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Probiotics Module Th1/Th17 and IFN Response in HIV Patients on Suppressive cART

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Introduction:

The complex relationship among the Th1/Th17 lymphocytes and innate antiviral defences in the intestinal mucosa during HIV-1 infection has not been well characterized. In advanced HIV-1 infection, the homeostatic balance between gastrointestinal indigenous bacteria and gut immunity fails and microbes are able to overcome the intestinal barrier and gain the systemic circulation *(Dillon SM. et al. 2016)*. Despite the effectiveness of Antiretroviral Therapy (ART) in maintaining undetectable viral load, the microbial translocation persists. Recent studies on probiotics benefit for people with HIV-1 were presented. Immunomodulatory mechanisms and the anti-inflammatory properties of probiotics were already described *(Tomas LV. et al. 2015).* We evaluated the immunomodulatory effect of six months of probiotics supplementation in chronic HIV-1 infected patients under ART (Fig.1).

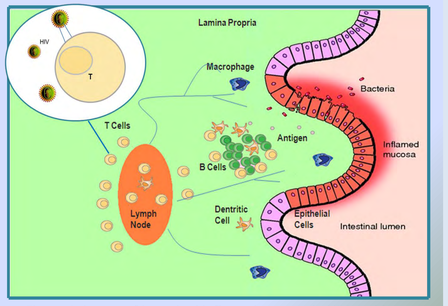


Figure 1: image of intestinal inflamed mucosa in HIV+ subjects

Aims:

To evaluate the impact of probiotics supplementation on Th1/Th17 cells balance, cellular immune activation and interferon response both in peripheral blood and intestinal mucosa of chronic HIV-1 infected patients under ART with suppressed HIV-RNA.

Methods:

Ten ART treated HIV-infected subjects underwent colonscopy procedures and blood collection prior to the probiotics supplementation (T0) and after six months of treatment (T6) (Trial #NCT02276326) (Table 1).
PBMCs (Peripheral Blood Mononuclear Cells) were obtained by Ficoll gradient centrifugation. We collected five different intestinal sites from the terminal ileum, cecum, ascending, transverse, and descending colon and consequently Lamina Propria Lymphocytes (LPLs) were isolated.
T-cell phenotypes (CD3, CD4, CD45RO, CD27) and activation markers (CD38 and HLA-DR) were detected on fresh samples. Th1 and Th17 cell phenotypes were detected by IFN-γ and IL-17A intra-cytoplasmic staining, respectively, after overnight PMA (3 ng/ml) and ionomycine (1 µg/ml) activation. Acquisitions were performed by Miltenyi Biotec flow cytometer-MACSQuant Analyzer and data were analyzed using MACSQuantify software 2.5 (Miltenyi Biotec, Bergisch Gladbach, Germany).
Total RNA was extracted from PBMCs and LPLs; IFN-α subtypes (n=12), IFN-γ mRNAs were quantified by RT-PCR with the LightCycler 480 instrument (Roche, Basel, Switzerland). Data were analyzed by Wilcoxon signed-ranked test (paired data) and Spearman's correlation using SPSS v22 (IBM, Somers, NY, USA).

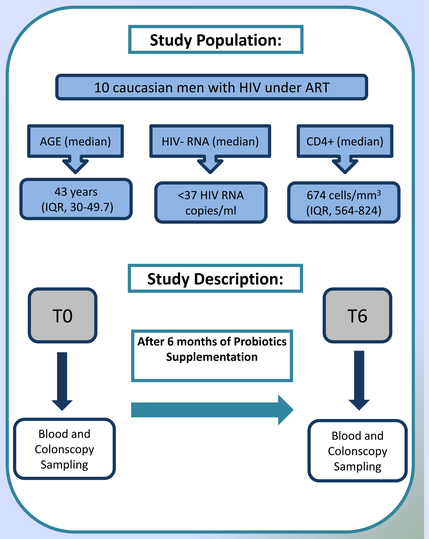


Table 1: characteristics of study population and timing of sample collection

Results:

Subjects assumed 1.8x10[12] live bacteria daily for six months; no adverse event was observed during the follow-up. All subjects had undetectable plasmatic viral load at T0 and T6. The median CD4+ T cells number at T0 was 674 cells/mm[3] and did not change at T6.

1) Six months of probiotics supplementation induced an increase in the frequencies of CD4+ cells expressing IL-17A (Th17) following PMA /ionomycin activation both in peripheral blood and intestinal mucosa; the increase was relevant in Central Memory (CM) and Effector Memory (EM) CD4+ subpopulations.
The ability of CD4+ cells to express IFN-γ (Th1) was restored by probiotics supplementation only in gut district (Figure 2).
After probiotics supplementation, we observed a reduction of CD38 and HLA-DR expression in CD4+ and in both Effector Memory and Central Memory subpopulations (data not shown).

