FUNCTIONAL RESTORING OF GUT BARRIER AFTER MODULATION OF INTESTINAL MICROBIOTA

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BACKGROUND

A complex series of events including enterocytes modifications, damage to the intestinal epithelial barrier, microbial translocation, a chronic systemic immune activation and mucosal immune dysfunction contribute to HIV disease progression. Of note, a gut compartmentalized IFN response during HIV infection is strongly related to the immunopathogenesis.

AIM OF THE STUDY

This study aimed to verify the potential role of probiotic administration to induce a recovery of the intestinal barrier integrity in chronically HIV-1-infected patients, focusing on cellular morphology, cellular apoptosis machinery, mitochondrial restoring and innate immunity response.

METHODS

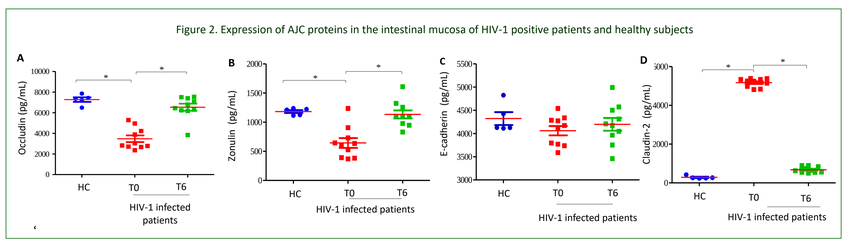
10 Caucasian cART-treated HIV-1 + patients controls underwent pancolonoscopy prior (T0) and after six months of probiotic supplementation (T6). 5 age and gender matched healthy were included in the study

Cellular morphology, cellular apoptosis machinery, Apical Junction complexes (AJC) proteins, mitochondrial restoring and IFN related response were analyzed in mucosal biopsies taken from different colonic tracts of intestine at T0 and T6.

RESULTS

❖ 10 successfully long term treated HIV-1-infected Caucasian men, with a median age of 42 years (range 22–53), had a CD4+ T cell count of 255 cells/mm^3 (range 42.75- 406.75 cells/mm^3) at enrolment. All HIV-1-positive subjects received antiretroviral therapy for a median of 6 years (IQR, 1.75–16.25 years). A control group of 5 gender- and age-matched HIV-1 seronegative healthy individuals was also included.

Figure 2. Expression of AJC proteins in the intestinal mucosa of HIV-1 positive patients and healthy subjects



❖Lower levels of Occludin (Figure 2, Panel A), Zonulin (Figure 2, Panel B) and higher levels of Claudin 2 (Figure 2, Panel D) were observed in HIV-1 infected patients compared to healthy individuals ($p<0.01$). Probiotic supplementation promoted an increase of Occludin (Figure 2, Panel A) Zonulin (Figure 2, Panel B) and a decrease of Claudin 2 levels in the gastrointestinal tract of HIV-1 infected patients (T0 vs T6 $p<0.01$).

❖Histology score analysis showed a difference between HIV-1 infected patients [7 (IQR: 5-9)] and healthy individuals [1 (IQR:0-1.5)] ($p<0.001$). In addition, a trend toward a general decrease in the histology score was found in HIV-1 infected patients [6 (IQR: 0-8)] (T0 vs T6, $p>0.05$).

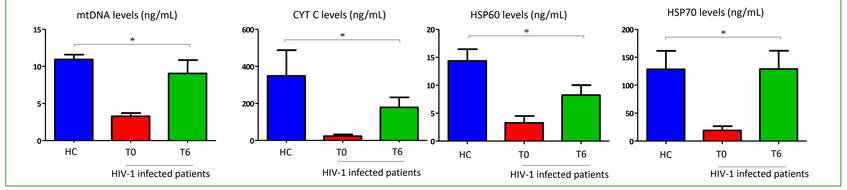
Table 1:mitochondrial number and size in enterocytes of HIV+ patients before and after the treatment and in healthy individuals

Variable	Healthy controls (A)	HIV-1 infected patients T0 (B)	HIV-1 infected patients T6 (C)	p value	
				A vs B	B vs C
Mitochondria (N)	65 (IQR:53-70)	5 (IQR:4-7)	24 (IQR:19-28)	**p<0.01**	**p<0.01**
Mitochondrial size (µm)	2.42 (IQR: 1.78-2.93)	0.88 (IQR:0.79-1)	1.50 (1.03-2.41)	**p<0.01**	**p<0.01**

❖Mitochondria number and size differed from HIV-1 infected patients and healthy individuals ($p<0.01$): samples taken at T6 showed significant increased number of larger mitochondria and the levels of these organelles were similar to healthy samples ($p<0.01$).

❖ The rate of enterocytes that undergoing apoptosis was significantly reduced, since the expression of cCK-18, a marker of epithelial apoptosis, was higher in HIV-infected patients (HIV: 355.9±109 pM vs HC: 83.7 ± 14 pM, $p=0.018$) but were reduced after probiotic treatment in this population 155.6±30 pM $p<0.05$).

Figure 3. mtDNA, Cytocrome C, HSP60 and 70 levels in gut samples of HIV-1 + patients before and after the treatment and in healthy individuals



❖Ultrastructural morphological analysis indicated an increase concentration of mitochondrial DNA in all the patients tested ($p<0.005$) and a similar trend for Cytochrome C (CYC C) concentration ($p<0.005$), with substantial reduction of HSP60 and 70 m-RNA expression in mucosal biopsies ($p<0.005$) after probiotic supplementation (Figure 3).

CONCLUSION

The modulation of intestinal microbiota ameliorates histopathologic alterations characterizing HIV-1-related enteropathy, reducing inflammation, the rate of vacuolated enterocytes undergoing apoptosis, damage of to the intestinal barrier integrity, and increasing larger mitochondria number, in ART-treated HIV-1-positive patients. These findings reinforced the concept on the beneficial impact of this specific probiotic intervention as a supplement to cART in the HIV-1 related pathogenesis.

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