Investigating chemerin/CMKLR1 signalling as a novel link between obesity and inflammatory bowel disease

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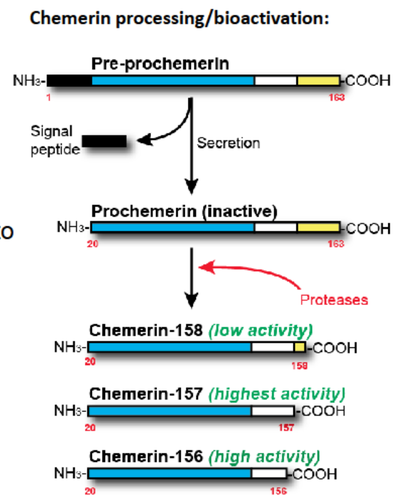
BACKGROUND

❖ Bioactive chemerin is a potent chemoattractant that recruits cells expressing the G-protein coupled receptor Chemokine-like receptor 1 (CMKLR1) to sites of inflammation

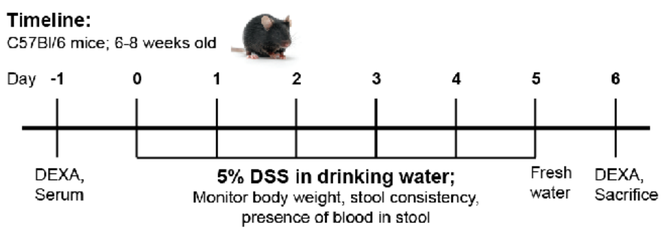
❖ Clinical studies demonstrate increased circulating levels of chemerin in several inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis; in murine models, chemerin injection has been reported to exacerbate colitis

❖ Chemerin is an adipocyte-secreted signaling molecule (adipokine) and circulating levels are correlated with adiposity

❖ Increased WAT mass negatively impacts severity and treatment of IBD; however, the link between adiposity and IBD is poorly understood

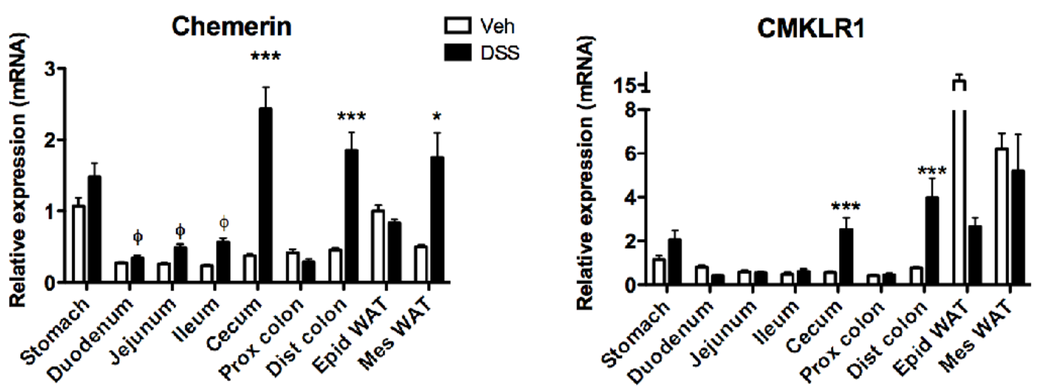


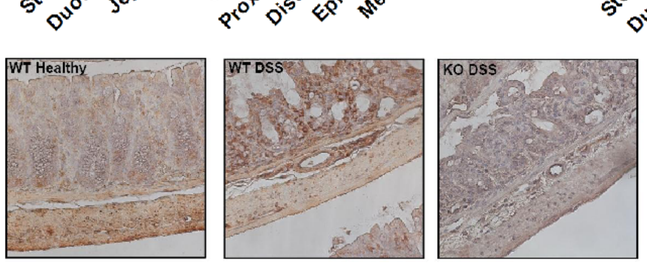
Hypothesis: *Chemerin plays a pro-inflammatory role in colitis development and loss of chemerin signaling will protect against disease severity*



