Intestinal Proteinase-Activated Receptor (PAR)-2 Is Up-Regulated In Active Celiac Disease and Co-Localize With Zot/Zonulin Receptor

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Background: Zonulin, the eukaryiotic Zonula occludens toxin (Zot) analogue that modulates intercellular tight junctions, is up-regulated during the acute phase of celiac disease (CD) and may be responsible for the increased intestinal permeability characteristic of the disease. Our recent data suggest that zonulin is structurally similar to mast cell proteinase (MCP)-II, an activator of proteinase-activated receptor (PAR)-2 which is expressed in human intestinal mucosa where plays a role in increasing intestinal permeability and fluid secretion. **Aim**: to investigate whether (1) PAR-2 and/or Zot/zonulin receptor are up-regulated during the acute phase of CD; (2) PAR-2 and Zot/zonulin receptor co-localize in human duodenal mucosa. **Methods**: human duodenal biopsies obtained from both patients with (N=15) and without (N=5) CD were incubated with either FITC-labelled Zot/zonulin binding antagonist FZI/0 peptide or with mouse monoclonal anti-human PAR-2 antibodies, followed by incubation with rhodamine-labelled anti-mouse IgG antibodies in single and double staining immunofluorescence experiments. **Results**: The immunofluorescent staining patterns visualized with FITC-FZI/0 and anti-PAR-2 antibodies showed over-expression of both Zot/zonulin receptor and PAR-2 in duodenal biopsies from CD patients compared to controls. Overlapping of the two images showed co-localization of the PAR-2 and FZI/0 peptide in both celiac and non-celiac intestinal specimens, suggesting that FZI/0 binds to a site very close to or synonymous with PAR-2 in human duodenal mucosa. **Conclusions**: Both Zot/zonulin receptor and PAR-2 are over-expressed during the acute phase of CD, which is characterized by increased intestinal permeability. Since it has been reported that pro-inflammatory cytokines, including TNFα, can up-regulate PAR-2 expression, it is logical to postulate that our findings can be related to the increased levels of TNFα characteristic of the acute phase of CD. The two receptors co-localize in human intestinal mucosa, suggesting that zonulin could represent an endogenous ligand of PAR-2. The elevated expression of both receptor (PAR-2) and ligand (zonulin) can be responsible of the sustained increased intestinal permeability described in CD.

Up-regulation and co-localization of intestinal proteinase-activated receptor (PAR)-2 and Zot/zonulin receptor in active celiac disease

