Ablation of Monocyte Chemoattractant Protein 1 from the Colonic Epithelium is Protective in the OA of Obesity

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Introduction: With a financial impact of more than $300 billion annually, osteoarthritis (OA) is the leading cause of disability in the United States. Given the lack of clinically accepted treatments that can modify the OA disease process, there is a critical unmet need for a therapeutic intervention. Obesity is one of the leading risk factors for OA development, with 66% of OA patients either obese or overweight. It is now appreciated that obesity induces OA progression via mechanisms that are independent of joint loading; one in particular related to obesity-induced gut microbiome dysbiosis that initiates a colonic, systemic, and joint-specific inflammatory cascade. More specifically, we have observed that Monocyte Chemoattractant Protein-1 (*Ccl2*, MCP-1) is upregulated in the colonic epithelium of obese mice, potentially promoting infiltration of pro-inflammatory macrophages into the colon. Moreover, correction of obesity-associated gut microbiome dysbiosis results in a decrease in MCP-1 in the colonic epithelium, which consequently halted macrophage infiltration into the colon, decreased systemic inflammation, and ultimately protected against progression of OA in the joints of obese mice. Here, we sought to test if knocking out *Ccl2* in the intestinal epithelium, can blunt colonic inflammation and, as a result, protect against systemic inflammatory shifts and obesity-associated OA.

Methods: Six-week-old tamoxifen-inducible *Vil1-CreERT2;Ccl2fl/fl* mice (*Vil-Ccl2* cKO) were placed on high-fat (HF) (60% of calories from saturated fat) or control diet. Tamoxifen was administered at 18 weeks of age for 5 days via IP injection to selectively ablate *Ccl2* in the intestinal epithelium. OA was then initiated via the surgical destabilization of the medial meniscus (DMM). Six weeks following injury mice were administered tamoxifen to ensure recombination. Fecal material was collected every 2 weeks for analysis of the gut microbiome, and 12 weeks following injury the colon, serum, and joints were collected for histologic and molecular analyses.

Results: Consumption of the high-fat diet led to obesity and alterations in the gut microbiome; however, the composition of the HF *Vil-Ccl2cKO* animals did not differ from that of high-fat control animals, suggesting that *Ccl2* deletion in the host did not influence the gut community in the obese context. Immunohistochemistry revealed that HF *Vil-Ccl2cKO* animals had a reduction in MCP-1 in the colon (Fig 1A). This decrease in MCP-1 led to a reduction in the number of macrophages, monocytes, and natural killer cells in the colon of HF *Vil-Ccl2cKO* animals. Serum cytokine analysis indicated that HF *Vil-Ccl2cKO* animals had evidence of reduced systemic inflammation, in particular reductions in the levels of circulating MCP-1 and IL-1β (Fig 1B). Finally, in DMM-injured joints, histomorphometry revealed that HF *Vil-Ccl2cKO* animals were protected from the accelerated OA phenotype (Fig 1C), with conditionally deleted mice displaying more total femur cartilage area compared to diet-matched genetic controls (Fig 1D). This increase in cartilage area coincided with a reduction in MMP13 expression in the femur of HF *Vil-Ccl2cKO* animals.

Discussion: Here we confirm that an obesity-associated gut microbiome dysbiosis drives colonic inflammation, which ultimately induces systemic inflammation and OA progression. Using the *Vil-Ccl2cKO* mouse model to knockout MCP-1 in the colonic epithelium, we demonstrate that despite persistence of gut microbiome dysbiosis in obese mice, a reduction in the infiltration of pro-inflammatory macrophages into the colon of obese mice can attenuate the inflammatory cascade that originates in the colon. This leads to a decrease in systemic inflammation and deceleration of the OA process that plays out in of obesity.

Significance: These findings suggest that approaches to reduce obesity-associated colonic inflammation may represent a novel strategy for addressing systemic and joint inflammation that leads to accelerated OA in the context of obesity.

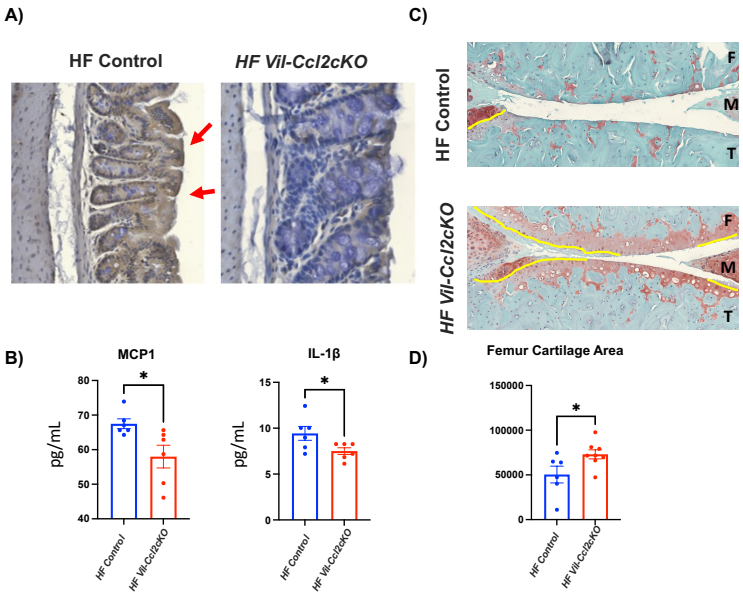

Figure 1: Conditional deletion of *Ccl2* in the intestinal epithelium is protective in the OA of obesity. A) Immunohistochemistry indicates that obese *Vil-Ccl2cKO* mice have a decrease in colonic MCP-1 staining compared to obese control animals. B) Serum cytokine analysis reveals that obese *Vil-Ccl2cKO* mice have a reduction in serum circulating levels of MCP-1 and IL-1β. C) Representative Safranin O/Fast Green-stained joints from obese *Vil-Ccl2cKO* and control mice. Yellow lines demarcate the tide mark. D) Histomorphometric analysis reveals that obese *Vil-Ccl2cKO* mice have an increase in total femur cartilage area.