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

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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.