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BACKGROUND

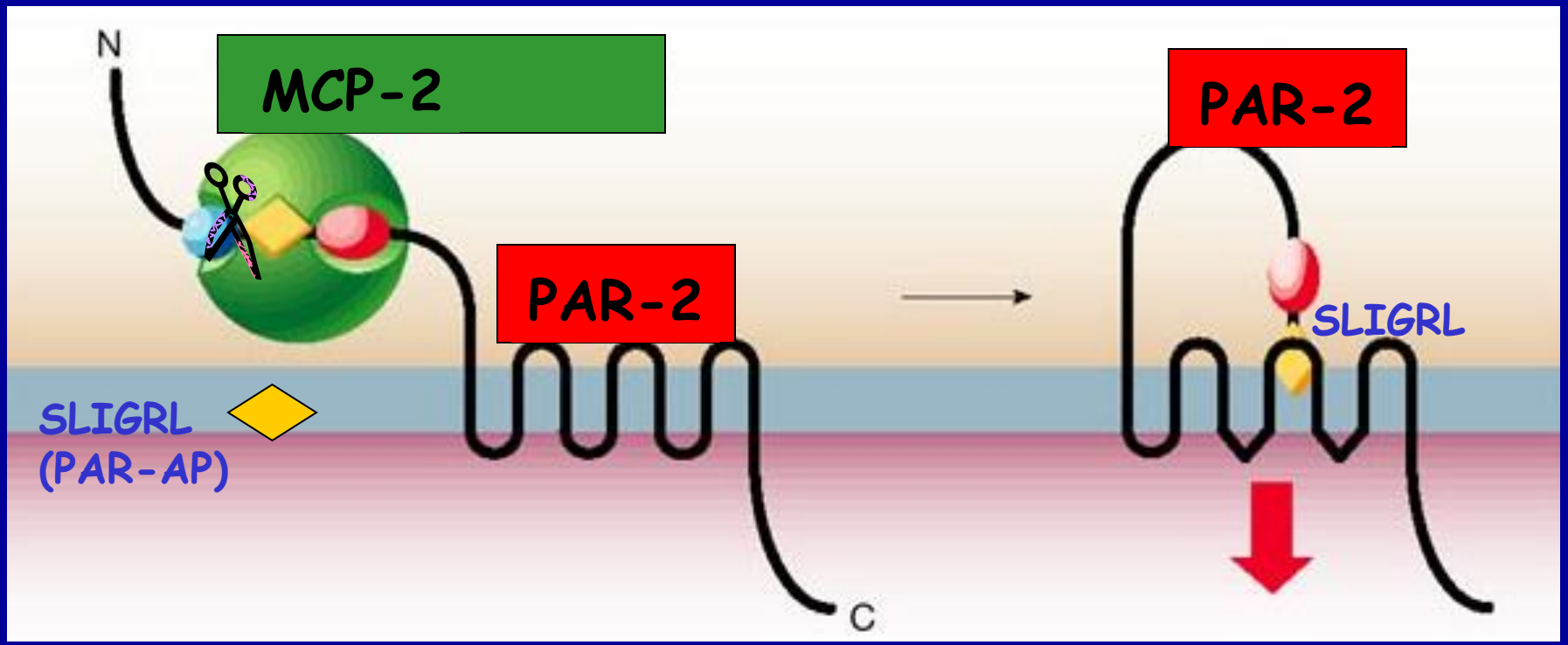
1 Our recent data suggest that **zonulin**, a molecule that dictates intestinal permeability during the acute phase of celiac disease, is structurally similar to **mast cell proteinase (MCP)-II**

2 MCP-II is an activator of **proteinase-activated receptor (PAR)-2** which is expressed in human intestinal mucosa where plays a role in increasing intestinal permeability and fluid secretion

3 **PAR-2 mRNA** is strongly expressed in the small intestine, colon, liver and pancreas and more weakly detected in the stomach

BACKGROUND

Mast cell proteinase (MCP)-II cleaves **PAR-2** within the extracellular amino terminus to expose the tethered **ligand SLIGRL**, which binds to and activates **PAR-2** to initiate multiple signaling cascades.



The active N-terminus **Zot** fragment **ΔG** contains the motif **FCIGRL**which is structurally similar to the SLIGRL ligand agonist of PAR-2 (see: **DDW 2004, abstract ID 103951)**

Aims

To investigate whether:

1 PAR-2 and/or Zot/Zonulin receptor **are up-regulated** during the acute phase of celiac disease

2 PAR-2 and Zot/Zonulin receptor **co-localize** in human duodenal mucosa

Methods

- Single and double staining fluorescence experiments on human duodenal biopsies obtained from 8 celiac patients and 8 non celiac patients:
  - FITC-labelled Zot/Zonulin binding antagonist FZI/0 peptide
  - monoclonal anti-human PAR-2 Abs followed by rhodamine-labelled secondary Abs

