

### **A031 Neutrophil Expressed CD47 Regulates CD11b/CD18-Dependent Neutrophil Transepithelial Migration in the Intestine In Vivo**

Veronica Azcutia, Matthias Kelm, Anny-Claude Luissint, Kevin Boerner, Sven Flemming, Miguel Quiros, Gail Newton, Asma Nusrat, Francis W. Luscinskas, and Charles A. Parkos  
*University of Michigan, Ann Arbor, MI; Brigham and Women's Hospital, Boston, MA*

**Introduction:** Dysregulated neutrophil transmigration across the intestinal epithelium (TEpM) results in crypt abscess formation and mucosal ulceration which are pathological hallmarks of ulcerative colitis. Despite such adverse clinical consequences, molecular events regulating neutrophil TEpM remain incompletely characterized. Previous *in vitro* studies have shown that neutrophil proteins including CD47 and CD11b/CD18 play discrete roles in regulating TEpM, however the importance of binding interactions between CD47 and CD11b/CD18 during neutrophil trafficking in the gut have not been explored. Here we show that CD47 physically interacts with CD11b/CD18 to regulate neutrophil trafficking across intestinal mucosa. **Methods:** Using total *Cd47*<sup>-/-</sup> knockout mice, epithelial-specific CD47 knockout mice (*Villin-Cre;Cd47<sup>fl/fl</sup>*) and novel neutrophil-specific CD47 knockout mice (*MRP8-Cre;Cd47<sup>fl/fl</sup>*), we determined the cell specific contributions of CD47 to neutrophil TEpM using an *in vivo* ileal loop assay. Complementary *in vitro* approaches were applied to determine the nature of CD47 association with CD11b/CD18 including chemotaxis assays, proximal ligation assays and flow cytometry analyses using both murine and human neutrophils. **Results:** Here, we report that neutrophil but not epithelial-expressed CD47 plays an important role in regulating neutrophil TEpM *in vivo*. We show that CD47 associates with CD11b/CD18 in the plasma membrane of both human and murine neutrophils. Importantly, loss of CD47 resulted in reduced CD11b/CD18 activation. Complementary *in vitro* and *in vivo* studies using function blocking antibodies further highlight the role of CD47 in regulating CD11b/CD18-dependent neutrophil TEpM and chemotaxis. **Conclusions:** These data support a role for CD47 in regulating neutrophil TEpM through activation of CD11b/CD18. Moreover, tissue-targeted CD47 knockout mice represent an important new tool to study the role of CD47 during inflammation *in vivo*. Taken together, these findings provide new insights for developing therapeutic approaches that will reduce neutrophil infiltration during chronic intestinal inflammation. Supported by: CCFA CDA454814, NIH 5R01DK079392-12 and R01HL125780.