IDENTIFICATION OF KEY GUT MICROBES INVOLVED IN AO AND DA RATS’ SUSCEPTIBILITY TO EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS

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Introduction:  
The development of effective therapy for multiple sclerosis (MS) that could alleviate the symptoms and stop the disease progression is the ultimate goal of MS research. It is becoming clear that gut microbiota represents an essential factor in development of gut associated lymphoid tissue (GALT). Studies on experimental autoimmune encephalomyelitis (EAE) indicated a role of altered gut microbiota in the MS development. Recent comparative studies on gut microbiota composition provided evidence of a moderate dysbiosis in the structure of gut microbiota in MS patients. Furthermore, 21 species showed significant differences in relative abundance between MS patients and healthy controls. These taxa comprised primarily of clostridial species belonging to Clostridia clusters XIVa and IV and Bacteroidetes. Moreover, it was found that Faecalibacterium was found to be lower abundant in MS patients.

Methods:  
In this study Albino Oxford (AO) and Dark Agouti (DA) rats were compared for their mesenteric lymph nodes (MLN) and Peyer’s patches (PP) cellular composition and their gut microbiota. AO rats are highly resistant to EAE induction, as they do not develop EAE in response to harsh immunization protocols that are efficient in other relatively resistant rat strains. On the other side, DA rats develop EAE after mild immunization, i.e. in the absence of any adjuvant. Housing of the rats was performed under conventional conditions. At least 6 rats were analyzed from each of the tested groups. EAE was induced with rat spinal cord homogenate in phosphate buffer saline mixed with equal volume of complete Freund’s adjuvant. GALT isolated cells were analyzed by cytofluorimetric analysis, while cytokine concentration was determined by sandwich ELISA. The microbial composition was determined by DGGE of PCR amplicons of 16S rRNA followed by nucleotide sequencing of bands of interest.

Results:  
Differences between AO and DA rats within GALT, including lower percentage of CD4+ T cells and generation of interleukin-17 and interferon- in MLN and PP of AO rats were found. Microbial analyses showed higher diversity of Lactobacillus spp. in EAE-resistant AO rats. In addition, an uncultivated species of Turicibacter genus was exclusively present in feces of non-immunized AO rats, but not in gut tissue samples. Some members of Firmicutes and Proteobacteria (Undibacterium oligocarboniphilum) were detected only in feces of DA rats between 12 and 15 days after induction (peak of the diseases). Interestingly, the members of Lachnospiraceae were detected in feces of healthy non-immunized DA rats, as well as in DA rats that remained healthy 16 days after induction together with an uncultivated species of Turicibacter genus.

Discussion:  
It was previously shown that members of the bacterial family Lachnospiraceae were dominantly present in gut microbial community of animals with symptoms of various diseases. In addition, it is tempting to assume that Turicibacter sp. contributes to the resistance of AO rats to EAE induction.

Keywords: Multiple sclerosis, Probiotics, Gut microbiota, Albino Oxford rats, Dark Agouti rats, Experimental autoimmune encephalomyelitis
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