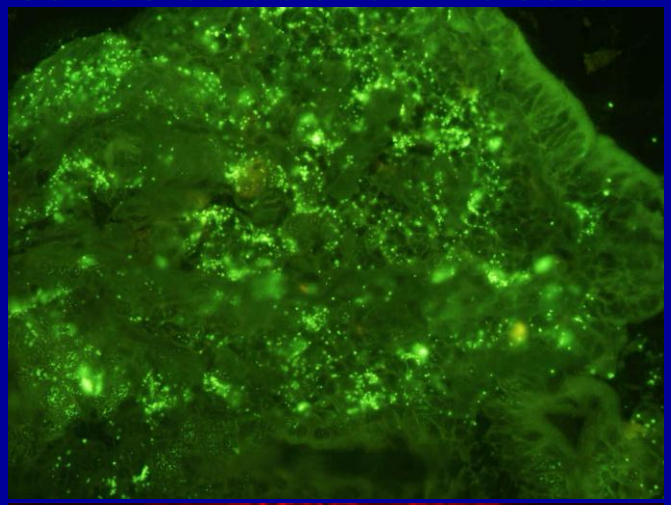
- **PAR-2 mRNA** expression by real time PCR in 5 biopsy specimens

RESULTS

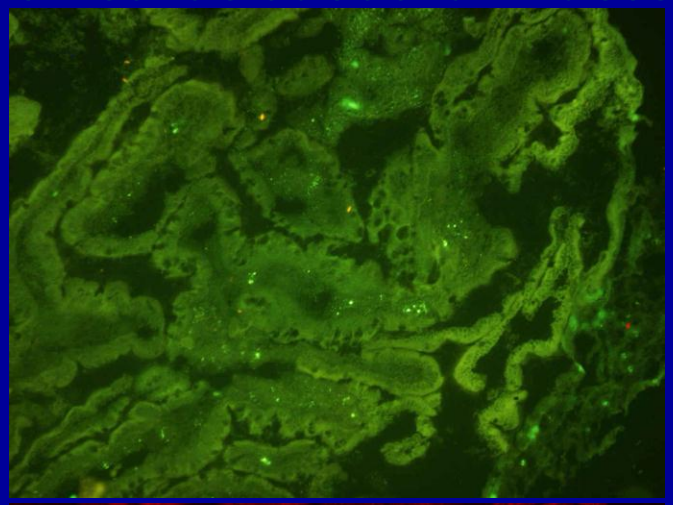
The fluorescent staining patterns showed over-expression of both Zot/Zonulin receptor and PAR-2 in duodenal biopsies from celiac patients compared to controls

