POWERED BY genOway's PRECLINICAL MODELS

Modeling Inflammatory Bowel Disease in mice with "Humanized" immune system

Bérengère Coupé[1], Alison Charton[1], Fanny Placide[1], Frederic Machet[1]

P0202

[1] *Evotec (France), Campus Curie, 195 route d'Espagne, 31036 TOULOUSE. Contact: berengere.coupe@evotec.com*

Background

Inflammatory bowel disease (IBD) is a recurrent chronic inflammatory disease with unknown etiology. Dextran sulfate sodium (DSS) induced colitis is a common preclinical mouse model in IBD research. DSS colitis involves activation of the submucosal immune system and can be used to study ulcerative colitis characteristics along different phases (acute, chronic and remission) and predict therapeutic efficacy. However, improving translation to human is still needed and the use of mice with Human Immune System (HIS) may provide a better model to study human immune system responses *in vivo*. HIS mice are created by transplanting human immune cells or their progenitors into highly immunodeficient recipient mice, thereby "humanizing" their immune systems. In this study we discuss colitis features under humanized conditions and benefits for testing therapeutics in preclinical studies.

Methods

BRGSF-HIS (Balb/c, Rag2-/-, IL-2Rγc-/-, SIRPαNOD, Flk2-/- mice) mice from GenOway (France) were used in this study. BRGSF-HIS mice were reconstituted with human CD34+ stem cells sorted from umbilical cord blood. Female 15-week-old BRGSF-HIS mice from 2 different donors were treated with 1.5%, 2% or 2.5% dextran sulfate sodium (DSS) in drinking water (ad lib.) for 5 days to induce acute colitis (Fig 1).

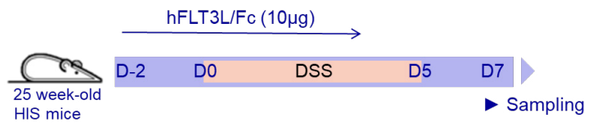


Figure 1: Study timeline

Group	Inducer (water bottle)	N
1	Water	4
2	DSS 1.5%	6
3	DSS 2%	6
4	DSS 2.5%	6

Table 1: Study groups

All mice were weighed daily, and the clinical score was measured daily based on body weight loss, feces consistency and presence of blood in feces. The DAI score (0-8) was determined by adding up the scores of the 3 individual parameters (Table 2).

Body Weight Loss (%)	Feces consistency	Blood in feces/rectum	Score
<1%	Normal	No	0
1-5%	–	Yes	1
5-10%	Soft	–	2
10-18%	Liquid	–	3
>18%	–	–	4

Table 2: Disease Activity Index scoring method

DSS was removed on Day 5. Mice were sacrificed on Day 7 to collect blood and colons to assess the following:

- Human cytokines concentration in colon and plasma were determined using V-PLEX proinflammatory Panel human kit.
- Colonic histological damage was assessed on colon Swiss roll after H&E staining. The scoring system used to score inflammation in the colon was based on three parameters: the loss of goblet cells, the loss of crypts architecture and the infiltration of immune cells at the level of the mucosa, submucosa and transmural wall of the colon.

Results

Immune cell characterization in BRGSF-HIS mice

- BRGSF-HIS mice exhibited 30% of mouse CD45+ cells and 55% of human CD45% cells in the spleen on Day 7.
- In agreement with GenOway's data, among human CD45+ cells, major human hematopoietic subsets, such as B cells (72%), T cells (25%), NK (<1%) and myeloid (<0.5%) cells were identified (Fig 2). With age, the balance between B and T cells tends to normalize.
- hFLT3L/Fc treatment increased myeloid cell number in the spleen (<1%).

