Altered Gut Microbiome and Immune Response in Crh-deficient Mice: a Novel Role for CRH in the Maintenance of Intestinal Homeostasis?

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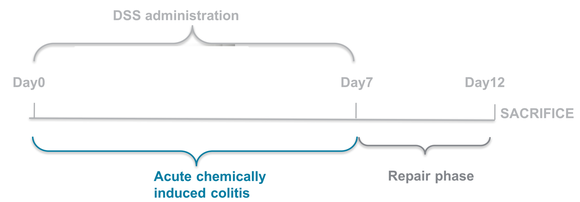
Background and Aims

Corticotropin Releasing Hormone (CRH), is the major mediator of the stress adaptive response in mammals and is implicated in the development and progress of intestinal inflammation. We have previously demonstrated that Crh-null (*Crh-/-*) mice are more susceptible to DSS- induced colitis, develop a more severe systemic disease and are unable to restitute the intestinal epithelial cell barrier, phenomena partially related to a defective regulation of autophagy.

Our aim was to investigate the role of CRH in the host-microbiome interaction in the mouse intestine.

Methods

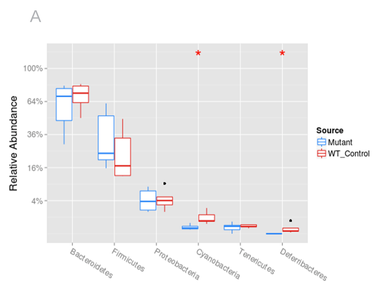
Crh-/- or wild-type (wt) adult male mice, of C57/Bl6J background were exposed to 3% DSS via their drinking water for 7 days and treated with a broad spectrum antibiotics mix (ciprofloxacin: 200mg/L, metronidazole: 500mg/l) . Mouse colon tissues were collected 7 days after initiation of DSS treatment as well as 4 days after the completion of DSS administration (repair phase) and processed for proteomic and RNA- seq analysis. Stool samples were collected and analyzed respectively to the alpha and beta diversity as well as the total 16S RNA expression. Some of the molecular targets were further evaluated using Western Blot, immunohistochemistry and Q-PCR.

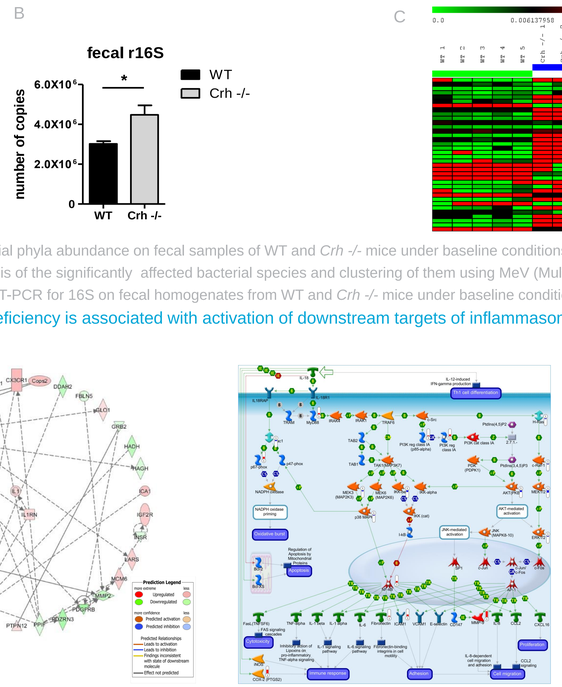


DSS induced colitis model- schematic representation

Results

Figure 1. Increased bacterial load and altered species distribution in *Crh-/-* mice under basal conditions





A.Relative bacterial phyla abundance on fecal samples of WT and *Crh -/-* mice under baseline conditions. *p < 0.05

B. In silico analysis of the significantly affected bacterial species and clustering of them using MeV (Multiple experiment Viewer) bionformatic tool.

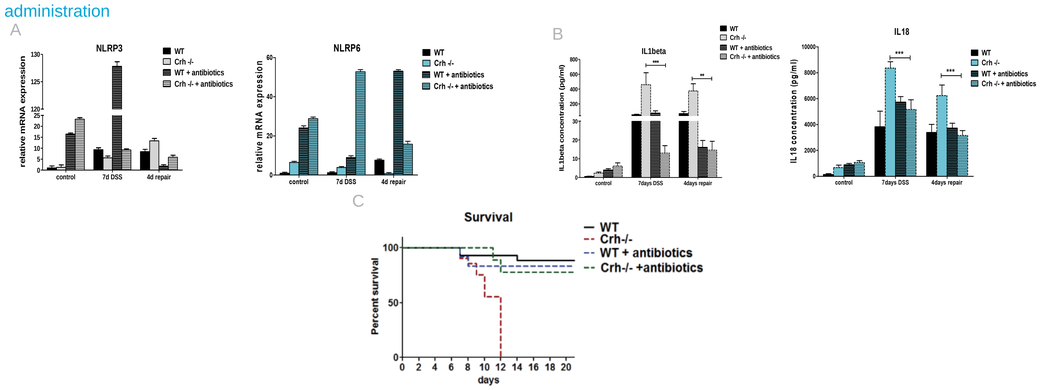
C. Quantitative RT-PCR for 16S on fecal homogenates from WT and *Crh -/-* mice under baseline conditions. *Crh -/-* mice exert a higher bacterial load. *p < 0.05

