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

KM Lammers, R Lu, J Brownley, A Sapone, B Lu, C Gerard, SN Vogel, A Fasano

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 persistant 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*)

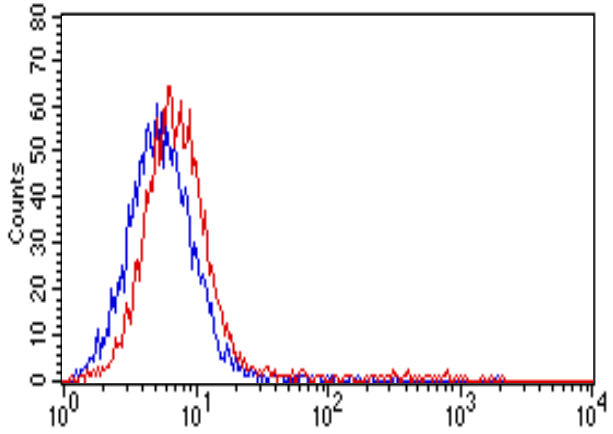
- We recently identified the chemokine receptor **CXCR3** as the receptor to which gliadin binds

Aim

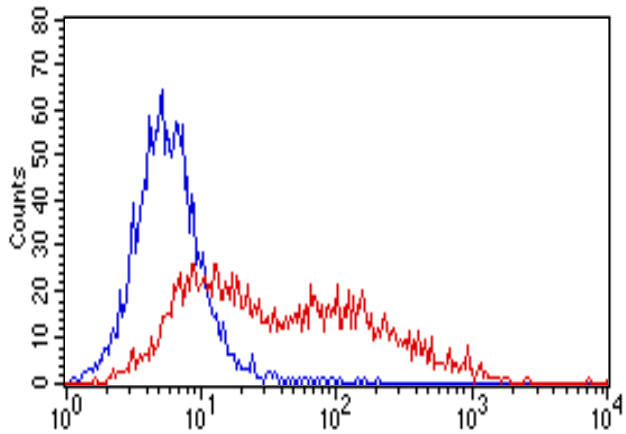
To explore the function of CXCR3 after gliadin binding

CXCR3 expression on transfected HEK293T cells

pcDNA-transfected HEK cells



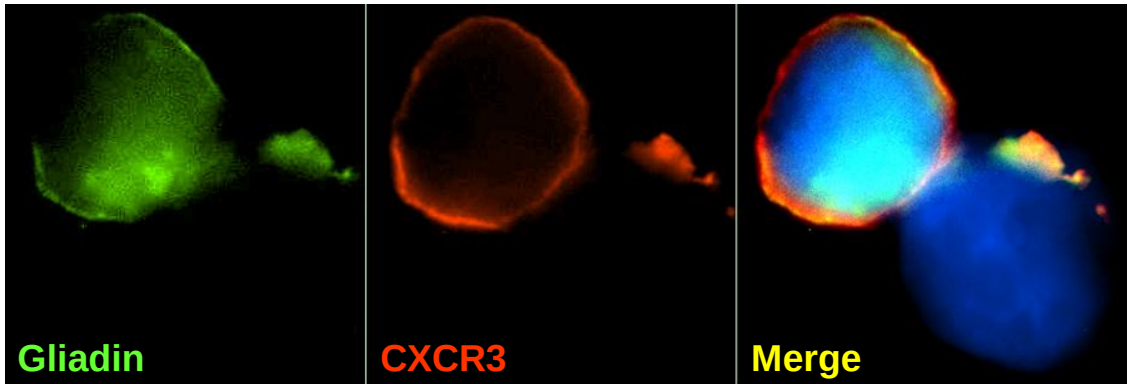
CXCR3-transfected HEK cells



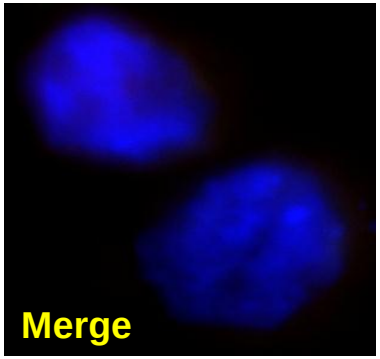
Isotype-matched control antibody (blue)
Anti-CXCR3 antibody (red)