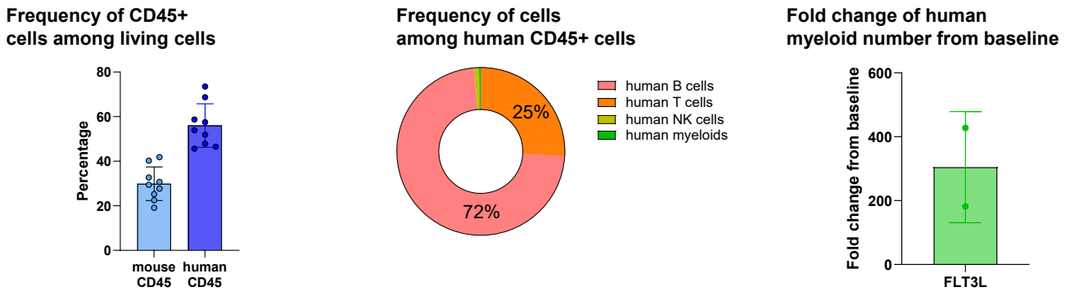


Figure 2: Human and mouse immune cell population in BRGSF-HIS mice and effect of FLT3L treatment on myeloid cells.

Effects on body weights & disease activity index scores

- 2.5% DSS treatment significantly reduced body weights starting from Day 5. Lower DSS doses, 1.5 and 2%, slightly reduced body weights but the effects did not reach statistical significance (Fig 3).
- DAI significantly increased in 2.5% and 2% DSS groups compared to Ctrl group (Fig 4).

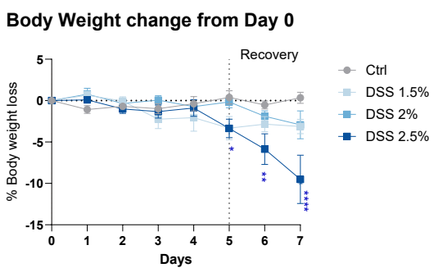


Figure 3: Effects of DSS on % body weights change from Day 0. Shown are means ± S.E.M. (n=4-6). *, **, ****: p < 0.05, p < 0.01, p<0.0001 vs. Ctrl, 2-way ANOVA followed by Dunnett's multiple comparisons test.

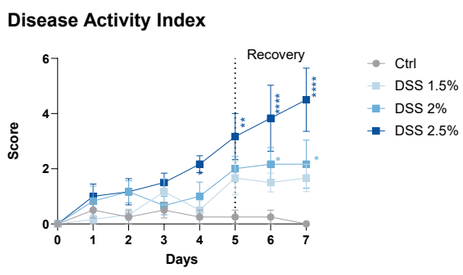


Figure 4: Effects of DSS on Disease Activity Index scores. Shown are means ± S.E.M. (n=4-6). *, **, ****: p < 0.05, p < 0.01, p<0.0001 vs. Ctrl, 2-way ANOVA followed by Dunnett's multiple comparisons test.

Effects on colon length and weight-to-length ratio

- 2.5% DSS decreased colon length compared to Ctrl group (Fig 5A). DSS increased colon weight to length ratio but reached significance at 1.5% (Fig 5B)

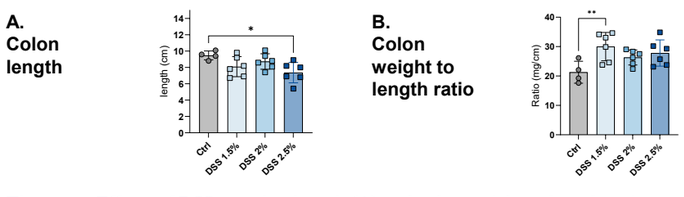
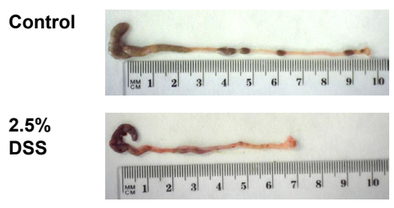


Figure 5: Effects of DSS on colon length (A) and colon weight to length ratio (B). Shown are. means ± SD (n=4-6). *, **; p < 0.05, p < 0.01, vs. Ctrl group, One-way ANOVA followed by Dunnett's multiple comparisons test.



Representative pictures of colon from Control and 2.5% DSS groups.

Effects on human cytokine levels in plasma

2% and 2.5% DSS increased human IL-18, IL-1β, IFNγ, IL-8, IL-6 and TNF plasma levels compared to Ctrl group (Fig 6). The effects were statistically significant only for human IL-8 for 2 and 2.5% DSS (Fig 6D).

