

**INTESTINAL PROTEINASE-ACTIVATED
RECEPTOR (PAR)-2 IS UP-REGULATED IN
ACTIVE CELIAC DISEASE AND CO-
LOCALIZES WITH ZOT/ZONULIN RECEPTOR**

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ABSTRACT

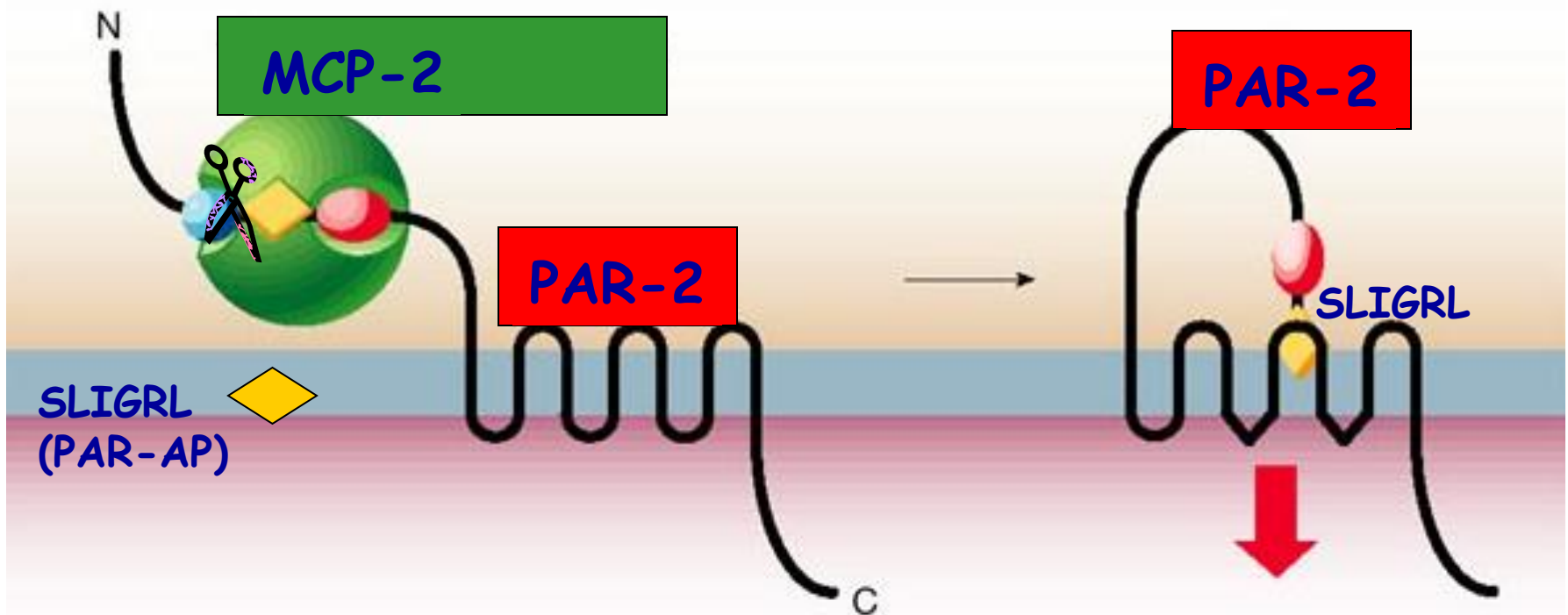
Background: Zonulin, the eukaryotic Zonula occludens toxin (Zot) analogue that modulates intercellular tight junctions, is up-regulated during the acute phase of celiac disease (CD) and may be responsible for the increased intestinal permeability characteristic of the disease. Our recent data suggest that zonulin is structurally similar to mast cell protease (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=8) and without (N=8) CD were incubated with either FITC-labeled 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 TNF α , can up-regulate PAR-2 expression, it is logical to postulate that our findings can be related to the increased levels of TNF α 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.

