Interleukin-1 Receptor 9 Gene Deletion Worsens Murine Colitis

Joseph M. Gaballa[1], Jesús Amo Aparicio[1], Carlo Marchetti[1], Shaikh M. Atif[1], Tania Azam[1], William S. Webber III[1], Makenna May[1], Suzhao Li[1], & Charles A. Dinarello[1]

[1]Department of Medicine, University of Colorado Anschutz Medical Campus

University of Colorado Anschutz Medical Campus
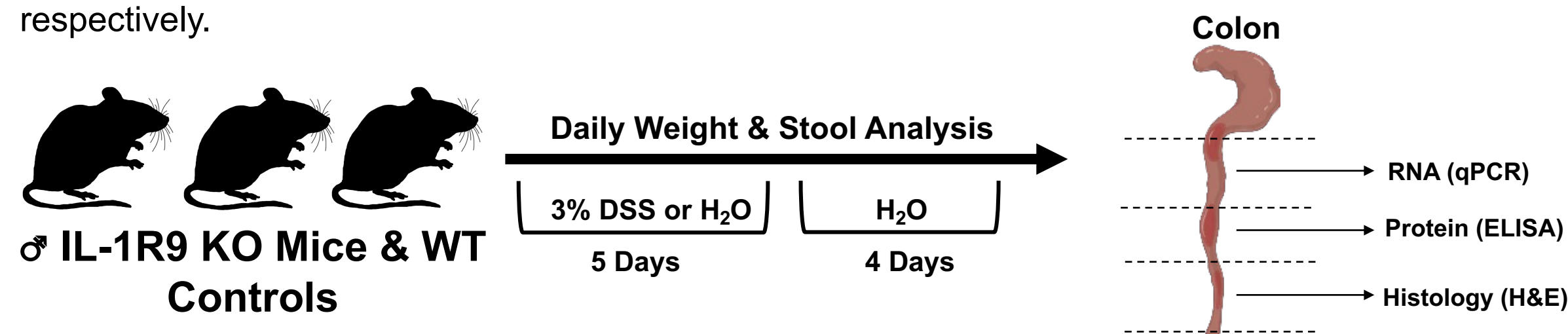
Introduction & Aims

Inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis, are characterized by chronic inflammation in the gastrointestinal tract. The pivotal role of the immune system in driving IBD pathogenesis is underscored by the current therapies which are designed to modulate immune responses. Several key immune cell subtypes, including macrophages, B cells, and T cells, have been implicated in disease progression. These cells employ intricate cytokine signaling networks to drive inflammation in IBD. Interleukin-38 (IL-38), a member of the interleukin-1 (IL-1) family, is an anti-inflammatory cytokine that has been demonstrated to contribute to the resolution of inflammation in ulcerative colitis. Previous research by our lab revealed that the genetic deletion of the Il38 gene (Il1f10) exacerbates colitis in a mouse model, however, the precise mechanism through which IL-38 acts to control inflammation in IBD has remained elusive. In this study, we aimed to characterize the role of an IL-38 ligand binding receptor, interleukin-1 receptor 9 (IL-1R9; IL1RAPL1) in regulating the severity of murine colitis.
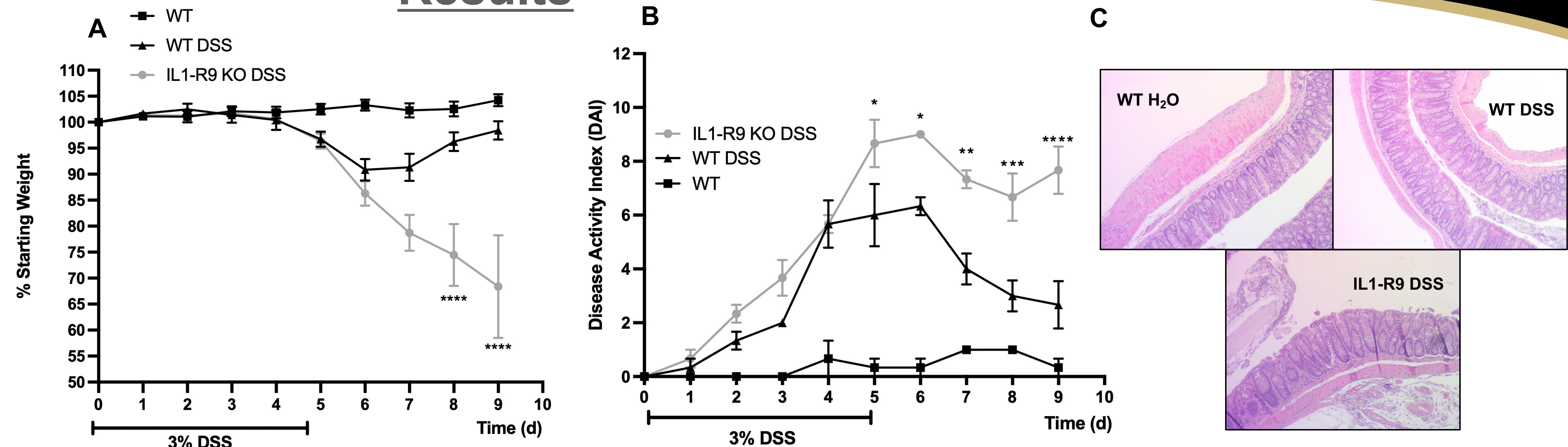
Background