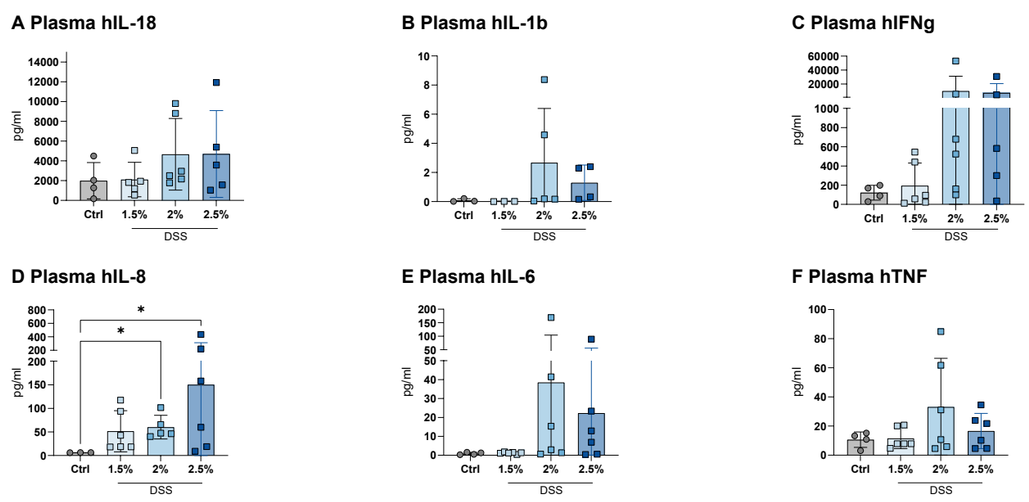


Figure 6: Effects of DSS on plasma levels of human IL-18 (A) IL-1β (B) IFNγ (C), IL-8 (D), IL-6 (E), TNF (F) on Day 7. Shown are means ± SD (n=4-6) for the groups indicated. *: p < 0.05 vs. Ctrl group, Kruskal-Wallis followed by Dunn's multiple comparisons test.

Effects on human cytokine levels in colons

2% and 2.5% DSS increased human IL-18, IL-1β, IFNγ, IL-8, IL-6 and TNF colon levels compared to Ctrl group (Fig 7). Significance was observed for human IL-18, IL-1β, IFNγ, and IL-8.

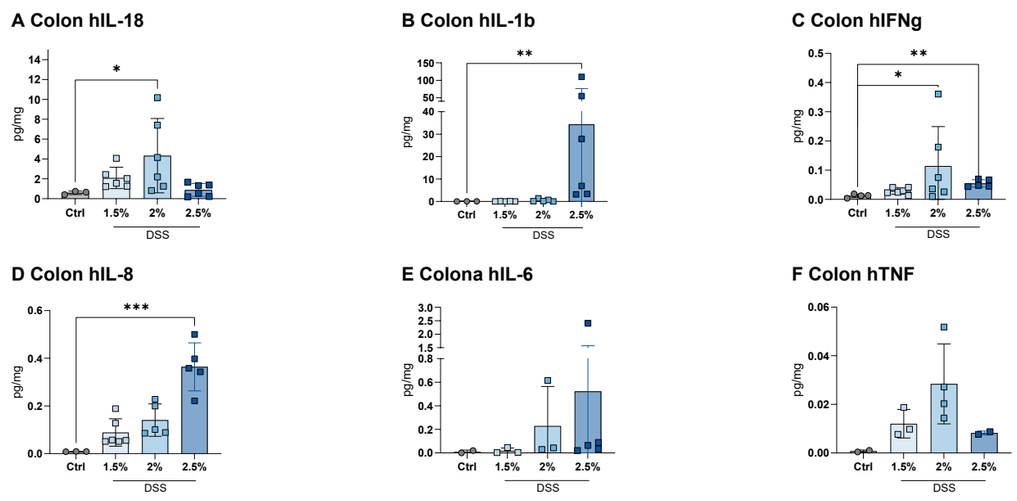


Figure 7: Effects of DSS on colon levels of human IL-18 (A) IL-1β (B) IFNγ (C), IL-8 (D), IL-6 (E), TNF (F) on Day 7. Shown are means ± SD (n=4-6) for the groups indicated. *, **, ***: p < 0.05, p < 0.01, p<0.001 vs. Ctrl group, Kruskal-Wallis followed by Dunn's multiple comparisons test.