BACKGROUND

- 1 Zonulin, a recent described protein that modulates intestinal tight junctions permeability, is up-regulated during the acute phase of celiac disease
- 2 Our recent data suggest that zonulin is structurally similar to mast cell proteinase (MCP)-II which is an activator of the proteinase-activated receptor (PAR)-2
- 3 PAR-2 is strongly expressed in human intestinal mucosa where plays a role in increasing intestinal permeability and fluid secretion

BACKGROUND

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 a motif which is structurally similar to the SLIGRL ligand agonist of PAR-2 (see: **DDW 2004, abstract ID 103951**)

BACKGROUND

PAR-2 and ZOT/Zonulin receptor similarities

PAR-2 and ZOT/Zonulin receptor are glycoproteins of similar mass (66-80 vs 65 kDa) that show:

- 1 - same mass reduction to 35-40 kDa by deglycosylation
- 2 - same distribution in the gut
- 3 - same intracellular signaling pathways, involving :
 - activation of phospholipase C
 - activation of protein kinase C
 - actin polymerization

Moreover, their activation results in increased intestinal permeability

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

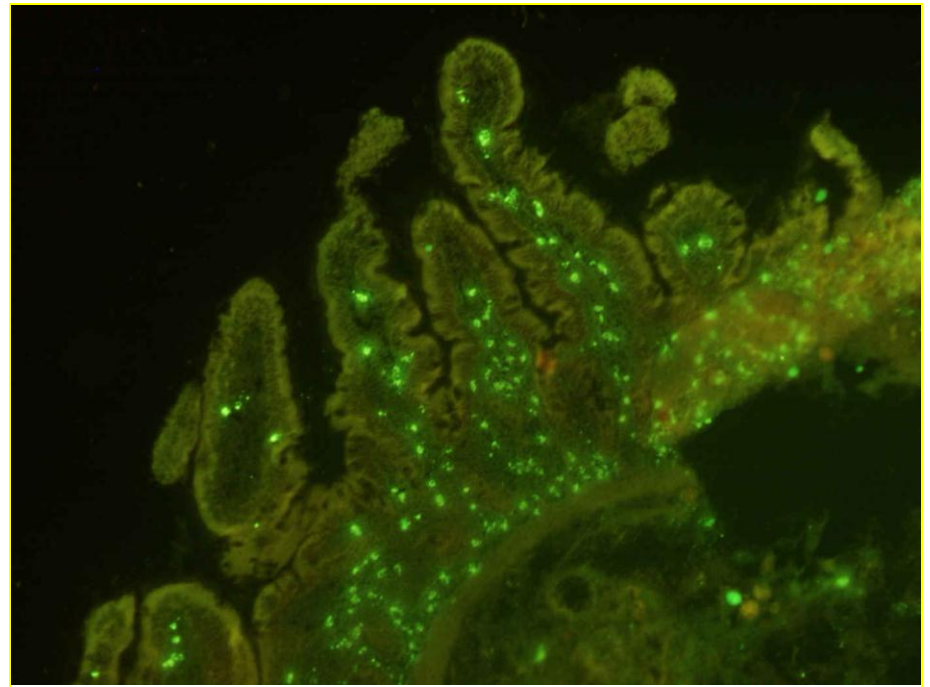
Single and double staining fluorescence experiments were performed on human duodenal biopsies obtained from 8 celiac patients and 8 non celiac patients:

- FITC-labeled FZI/0, a synthetic octapeptide that binds to Zonulin receptor
- the commercially available anti-human PAR-2 monoclonal antibody followed by FITC-labeled (single staining experiments) or rhodamine-labeled secondary antibody (double staining experiments)

