Innate Immune Mechanisms by which TLR4 SNPs Contribute to Severe Murine Colitis

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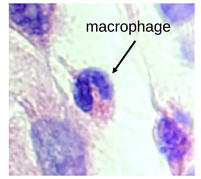

BACKGROUND

Toll-Like Receptor 4 (TLR4)

•TLR4 is a genomically-encoded Pattern recognition Receptor (PRR) of the innate immune system that responds to Gram negative bacterial lipopolysaccharide (LPS)

TLR4 Single Nucleotide Polymorphisms (SNPs)

•~10-18% of Caucasians express D299G/T399I SNPs (exon 3, TLR4 extracellular domain, rs4986790 and rs4986791)
•These mutations affect the functionality of the receptor and are correlated with hyporesponsiveness to LPS, increased susceptibility to Gram negative sepsis, and IBD
•The Vogel laboratory engineered knock-in mice that homozygously express the murine homologues (D298G/N397I) of the human TLR4 D299G/T399I SNPs
•TLR4-SNP mice were shown to exhibit **LPS-hyporesponsiveness** *in vivo* and *in vitro*[1]

TLR4 SNPs in Inflammatory Bowel Disease (IBD)

•LPS-unresponsive mouse strains (C3H/HeJ, C57BL/10ScCr, MyD88$^{-/-}$ and TLR4$^{-/-}$) are highly susceptible to DSS-induced colitis[2,3]

IBD – Including Crohn's Disease and Ulcerative Colitis

•Pathogenesis of IBD is thought to involve a combination of genetics, the environment, immune system dysregulation, and microbiome alteration (dysbiosis)

M2 Macrophages (Mϕ) in IBD

•Defects in intestinal tissue repair have been causally linked to altered Mϕ polarization[4]
•Rosiglitazone, a PPARγ ligand, has been shown to upregulate M2 macrophages and has been used to ameliorate IBD in humans[5]

HYPOTHESIS

The TLR4 SNPs lead to impaired innate immune responses that underlie impaired colonic functioning and more severe mucosal damage in this subset of IBD patients.

METHODS

Alternating days measure:
- Stool consistency, fecal occult blood

Harvest days:
- Measure colon length, 1 cm distal colon for H&E, flash freeze remaining colonic tissue

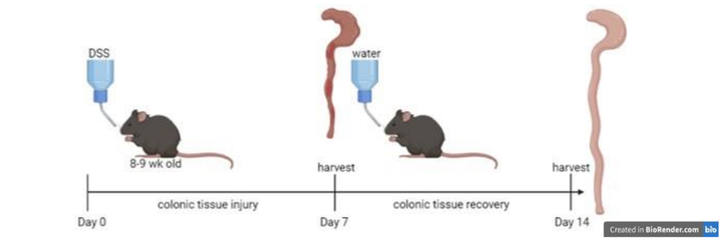

Figure 1. Establishment of DSS model in WT mice. Male and female (8 wk old) C57BL/6J mice were administered 3% DSS in their drinking water for 7 days, followed by 7 days of water only. Mice were weighed daily and the % change recorded. Results are from 4 experiments (males, n=26; females, n=27). To insure reproducibility, subsequent DSS lots were titrated to induce comparable kinetics and magnitude of weight loss. Mean starting weights of male and female mice were 24.8±0.3 and 19.1±0.2 g, respectively. *, p<0.05, 2-way ANOVA with Sidak's multiple comparisons.

RESULTS

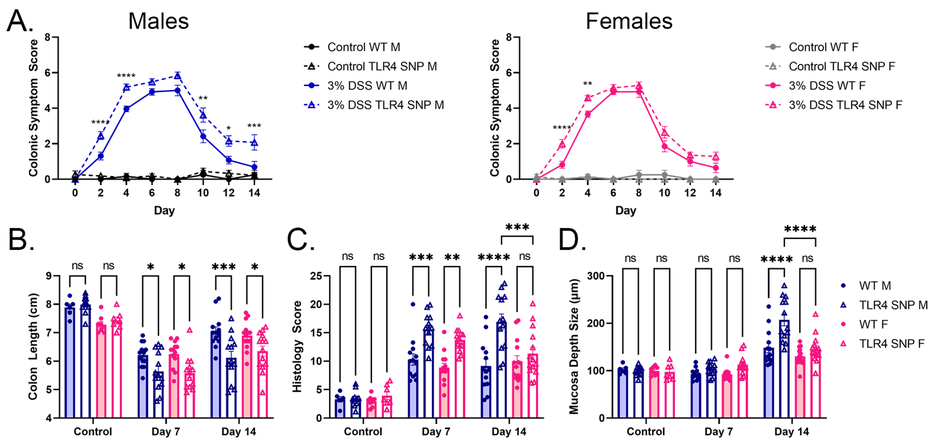

Figure 2. Genotype- and sex-specific differences in WT and TLR4-SNP mice treated with DSS. (A) Colonic symptom scores, (B) colon lengths, (C) histology scores, and (D) mucosal depth measurements. WT and TLR4-SNP mice, n=26/strain; female mice, n=27/strain from 4 separate experiments. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001 by one-way ANOVA with Tukey's multiple comparisons test, comparing at each time point WT and TLR4-SNP groups. Error bars are SEM.

