The Role of Microbes in HMGB1-mediated Cytoprotection of Intestinal Epithelial Cells During Inflammatory Bowel Disease

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**Background:** HMGB1 is well-characterized as an extracellular, pro-inflammatory protein, but far less is known about its intracellular functions or regulation. Our group recently reported that intracellular HMGB1 protects intestinal epithelial cells (IEC) from death during microbe-associated inflammation by regulating the calpain-mediated switch between autophagy and apoptosis. This protection is compromised in the diseased mucosa of IBD patients since they have decreased levels of intracellular HMGB1. Acute, high-level exposure to microbial components activates inflammation in IEC. Conversely, low level exposure to the bacterial cell wall component muramyl dipeptide (MDP) activates cytoprotective and anti-inflammatory pathways that regulate cell death during inflammation. This led us to hypothesize that chronic, low-level microbial stimulation through innate immune receptors increases intracellular HMGB1, improving IEC survival during microbe-associated stress.

**Methods:** We utilized a novel mouse model conditionally deficient in IEC HMGB1 and intestinal tissues derived from patients with IBD to test this hypothesis.

**Results:** Immunoblotting for HMGB1 in gnotobiotic mice showed that the level of intracellular HMGB1 is regulated by exposure to microbes. MDP treatment of small-intestine derived mouse organoids or colonic IEC derived from normal patient endoscopic biopsies increased intracellular HMGB1 protein concentrations. MDP treatment also increased cellular ATP production in mouse organoids in an HMGB1- and calpain-dependent manner.

**Conclusions:** Our data show that microbial components regulate intracellular HMGB1 expression in IEC. We have also identified HMGB1 as a cytoprotective factor produced in response to MDP and shown that HMGB1 is essential for an increase in ATP production in IEC challenged by MDP. Compromise of these HMGB1-mediated survival and homeostatic pathways likely contributes to the increased IEC death and dysfunction seen in IBD patients since they exhibit decreased intracellular HMGB1 in diseased tissues. Therefore, therapeutic strategies targeting intracellular HMGB1 could be a novel method to prevent IEC death and promote mucosal healing in IBD patients.