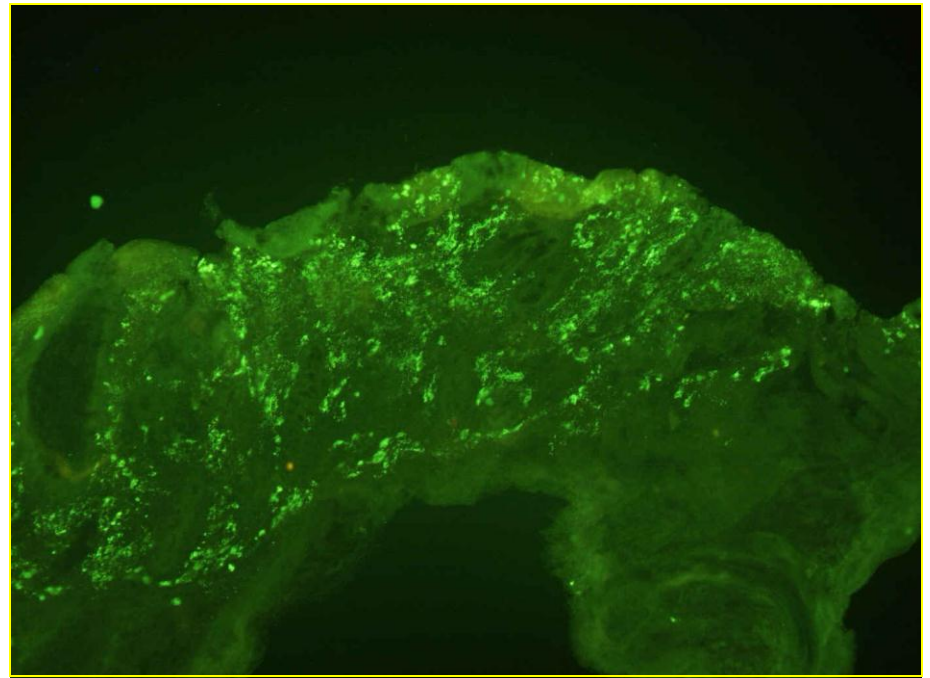

RESULTS

FZI/0 fluorescent
staining patterns of
human duodenal biopsies:

Non-celiac mucosa



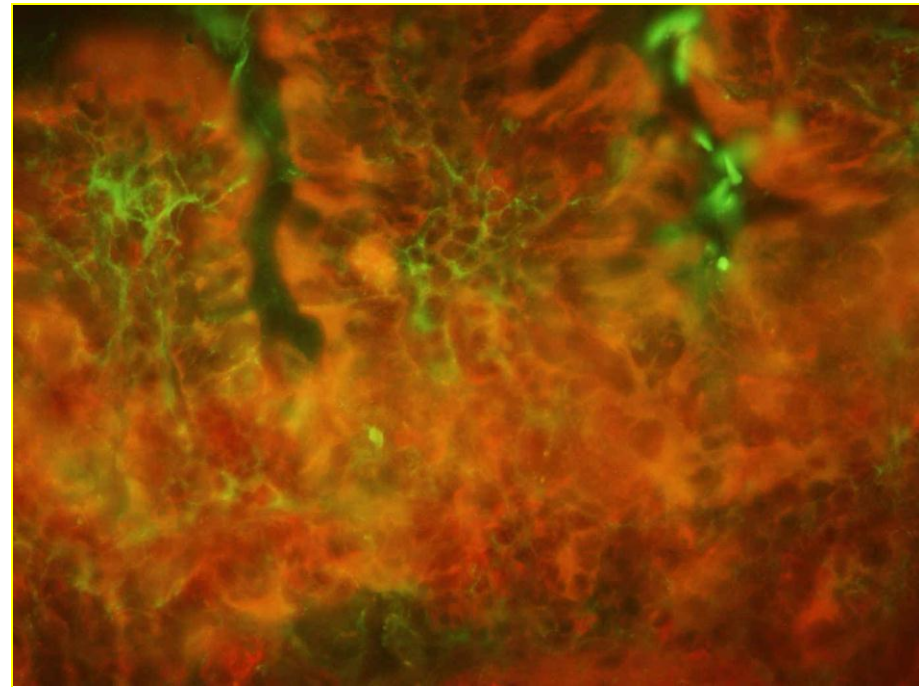
*celiac mucosa with
total villous atrophy
showing
over-expression
of zot/zonulin receptor*