Co-localization of PT-gliadin and CXCR3

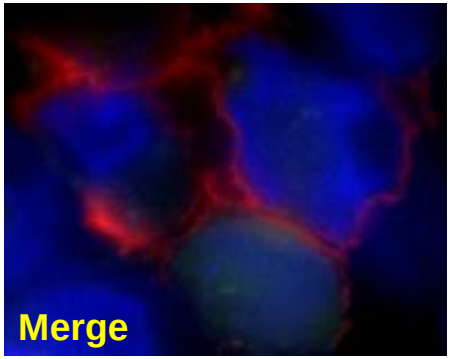
CXCR3-transfected HEK cells
PT-gliadin treatment



pcDNA-transfected HEK cells
PT-gliadin treatment



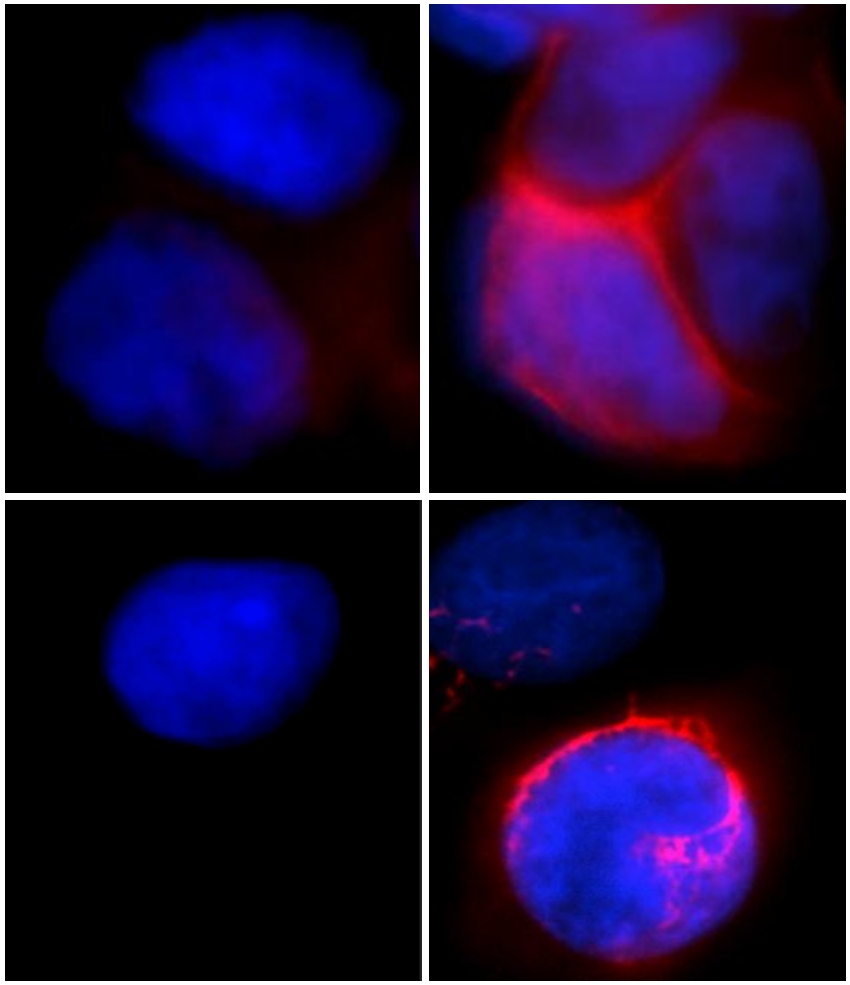
CXCR3-transfected HEK cells
BSA treatment



