Analyzing the Role of Gut Microbiota in the Onset of Autoimmune Diseases Using the TNF$^{\Delta ARE}$ Murine Model

Vivienne Edwards[1], Dylan Smith[1], Sarah Poncet[1], Francoise Meylan[4], Linda Tiffany[1], Wells Wu[2], Je-Nie Phue[2], Luis Santana-Quintero[3], Kathleen A. Clouse[1] and Odile Gabay Engel[1]
[1]FDA/CDER/OBP/Office of Pharmaceutical Quality Silver Spring MD 20903 [2]FDA/CBER/Facility for Biotechnology Resources Silver Spring MD 20903 [3]FDA/CBER/OBE/HIVE Silver Spring MD 20903 [4]NIH/NIAMS Bethesda MD 20814

Abstract

We hypothesized that transplanting fecal bacteria from TNF$^{\Delta ARE}$ mice, who overexpress TNF-α and spontaneous develop Rheumatoid Arthritis (RA) and Inflammatory Bowel Disease (IBD), into germ-free mice (GF) would lead to dysbiosis and possible disease manifestation.
Fecal samples from conventional control (C) mice and sick TNF$^{\Delta ARE}$ mice were collected under anaerobic conditions and transplanted into recipient GF and C C57BL-6 mice. Gut microbial profile and inflammation markers were assessed.
We observed disease features consistent with transmission of RA and IBD in the GF mice cohort, with degradation of cartilage and disruption of the gut tissue, elevated inflammatory mediators in the tissues, activation of CD4/CD8+ T cells, and colonization and transmission of the gut microbiome, similar to the donors' profile. When TNF$^{\Delta ARE}$ mouse fecal matter was used for FMT into healthy C mice, the disease phenotype was not observed.
C mice did not develop disease phenotype after FMT, suggesting that a healthy microbiome might normalize the introduction of pathological bacteria into the gut environment. Further studies are needed to better understand the role of gut microbiome in autoimmune diseases and how this interaction can potentially facilitate identification of biomarkers predictive of treatment response.

Introduction

The human gut microbiome is composed of a diverse community of microorganisms that includes bacteria, viruses, fungi, and parasites. Dysbiosis in the gut microbiome has been linked to autoimmune diseases, such as RA and IBD, which are driven by the major proinflammatory cytokine, TNF-α. TNF-antagonists that neutralize the biological activity of TNF-α have been widely used to treat these diseases. However, studies have shown that the response to these therapeutics can vary, and the treatment can become inefficient or ineffective overtime. We performed a pilot study to determine the effects of TNF antagonists on healthy microbiota. Our results revealed significant immunological variations and changes in the taxonomic distribution in C and GF mice exposed to TNF-antagonists.

** Gabay O. et al., JBS, 2020:* https://doi.org/10.1016/j.jbspin.2019.08.001

Hypothesis

We hypothesized that transmission of the gut microbiome from sick TNF$^{\Delta ARE}$ mice, who model IBD and RA, through fecal matter transplantation (FMT) into GF or C mice could lead to dysbiosis in the gut and the development of disease etiology.

Healthy Control (C) Donors **Sick TNF$^{\Delta ARE}$ Donors**

Materials and Methods

Germ-Free Facility

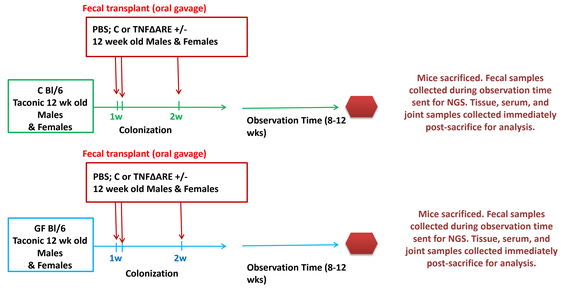
Anaerobic Chamber

GF mice housed in sterile GF facility. The anaerobic chamber is used to process fecal samples, preserving as much anaerobic bacteria as possible.

Materials and Methods

Study design (including various controls)

- Mice are co-housed; Panel of controls [sentinels (mice FMT with PBS), C donors]



Fecal Matter Transplant (FMT) samples from C and TNF$^{\Delta ARE}$ donor mice are transplanted via oral gavage. Both male and female mice used.