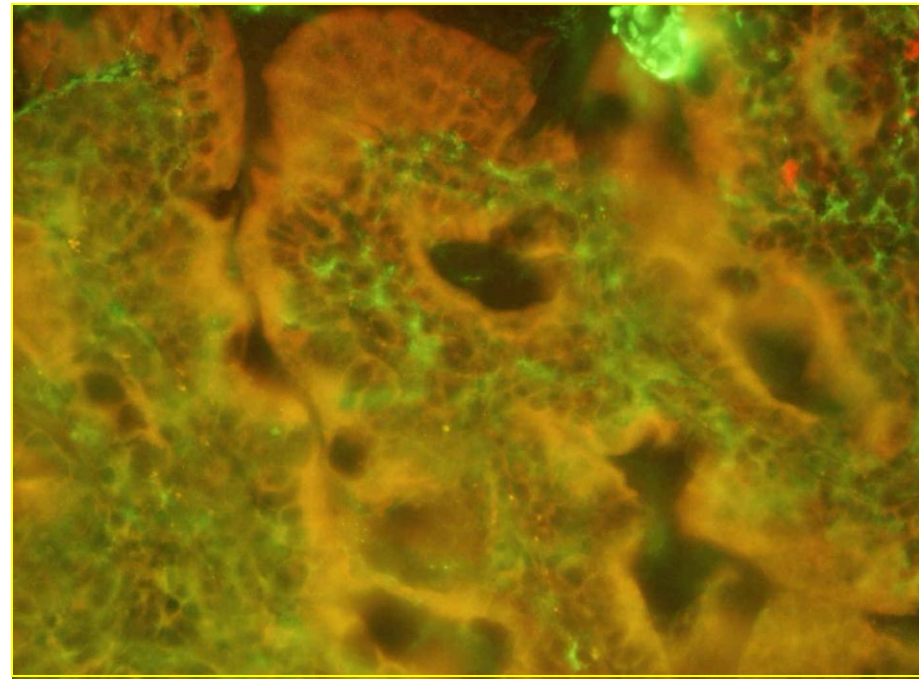

RESULTS

PAR-2 fluorescent staining patterns of human duodenal biopsies:

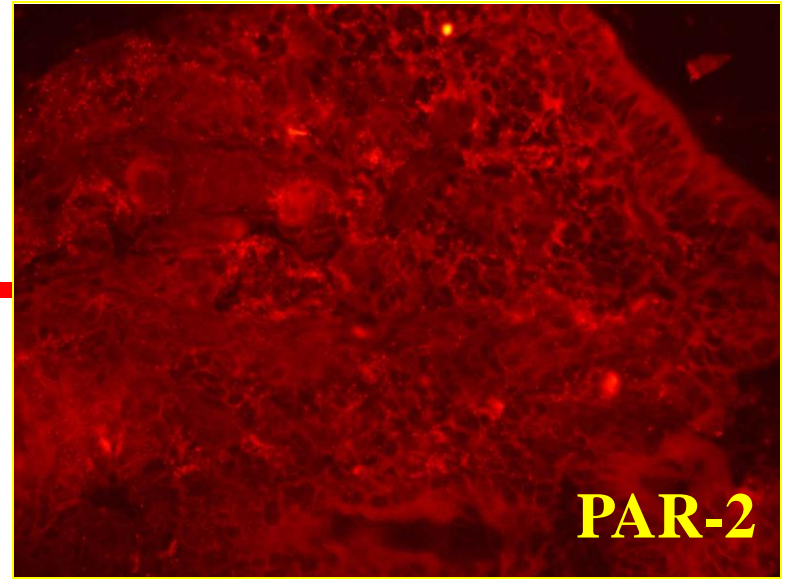
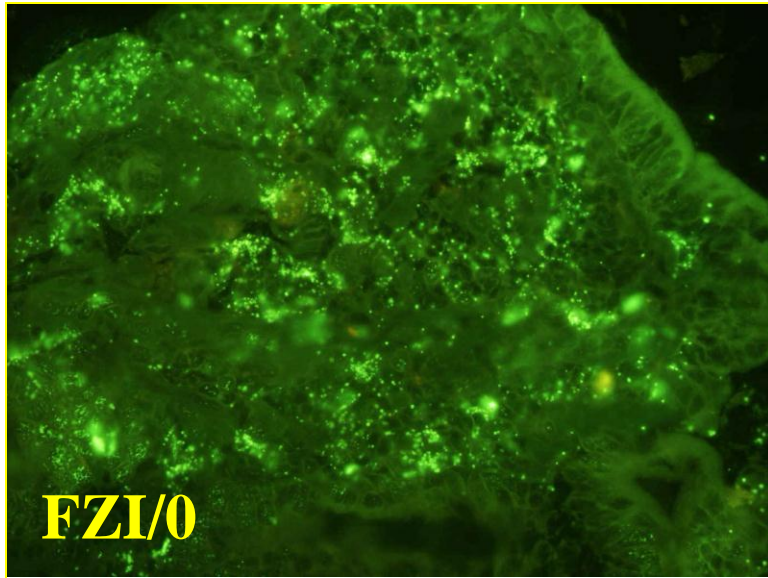
non celiac mucosa