Basal CXCR3 expression on intestinal epithelial cell lines

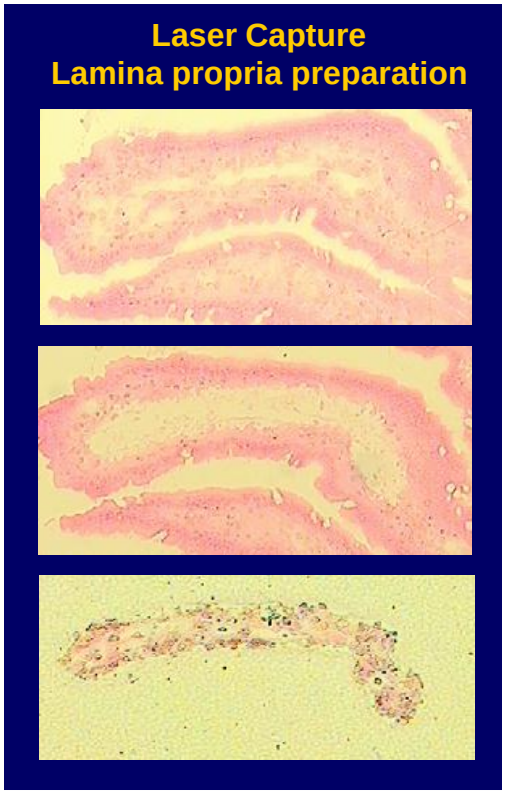
Caco-2

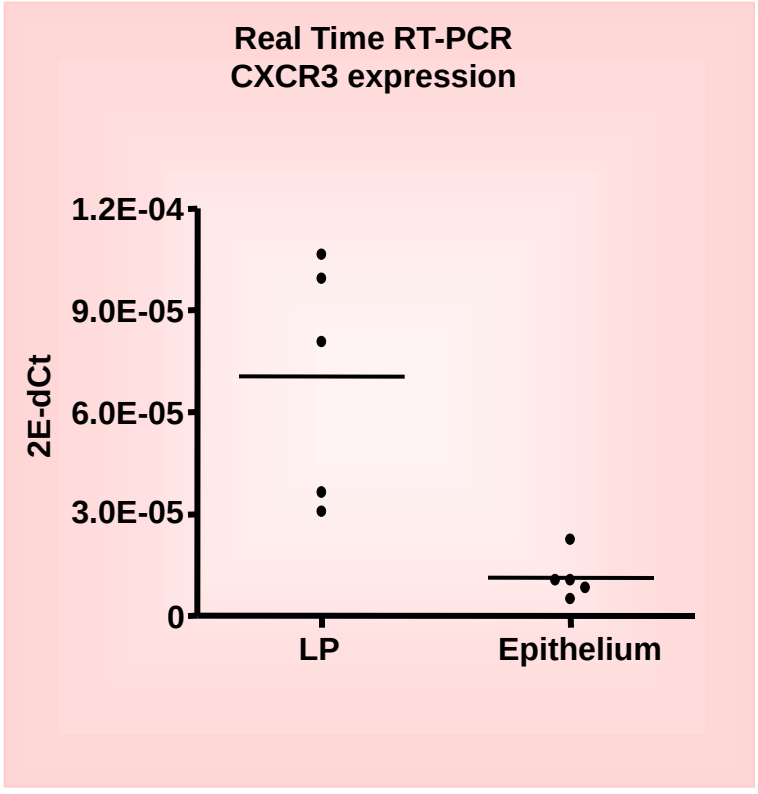
IEC6

