GLIADIN BINDING TO CXCR3 INDUCES A MyD88- AND G-COUPLED PROTEIN RECEPTOR-DEPENDENT INCREASED INTESTINAL PERMEABILITY AND ZONULIN RELEASE

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Background & premises

• Celiac disease (CD) is an auto-immune enteropathy triggered by the ingestion of gluten

• Gliadin, a component of the grain protein gluten, is known to induce increased intestinal permeability, which is considered an early crucial biological event in the pathogenesis of CD

• Zonulin induces tight junction disassembly. It is therefore considered to be involved in CD

• In CD:
An increased and persistent release of zonulin and a significant increase in intestinal permeability (S. Drago et al. Scand J Gastroenterol. 2006)
Apical, but not basolateral, exposure to gliadin led to zonulin release (MG Clemente et al. Gut 2003)

• We recently identified the chemokine receptor CXCR3 as the receptor to which gliadin binds
Aim

To explore the function of CXCR3 after gliadin binding
CXCR3 expression on transfected HEK293T cells

pcDNA-transfected HEK cells  CXCR3-transfected HEK cells

Isotype-matched control antibody (blue)
Anti-CXCR3 antibody (red)
Co-localization of PT-gliadin and CXCR3

CXCR3-transfected HEK cells
PT-gliadin treatment

pcDNA-transfected HEK cells
PT-gliadin treatment

CXCR3-transfected HEK cells
BSA treatment
Basal CXCR3 expression on intestinal epithelial cell lines

Caco-2

IEC6

Isotype control

CXCR3
CXCR3 is expressed in mouse intestinal epithelium and in lamina propria
CXCR3 expression in human intestinal tissues

Active CD

Non-celiac

100x
Intestinal CXCR3 expression is elevated in active CD and returns to baseline during GFD.
PT-gliadin induces increased permeability in wild-type-, but not in CXCR3⁻/⁻, intestinal segments.
PT-gliadin induces increased zonulin release in wild-type-, but not in CXCR3−/−, intestinal segments.
Pre-incubation with *Pertussis toxin* prevents PT-gliadin induced increase of intestinal permeability.
Physical association of CXCR3 and MyD88 after PT-gliadin Binding Dose- and Time-dependent

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<th>PT-gliadin (mg/ml)</th>
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<th>0.25</th>
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Conclusions

• We have shown colocalization of CXCR3 and gliadin.

• CXCR3 was expressed at intestinal epithelial level, and staining showed an apical localization.

• CXCR3 gene expression in biopsy specimens was enhanced in active CD compared to controls, and this expression seemed to return to base-line with GFD.

• In functional studies, binding of gliadin to CXCR3 induced enhanced zonulin release and increased intestinal permeability, an early event in CD pathogenesis.

• The effect of gliadin on tight junction disassembly appeared to be MyD88-dependent.
Hypothesis

• In genetically predisposed individuals binding of gliadin to CXCR3 is the first step leading to tight junction disruption and subsequent increase in intestinal permeability.

• This allows the passage of gliadin into the intestinal mucosa leading to a cascade of immune events that eventually lead to autoimmunity.