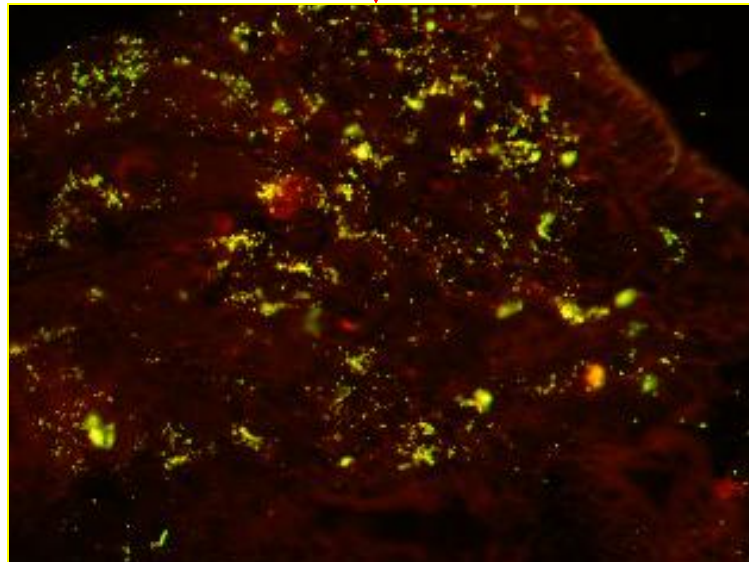
celiac mucosa showing over-expression of PAR-2 receptor



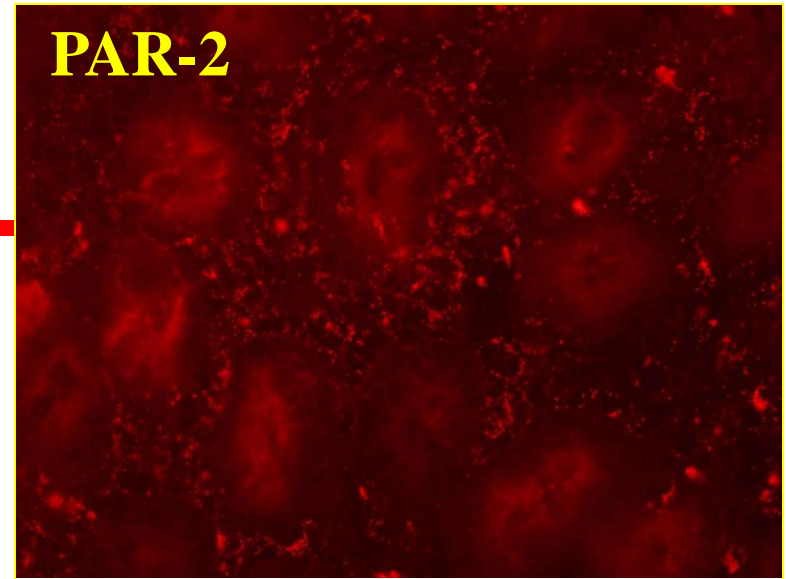
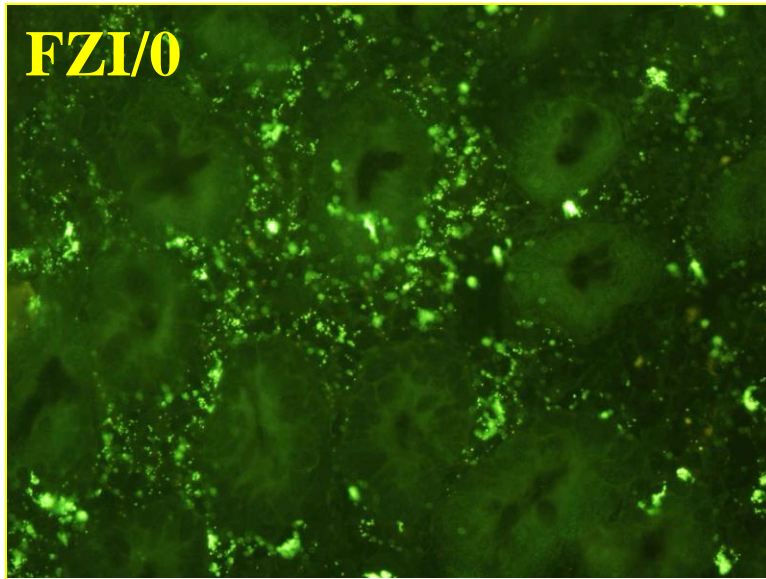
RESULTS : co-localization of the PAR-2 and FZI/0 peptide (microscope images, 10x magnification)



