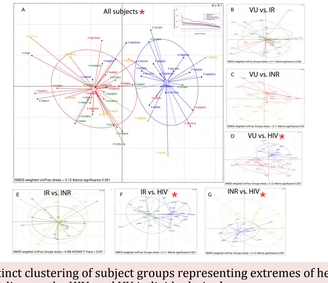
In the active arm, the nutritional intervention did not result in significant variation of alpha diversity parameters, being the microbiota of VU individuals still the more diverse at the end of the study, and that of INR individuals the less diverse

Beta diversity (compositional heterogeneity)



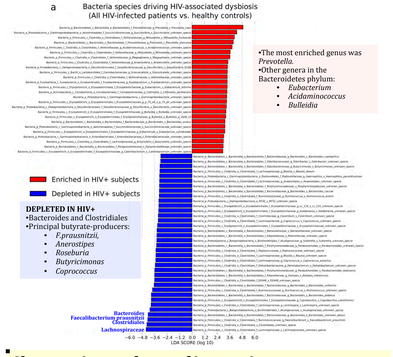
Weighted Unifrac distance: INR individuals had the highest bacterial heterogeneity.

Gut microbiota is representative of the disease status. Partitioning Around Medoids (PAM) analysis



Distinct clustering of subject groups representing extremes of health and disease, the HIV$^-$ and VU individuals, is shown.
* p value<0.05 in the Adonis test.

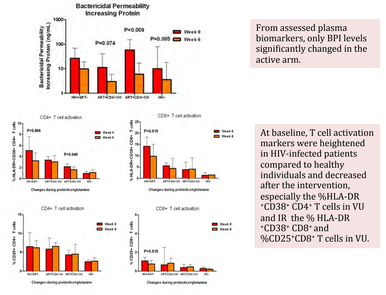
HIV infection alters microbiota structure and introduces depletion of butyrate producers



- The most enriched genus was *Prevotella*.
- Other genera in the Bacteroidetes phylum:
  - *Eubacterium*
  - *Acidaminococcus*
  - *Bulleidia*

DEPLETED IN HIV+
- Bacteroides and Clostridiales
- Principal butyrate-producers:
  - *F.prausnitzii*,
  - *Anerostipes*
  - *Roseburia*
  - *Butyricimonas*
  - *Coprococcus*

Changes in markers of innate immune activation, T cell activation and thymic funtion in the active arm

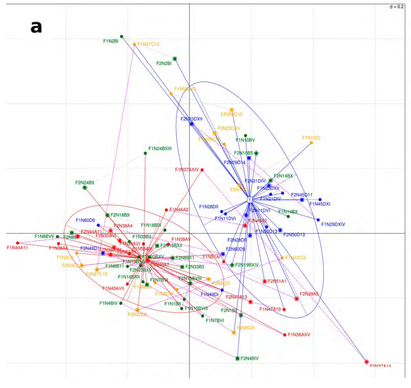


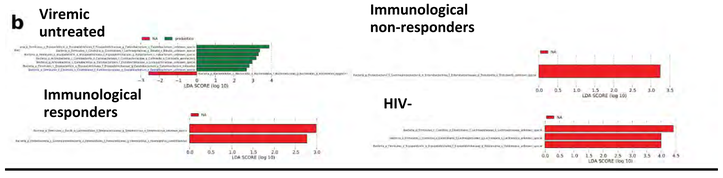
From assessed plasma biomarkers, only BPI levels significantly changed in the active arm.

At baseline, T cell activation markers were heightened in HIV-infected patients compared to healthy individuals and decreased after the intervention, especially the %HLA-DR$^+$CD38$^+$ CD4$^+$ T cells in VU and IR the % HLA-DR$^+$CD38$^+$ CD8$^+$ and %CD25$^+$CD8$^+$ T cells in VU.

Thymic output improved in all group of HIV$^+$ individuals, although remained impaired compared with HIV$^-$ controls. The increase only reached the statistical significance in VU individuals.

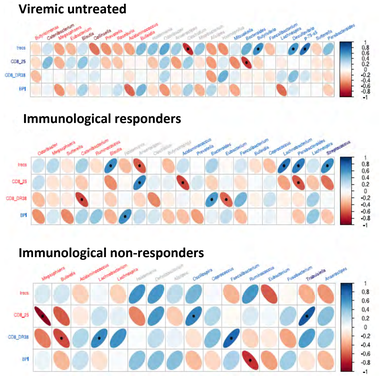
The nutritional intervention elicited a compositional shift that was not appreciated in the placebo arm





Changes in gut microbiota composition during the intervention.
a) NMDS of the unifrac distance matrix of the OTU bacterial composition in VU (red dots), IR (green dots), INR < (orange dots) and HIV$^-$ (blue dots). *The centroids are represented by numbers, while ellipses represent 70% of the samples belonging to each condition. Each point is connected with a dash line to another dot corresponding to the sample at the end of follow/up. Purple dash lines indicates those samples that significantly migrated from their baseline group, black dash lines indicate those samples which migration did not reach the level of statistical significance based on the weighted unifrac significance test. Dots framed by a rectangle represent individuals after treatment in the active arm; dots framed by a diamond represent individuals after treatment in the placebo arm.*
Although in IR individuals the intervention failed to approximate the bacterial communities towards the HIV$^-$ group, VU individuals in the active arm and, to a lesser extent, INR individuals, experienced a compositional shift that was not appreciated in the placebo arm. From 6 VU subjects whose samples migrated from their group to the HIV$^-$ cluster, 5 were in the active arm, while 2 of 2 samples in the INR group migrating to the HIV$^-$ cluster were in the active arm.
b) LEfSe analysis revealed that the nutritional supplement increased the abundance of *Faecalibacterium* in VU individuals, and also other genera included in the Firmicutes phylum such as *Catenibacterium, Eubacterium* and *Blautia*

Potential microbial targets for treatment of immune dysfunction



Correlations between changes in the abundance of bacterial biomarkers and BPI, thymic function and CD8+ T cell activation.
Variations in *Butyrivibrio*, *Desulfovibrio* and *Blautia* strongly correlated with significant improvements of thymic output (sj/β TREC ratio), while changes in *Catenibacterium* and *Eubacterium* strongly correlated with significant declines of activated (%HLA-DR+CD38+) T cells in both VU and AT (all, P<0.05).

Bacterial genera which abundance increased after the intervention are represented in red, those which abundance decreased are represented in blue. Dark/blue color denotes a p value <0.05 for the difference between abundance before/after the intervention. Blue ellipses represent positive spearman's correlation coefficients, red ellipses represent negative correlations. Dots mark the correlations holding statistical significance after adjustment for multiple comparisons.

CONCLUSIONS

- We observed a spectrum of HIV-associated dysbiosis, reflecting the disease status of the study participants determined by the extent of immunovirological control.
- A short dietary supplementation with prebiotics and glutamine ameliorated gut microbiota structure in ART naïve and treated patients, although gut microbiota of IR individuals was found more resilient.
- We identified several bacteria correlated with improvements of markers of immune reconstitution and T cell activation, and hence, potential targets for interventions.
- Metagenomic and metabolomic approaches, and personalized therapies based on microbiota structure would help to understand how we might exploit the microbial-host interactions to restore normal health of individuals living with HIV.

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