Commensal microbes, immune reactivity and childhood inflammatory bowel disease

AKADEMISK AVHANDLING

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I. Barkman C., Saalman, R., Lindberg E., Wolving M., Ahrné S., Molin G., Adlerberth I., Wold A.E. **Intestinal microbiota at début of childhood inflammatory bowel disease.** In manuscript

II. Barkman C., Saalman, R., Rudin A., Wold A.E. **Activation, homing and memory markers on blood circulating B and T lymphocytes at début of childhood inflammatory bowel disease.** In manuscript.

III. Barkman C., Martner A., Hessle C., Wold A.E. **Soluble bacterial constituents down-regulate secretion of IL-12 in response to intact Gram-positive bacteria.** Microbes and Infection 10 (2008) 1484-93

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Commensal microbes, immune reactivity and childhood inflammatory bowel disease

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Abstract

Inflammatory bowel disease (IBD) is characterized by chronic and relapsing intestinal inflammation of unknown etiology, but immune activation by the commensal microbiota probably plays a major role. The two major categories of IBD are ulcerative colitis and Crohn's disease. One fifth of the cases present in childhood and Sweden has a high and rising incidence of pediatric IBD. The aim of this thesis was to study the composition of the small and large intestinal microbiota and signs of activation on lymphocyte subsets in the blood circulation in children at the début of IBD, before initiation of treatment. Further, the requirements of commensal Gram-positive bacteria to initiate production of IL-12, a cytokine stimulating Th1 reactions in innate immune cells, was studied using blood obtained from healthy donors.

Blood, faecal and duodenal samples were obtained from children referred to a pediatric gastroenterology centre due to suspected IBD. Samples of the microbiota were cultivated quantitatively for aerobic and anaerobic bacteria. After establishment of diagnosis, the composition of the microbiota and lymphocyte subsets were compared between children with ulcerative colitis, Crohn's disease, symptomatic children found not to have IBD (diseased controls) and healthy controls. The microbiota mainly in children with ulcerative colitis was shown to be altered, with decreased counts of anaerobic Gram-positive bacteria such as bifidobacteria and clostridia. Whereas bifidobacterial counts normalized with treatment, clostridial populations remained low. Both children with ulcerative colitis and Crohn's disease had an increased fraction of Gram-negative bacteria in the stools, compared with controls. Blood cell subsets were analysed by flow cytometry for activation and memory markers. Children with ulcerative colitis displayed strong activation of circulating T cells, especially manifested as increased expression of β1-integins. Children with Crohn's disease had few memory B-cells, suggesting immunological immaturity.

Our studies further revealed that Gram-positive bacteria are major stimuli for IL-12 production in monocytes as long as they remain intact, but that fragments of Gram-positive bacteria inhibit IL-12 production in blood cells. This may be a physiological feedback circuit, since such fragments may signal that further activation of the phagocyte via the IL-12/IFN-γ loop is unnecessary. IL-12 production in response to intact Gram-positive bacteria required phagocytosis, activation of TLR2- and Nod2-receptors, demonstrated by chemical blocking, anti-human TLR antibodies, and binding of synthetic or natural ligands. Further, IL-12 production induced by intact Gram-positive bacteria required signalling via PIP3, NF-κB and JNK. These pathways differed from those inducing IL-12 production in response to LPS in interferon-γ primed monocytes. Thus, Gram-positive bacteria have unique and important immunomodulating properties that may influence IBD development.

Key words: inflammatory bowel disease, children, ulcerative colitis, Crohn's disease, microbiota, T cells, B cells, cytokine, IL-12, monocytes, phagocytosis, intracellular signalling pathways