

A Specific Mixture of Prebiotic Oligosaccharides Reduces Hyper-Immune Activation and Improves NK Cell Cytolytic Activity in HAART-naïve HIV Positive Adults

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Introduction

HIV-1 infection is characterized by progressive deterioration of the immune system manifested by depletion of CD4⁺ T-cells, chronic immune activation and altered effector functions. In contrast to the hyper-activation of T-cells, the cytolytic activity of NK cells from viremic patients is reduced compared to uninfected individuals, HAART-treated aviremic patients, or long term non-progressors. Emphasizing the role and early deterioration of the gastrointestinal tract in HIV (Gori A et al., *JCM in press*) and acknowledging the relationship between nutritional intervention and immune function, we hypothesized that prebiotic intervention i.e. stabilization of the impaired gut flora could induce beneficial immunologic changes during HIV infection.

Methods

In a double blind, placebo controlled pilot study (COPA trial), 57 HAART-naïve HIV positive adults (baseline median (range) of CD4⁺ T-lymphocyte count 451 (300-1080) cells/μl and HIV RNA levels 11014 (49-196524) copies/ml) were randomized to receive either placebo (maltodextrin) or a unique prebiotic mixture (15g (single dose) or 30g (double dose) per day) consisting of short-chain Galactooligosaccharides (scGOS), long-chain Fructooligosaccharides (lcFOS) and Acidic Oligosaccharides from pectin hydrolysate (AOS) (ratio 9:1:10) for 12 weeks. Immunologic and prebiotic effects were examined at baseline and during the 12 weeks of product intake. FACS analyses of CD4⁺/CD25⁺, CD4⁺/CD25⁺/Fox-p3⁺ CD8⁺/CD38⁺/CD45RO⁺, and CD14⁺/B7-H1⁺ were performed on freshly isolated PBMCs. In addition, spontaneous cytolytic activity of NK cells at effector:target (E/T) ratios of 50:1, 25:1 and 12.5:1 were analyzed. Results obtained at week 12 compared to baseline are reported and statistically analyzed using Mann-Whitney or ANOVA.

Results

In addition to a clear prebiotic effect of this oligosaccharide mixture in HIV positive individuals (*data shown elsewhere*) immune modulatory effects were observed. A dose dependent reduction of activated CD4⁺/CD25⁺ T-cells was detected in the groups receiving scGOS/lcFOS/AOS. As shown in **Figure 1** subjects receiving 15g/d had a clear trend towards reduced percentages of activated CD4⁺/CD25⁺ T-cells after 12 weeks (p=0.09). Subjects receiving 30g/d had a statistically significant reduction of the percentage of

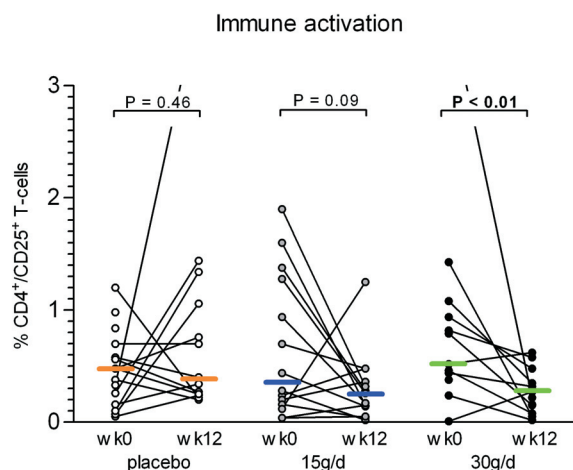


Figure 1: T-cell activation is reduced after 12 weeks of scGOS/lcFOS/AOS intake. Individual percentages of CD4⁺/CD25⁺ T-cells are shown as dots, with Median values displayed per group as lines. P values indicate statistical significant changes from baseline as tested with Mann-Whitney ($\alpha=0.033$ for multiple comparisons).

CD4⁺/CD25⁺ T-cells in circulation after 12 weeks of product intake (p<0.01). Also the number of subjects displaying a reduction in CD4⁺ T-cell activation after 12 weeks of product intake increased dose dependently with 64% vs 77%, respectively.

