Gliadin Binding to CXCR3 Causes Zonulin Release and Increased Intestinal Permeability

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Background

• Celiac disease is an immune-mediated enteropathy triggered by the ingestion of gluten-containing grains;

• Celiac disease is unique since it is the only autoimmune disease for which the triggering environmental factor, gliadin, is known;

• Gliadin induces the release of zonulin (an intestinal protein involved in the regulation of intestinal permeability) that initiates the onset of the autoimmune process;

• Epithelial zonulin release and the parallel increase in intestinal permeability occur after apical, but not basolateral, exposure to gliadin
AIM

To identify the gliadin intestinal epithelial apical receptor involved in zonulin release and loss in intestinal barrier function.
### Gliadin Affinity Column Results: Gliadin Receptor Binding

**Gliadin Receptor Binding**

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<th>Measured Mass (M)</th>
<th>Avg/Mono</th>
<th>Computed Mass</th>
<th>Error (ppm)</th>
<th>Residues Start To</th>
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<th>Peptide sequence</th>
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</table>

*Similar to interferon-inducible protein 10 (IP-10, CXCR3) receptor - mouse*
Gliadin Binds to CXCR3

CXCR3-transfected HEK293T cells express CXCR3

- 4.42% MFI 11.9
- 61.78% MFI 95.5
Gliadin Binds to CXCR3

Co-localization of PT-gliadin and CXCR3 in CXCR3-transfected HEK293 T cells:

Cells transfected with vector + CXCR3

Cells transfected with vector alone

Gliadin FITC

CXCR3 TRITC

Nucleus DAPI

Merge

Magnification 100x

Magnification 100x
CXCR3 is Expressed on Intestinal Epithelial Cells

CXCR3 expression in intestinal epithelial cell lines

CaCo-2

IEC6

Magnification 100x
CXCR3 is Expressed on Intestinal Epithelial Cells

CXCR3 mRNA expression in CaCo-2 cells and CXCR3-transfected HEK293T cells
CXCR3 is Expressed on The Enterocytes Brush Border and in the Intestinal Lamina Propria

Red: CXCR3
Green: Cytoskeleton
Blue: Nuclei
CXCR3 is Expressed on Intestinal Epithelial Cells

Laser capture microdissection of mouse intestinal tissue: CXCR3 mRNA expression

Laser Capture Lamina propria preparation

Real Time RT-PCR

Laser Capture Epithelial tissue preparation

CXCR3 expression

2.\text{-}dCt

LP

Epithelium

0

3.0 \times 10^{-5}

6.0 \times 10^{-5}

9.0 \times 10^{-5}

1.2 \times 10^{-4}
CXCR3^{-/-} Mice do not Release Zonulin Following Intestinal Challenge with PT-gliadin

PT-gliadin induces increased zonulin release in wild-type but not CXCR3^{-/-} mice

* p < 0.05
PT-gliadin Induces TEER Changes in Wild-type but not CXCR3−/− mice

**p<0.02
*p<0.05
CXCR3 Expression in Human Intestinal Tissue is Up-Regulated by Gliadin Exposure in Celiac Disease (CD) Patients

CXCR3 mRNA expression in human intestinal tissues

*\(p=0.004\)
Conclusions

• The chemokine receptor CXCR3 is the gliadin intestinal mucosal receptor that leads to activation of the zonulin pathway;

• CXCR3 is expressed on the surface of enterocytes;

• CXCR3 is over-expressed in celiac disease patients when ingesting gluten and returns to baseline expression when patients embrace a gluten free diet.