Figure 2. Crh deficiency is associated with activation of downstream targets of inflammasome

A.Proteomic analysis of whole protein lysates from from WT and *Crh -/-* colons immediately (7 days) after 3% DSS administration. Both IL18 and IL1beta pathways are upregulated.

B. Western blot analysis of inflammasome downstream targets, cytokines IL1beta and IL18. Whole protein lysates were extracted from WT and *Crh -/-* colons under baseline conditions (control), immediately (7 days) or 4 days after (repair) 3% DSS administration. *p < 0.05

Figure 3. Increased levels of IL1beta and IL18 and inflammation induced lethality of *Crh-/-* mice are rescued after antibiotic administration

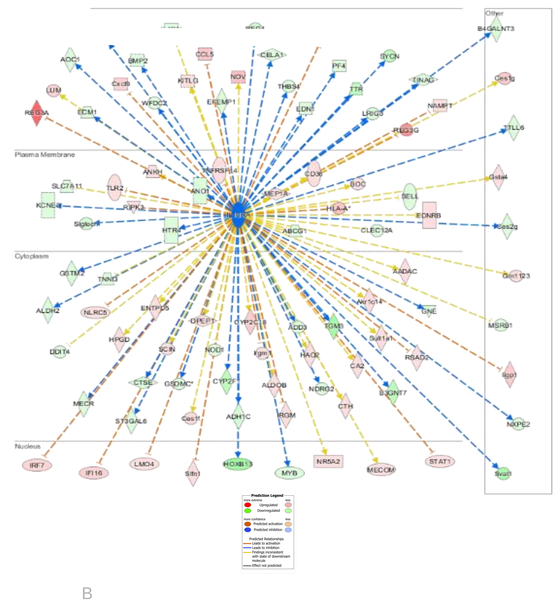


A. Quantitative RT-PCR for NLRP3 and NLRP6 conducted on colon homogenates from WT and *Crh-/-* in baseline conditions (control), immediately (7 days) or 4 days following (repair) 3% DSS administration, before and after antibiotics administration. Notably there is increasing activation of NLRP3 following DSS colitis in contrast to the downregulation of the expression of NLRP6.

B. Excessive secretion of IL1beta and IL18 following completion of DSS treatment in the *Crh-/-* mice. Cytokines secreted from WT and *Crh-/-* colonic tissue explants isolated following at the indicated times after the completion of DSS administration. Notably, *Crh-/-* mice had increased basal levels of IL1beta and IL18 during the remission phase which are normalized after the administration of antibiotics. **p < 0.01, ***p < 0.001

C. Kaplan-Meier survival curve from WT (n=52) and *Crh-/-* (n=42) mice with DSS, WT (n=) and Crh-/- (n=) mice with DSS and antibiotics administration.

Figure 4. *Crh -/-* mice exert decreased expression of IL10 receptor (IL10Ra) and its downstream targets despite the elevated IL10 levels



A. Transcriptomic analysis (RNAseq) of colon homogenates from WT and *Crh -/-* mice immediately (7 days) after 3% DSS administration. IL10Ra signaling pathway is downregulatedin the absence of Crh.

B. Quantitative RT-PCR for IL10, IL10Ras and its downstream targets STAT3 and SOCS3 conducted on colon homogenates from WT and *Crh-/-* in baseline conditions (control).Notably there is increasing expression of IL10 in contrast to the downregulation of the expression of IL10Ra. *p < 0.05, **p < 0.01, ***p < 0.001

Conclusions

Our findings suggest the intriguing possibility for the interaction of Crh, inflammasome and IL-10 signaling pathway in the maintenance of intestinal epithelial homeostasis. This is the first evidence for a potential link between the main mediator of the adaptive response to stressors, the gut microbiome and mechanisms driving resolution of inflammation and epithelial regeneration following injury. Finally, our findings suggest the intriguing possibility for a therapeutic potential of CRH and its agonists in the resolution of inflammation, epithelial regeneration and intestinal barrier restitution in colitis. Our ongoing studies aim to translate our findings in a human in vitro system.

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