Universitat Autònoma de Barcelona

Changes in intestinal microbiota during development of intestinal inflammation in interleukin 10-deficient mice

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GOAL

To determine and describe changes in intestinal microbiota of interleukin 10-deficient mice during development of intestinal inflammation and after antibiotic treatment using high-throughput DNA sequencing.

INTRODUCTION

Inflammatory Bowel Disease (IBD) is characterized by aberrant immune responses against microorganisms that are present in the intestine. Changes in diversity and composition of the intestinal microbiota have been described and associated with intestinal inflammation in both human patients and animal IBD models.

IBD's etiology and pathogenesis are still unclear, it is proposed that the development of the disease requires three factors *(Figure 1):*

Figure 1. *Factors implicated in development of IBD*

Interleukin 10-deficient mice (IL-$10^{-/-}$) raised under conventional conditions develop chronic enterocolitis by 2 – 3 months of age, but mice will remain healthy when they are housed under germ-free conditions or when they receive antibiotic treatment. These features indicate that the inflammation is originated from uncontrolled immune responses stimulated by enteric antigens.

MATERIAL AND METHODS

20 IL-$10^{-/-}$ mice & 20 wild-type (WT) mice

Study 1

Goal: To determine the changes in intestinal microbiota during development of intestinal inflammation.

10 IL-$10^{-/-}$ mice & 10 WT mice

- 4 IL-$10^{-/-}$ mice + 4 WT mice → Euthanasia at 6 weeks of age
- 6 IL-$10^{-/-}$ mice + 6 WT mice → Euthanasia at 20 weeks of age

Study 2

Goal: To determine the changes in intestinal microbiota after 7 weeks of antibiotic treatment (vancomycin, neomycin, metronidazole and ampicillin).

10 IL-$10^{-/-}$ mice & 10 WT mice

- 6 IL-$10^{-/-}$ antibiotic + 4 IL-$10^{-/-}$ control
- 6 WT antibiotic + 4 WT control

→ Euthanasia at 12 weeks of age

❶Sample collection (cecal content) → ❷DNA extraction → ❸16S rRNA gene amplicon sequencing → ❹Bioinformatics and biostatistics analysis

RESULTS & DISCUSSION

Study 1

Intestinal microbiota richness increased in 20 weeks of age mice. → Change in housing conditions (Highly controlled → Conventional).

20 weeks of age WT mice presented lower α-diversity than the other groups. → In contradistinction to IL-$10^{-/-}$ mice, in WT mice the microbiota has stabilized correctly.

Alterations in the abundances of bacterial phyla. (*Figure* 2):

- Lower abundance of Verrucomicrobia in 20 weeks of age IL-$10^{-/-}$ mice compared with 20 weeks WT mice.

  Mouse model associated change VS Consequence of enteric inflammation (↓*Akkermansia muciniphila* in IBD patients).

- Increased levels of TM7 bacterial division in 20 weeks IL-$10^{-/-}$ mice

  TM7 may contribute to a pro-inflammatory shift of the intestinal microbiota by modulation of the local growth conditions.

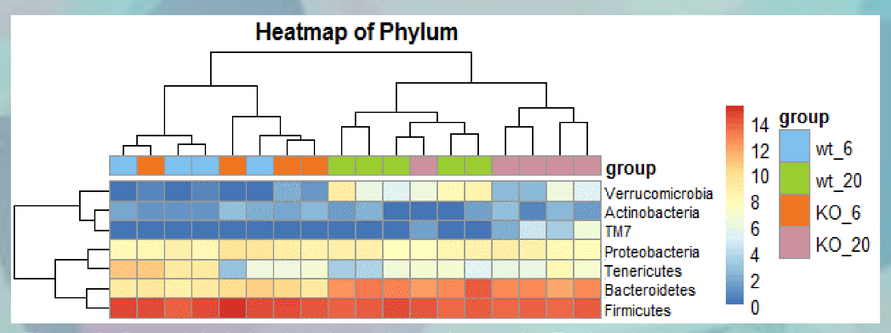

Figure 2*. Bacterial phyla abundance in each sample. Each column represents a sample and each row a bacterial phylum. In the color scale, cold tones are indicative of low abundance and warm tones are indicative of high abundance.*

Study 2

In antibiotic-treated IL-$10^{-/-}$ mice didn't decrease microbiota richness.

Alterations in the abundances of bacterial phyla (*Figure 3*):

- Increased levels of Proteobacteria in antibiotic-treated WT mice but not in antibiotic-treated IL-$10^{-/-}$ mice.
- Lower abundance of Bacteroidetes and Verrucomicrobia.
- Lower abundance of Tenericutes in antibiotic-treated WT but not in antibiotic-treated IL-$10^{-/-}$ mice.

In antibiotic-treated WT mice decreased all the beneficious bacterial phyla and Proteobacteria phylum increased.

↓

Inflammatory intestinal microbiota.

↓

Normal response to antibiotic treatment.

Antibiotic-treated IL-$10^{-/-}$ mice microbiota remains similar to control groups → active immune response due to lack of IL-10

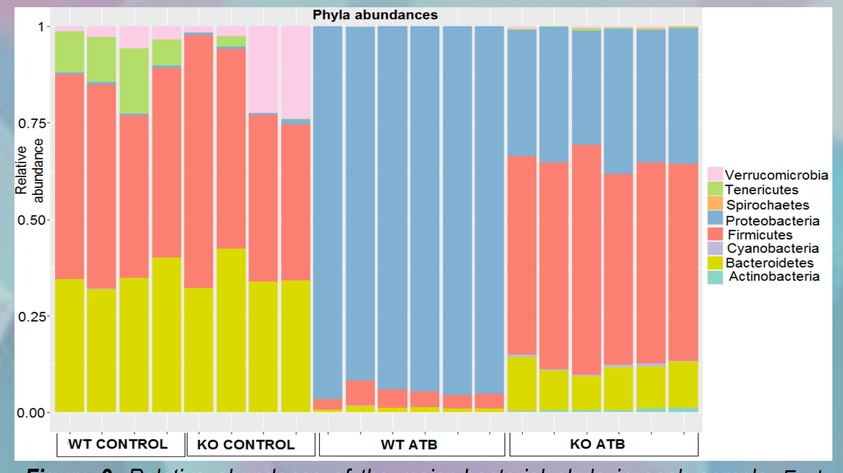

Figure 3*. Relative abundance of the major bacterial phyla in each sample. Each color represents a bacterial phylum and each column represents a sample.*

CONCLUDING REMARKS

- ✓ Intestinal dysbiosis plays an important role in the pathogenesis of IBD.
- ✓ There is correlation between changes observed in IL-$10^{-/-}$ mice and in human patients → valid animal model for IBD investigation.
- ✓ IL-$10^{-/-}$ mice show a paradoxical response to antibiotic treatment, confirming the role of bacteria in the onset and/or progression of IBD.
- ✓ Further studies are needed to know the usefulness of antibiotics to prevent or treat this disease.