RESULTS

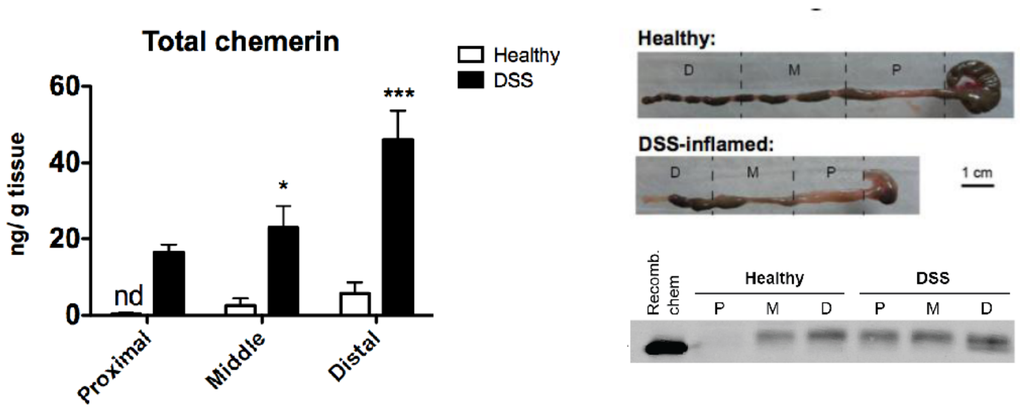
1. Chemerin and CMKLR1 expression both increase in the cecum and distal colon following DSS treatment





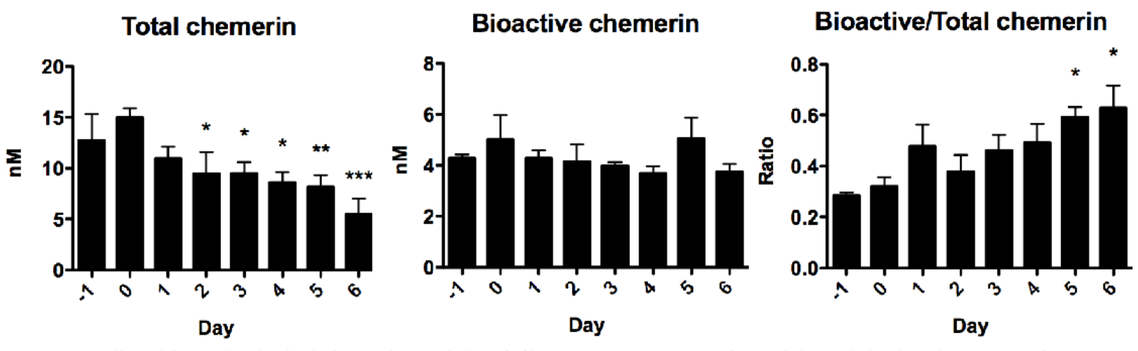
Above: RNA from the indicated tissue was isolated at sacrifice from mice that received normal drinking water (Veh) or 5% DSS treatment. qPCR analysis was performed to look at changes in chemerin and CMKLR1 expression. Φ p<0.05 compared to stomach for Veh treated animals; *p<0.05 compared to Veh.
Left: CMKLR1 expression in the colon was visualised by immunohistochemistry (40X magnification)

2. Local chemerin secretion from the colon increases in a proximal-distal gradient following DSS treatment



Colons were isolated from mice exposed to DSS or vehicle treatment for 5 days, flushed with PBS, and dissected into regions indicated. Colon explants were cultured for 24h, and chemerin levels in the supernatant were evaluated using ELISA or Western blot. *p<0.05 relative to healthy control.