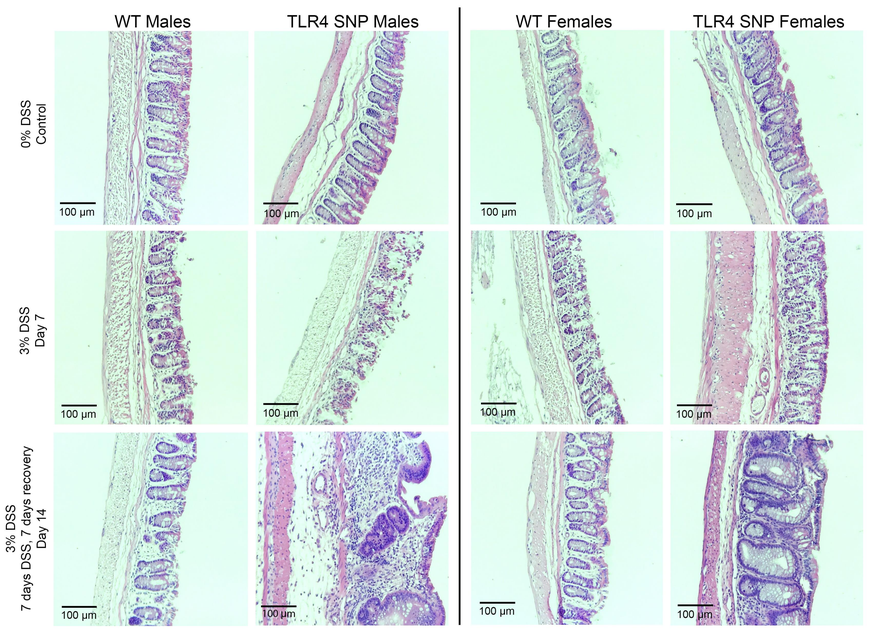

Figure 3. H&E-stained colon sections of male and female WT and TLR4-SNP mice exposed to DSS. ≥6 fields/mouse/experiment were analyzed. Histology score (**Fig. 2C**) is the sum of epithelial damage (0 = intact epithelia, 1 = mild damage, 2 = moderate damage, 3 = severe damage), inflammatory infiltrate into the mucosa/crypt loss (0 = no infiltration/no crypt loss, 1 = mild infiltration/loss of basal 1/3 of crypt, 2 = moderate infiltration/loss of basal 2/3 of crypt, 3 = complete infiltration/entire loss of crypt), inflammatory infiltrate in the submucosa/edema (0 = none, 1 = mild, 2 = moderate, 3 = complete), and inflammatory infiltrate in the muscularis mucosa (0 = none, 1 = mild, 2 = moderate, 3 = complete infiltration). Extent of damage was used as a multiplier for each of the 4 regions analyzed (1 = focal, 2 = patchy, 3 = diffuse).

RESULTS (continued)

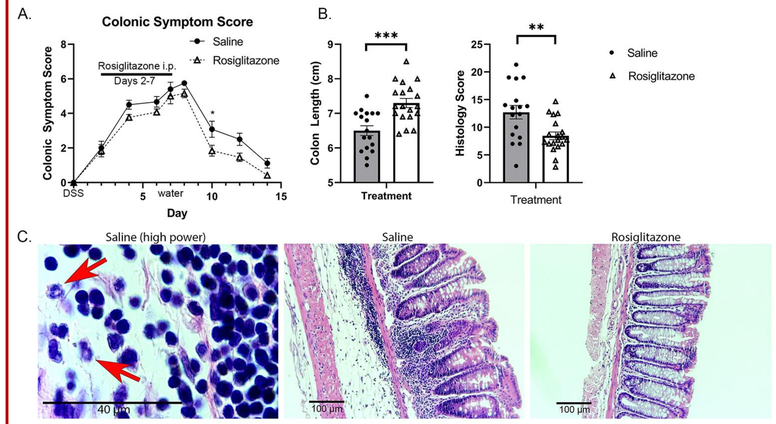

Figure 4. Rosiglitazone (25 mg/kg i.p.) treatment on Days 2-7 after improves DSS-induced colitis in male TLR4-SNP mice. (A) Colonic symptom score, (B) colon length and histology score, and (C) H&E-stained distal colon segments (left panel: arrows point to rare PMN). Saline-treated TLR4-SNP mice, n=17 and rosiglitazone-treated TLR4-SNP mice, n=19 from 3 separate experiments. *p<0.05, **p<0.01, ***p<0.001, by (A) two-way ANOVA with Tukey's multiple comparisons test, and (B, C) unpaired t test. Error bars are SEM.

CONCLUSIONS

- TLR4-SNP mice exhibit **more severe colitis** than WT mice (*e.g.*, weight loss, disease score, histopathology).
- TLR4-SNP **male** mice exhibit more severe DSS-induced colitis than TLR4-SNP female mice.
- TLR4-SNP mice have a **delay in recovery** at Day 14.
- Rosiglitazone **significantly enhanced recovery** from DSS-induced colonic damage in TLR4-SNP male mice by Day 14.
- Majority of infiltrating cells are **mononuclear**, supporting a critical role for macrophages in recovery.

FUTURE DIRECTIONS

•Evaluate the role of specific innate immune dysregulations that influence colitis severity:
  - M1 *vs* M2 Mϕ marker expression analysis
  - Colitis severity in mice lacking PPARγ in macrophages and neutrophils
  - Colitis severity in mice lacking the IL-4 and IL-13 shared receptor IL-4Rα

•Evaluate the role of Tlr4 SNPs in establishing or maintaining tight junction integrity
•Analyze the role of the microbiome in the WT *vs* TLR4 SNP colitis severity difference
•Compare chemical-induced colitis to infection-induced colitis (*Citrobacter rodentium*)

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References
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