ENGINEERING LACTIC ACID BACTERIA WITH CYTOKINE/CHEMOKINE BINDING ABILITY AND INFRARED FLUORESCENCE FOR IMAGING-GUIDED TREATMENT OF INFLAMMATORY BOWEL DISEASE

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Introduction:
Inflammatory bowel diseases (IBD) are idiopathic chronic intestinal inflammations that include ulcerative colitis and Crohn’s disease. The disease represents a significant health burden and its treatment costs exceed 1.7 billion dollars annually in the US. The exact etiology of IBD is unknown, but it is hypothesized to be a combination of genetic factors and alterations of the microbiota which together cause a dysregulation in the immune response and intestinal inflammation. Altered pattern of cytokine production offers an opportunity for therapeutic intervention by neutralization of pro-inflammatory cytokines, as already successfully demonstrated by monoclonal antibodies against TNFα. Lactic acid bacteria (LAB) are natural members of intestinal microbiota and are used for the treatment of IBD. Their probiotic activity can be upgraded by introducing cytokine/chemokine binding ability with genetic engineering or heterologous protein coating.

Methods:
Small protein cytokine/chemokine binders (TNFα-binding affibody, IL-17-binding fynomer, IL-23-binding adnectin and chemokine-binding evasin-1, evasin-3 and evasin-4 with the ability to bind CXCL-2, CXCL-3, CXCL-8, CCL-3, CCL-4 and CCL-5) were fused to secretion signal and cell wall anchor domain and inducibly expressed in model LAB Lactococcus lactis. Fusion proteins were secreted to the growth medium and enabled display of binding proteins on recombinant Lactococcus lactis and, upon producer cell removal, coating of non-recombinant species of LAB, thus providing a non-genetically modified organism alternative. Surface display was confirmed with flow cytometry and fluorescent microscopy, while the amount of cytokine/chemokine binding was assessed with whole-cell ELISA and Luminex. Infrared fluorescent protein (IRFP) was expressed in L. lactis and Lb. plantarum. Infrared bacteria were tracked in vivo in mice using Ivis spectrum imager.

Results:
Several non-recombinant species of Lactobacillus were coated with small model protein binder. Coating was particularly effective on Lactobacillus salivarius ATCC 11741, which has been suggested as an optimal non-recombinant host for surface display. Lb. salivarius coated with individual binders removed up to 78.3% of IL-17, 79.0% of TNFα and 26.7% of IL-23. Heterologous display of multiple binders on Lactobacillus salivarius enabled the construction of bacteria with concomitant IL-17-, IL-23- and TNFα-binding ability. Evasin-displaying bacteria bound from 37.5% to 67.0% of chemokines in vitro and neutralized CXCL-8 in colon epithelial cell model. IRFP-expressing L. lactis and Lb. plantarum were administered to healthy mice and imaged in vivo to demonstrate the ability of quantifying the bacteria and determining their transit time. IRFP-expressing bacteria that were coated with cytokine binder will enable concomitant in vivo imaging and therapeutic cytokine binding.

Discussion:
We have shown that engineered probiotics can bind various cytokines/chemokines, including their mixtures, and can interfere with cytokine signaling. We have also established a platform for in vivo fluorescent imaging of IRFP-expressing LAB. These two modalities were combined to engineer LAB that will concomitantly enable amelioration of excessive immune response in inflammatory bowel disease, as well as in vivo monitoring of bacterial fate.

Keywords:
Inflammatory bowel disease, Chemokine binding, Cytokine binding, Fluorescence animal imaging, Lactococcus lactis, Lactobacillus salivarius, Probiotics
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