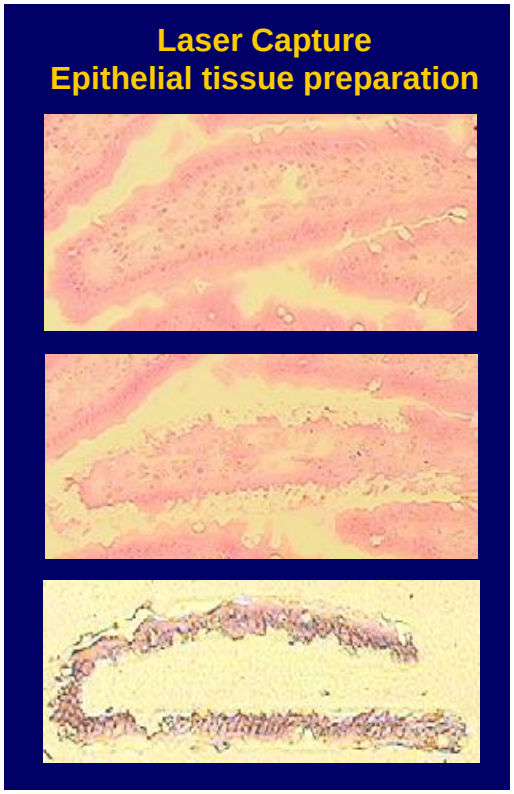


Isotype control CXCR3

CXCR3 is expressed in mouse intestinal epithelium and in lamina propria

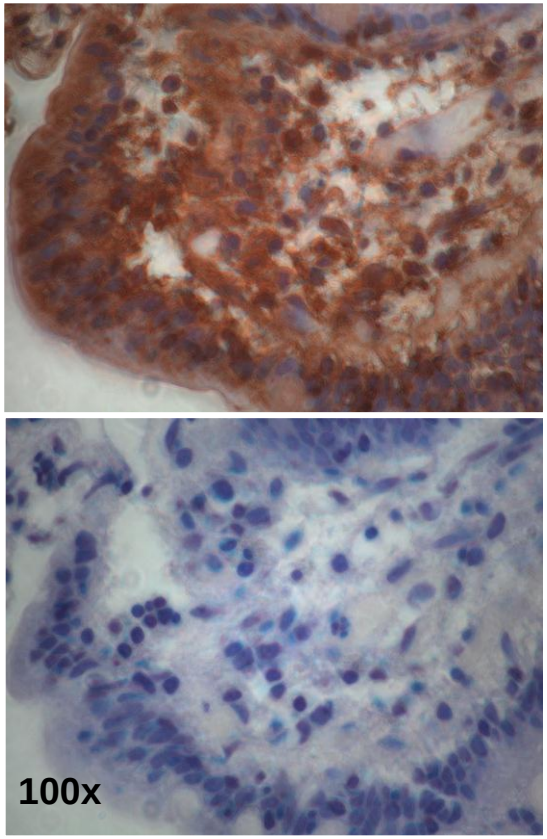