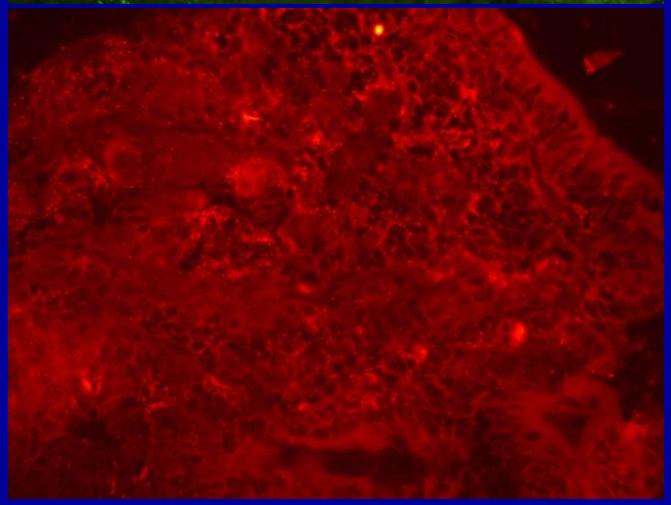
celiac duodenal mucosa

non celiac duodenal mucosa

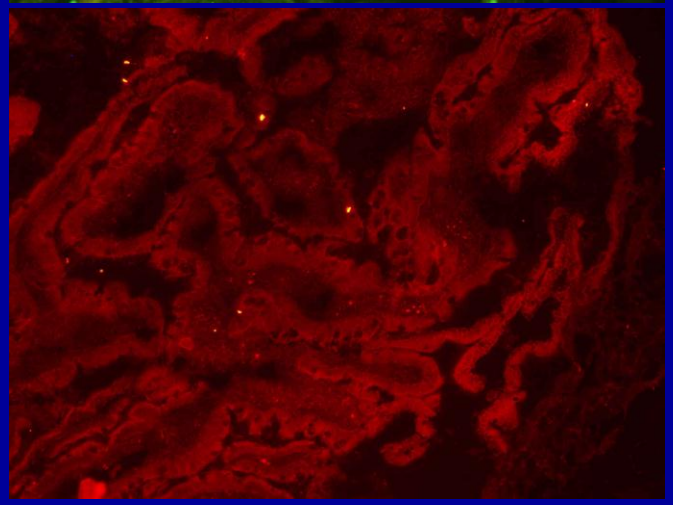


← **FZI/0** →



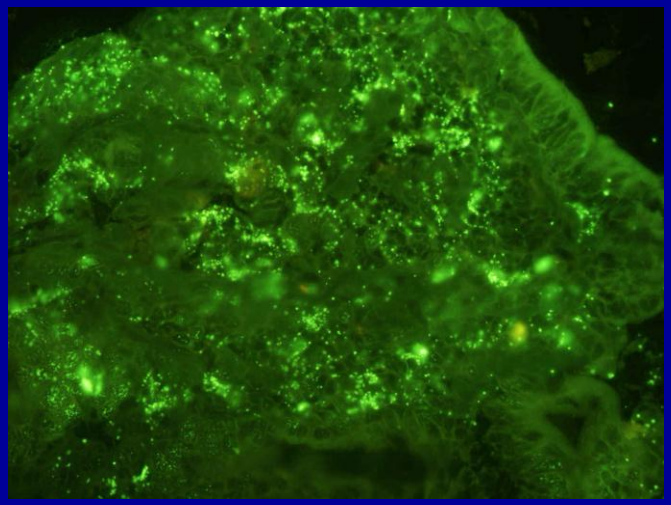


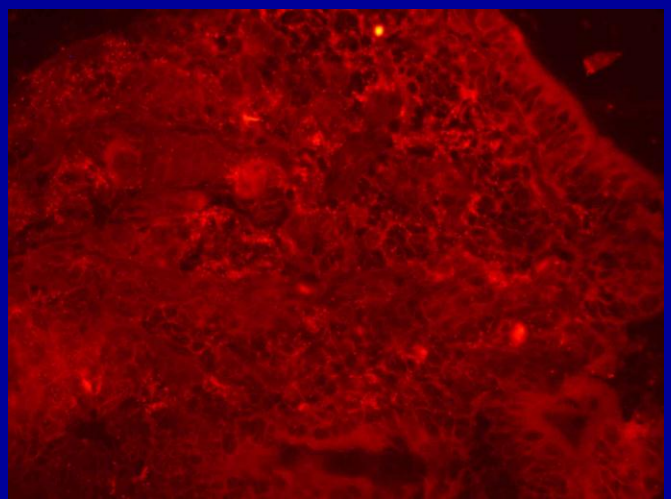
← **PAR-2** →

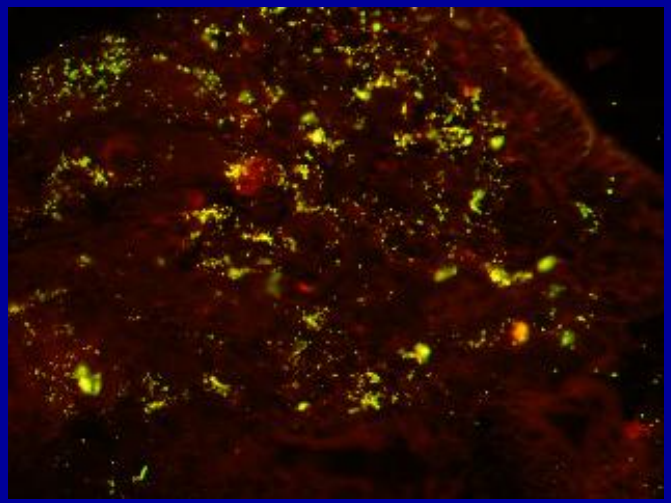


RESULTS

Overlapping of the two images showed co-localization of the PAR-2 and FZI/0 peptide in both celiac and non celiac intestinal specimens







