Gliadin binding to CXCR3 induces a MyD88- and G-coupled protein receptor-dependent increased intestinal permeability and zonulin release

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Background: Increase in both zonulin release and intestinal permeability (IP) occur after apical but not basolateral intestinal epithelial gliadin exposure, suggesting that gliadin interacts with a luminal gut receptor. Recently, we identified the chemokine receptor, CXCR3, as the receptor for gliadin. **Aim:** to explore the function of CXCR3 after gliadin binding. **Methods:** CXCR3 expression in intestinal epithelial cells and intestinal mucosa, gliadin binding to CXCR3, and signaling pathways activated by this binding were studied *in vitro*, using immuno-fluorescence microscopy, real time (rt) RT-PCR and Western blot analysis. The role of CXCR3 in gliadin-mediated zonulin release and intestinal transepithelial electrical resistance (TEER) changes were studied *ex vivo* using C57BL/6 wild type (WT)- and $CXCR3^{-/-}$-mouse small intestine in microsnapwell assays, laser capture microdissection, rtRT-PCR, and immuno-histochemistry.
Results: *In vitro* experiments showed specific co-localization of gliadin with CXCR3. CXCR3 was expressed in intestinal epithelium and lamina propria (LP) and was up-regulated during active celiac disease (CD) but not after remission following gluten-free diet (GFD). Gliadin exposure led to physical association of CXCR3 with MyD88. Increased zonulin release and decreased TEER were detected in WT, but not in $CXCR3^{-/-}$ intestinal segments after gliadin exposure. Pre-incubation of WT gut tissue with G-coupled protein receptor inhibitor, pertussis toxin (PT), prevented the gliadin-induced drop in TEER (1.5%, p=NS) compared to media controls (33.5%, p=0.034) and inactive genetic mutant of PT (31.2%, p=0.06). **Conclusion:** Gliadin binding to CXCR3 leads to activation of the zonulin pathway and IP increase in a MyD88- and G-coupled protein receptor-dependent fashion. CXCR3 intestinal expression was detected at epithelial and LP level and was up-regulated during active CD but not after CD remission.

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