A030  Fusobacterium Nucleatum Metabolites and Outer Membrane Vesicles Drive Intestinal Inflammation In Vitro and In Vivo

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Introduction: Inflammatory bowel disease (IBD) is a group of chronic inflammatory conditions that has increased rapidly in incidence in recent decades. While the exact cause of IBD is unknown, the intestinal microbiota has been proposed to participate in both initiation and perpetuation of IBD. It has also been speculated that oral cavity microbes may translocate and become intestinal pathobionts. Bacteria in the genus Fusobacterium, an oral cavity microbe, have been identified in the intestinal mucosa of IBD patients. However, a causative link between Fusobacterium and intestinal inflammation has not been firmly established. We hypothesized that F. nucleatum promotes epithelial inflammation. Methods and Results: To test this hypothesis, F. nucleatum sbsp polymorphum was grown in BHI for 48 hrs and cell-free supernatant was fractionated by size. Application of >50 kD F. nucleatum metabolites to colonic HT29 cells results in secretion of pro-inflammatory cytokines IL-8 and TNF. Additionally, purified F. nucleatum outer membrane vesicles (OMVs), which are present in the >50 kD fraction, stimulated IL-8 and TNF. No secretion of IL-8 or TNF was observed with metabolites <50 kD or uninoculated BHI. Pharmacological inhibition of Toll-Like Receptor 4 (TLR4) ablated the production of IL-8 and TNF by >50 kD and OMV stimulation. Western-blot analysis revealed increased p-ERK, p-CREB, and NF-κB in HT29 cells treated with F. nucleatum >50 kD and OMVs; suggesting that these signaling molecules are downstream effectors of TLR4. F. nucleatum metabolites also stimulated TNF secretion, p-ERK, p-CREB and NF-κB activation in human colon organoid monolayers. Finally, to examine inflammation in vivo, mice harboring a human microbiota were pretreated with antibiotics and then exposed to a single oral gavage of F. nucleatum (10^8 CFU). Compared to mice receiving PBS, mice treated with F. nucleatum showed disruption of the colonic architecture, increased immune cell infiltration and depleted mucus layers. Analysis of colonic gene expression revealed increased levels of pro-inflammatory cytokines at day 3 and day 5 in F. nucleatum-treated mice. These pro-inflammatory effects were absent in mice who received F. nucleatum without pretreatment with antibiotics; suggesting that an intact microbiome is protective against F. nucleatum-mediated immune responses. Conclusions: These data indicate that F. nucleatum drives inflammation in the context of a depleted intestinal microbiome.