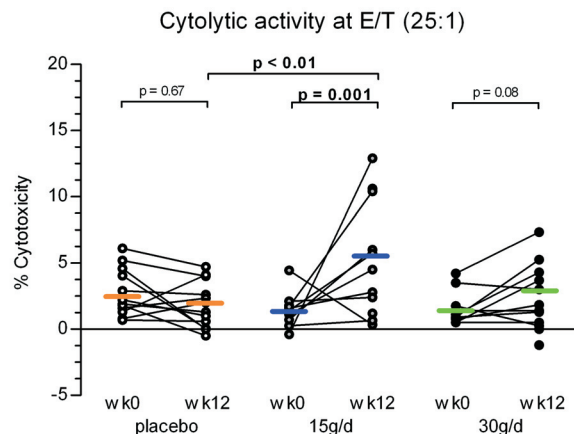


Figure 2: Percentage cytotoxicity is increased after 12 weeks of scGOS/lcFOS/AOS intake. Individual percentages of target cell lysis at E/T ratio of 25/1 are shown as dots, with estimated marginal means displayed per group as lines. P values indicate significant changes from baseline (ANOVA)

Paralleling the reduced activation of CD4⁺ T-cells, cytolytic activity of NK cells was significantly improved as shown in **Figure 2** for example the E/T of 25:1. The strongest effect was observed in subjects receiving 15g/d of scGOS/lcFOS/AOS. Significant increases were detected at E/T ratios of 50:1, 25:1, 12.5:1 with 3.44 (p=0.002), 3.63 (p=0.001), and 4.25 (p<0.001) fold respectively. In subjects receiving 30g/d increases of 2.15-1.93 folds were observed, indicating a clear trend towards improved NK cell cytotoxicity. Moreover, in subjects receiving 15g/d of scGOS/lcFOS/AOS for 12 weeks a significant improved cytolytic activity was detected compared to placebo at E/T ratios of 25:1 (p<0.01) and 50:1 (p<0.01). Immune parameters, including total CD4⁺ T-cell count, HIV-1 RNA levels, the percentage of activated CD8⁺ T-cells, regulatory T-cells (Fox-p3⁺) or CD14⁺/B7-H1⁺ expressing monocytes were comparable between groups and no statistical significant changes were observed during the 12 week period (**Table 1**).

Table 1: Immunologic parameters at baseline and week 12

Parameter	Placebo		15g/day		30g/day	
	Wk0	Wk 12	Wk0	Wk12	Wk0	Wk12
Viral load (log ₁₀)	4.1 (0.2)	4.1 (0.2)	4.0 (0.2)	3.7 (0.2)	4.3 (0.2)	4.0 (0.2)
CD4 count (cells / μL)	497 (50)	548 (67)	531 (50)	478 (71)	501 (50)	520 (75)
CD8 ⁺ /CD38 ⁺ /CD45RO ⁺ (%)	12.9 (1.1)	9.8 (2.5)	8.6 (1.3)	9.4 (2.8)	10.3 (1.2)	8.8 (2.6)
CD4 ⁺ /CD25 ⁺ /Fox-p3 ⁺ (%)	1.1 (0.4-4.4)	0.7 (0.7-8.9)	0.9 (0.2-6.1)	1.0 (0.1-2.4)	0.7 (0.2-2.7)	0.8 (0.2-4.7)
CD14 ⁺ /B7-H1 ⁺ (%)	17.0 (3.8)	11.7 (3.3)	21.5 (4.1)	15.6 (3.5)	26.7 (4.3)	15.8 (3.8)

Values are shown in Estimated Marginal Mean (EMM (SE)) or in Median (Median (range)). No significant statistical changes were observed in these parameters.

Conclusions

This study shows a promising role for prebiotic intervention in early HIV infection. A short cycle of treatment with the unique mixture of oligosaccharides in HAART-naïve HIV positive individuals results in improvement of NK cell cytolytic activity and reduction of hyper-immune activation. These data indicate that intake of scGOS/lcFOS/AOS can modulate both the innate and adaptive immune compartment. This finding warrants further investigation towards effects of prolonged prebiotic intervention on the gastrointestinal tract, disease progression and clinical outcome in HIV infection.