Effects on histopathology scores

Examination of H&E-stained colon Swiss rolls showed that 2% and 2.5% DSS significantly increased immune cells infiltration, and induced loss of both crypt architecture and goblet cells, resulting in a significant increase in mean histopathology scores compared to the Ctrl group (Fig 8).

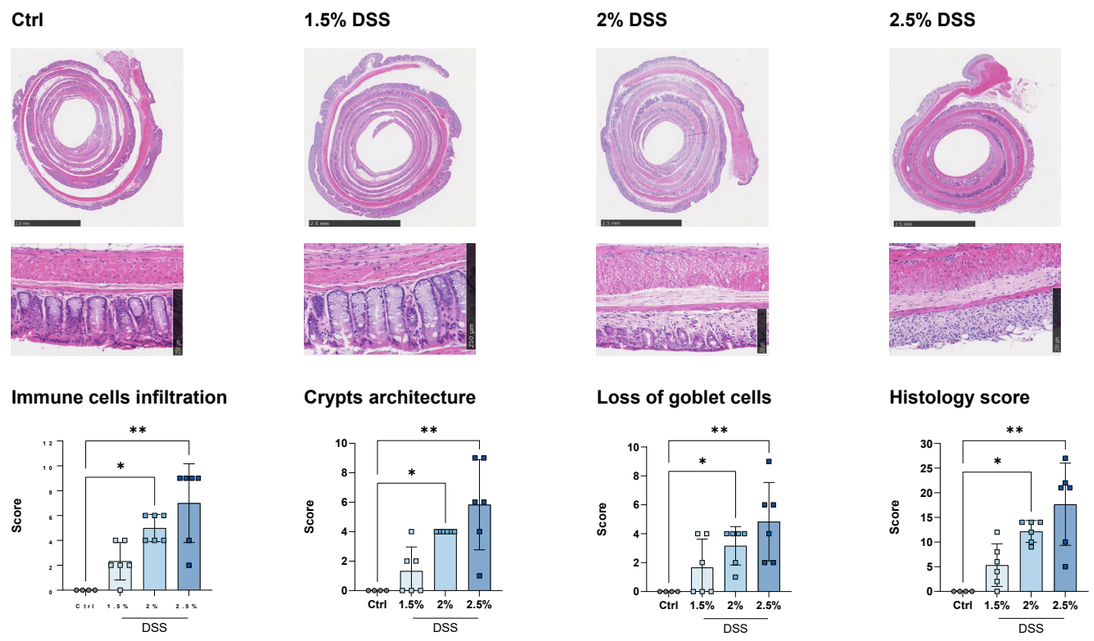


Figure 8: Representative images of H&E-stained colon Swiss rolls sections. Effects of DSS on colon histopathology scores at Day 7. Shown are means ± SD (n=4-6) for the groups indicated. *, **: p < 0.05, p<0.01 vs Ctrl group, Kruskal-Wallis followed by Dunn's multiple comparisons test.

Conclusions

- For the first time, we report here that BRGSF-HIS mice, a humanized model of BALB/c genetic background, that underwent cytokine treatment to boost the myeloid compartment, develop robust signs of colitis after 5-day 2.5% DSS treatment.
- DSS treatment in BRGSF-HIS mice induced dose-dependent effects on DAI scores, human pro-inflammatory cytokines colon levels and histopathology scores. The magnitude of effects was comparable to that typically observed in normal mice.
- Despite the low percentage of myeloid cells in BRGSF-HIS mice (<1% after hFLT3L/Fc boost), DSS treatment induced and maintained colitis until Day 7.
- BRGSF-HIS mice provide a valuable solution for directly assessing the efficacy of human-specific antibodies and other biologics, but also increase clinical translation, for example by adding IL-8 to the pathways.