Proteinase-Activated Receptor 2 (PAR-2) Involvement In The Zot/zonulin-Mediated Regulation Of Intestinal Tight Junctions

Maria Grazia Clemente, Mucosal Biology Research Center, University of Maryland School of Medicine, Stefanie Vogel, Department of Microbiology and Immunology and Mucosal Biology Research Center, University of Maryland School of Medicine, Morley D. Hollenberg, Department of Pharmacology and Therapeutics and Department of Medicine, University of Calgary Faculty of Medicine, and Alessio Fasano, Mucosal Biology Research Center, University of Maryland School of Medicine,

Background: We have recently showed that zonulin, the eukaryiotic Zonula occludens toxin (Zot) analogue that modulates intercellular tight junctions (tj), is structurally similar to mast cell proteinase (MCP)-II. Further, the active Zot fragment ΔG N-terminus contains a motif structurally similar to the tethered ligand agonist of proteinase-activated receptor (PAR)-2, SLIGKV-amide. **Aim:** To establish whether PAR-2 (or a related receptor) represents the target receptor for both Zot and zonulin. **Methods:** Human intestinal epithelial cells (Caco2) 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-labeled anti-mouse IgG antibodies. Similar experiments were conducted in situ on duodenal biopsies obtained from both patients with active celiac disease and controls. The effect of SLIGKV-amide on the intestinal cell cytoskeleton was assessed both in the presence and absence of FZI/0 in Caco2 cells stained with FITC-phalloidin. The effect of FZI/0 on SLIGKV-amide -induced changes in transepithelial electrical resistance (TEER) was also tested on rat intestinal tissues mounted in the microsnapwell system. **Results:** Immunofluorescent particles were visualized in Caco2 cells with both FITC-FZI/0 and anti-PAR-2 antibodies. Overlapping of the two images showed colocalization of the PAR-2 receptor and FZI/0. Similar results were obtained with in situ immunofluorescence experiments performed on human intestinal biopsies. In competitive experiments, cells exposed to an excess of SLIGKV-amide ligand showed a significant reduction of FZI/0 immunofluorescent staining particles compared to monolayers exposed to a scrambled peptide, suggesting that FZI/0 binds to a site very close to or synonymous with PAR2. Activaton of PAR2-PR with SLIGKV-amide induced a significant intracellular cytoskeleton reorganisation in Caco2 cells that was prevented by pre-treatment with FZI/0 peptide. Addition of SLIGKV-amide to the luminal aspect of the intestine decreased TEER compared to untreated tissues and these changes were completely prevented by pretreatment with FZI/0. **Conclusions:** Our findings suggest that PAR-2 (or a closely related moiety) could be the target receptor for both Zot and zonulin and is involved in the regulation of intercellular tj.