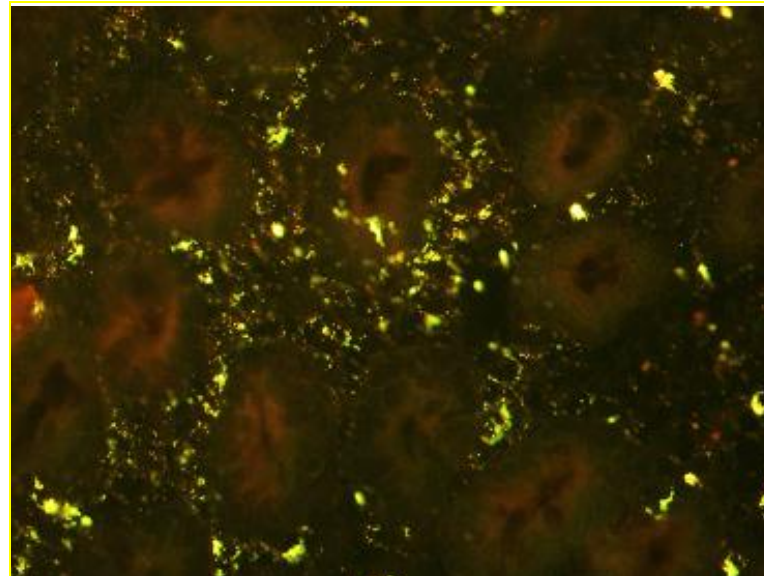
Duodenal mucosa
from a celiac
patient with total
villous atrophy and
crypt hyperplasia