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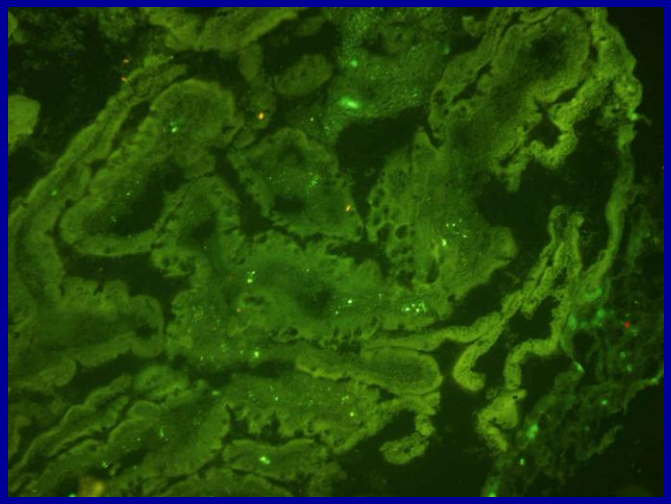

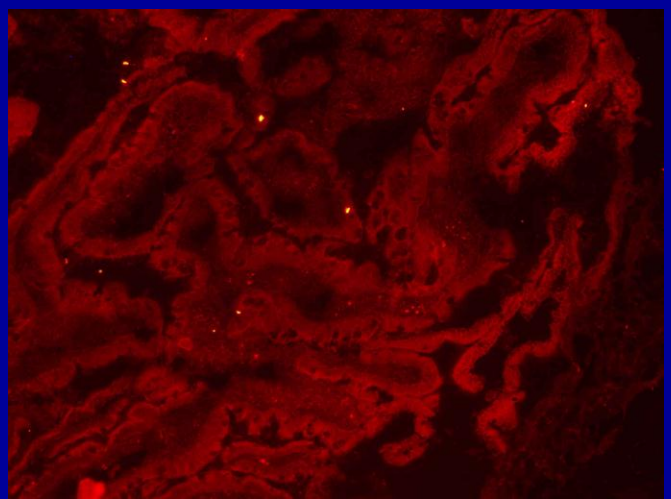