Analyses Performed:

- Histology and immunohistochemistry (anti-TNF) on gut tissue and knee joints
- Luminex (ELISA Multiplex) assays on serum and gut tissue
- Flow cytometry on spleen cells
- 16 S Next-generation sequencing (NGS) and HIVE Censuscope bioinformatic analysis on fecal samples collected from donors and recipient mice post-FMT

Results**

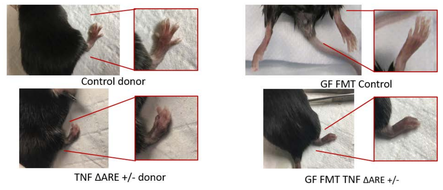


Figure 1. Phenotype of GF mice FMT with TNF$^{\Delta ARE}$ mice exhibited signs of IBD (blood in feces, difficulty giving fecal samples) and RA (pain, aggression, swelling/tenderness of joints, ulnar deviation). This was not observed in GF FMT C, nor in conventional mice FMT with either C or TNF$^{\Delta ARE}$.

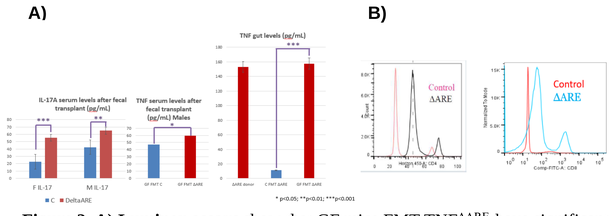


Figure 2. A) Luminex assays show that GF mice FMT TNF$^{\Delta ARE}$ have significant increase in IL-17A and TNF production in serum and TNF production in gut, but C mice FMT C or TNF$^{\Delta ARE}$ do not. Two-sample T test analysis. **B) Flow cytometry** of spleen cells show an activation and expansion of CD4+ and CD8+ T-cells in GF mice FMT TNF$^{\Delta ARE}$.