- IL-38 is an anti-inflammatory cytokine that plays a crucial, protective role in several autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis, and IBD.

- Levels of IL-38 are increased in the intestinal mucosa of IBD patients.[1]

- At the molecular level, IL-38 acts to block signaling of IL1-R6 (pro-inflammatory) by functioning as a receptor antagonist.[2]

- IL-38 has also been shown to bind to IL1-R9, however the functional relevance of this interaction is largely unknown.

Methods

In this study, we induced colitis in IL1-R9 knockout mice and age and sex matched wild-type controls by supplementing drinking water with 3% dextran sodium sulfate (DSS) for 5 days. After 5 days, we allowed them to recover with fresh water for 4 days before sacrifice. To quantify the disease activity index (DAI), we obtained body mass measurements, assessed stool consistency, and scored rectal bleeding during each day of the study. Upon sacrifice, colons were isolated, and inflammation was assessed through histologic analysis and colon length measurements. In addition, we measured inflammatory gene and protein expression through quantitative PCR and ELISA, respectively.

♂ IL-1R9 KO Mice & WT Controls — Daily Weight & Stool Analysis — 3% DSS or H2O (5 Days) — H2O (4 Days) — Colon → RNA (qPCR), Protein (ELISA), Histology (H&E)

Results

Figure 1: IL1-R9 KO mice develop a more severe colitis phenotype. A) Daily disease activity index was calculated by assigning 4 points to each of the following parameters: weight loss, rectal bleeding, and stool consistency. Maximum DAI was 12. B) Weight loss of IL1R9 KO and WT mice subjected to DSS. C) Representative histology of the rectum highlighting increased mucosal inflammation and crypt destruction in the rectum of IL1-R9 KO mice subjected to DSS colitis. Slides were stained with hematoxylin and eosin. $n=3$ mice per condition. