Human salivary microbes stably colonizing the gut implicate high carbohydrate metabolism and colonic T cells activity in mice

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Abstract

In this study, I made gnotobiotic mice, designated HSM mice that were colonized by human salivary microbiota to explore salivary microbes that stably colonize the gut. Metagenomic and 16S rRNA gene analysis of the gut microbiota in HSM mice revealed the existence of human salivary bacteria having the ability to colonize the mouse gut with the abundant carbohydrate metabolism pathway. In addition, I found several salivary microbes that were associated with the differentiation of several T cells including type 1 helper T (T_H1), type 17 helper T (T_H17), $CD8^+IFN\gamma^+$ NK T cells and $CD4^+FOXP3^+$ regulatory T cells (T_{reg}) in the colonic Lamina Propria (LP). These findings suggested the association between the intestinal and salivary microbiota, some of which might be involved in the intestinal inflammation.

Methods

Gnotobiotic mice (HMS mice) colonized by human salivary microbiota

Five weeks old germ-free (GF) C57BL/6 and BALB/c (n=3-4/sample) mice were colonized with salivary microbiota of IBD patients or healthy subjects by oral gavage, respectively. These gnotobiotic mice were kept under separate isolators for 6-9 weeks (Figure 1).

Meta 16S rRNA gene sequencing and data analysis

The V1–V2 region of the bacterial 16S rRNA gene was amplified using forward primer 27Fmod and reverse primer 338R. Obtained 16S rRNA gene amplicons were subjected to 454 GS FLX Titanium or 454 GS JUNIOR (Roche Applied Science) sequencing according to the manufacturer's instructions. 16S rRNA gene sequences with the quality value < 25, not having primer sequences at the both ends or possibly chimeric reads were removed. These filter-passed reads were used for further analysis by trimming off both primer sequences. Of the filter passed reads, only 2000 reads for each sample was used and subjected to OTU analysis. The total reads were then sorted by read quality value and grouped into operational taxonomic units (OTUs) using UCLUST with sequence identity threshold of 96%. Reads Taxonomic assignments were made according to the best BLAST hit taxon.

Immunological analysis

HSM mice were sacrificed and lymphocytes of colonic LP was isolated, stained with fluorophore-conjugated mouse antibodies and performed with flow cytometry at 6-9 weeks after the transplantation. The obtained data were analyzed by Flowjo software.

Results and Discussion

The meta 16S rRNA gene analysis showed the existence of bacterial species in the gut of HSM mice (Figure 2). Four phyla, Firmicutes, Actinobacteria, Fusobacteria and Proteobacteria were stably colonized the gut of mice. I showed that *Streptococcus parasanguinis* and *Bifidobacterium dentium* have especially high potential to colonize the intestinal environment.

To determine whether the salivary microbes colonizing the gut are associated with the colonic immune system, I analyzed the accumulation of pro-inflammatory T cells in the colonic LP of HSM mice. In several HSM mice, the frequency of IFN-γ^+, IL17$^+$ (T_H1, T_H17) and IFN-γ^+/IL17$^+$ double producing cells within CD4$^+$ cell population were increased (Figure 3). These findings demonstrated the existence of human salivary bacteria that induce T_H1, T_H17 and IFN-γ/IL17 double expressing T cells in the colonic LP. In addition, I showed that *Veillonella* and *Fusobacterium* species may be inducers of T_H1 and T_H17, IFN-γ/IL17 double producing T cells in the colonic LP, respectively.

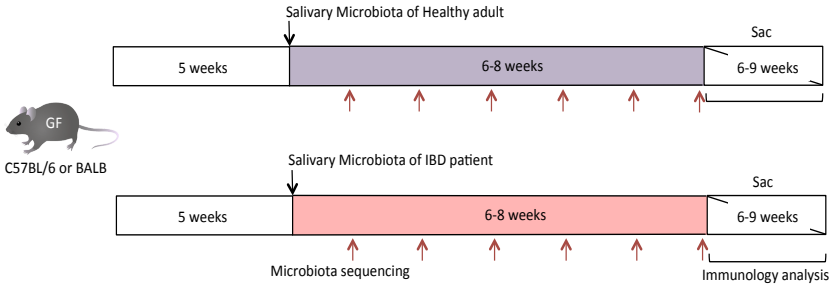

Figure 1. Schematic of colonization model. C57BL/6 or BALB mice were transplanted with human salivary microbiota at 5 weeks old. Red arrows indicate fecal collection for Meta 16S rRNA gene sequencing. HSM mice were sacrificed for immune analysis at 6 to 9 weeks after transplantation.

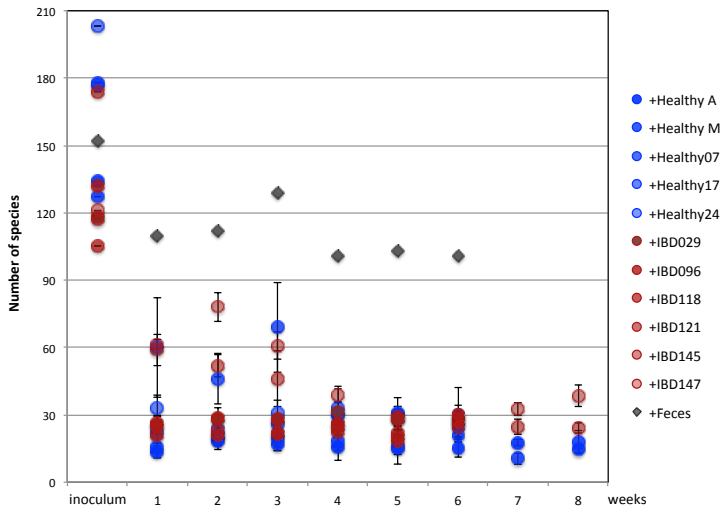

Figure 2. Observed number of species (OTUs) in fecal samples. The inoculum indicates the number of OTUs in salivary or fecal microbiota of human donors. OTUs number of feces of each HSM mouse is indicated in 1-8 column. Each number of bottoms indicates time point after the transplantation. Data are presented as mean ± SD.

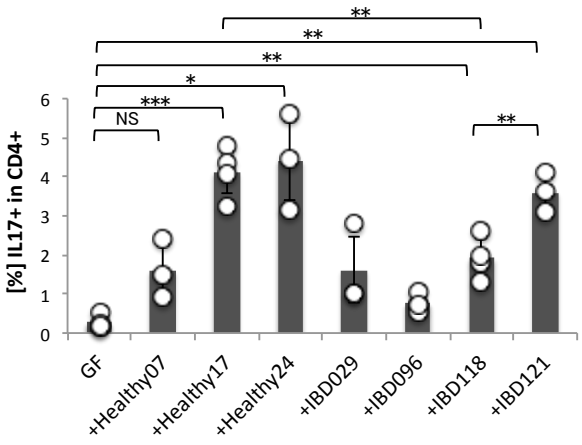

Figure 3. T_H17 cells accumulation in HSM and GF mice. The percentage of IL17$^+$ cells within CD4$^+$ cells in the colonic LP. Circles indicate individual mice. *p<0.05, **p<0.01, ***p<0.001, NS, not significant. Student's t-test. Data are presented as mean ± SD.