3. Circulating levels of relative bioactive chemerin increase throughout DSS treatment

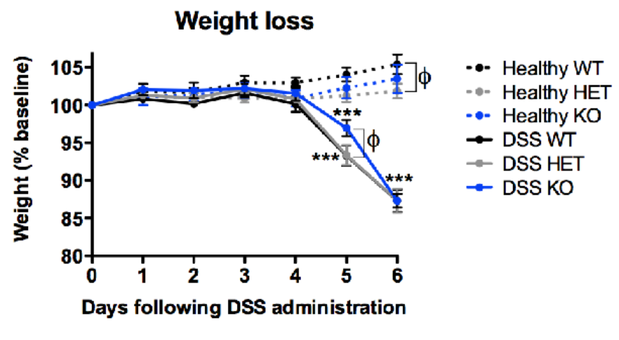


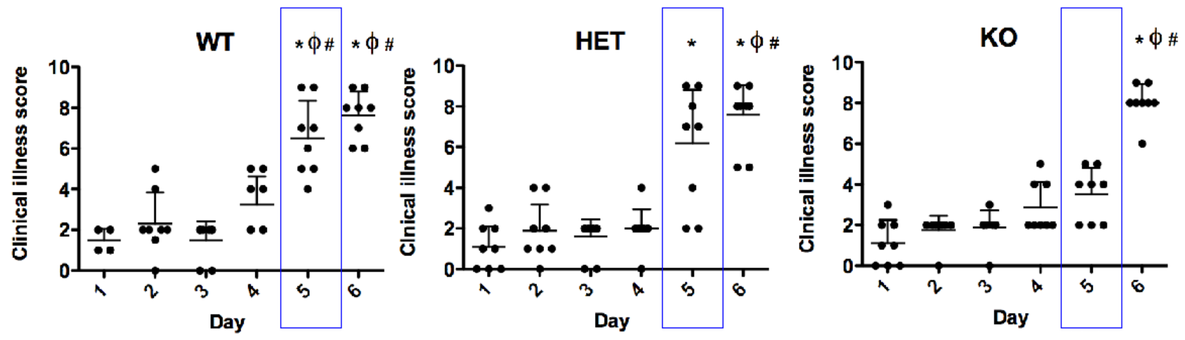
Serum was collected from mice the day before and on each day of 5% DSS treatment. An ELISA for total chemerin levels and Tango assay for mCMKLR1 activation were performed, and the ratio of bioactive:total chemerin levels calculated. *p<0.05 compared to the day before DSS treatment was initiated.

4. CMKLR1-null mice have a slower onset of disease

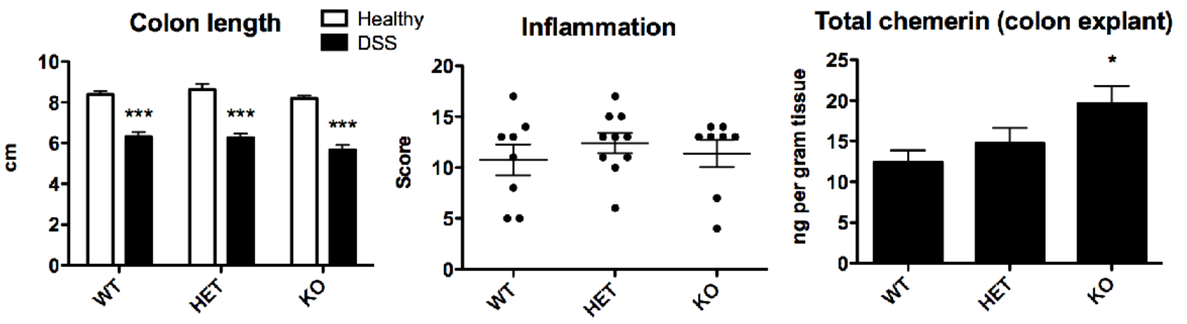
Right: Body weight of mice that received normal drinking water (healthy) or 5% DSS was monitored daily and expressed relative to initial weight on day 0. *p<0.05 relative to day 0, Φp<0.05 compared to WT within a treatment group.