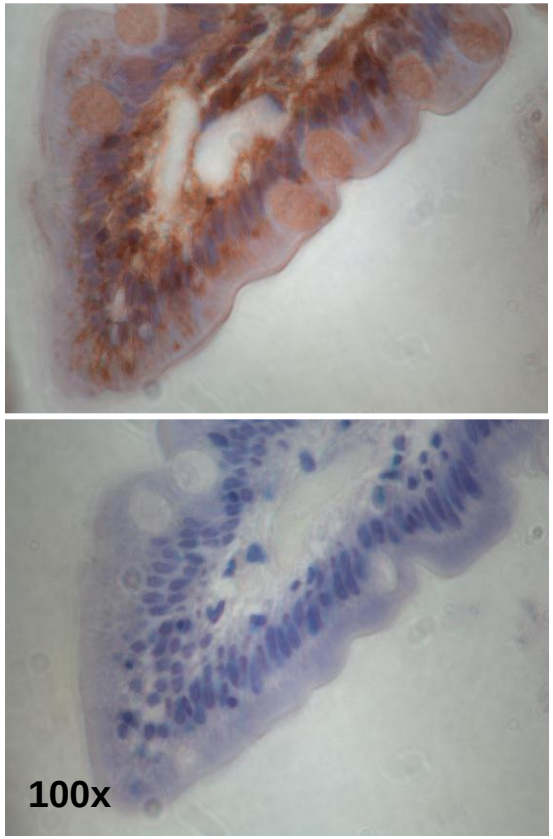


CXCR3 expression in human intestinal tissues

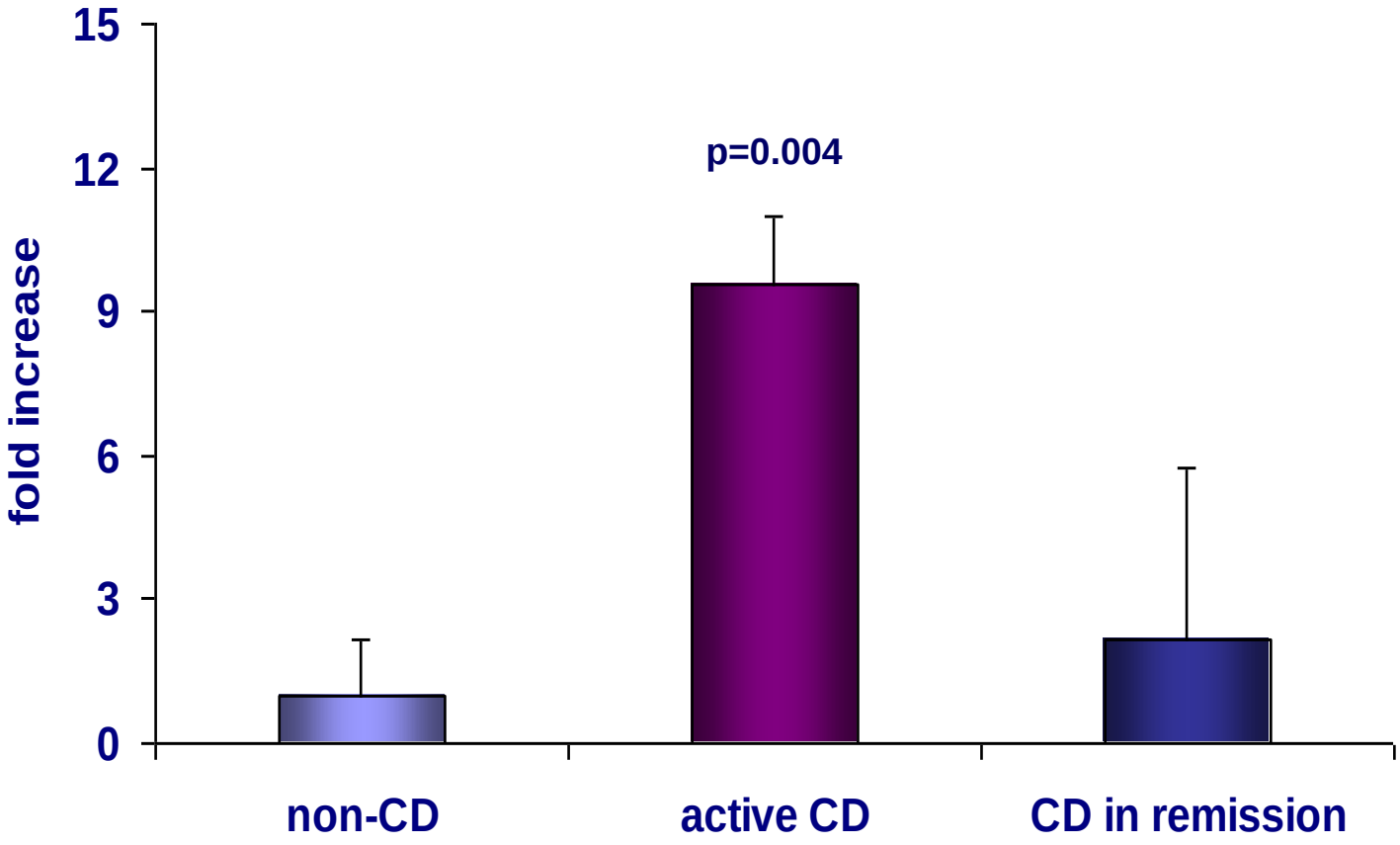
Active CD



