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In vitro models of inflammation-damaged intestinal barrier for screening the efficacy of probiotic strains

The integrity of the intestinal barrier is fundamental to gut health and homeostasis. Various inflammatory molecules, such as cytokines or reactive oxygen species, induce damage to tight junction proteins, leading to increased intestinal permeability, translocation of bacteria and/or endotoxins from the gut, and the development of diverse intestinal diseases, including inflammatory bowel disease (IBD). IBD can be simulated in a variety of in vitro models, ranging from simplified 2D to complex 3D models of intestinal organoids, each with its own advantages and disadvantages. Certain strains of probiotics, which are commonly present in the indigenous gut microbiota or found in fermented foods and dairy products, are known for their anti-inflammatory properties and their ability to protect and strengthen the intestinal barrier function. In vitro models serve as a starting point for biological and medical research and are also important for screening and selecting probiotic strains targeting IBD. Therefore, it is important to use the most relevant in vitro model for primary screening the ability of a probiotic strain to alleviate a compromised intestinal barrier. The lecture focuses on the comparison of 2D in vitro models of intestinal mucosa damaged by different inflammatory stimuli, which are commonly used to test the anti-inflammatory and protective properties of probiotic bacteria on the intestinal barrier. The differentiated human epithelial cell line Caco-2 has been widely used as a model of the intestinal epithelial barrier. The main limitation of Caco-2 cells is lack of mucus production, which is overcome by co-culturing them with HT29-MTX goblet cells, and such a model more closely resembles the properties of the human intestine in vivo. Various immune cells, such as macrophages, dendritic cells or T-lymphocytes, are found in the intestinal mucosa and play an essential role in intestinal homeostasis and can determine the flare-up or alleviation of disease. Therefore, incorporating immune cells into a model of the intestinal barrier may provide a more accurate simulation of human disease and also validate the effect of probiotic strains. In this work, we compared three different methods of intestinal barrier disruption (pro-inflammatory cytomix, pro-inflammatory macrophages and H_2O_2) and tested the efficacy of well-characterized and clinically relevant probiotic strains *Lacticaseibacillus rhamnosus* GG and *Lactiplantibacillus planatrum* 299v on parameters of intestinal barrier established by co-culture of Caco-2 and HT-29 MTX cell lines growing on the membrane of Transwell inserts. The aim of this talk is to present an overview of several in vitro models of IBD and provide insights into their advantages and limitations and their importance in testing the potential of probiotic bacteria.