ABSTRACT

Gut microbiota is important for our health and well-being, but when its composition is disrupted, it can induce or perpetuate several chronic inflammatory disorders, including inflammatory bowel diseases (IBD). The mechanisms which distinguish protective microbes from the deleterious or indifferent ones are largely unknown. The aim of this thesis was to study the interaction of the immune system with microbes that have different relationships to IBD pathogenesis.

Escherichia coli is a predominant aerobic microorganism of the gastrointestinal tract. This species includes microbes implicated in induction of IBD as well as in its therapy. Four *E. coli* strains with different relations to IBD were selected for our experiments: *E. coli* Nissle 1917 (EcN), which has been successfully used in IBD therapy, *E. coli* strains LF82 and p19A, which have been implicated in the pathogenesis of IBD, and *E. coli* strain K6, which has neither been implicated in pathogenesis nor in protection from this disease.

The experiments were performed both with living bacteria and inactivated ones. As the mode of inactivation may change the microbial antigenic structure, we measured how different methods of inactivation, i.e. 1% formaldehyde, exposure to heat or UV irradiation, influence the microbe's immunogenicity.

First, we analyzed the serum IgA and IgG against *E. coli* in sera of patients with IBD and healthy controls using indirect ELISA. The different mode of inactivation did not change the serum reactivity to any of the *E. coli* strains. There were no differences in the antibody responses among tested groups, except for the increase in IgA against the potentially pathogenic *E.coli* strain p19A in IBD patients.

Next, we cultivated spleen cells or cells isolated from mesenteric lymph nodes from either healthy mice or mice with active intestinal inflammation with inactivated bacteria, and measured the early cell activation (expression of CD69) by flow cytometry. In addition, we stimulated murine macrophage cell line (RAW264.7) with inactivated bacteria and measured the cell activation by Griess assay (nitrite production) and flow cytometry (CD40 expression). Overall, there were no significant differences among the stimuli.

Since the disruption of the epithelial cell layer is an important step in IBD pathogenesis, we measured the detachment of intestinal epithelial cells (murine MODE-K or human Caco-2) after their 4h cultivation with live *E. coli* by flow cytometry. In both cell lines, p19A detached the most epithelial cells, while EcN did not disrupt the cell monolayer at

all. In all cases, almost all detached cells were either dead (Hoechst+) or undergoing apoptosis (Annexin V+).

In conclusion, neither of the inactivation types induced significant changes in bacteria immunogenicity. The antibody avidity to both probiotic and pathogenic microbes was very similar in IBD patients and controls, except for p19A. We could not find any significant changes in cellular response to different *E. coli*, but both used pathobionts damaged the epithelial layer *in vitro*. Strain p19A caused the most extensive damage to epithelial cells, which suggests that this could be the major factor of virulence of this bacterium engaged in IBD pathogenesis.

Keywords: inflammatory bowel diseases, *E. coli*, inactivation, immune response