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Engineering of the human commensal *Bacteroides ovatus* for the controlled in situ delivery of immunomodulatory proteins

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Soluble growth factors that promote epithelial repair and can suppress inflammation are of clinical interest as therapeutic agents for chronic intestinal inflammation that is characteristic of inflammatory bowel disease. However, when administered orally as recombinant proteins they are unstable and systemic administration increases the risk of unwanted side effects. Alternative means of delivery have been considered of which delivery via live microorganisms has shown real promise. The aim of this work was to genetically engineer the human commensal colonic bacterium *Bacteroides ovatus* to produce and secrete mammalian cytokines under the control of the xylanase promoter. The xylanase promoter was cloned and sequenced using an inverse-PCR approach. The coding sequence of the mature human cytokines TGF-β or KGF was PCR amplified from cDNA and cloned downstream of the xylanase promoter in the *E. coli-Bacteroides* suicide vector pBT2 that was introduced into *B. ovatus* by conjugation. Resulting transconjugants were tested for cytokine gene expression by reverse transcription-PCR and protein production by enzyme-linked immunosorbent assay (ELISA) and bioassay. Recombinant strains of *B. ovatus* secreted high levels of human TGF-β or KGF in a xylan dependent manner. Cytokine expression was minimal in the absence of xylan. Studies to assess the efficacy of these strains in the treatment and prevention of chemically induced colitis in mice are ongoing.

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