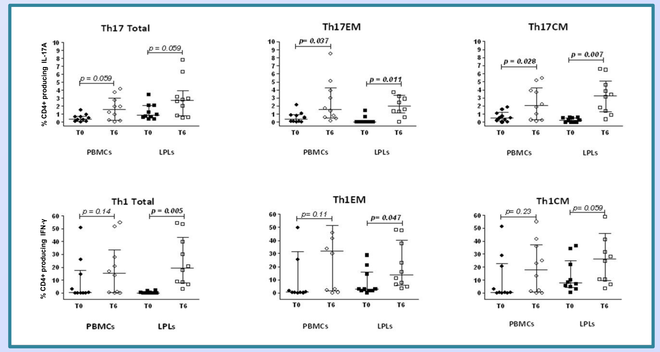


Figure 2: percentages of IL-17A and IFN-γ CD4+ cells In Th17 and Th1 memory subsets

2) The most statistically significant increase in Th17 frequencies in gut was observed in CM population and this level at T6 was inversely correlated to the percentages of CD4+ cells expressing HLA-DR in EM subpopulation (r=-0.79 *p*=0.006) (Figure 3).

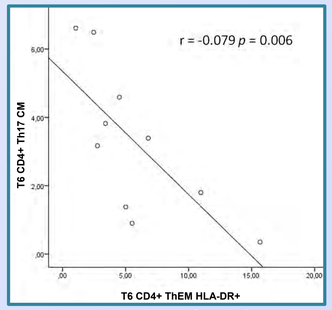


Figure 3: correlation of CD4+ Th17CM and ThEM at time 6

3) A decrease in IFN-γ expression and an increase in IFN-α subtypes (10,14,17 and 21) expression were observed at T6 in gut (*p* = 0.063) (Figure 4), whereas no differences were observed for the other IFN genes analyzed in gut and peripheral blood compartments.

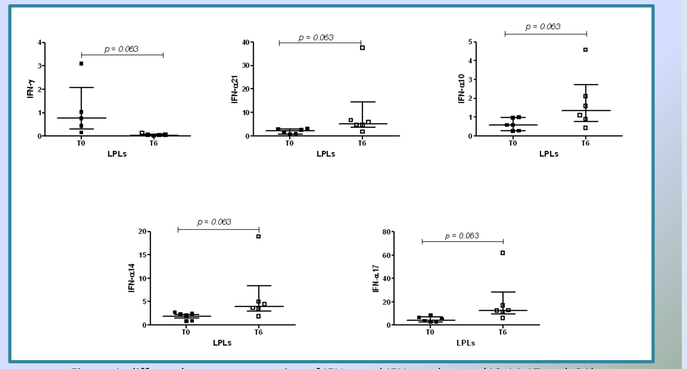


Figure 4: diffence between expression of IFN-γ and IFN-α subtypes (10,14,17 qnd 21) in LPLs at time 0 and 6

Conclusions:

Interaction between HIV-1 and the host's immune system plays a pivotal role in the gut mucosa compartment. The antiretroviral treatment improves Gut-Associated Lymphoid Tissue (GALT) immune function despite the persistence of immune activation. Probiotics supplementation for six months in HIV-1+ individuals on effective ART induced a marked recovery in the levels of both Th17 and Th1 cells in GALT and in peripheral blood; this recovery was paralleled by a significant reduction of cellular markers of activation. Furthermore, the use of probiotics was associated with a specific modulation of IFN-mediated immunity in gut.

Research group Sapienza University of Rome: Carla Selvaggi, Giuseppe Corano, Andrea Mastrangelo, Gianfranco Fanello, Fausto Fiocca, Marileda Indinnimeo.

Bibliography: Dillon SM, Lee EJ, Donovan AM, Guo K, Harper MS, Frank DN, McCarter MD, Santiago ML, Wilson CC. Enhancement of HIV-1 infection and intestinal CD4+ T cell depletion ex vivo by gut microbes altered during chronic HIV-1 infection.Retrovirology. 2016 Jan 14;13(1):5. doi: 10.1186/s12977-016-0237-1;
Thomas LV, Suzuki K, Zhao J. Probiotics: a proactive approach to health. A symposium report. Br J Nutr. 2015 Dec;114 Suppl 1:S1-S15. doi: 10.1017/S0007114515004043.