Below: Mice were evaluated daily for signs of clinical illness and a score was assigned based on weight loss, stool consistency, and the presence of blood in the stool. *p<0.05 compared to day 1, Φ p<0.5 compared to day2, and #p<0.05 compared to day 3.



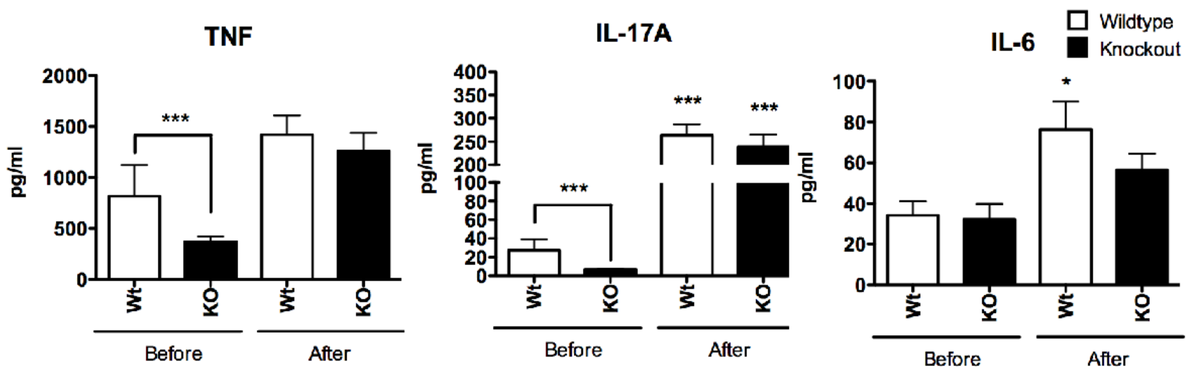


5. CMKLR1-null mice have similar levels of colon inflammation but increased local chemerin secretion



Colons were isolated from mice exposed to DSS or vehicle treatment for 5 days, measured, and processed for hematoxylin and eosin staining or colon explant culture. A blinded investigator assessed the colon for signs of inflammation based on the presence of edema (0-1), ulceration (0-3), hyperplasia (0-3), crypt damage (0-5), and inflammatory infiltrate (0-5). Chemerin levels in the supernatant of colon explant cultures were measured via ELISA. ***p<0.001 compared to healthy animals *p<0.05 compared to WT.

6. Loss of CMKLR1 alters markers of systemic inflammation



Circulating levels of pro-inflammatory cytokines were measured in the serum of mice before and after 5% DSS treatment using a Multiplex assay. ***p<0.05 compared to healthy animals of the same genotype or as indicated.

CONCLUSIONS & FUTURE STUDIES

❖ Chemerin expression, secretion, and processing increase along a gradient positively associated with the severity of colon inflammation, and circulating bioactive chemerin levels increase, following DSS treatment

❖ 6-8 and 14-16 (not shown) week-old CMKLR1 KO mice develop signs of clinical illness more slowly and have altered markers of systemic inflammation than wildtype mice, but ultimately develop colitis

❖ Systemic injection of bioactive chemerin does not alter severity of DSS-induced inflammation (not shown)

❖ Considered altogether, bioactive chemerin is a novel biomarker for the severity of colitis

❖ Strategies to modulate chemerin signalling other than chronic CMKLR1 loss are necessary to exploit chemerin as a therapeutic target for the treatment of IBD

❖ Future studies will investigate:
- ❖ Differences in populations of infiltrating cells in the colon following DSS-induced inflammation
- ❖ Source of chemerin bioactivation in DSS-induced colitis
- ❖ Effect of local chemerin administration in DSS-induced colitis
- ❖ Morphology and immune cell infiltration of mesenteric WAT in CMKLR1 KO mice
- ❖ Role of other chemerin receptors (GPR1, CCRL2)

ACKNOWLEDGEMENTS

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