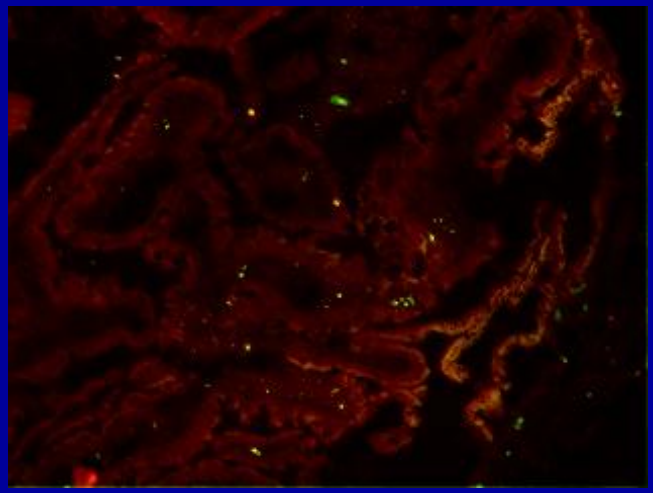

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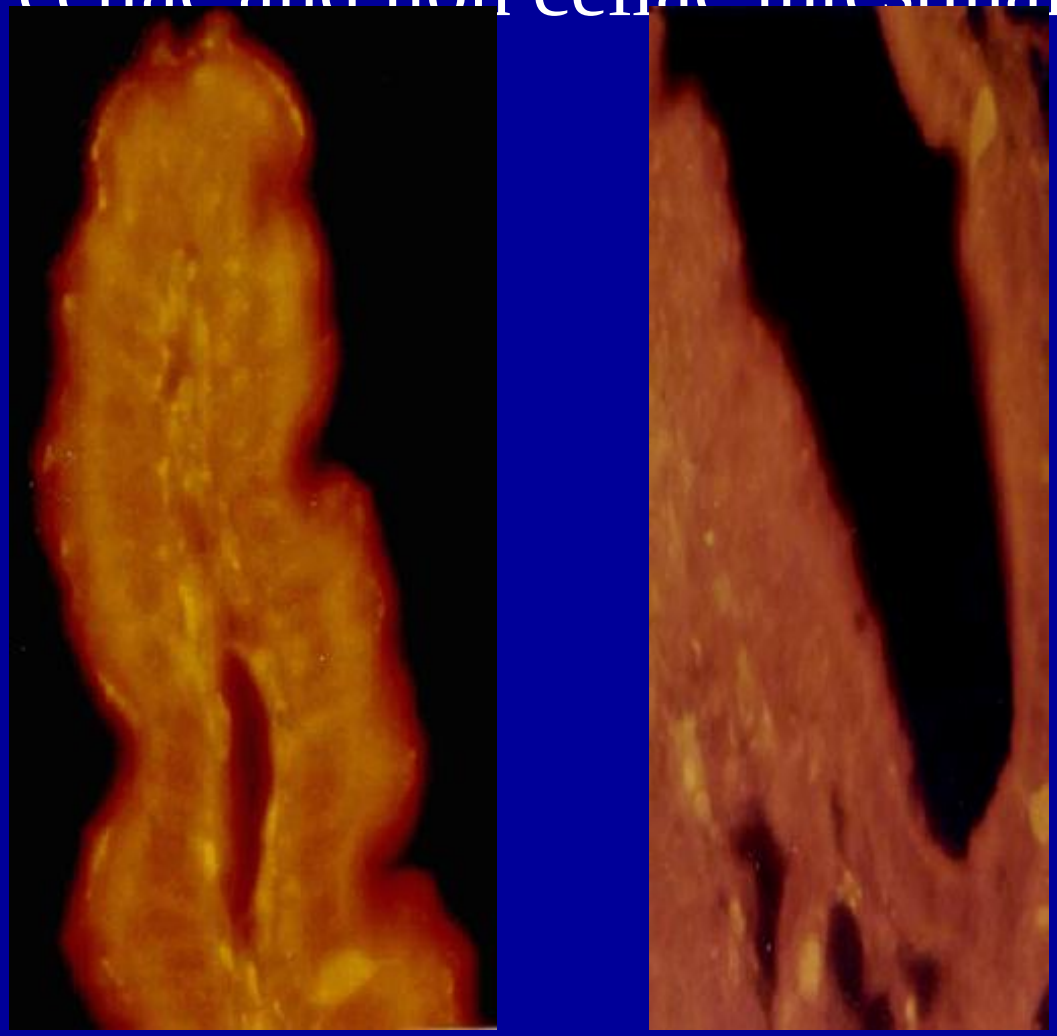

