

Microbiome in Multiple Sclerosis: Role of B-cells and Microglia

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Background: The gut microbiota is hypothesized to affect in MS by modulating the central and peripheral immunity.

Objective: (i) To assess the effect of Anti-CD-20 therapies on microbiome in MS; (ii) To investigate whether MS-associated microbiota affects microglia.

Methods: (i) To investigate the effect of therapy, we used stool samples from patients treated with Anti-CD20 therapies (n=16) and compared them untreated patients (n=34) and healthy controls (n=49). Bacterial DNA was extracted and the V4 region of the 16S rRNA gene was amplified and sequenced on the MiSeq platform (Illumina). QIIME2 was used to analyze and visualize the data. (ii) to investigate the effect of MS-associated microbiota on microglia, we selected fecal samples (n=3) from untreated relapsing and progressive MS patients and controls and transferred into antibiotic treated mice by oral gavage three times/week for two weeks. Microglia were then FACS sorted using the Fcrls microglial specific antibody. Transcriptional profiles from microglia were sequenced using Smart-Seq2 on an Illumina platform and results analyzed using DESeq2 software.

Results: We found that anti-CD20 treatment normalized several species altered in untreated MS patients and returned them to healthy control levels, including increasing *Blautia*, *Roseburia* and *Paraprevotellaceae*, and depleting *Eubacterium dolichum* and *Streptococcus anginosus*. We also found that fecal microbiome transfer from MS subjects differentially affected the transcriptional profile of microglia in naïve mice compared to healthy controls. 16 genes were upregulated and 20 genes down-regulated following fecal transfer. The down-regulated genes consisted primarily of the genes in the IL-10 pathway.

Conclusion: B-cell depleting therapies normalize several bacterial species that are altered in MS, which implies an association between B-cells and microbiome in MS. Moreover, microbiota may impact MS by shaping the microglia phenotype. These data together suggest the important effects of microbiome on both peripheral and central immune system in MS.