Results**

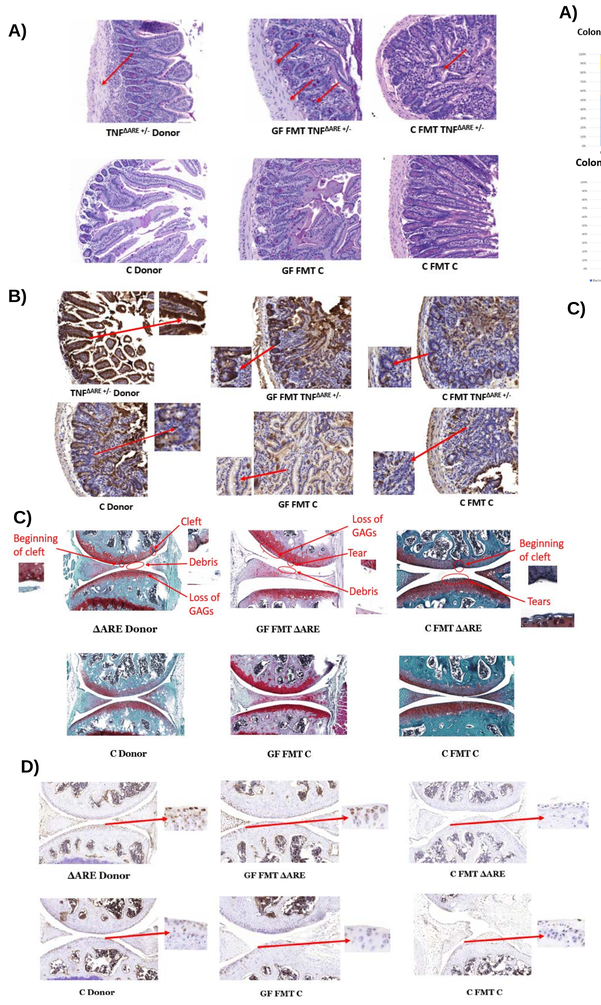


Figure 3. A) Gut histology (PAS stain) shows GF mice FMT with TNF$^{\Delta ARE}$ exhibit thickening of the intestinal wall and disorganization of the villi, similar to TNF$^{\Delta ARE}$ donors, C mice do not. **B) Gut immunohistochemistry** (anti-TNF) shows significantly increased TNF production in TNF$^{\Delta ARE}$ donors and GF mice FMT with TNF$^{\Delta ARE}$; C mice do not. **C) Knee histology** (Safranin O/Light green stain) shows TNF$^{\Delta ARE}$ donors and GF mice FMT with TNF$^{\Delta ARE}$ exhibit signs of degradation seen in RA, including formation of clefts, debris in synovial fluid, and loss of glycosaminoglycans in cartilage scaffolding; C mice do not. **D) Knee immunohistochemistry** (anti-TNF) shows significantly increased TNF production in TNF$^{\Delta ARE}$ donors and GF mice FMT with TNF$^{\Delta ARE}$; C mice do not. **Note**: All samples are male and representative of their respective cohorts.

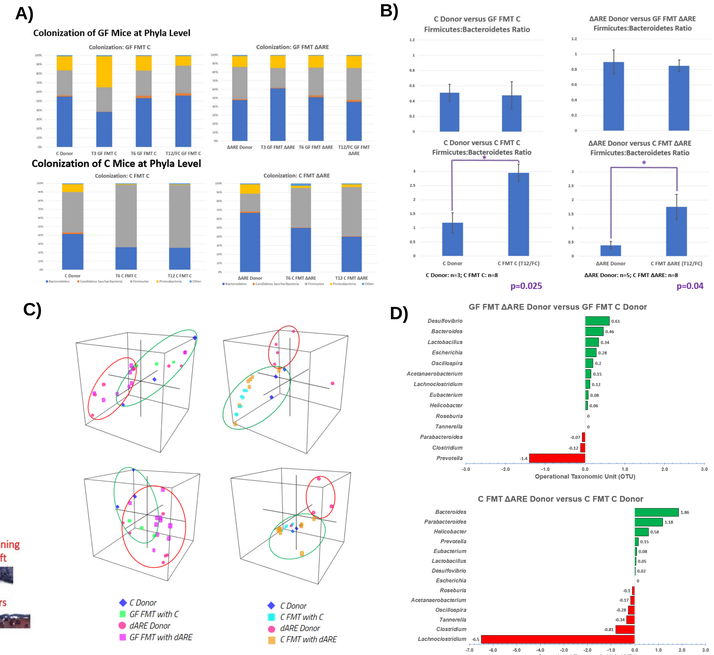


Figure 4. A) Taxonomic analysis of male mice at phyla level; colonization observed by donor mouse microbiome in GF mice but not C mice by 12 weeks post-FMT. (n=3-10) **B)** Alteration in the Firmicutes-Bacteroidetes ratio a dysbiosis feature in the gut, indicative of disease state. F/B ratio confirm GF mice were colonized, but C mice were not. Dysbiosis (alteration in ratio) seen in sick TNF$^{\Delta ARE}$ mice donors, as well as GF FMT TNF$^{\Delta ARE}$, compared to controls. Independent two-sample T-test statistical analysis. **C)** 3D vectorization of gut profile (each dot representing one mouse) shows clustering for GF mice and their respective donors, but not for C mice and their respective donors, at both the phyla level (top) and genus level (bottom). **D)** Taxonomic analysis at genus level identified the presence of key genus players- *Lactobacillus*, *Prevotella*, *Bacteroides*, *Parabacteroides*, *Clostridium*, and *Lachnoclostridium*.

***Gabay O et al. , Manuscript in preparation, 2021*

Conclusion

- Our study results suggest that the gut microbiome plays a significant role in the development of autoimmune diseases, such as IBD and RA
- TNF$^{\Delta ARE}$ mice, which overexpress TNF-α and exhibit RA and IBD, have a specific gut microbiome profile that can be transmitted into a GF model
- The transmission of their gut microbiome into GF animals leads to the development of an auto-immune inflammatory phenotype in the transplanted mice compared to controls
- We did not observe the same significant transmission into control (C) mice carrying a healthy, conventional gut microbiome

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