Innate immunity of human intestinal epithelium in childhood celiac disease
- Influences from celiac disease associated bacteria and dietary oats

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Akademisk avhandling

som med vederbörligt tillstånd av Rektor vid Umeå universitet för avläggande av filosofie/medicine doktorsexamen framläggs till offentligt förvar i A5_R0 (Farmakologens seminarierum), byggnad 6A, torsdagen den 23 november, kl. 09:00.
Avhandlingen kommer att förvaras på engelska.

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Background & Aims: Celiac disease (CD) is a chronic inflammatory small-bowel enteropathy caused by permanent intolerance to gliadin in wheat gluten, and related proteins in rye and barley. It is disputed whether CD patients tolerate oats. The only treatment of CD is lifelong gluten-free diet (GFD). Only individuals that carry the HLA-DQ2 and/or DQ8 alleles, and eat gluten can develop CD. Dysbiosis in the gut microbiota is a suggested risk factor for CD. T cells in small intestinal mucosa, including intraepithelial lymphocytes (IELs), are known to be important in the pathogenesis of CD. In contrast, the role of intestinal epithelial cells (IECs) is poorly understood. In this thesis we investigated the role of IECs in the immune pathology of CD from duodenal mucosa of children with CD, clinical controls and treated CD. We also investigated the role of CD associated bacteria and oat supplemented GFD on the mucosal immune system.

Results: A new CD-associated bacterium, *Prevotella jejuni*, was isolated and characterized. It is a saccharolytic and proteolytic anaerobe. More than 25 defense-related genes, including IRF1, SPINK4, ITLN1, OAS2, CIITA, HLA-DMB, HLA-DOB, PSMB9, TAP1, BTN3A1, and CX3CL1, were upregulated in IECs in active CD. In two *in vitro* models for intestinal epithelium, small intestine enteroids and T84 polarized tight monolayers, we showed that 70% of these genes were upregulated by interferon (IFN)-γ via the IRF1 pathway. IRF1 was also upregulated by the CD-associated bacteria *P. jejuni* and *Actinomyces gravenitzii*. IECs expressed the NLRP6/8 inflammasome yielding CASP1 and biologically active interleukin (IL)-18, which induces IFN-γ in IELs. *P. jejuni* bound the intestinal epithelial cell lines T84, Caco2, HT29, and INT407, while *Lachnoanaerobaculum umeaense* preferentially bound Caco2. *P. jejuni* caused decreased transepithelial resistance over tight monolayers, while *L. umeaense* caused an increase. *P. jejuni* upregulated mRNAs for the detoxification molecules CYP1A1, CYP1A2, CYP1B1, and TIPARP, the chemokines CX3CL1, CXCL1, and CXCL10, the sialyltransferase ST3GAL4, and the inflammation promoting protein S100A3 in tight monolayers. *L. umeaense* upregulated the chemokines CCL20 and CXCL10, and down-regulated TLR2. In a randomized, double-blinded intervention trial comparing two study-groups, standard GFD and oat-containing GFD, we found that mRNAs for several immune effector molecules and tight junction proteins were only reduced in patients receiving GFD, but not in a substantial fraction of patients on GFD with oats. The down-regulatory cytokines IL-10 and TGF-β1, the cytotoxicity-activating NK-receptors NKG2C and NKG2E, and the tight junction protein claudin-4 remained elevated in the study group on GFD with oats.

Conclusions: IECs are far from inactive in CD. A key factor in the epithelial reaction in CD appears to be over-expression of IRF1 in IECs. Dual activation of IRF1 and IRF1-regulated genes, both directly by *P. jejuni* and indirectly by IFN-γ via the IL-18-inflammasome, would drastically enhance the inflammatory response and lead to the pathological situation seen in active CD. *P. jejuni* harms the intestinal epithelium, i.e., it is a likely risk factor for CD, while *L. umeaense* strengthen barrier function and local immunity, possibly acting as a protective. A fraction of CD patients should avoid oats in the diet.

Keywords: Celiac disease, dietary oats, gut microbiota, intestinal epithelium, IEL, IFN-γ, IRF1, IL-18, CX3CL1, inflammasome, permeability, *Prevotella jejuni*, *Lachnoanaerobaculum umeaense*