A039 Mucosal Defenses Against Giardia Duodenalis: A Role for PAR2

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Background: Disruption of the intestinal mucus barrier is associated with increased risk of infection and inflammation in the gut. *Giardia duodenalis* has been shown to disrupt both mucus production and secretion in the small and large intestines via unknown mechanisms. We aim to determine the role of protease-activated receptor-2 (PAR2) in disrupted mucus production and secretion during *Giardia* infection. **Methods:** The goblet-like cell line LS174T was infected with *Giardia* trophozoites (isolates *NF*, *WB*, *S2*, and *GSM*). Prior to infection, trophozoites were treated with E64, a broad-spectrum cysteine protease inhibitor, and LS174T were treated with a PAR2 antagonist, a calcium chelator, or an ERK1/2 inhibitor. MUC2 mucin gene expression was assessed using quantitative PCR (qPCR). Chinese hamster ovary cells transfected with nano-luciferase-tagged PAR2 were incubated with *Giardia* trophozoites. Release of enzymes due to cleavage at the receptor N-terminus by *Giardia* resulted in a luminescent signal proportional to the number of cleaved receptors. Wild type (WT) and PAR2 deficient (PAR2-/-) mice were infected with *Giardia* trophozoites. The colonic mucus layer was stained using fluorescein-coupled wheat germ agglutinin (WGA), and qPCR was performed for Muc2 and Muc5ac in the small and large intestines. **Results:** *Giardia* isolates *NF*, *S2*, and *WB*, but not *GSM*, upregulate MUC2 expression in LS174T cells. This increase was attenuated by inhibition of *Giardia* cysteine proteases and by antagonism of PAR2 or inhibition of calcium release or MAPK pathways in LS174T cells. *Giardia* trophozoites cleaved PAR2 within N-terminal activation domains. The amount of cleavage was isolate-dependent, reflecting differences in protease activity, and cleavage was significantly reduced by E64 treatment for all isolates. *Giardia* infected WT mice show altered mucin gene expression in the large and small intestines that is not observed in PAR2-/- mice. Both WT infected and PAR2-/- non-infected mice showed thinning of the colonic mucus layer compared to WT controls. There was some recovery in thickness in PAR2-/- infected mice. **Conclusions:** PAR2 plays a significant role in the regulation of mucin gene expression in mice and a human colonic cell line. Proposed mechanisms involve cleavage of PAR2 by *Giardia* cysteine proteases to activate canonical PAR2 signaling, which involves both calcium release and MAPK activation.