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Protease Activated Receptor-2 Mediates *Giardia*-Induced Disruptions of the Intestinal Mucus Barrier

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Background: *Giardia duodenalis* has been associated with disruptions of the mucus layers of the small and large intestines, in part via *Giardia's* cysteine proteases. Disruption of the intestinal mucus barrier has been implicated in a variety of intestinal disorders, but mechanisms currently remain unclear.

Hypothesis: Goblet cell function and mucus disruptions are modulated by activation of protease activated receptor 2 (PAR2), which is highly expressed through the gastrointestinal tract and is known to regulate mucus production in the airway and stomach. *Giardia's* cysteine proteases cleave and activate PAR2 on intestinal goblet cells.

Methods: MUC2 mucin and PAR gene expression were assessed in the human colonic epithelial cell line LS174T using quantitative PCR (qPCR). LS174T were pre-treated with the PAR2 antagonist p2Pal-218S pepducin, and incubated with *Giardia* trophozoites or the PAR1 and PAR2 agonist peptides TFLLRN and 2fLIGRLO, respectively. Ability of *Giardia* to cleave PAR1 and PAR2 at the N-terminal domain was determined using CHO cells transformed with N-luciferase tagged PAR1 or PAR2. Following incubation with *Giardia*, culture supernatant was collected, reacted with luciferin, and luminescence was measured. Wild-type and PAR2 deficient mice were infected with *Giardia* trophozoites. Thickness of the mucus layer was measured by fluorescently staining mucus with fluorescein-coupled WGA, and expression of Muc2 and Muc5ac genes were determined in the colon and jejunum via qPCR.

Results: LS174T cells express functional and responsive PAR2, but little or no PAR1. Treatment of cells with 2fLIGRLO PAR2 agonist, but not TFLLRN PAR1 agonist, increased MUC2 gene expression. Incubation of cells with *Giardia* trophozoites increased MUC2 mRNA production, and this increase was abolished by pre-treatment of cells with a PAR2 antagonist. *Giardia* also caused decreased PAR2 expression in LS174T. *Giardia NF* and *Giardia GSM* are both able to cleave PAR1 and PAR2 at the N terminal domain. Cleavage by *NF* was more efficient than *GSM*, and PAR1 was cleaved more extensively than PAR2 by both strains. *Giardia* infection increased Muc2 and Muc5ac expression in the colon and increased Muc5ac expression in the jejunum of wild-type mice compared to uninfected controls. In PAR2-/- mice, *Giardia* infection reduced expression of Muc5ac in the jejunum, and had no impact on either gene in the colon. *Giardia* infection caused thinning of the mucus layer in wild-type mice. In contrast, infection induced thickening of the mucus layer in PAR2-/- mice.

Conclusions: Using a model of *Giardia* infection, the findings demonstrate that PAR2 plays a significant role in mucin gene regulation and function in mice and in a human colonic goblet cell line, and that *Giardia* is capable of cleaving both PAR1 and PAR2 receptors with strain-specific efficiencies.

References: Amat, C. *et al*. Am. J. Pathol. 2017.

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