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 the 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.