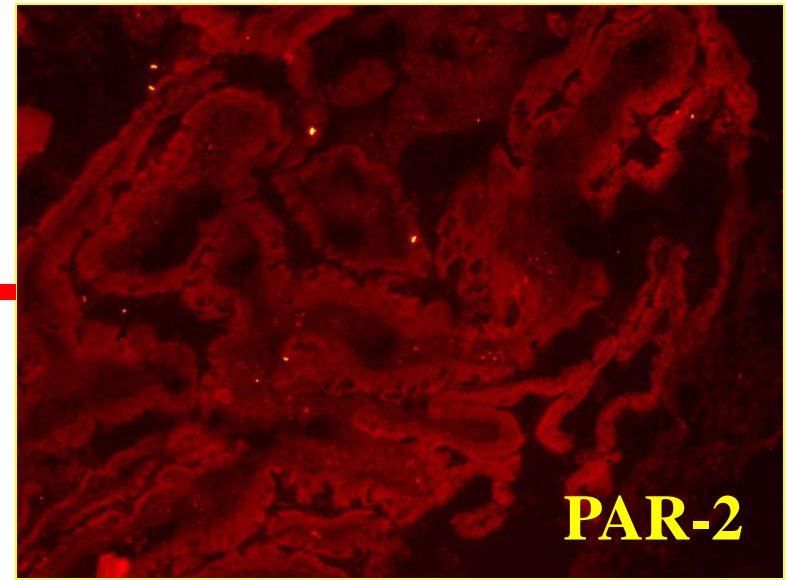
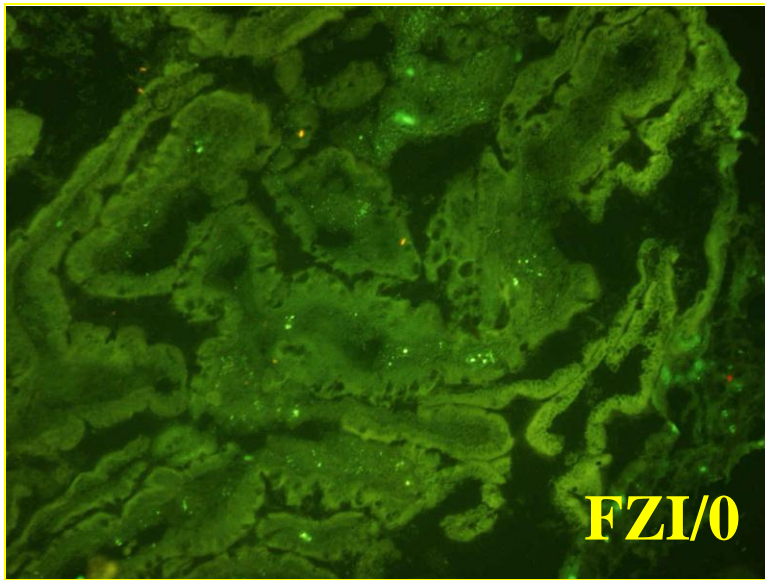
RESULTS : co-localization of the PAR-2 and FZI/0 peptide (microscope images, 40x magnification)



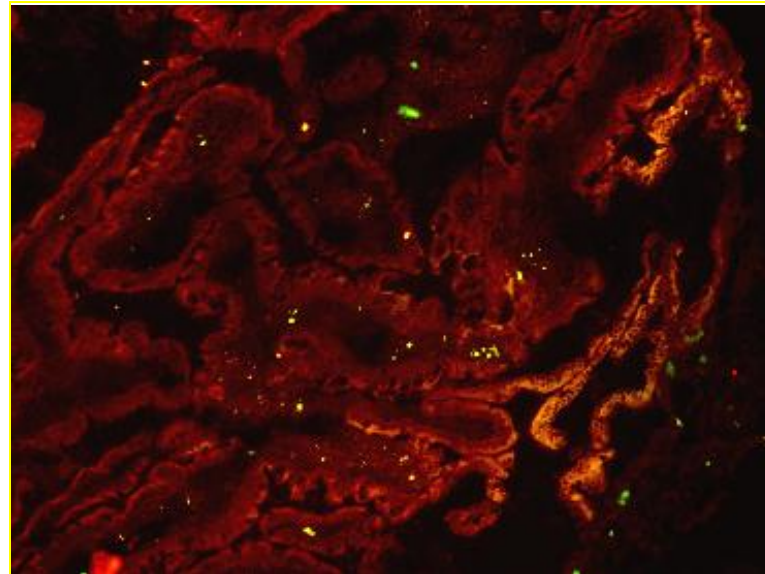
Duodenal mucosa
from a celiac
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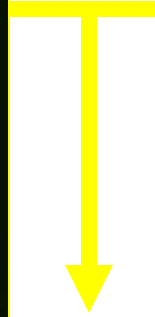
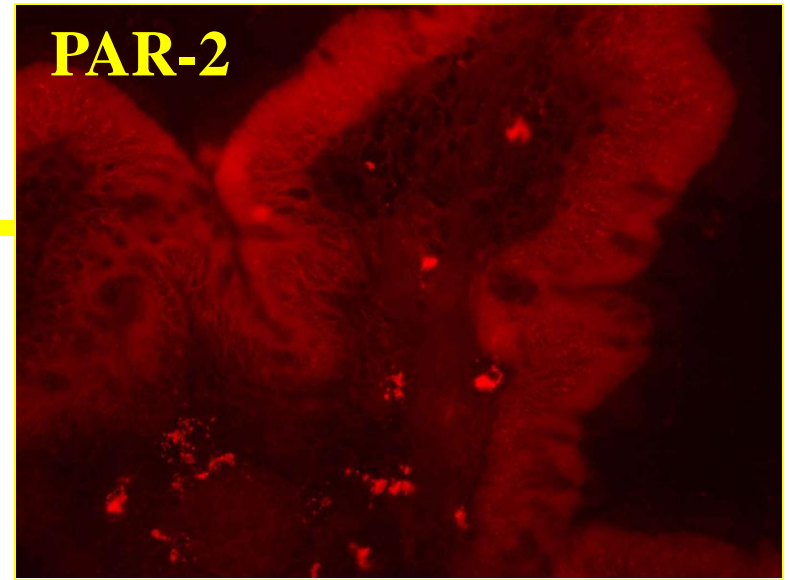
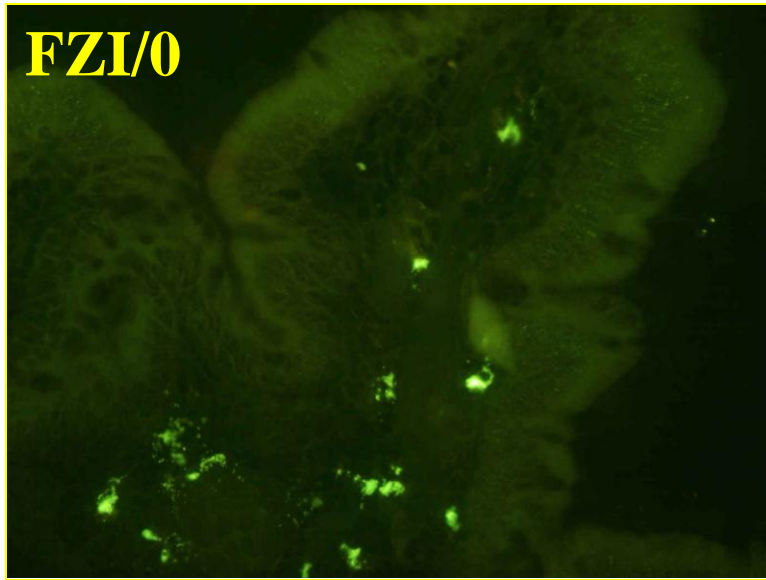
RESULTS : co-localization of the PAR-2 and FZI/0 peptide (microscope images, 10x magnification)