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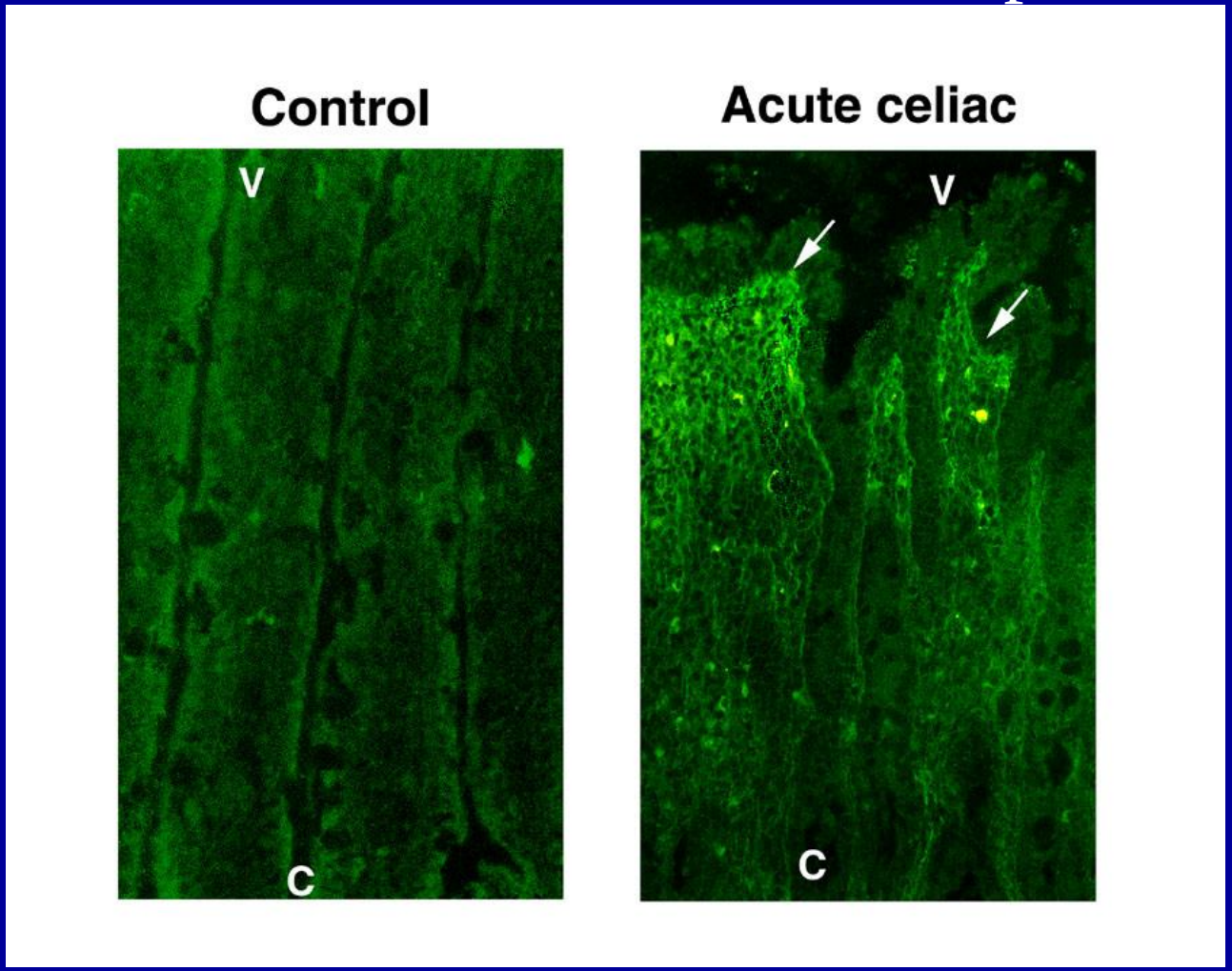

CONCLUSIONS

- Both Zot/Zonulin receptor and PAR-2 are over-expressed during the acute phase of celiac disease, which is characterized by increased intestinal permeability
- the co-localization experiment results suggest that FZI/0 binds to a site very close to or synonymous with PAR-2 in human duodenal mucosa
- the elevated expression of both receptor (PAR-2) and ligand (zonulin) can be responsible of the sustained increased intestinal permeability described in celiac disease.