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Anti-inflammatory effects of a non-commensal soil bacterium in inflammatory bowel disease

Inflammatory bowel disease (IBD) refers to a spectrum of diseases characterized by chronic, relapsing inflammation of the gastrointestinal tract. The exact cause of IBD remains unknown, but available evidence suggests that an abnormal immune response against the microorganisms of the intestinal microbiota is responsible for the disease in genetically susceptible individuals. The increasing prevalence of IBD and other autoimmune diseases in the Western world has been associated with reduced exposure to environmental microorganisms. The "Old-friends hypothesis" suggests that the striking increase in chronic inflammatory disorders is largely due to a depletion of microorganisms that we have coevolved with, and that we depend on for proper development and regulation of the immune system. In this context both the commensal microbiota, pseudo-commensals and even the environmental microbiota can be thought of as essential immunoregulatory elements in mammals.

We have previously shown that the methanotrophic soil-bacterium *M. capsulatus* (Bath) can alleviate inflammation in the murine dextran sulfate sodium (DSS)-model of IBD. Furthermore, *M. capsulatus* (Bath) shows tropism towards murine and human *in vitro*-generated dendritic cells (DCs). Interactions with microorganisms are known to affect DC maturation, expression of co-stimulatory molecules and thus, their function as antigen presenting cells, and cytokine release, explaining the significance of DCs in the polarization of distinct effector T-cell subsets.

To scrutinize the anti-inflammatory effects observed in the DSS-colitis model, we want to investigate the ability of *M. capsulatus* to induce maturation and activation of human DCs. $CD14^+$ human peripheral blood monocytes will be isolated and differentiated to DCs using recombinant human cytokines rh IL-4 and rh GM-CSF. DCs will be co-incubated with *M. capsulatus* (Bath) and relevant control bacteria including immunoregulatory commensals from the gut microbiota and established probiotic bacteria. DCs matured by a cocktail of rh TNFα, PGE2 and LPS will be used as control. The resulting DCs will immunophenotyped by flow

cytometry using antibodies against surface markers associated with maturation/activation of human DCs: HLA-ABC, HLA-DR, CD40, CD80, CD86.

To examine DC cytokine release, the cytokine profile of culture supernatants from DCs stimulated with *M. capsulatus* (Bath) or control bacteria will be determined using human ELISA-sets to detect human IL-4, human IFN-gamma, IL-12p70 total, human TNF-a and human IL-10. DC-mediated effects on T-cell polarization will be investigated by co-cultivation of monocyte-derived DCs matured by the different bacteria in the presence of anti-CD3/CD28 stimulated human peripheral blood T cells. Again, the cytokine profile of the culture supernatants will be determined using the same human ELISA sets. T effector cells subsets will then be analyzed by multiparameter flow cytometry using CD4 FITC/CD3 PE/CD8, CD25 and antibodies against Tbet, GATA3, FoxP3 and RORgt. Accumulation of $CD4^+$ T cells in the intestinal mucosa is associated with IBD. The α4β7 integrin that binds to the mucosal homing receptor MAdCAM is of particular interest in this context, and T cells primed by DCs stimulated with *M. caps*ulatus (Bath) or control bacteria will be examined for expression of CD49d, the a-chain of the a4b7 integrin.