

GUT MICROBIOTA COMPOSITION MODIFIES TNBS COLITIS SEVERITY

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Objectives: Animal model of TNBS colitis depends also on gut microbiota composition. We used mice from two animal facilities – Institute of Microbiology (IMIC) and 1st Faculty of Medicine (FoM) for our experiments. Our main goal was to compare the sensitivity of those mice to TNBS-induced colitis, both toxic and hapten-type. **Methods:** Intra-rectal application of TNBS dissolved in 50% ethanol was used to induce toxic-type of colitis. Using the skin pre-sensitization with TNBS dissolved in olive oil 7 days before intra-rectal induction is essential for hapten-type colitis. Gut microbiota composition of FoM and IMIC mice was characterized by 16S rRNA sequencing. We also tested the ability to transfer the phenotype by colonizing germ-free (GF) mice. The cells isolated from mesenteric lymph nodes and spleens were analyzed by flow cytometry. **Results:** Based on our results, FoM mice are significantly more sensitive to toxic TNBS colitis according to Wallace score. Pre-sensitization increased colitis severity in IMIC mice, but not in FoM mice. Differences in gut microbiota composition were also observed. The main classes like *Bacilli*, *Clostridia* and *Bacteroidia* are similarly abundant, but classes *Campylobacteria* and *Verrucomicrobiae* are more abundant in both conventional and colonized IMIC mice, while class *Saccharimonadia* is more abundant in FoM conventional and also colonized mice. Representation of individual groups of T cells and cytokine production were also different. We found out that conventional mice from FoM have significantly more IFN- γ +CD4+T cells and less naive T cells in their spleen than mice from IMIC. Ex-GF mice colonized with microbiota of FoM mice have more TNF- α +CD4+ T-cells in their mesenteric lymph nodes than mice colonized with IMIC microbiota. FoM conventional mice also have significantly more effector memory T-cells (TEM) in their spleen. **Conclusion:** TEM cells might have essential role in animal model of TNBS colitis since they react fast to pathogen, which may be represented by bacteria translocated by induction itself. This suggests that gut microbiota modifies acute TNBS colitis severity by modulating T-cell functions.

Keywords: colitis, microbiota, sequencing, flow cytometry