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$
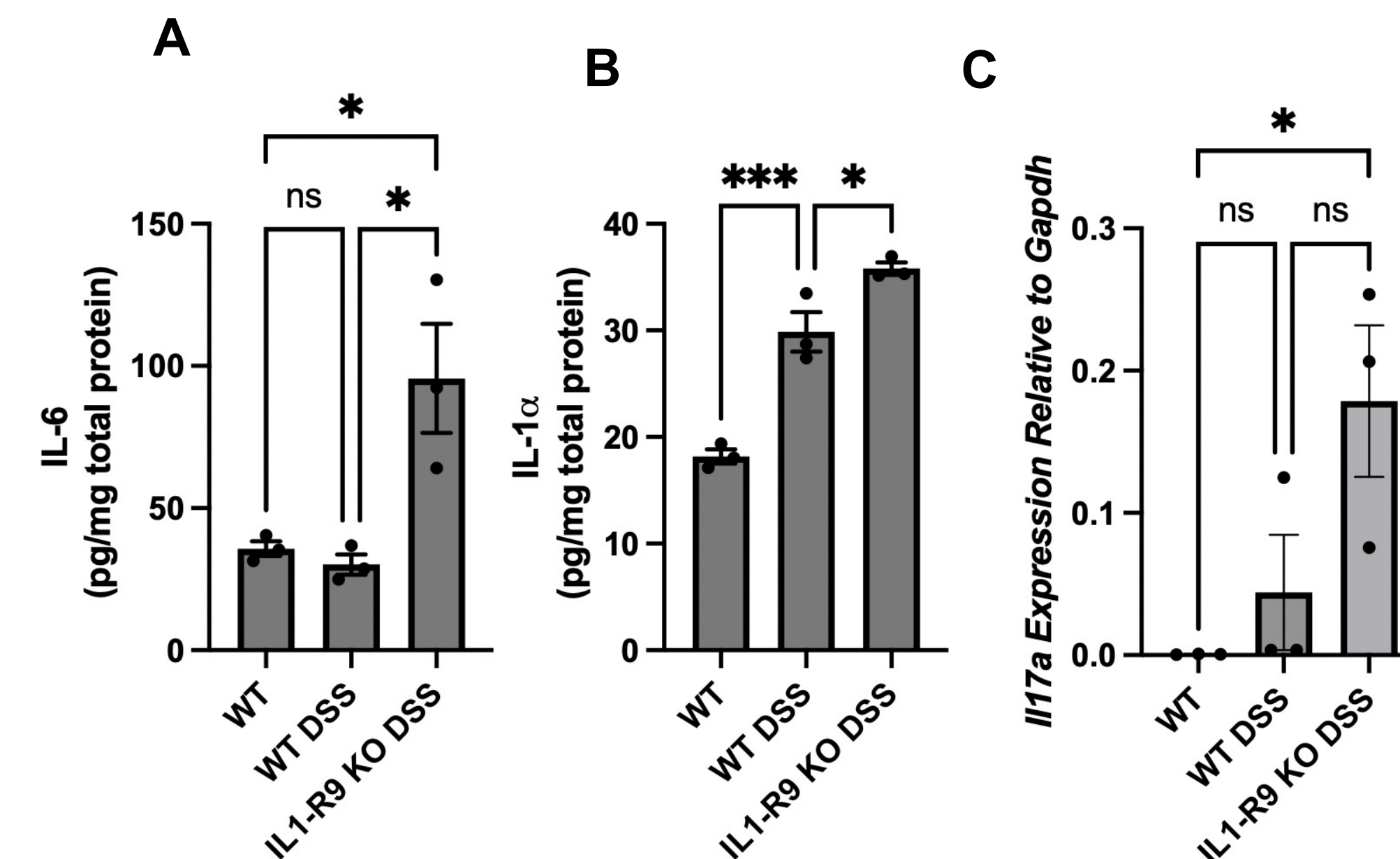
Figure 2: Mice lacking IL1-R9 have increased inflammatory cytokine expression in the colon during colitis. A,B) Measurement of IL-6 and IL-1α levels in the colon during colitis. C) Comparison of relative Il17a expression in the colon during colitis. $n=3$ mice per condition. *=$p<0.05$, **=$p<0.01$, ***=$p<0.001$, ****=$p<0.0001$

Conclusions

We observed a more severe colitis phenotype in the IL1-R9 knockout mice compared to the wild type mice. Gene and protein analysis in the colon revealed a prominent Th17 signature as indicated by heightened IL-6 and IL-17A expression. These findings shed light on the intricate interplay of cytokines and receptors of the interleukin-1 family in the regulation of IBD-related inflammation and suggests potential targets for future therapeutic interventions.

Future Directions

- Attempt to rescue colitis model with recombinant IL-38. We would expect the IL1-R9 KO mice to have no improvement in colitis upon IL-38 treatment.

- Characterize the role of IL1-R9 in CD4+ T cell differentiation using an in-vitro model. There is data in the literature that suggests that IL-38 plays an important role in regulating Treg/Th17 differentiation.

- Isolate CD4+ T cells from the colon of IL1-R9 KO mice and measure IL-17A production in comparison to wild-type.

References

[1]Ohno et al. J Clin Biochem Nutr. (2018)
[2]van de Veerdonk et al. PNAS. (2012)