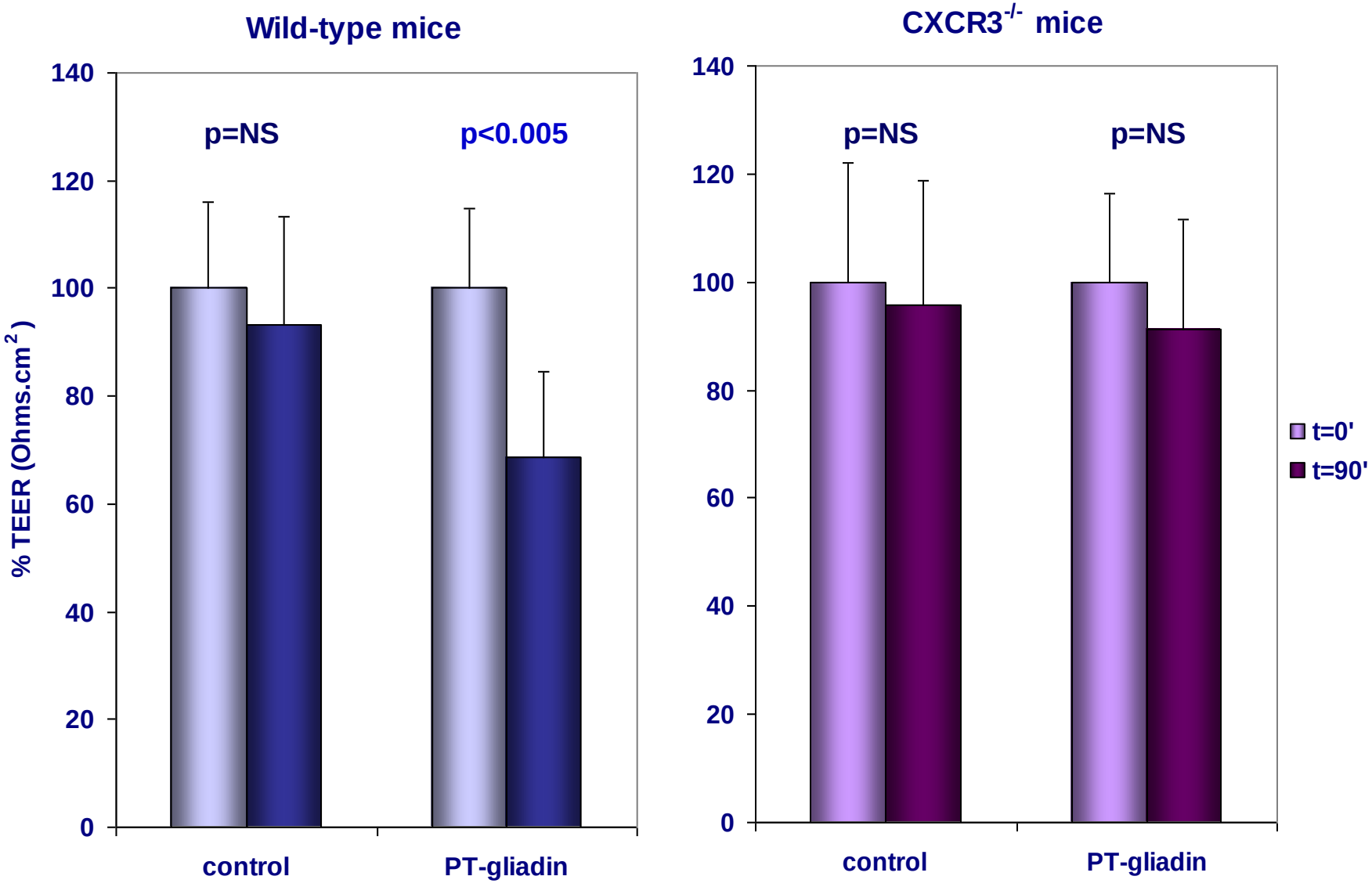
Non-celiac



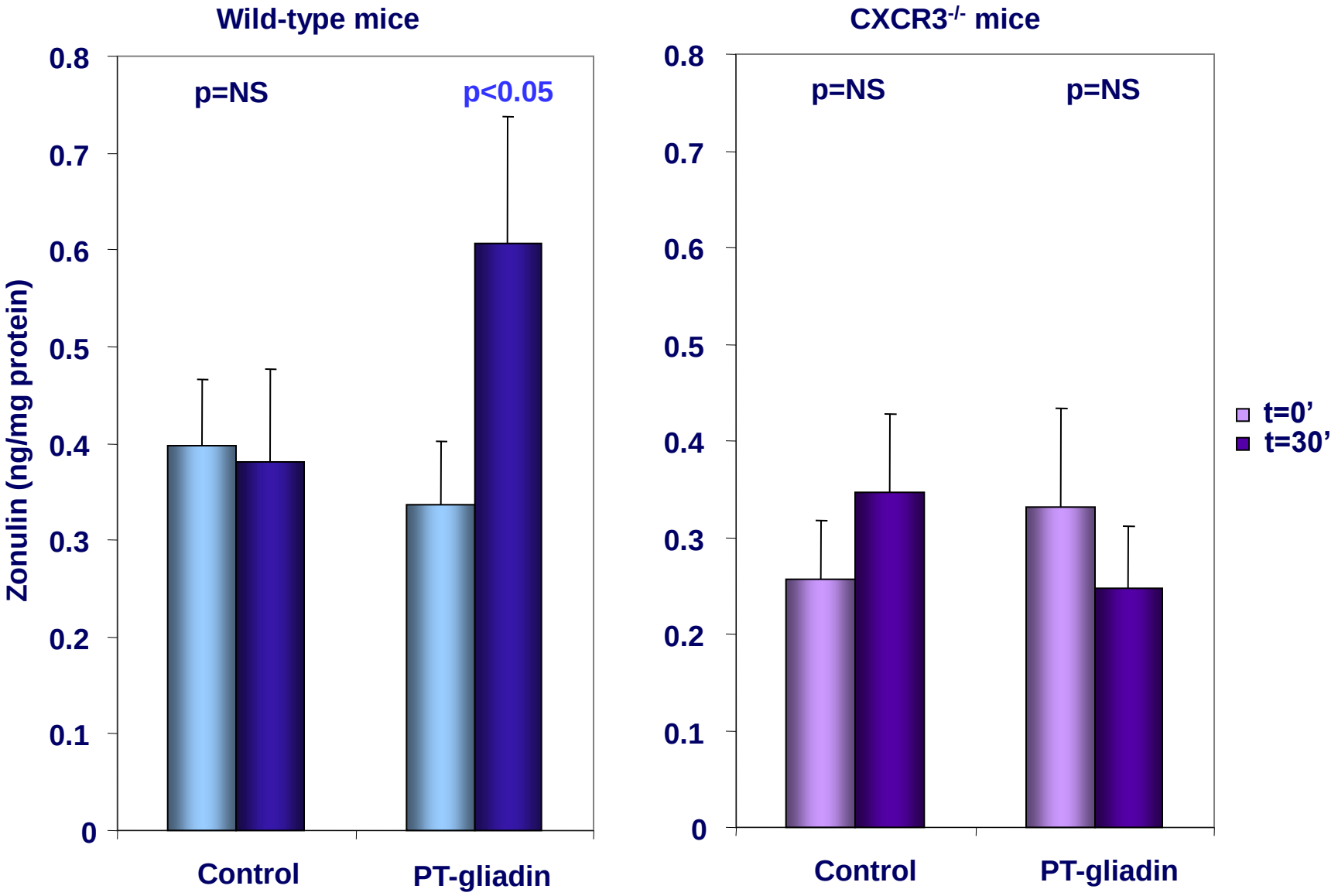
Intestinal CXCR3 expression is elevated in active CD and returns to baseline during GFD