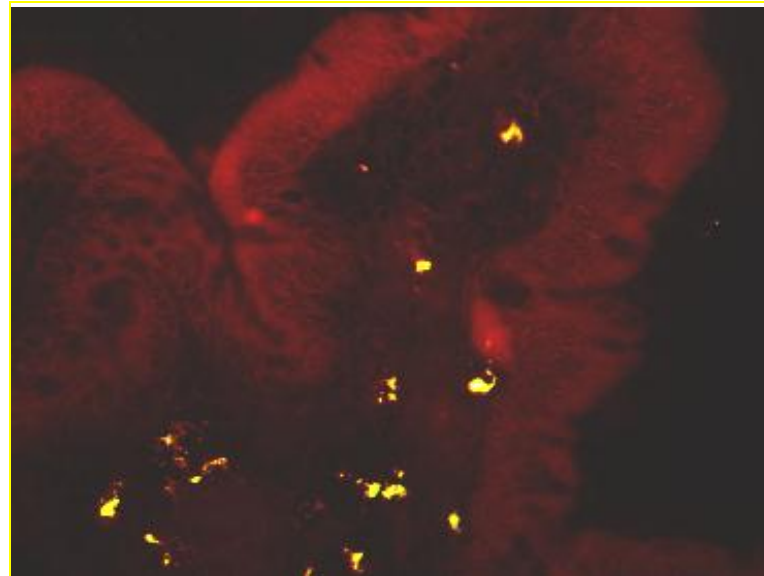
Duodenal mucosa
from a non celiac
patient.



RESULTS : co-localization of the PAR-2 and FZI/0 peptide (microscope images, 40x magnification)



Duodenal mucosa
from a non celiac
patient



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 for the sustained increased intestinal permeability described in celiac disease
- Experiments are in progress to fully characterize the receptor recognized by the anti-PAR2 antibodies and that co-localizes with FZI/0 binding.