Luminal protease activity is increased in pouch inflammation of ulcerative colitis patients

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Introduction: Up to 50% of the ulcerative colitis (UC) patients that undergo restorative ileal pouch anal anastomosis (IPAA) surgery develop pouch inflammation (pouchitis). The mechanisms triggering enteric inflammation are diverse, probably involving a compromise of the epithelial barrier function through disruption of tight junction (TJ) proteins and exposure of the immune system to microbial antigens. Aim: To assess whether fecal proteolytic activity of pouchitis patients is elevated and whether it mediates increased epithelial permeability. Methods: Fecal protease activity was measured in patients with normal pouches (NP, n=6), active pouchitis (AP, n=10), and in healthy controls (HC, n=9), using FITC-casein florescence assay. Human epithelial cell line (Caco-2) monolayers were exposed to the fecal supernatants. FITC - Dextran 4 (FD4) was used as a fluorescent probe to study cell permeability. Tight junction (TJ) proteins integrity was assessed by Western-blot and immunofluorescence. Results: AP patients exhibited 4.3-fold (P0.05) greater fecal proteolytic activity compared to NP patients and HC. Exposure of Caco-2 cells monolayers to AP supernatants resulted in increased epithelial permeability to FITC-dextran by 18.36 (P0.05) and 73 fold (P0.01), compared to fecal sups from NP and HC, respectively. Fecal sups from AP disrupted TJ proteins (Occludin and Zonula occludens) of Caco-2 cell monolayers, as assessed by Western blot and immunofluorescence. Conclusions: Pouch inflammation is associated with increased luminal proteolytic activity that can disrupt TJ proteins of Caco-2 cell monolayers and increase epithelial permeability, implicating a mechanism through which pouch inflammation may be initiated.