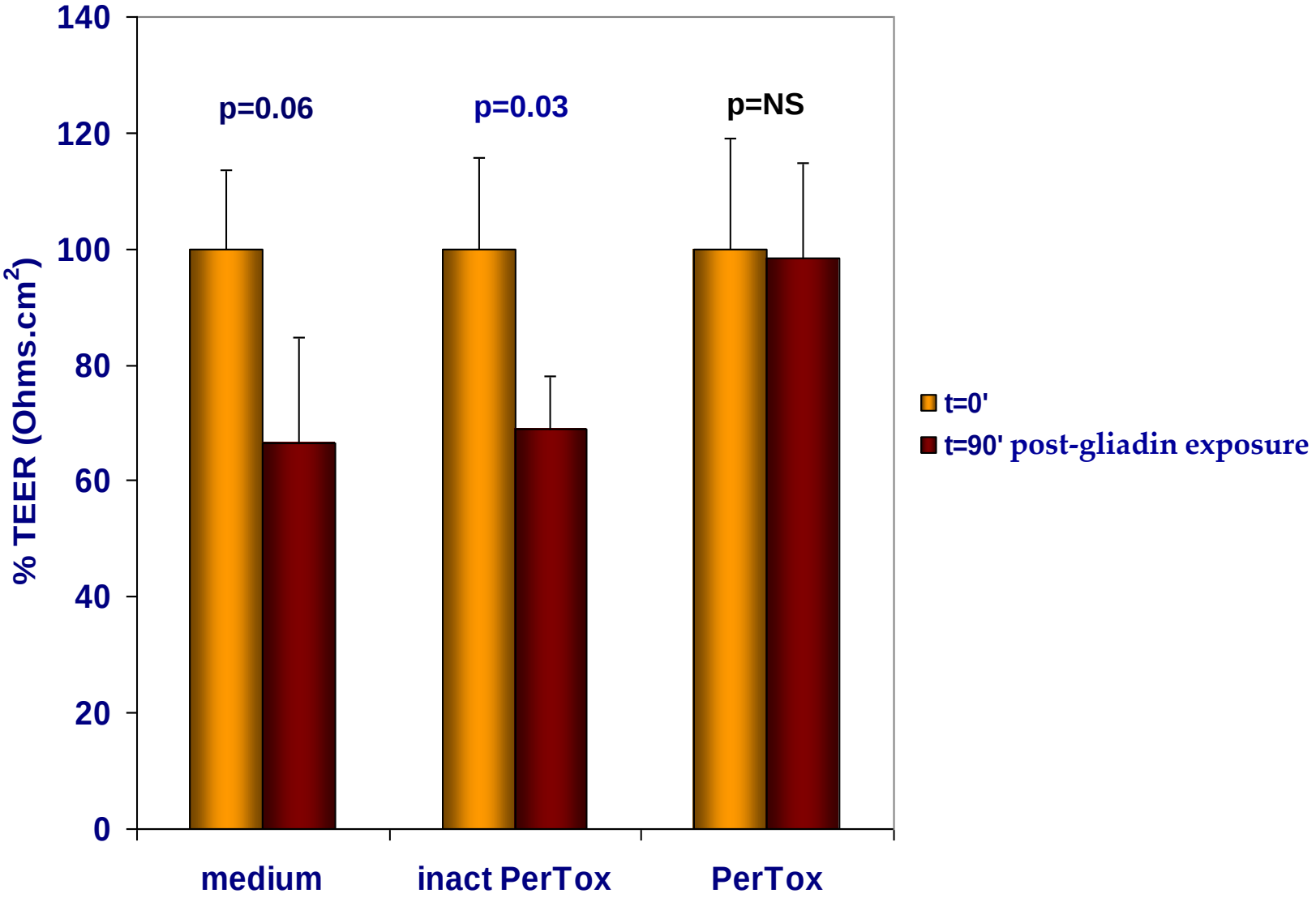
PT-gliadin induces increased permeability in wild-type-, but not in CXCR3$^{-/-}$, intestinal segments



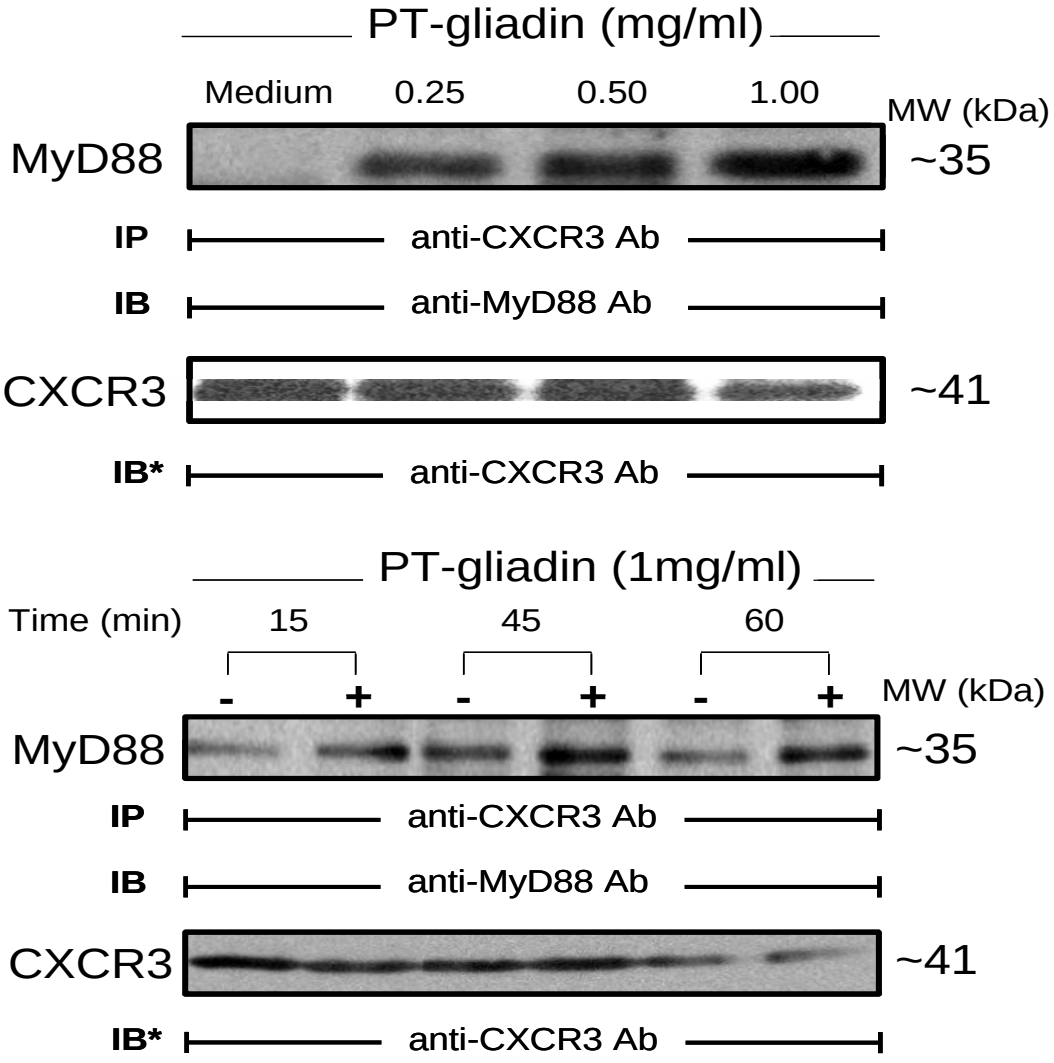
PT-gliadin induces increased zonulin release in wild-type-, but not in CXCR3$^{-/-}$, intestinal segments



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



Conclusions

- We have shown colocalization of CXCR3 and gliadin

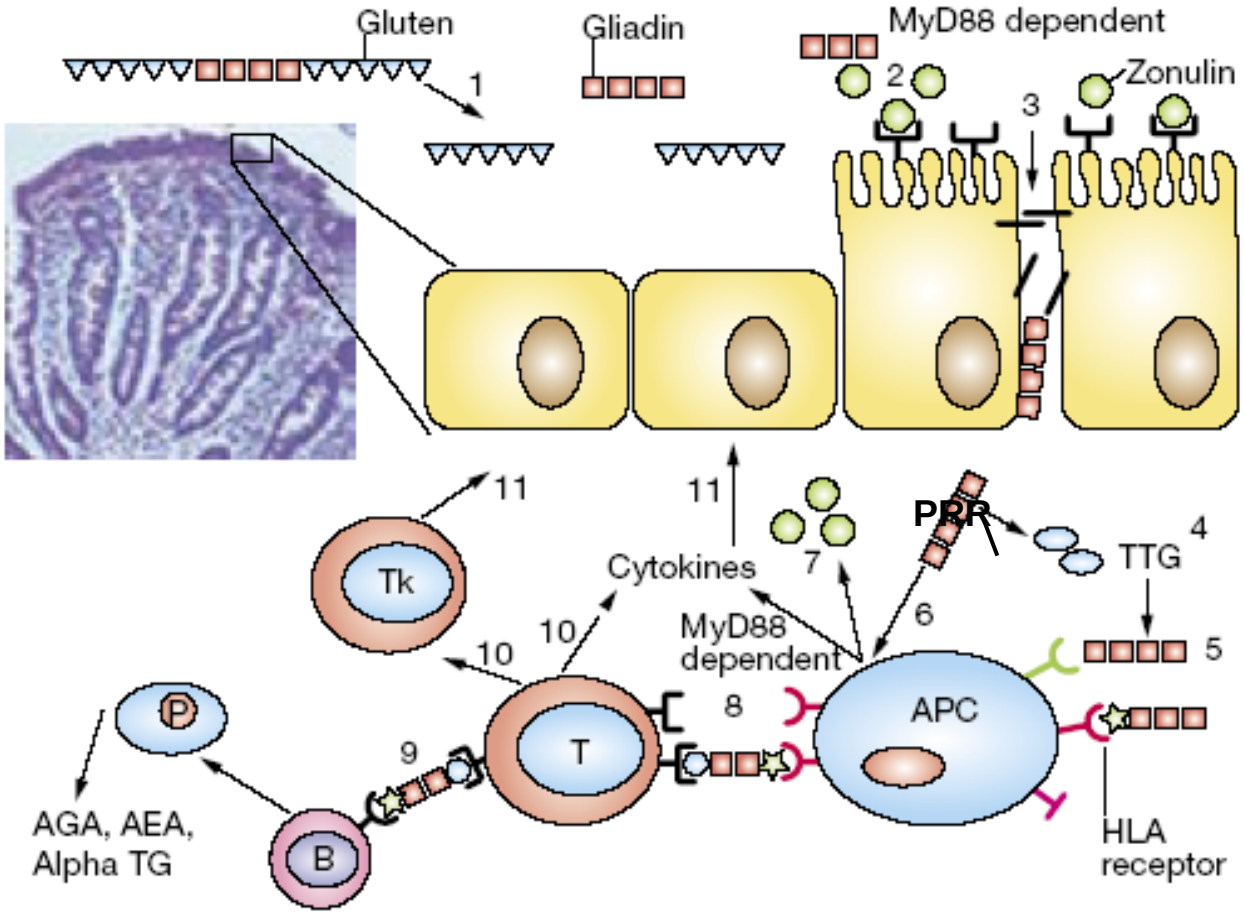
- CXCR3 was expressed at intestinal epithelial level, and staining showed an apical localization

- 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.

A. Fasano and T. Shea Donohue, Nature Clin Pract Gastroenterol Hepatol 2005