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The Role of SP-D in Human Colonic Inflammatory Bowel Disease and in Murine Dextran Sodium Sulfate Induced Colitis

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BACKGROUND

SP-D is a collectin widely distributed on mucosal surfaces of the body including the gastrointestinal tract (1). It is part of the innate immune system and mediates control of pro-inflammatory mechanisms.

Genetic variations of SP-D have recently been associated to inflammatory bowel disease (IBD) in a Japanese population and showed association to ulcerative colitis (UC) (2). In contrast, an American case-control analysis revealed a significant association between a specific SP-D genetic variant and susceptibility to Crohn's disease (CD), but not to UC (3).

Together these two studies suggest that SP-D is involved in IBD and warrant *in vivo* studies of the related pathophysiological mechanisms.

AIM

We aimed to investigate the expression of SP-D in IBD patients and compared it to the intestinal inflammatory activity, and we compared the inflammatory response between *Sftpd*$^{+/+}$ (WT) and *Sftpd*$^{-/-}$ (KO) mice in a dextran sodium sulfate (DSS) model of colitis.

We hypothesized that SP-D dampens intestinal inflammation by reducing innate inflammatory signaling and facilitate clearance of apoptotic cells.

METHODS

Surgical specimens from IBD patients (n=27) including Crohn's disease (CD) (n=9) and ulcerative colitis (UC) (n=18) were immunohistochemically stained for SP-D. The tissue specimens was scored for SP-D expression and inflammatory activity.

Colitis was induced in WT and KO littermates by supplementing the ad libitum water with DSS.

Day 0:
Start of treatment with DSS

Day 7:
Mice receiving 1.5% DSS are terminated

1% DSS is replaced with water

Day 10:
Mice are terminated after receiving water for 3 days

Colon tissue were stained with hematoxylin & eosin to estimate the histological damage, and immunohistochemical staining's were used to analyze levels of apoptosis, necrosis and inflammatory infiltration.

Colon tissue samples were analyzed for inflammatory markers (TNF-α, IL-6, INF-γ and CCL-2) by ELISA analysis.

RESULTS

- We found a significant correlation between the expression of intestinal SP-D and inflammatory activity in IBD patients (**Fig. 1**).
- Mice treated with DSS did experience a notable weight loss and increased disease activity index (DAI) score **(Fig. 2)**, but there were no difference between WT and KO.
- ELISA analysis showed a significant difference in the levels of TNF-α between WT and KO in the restitution phase and qRT-PCR did show a tendential upregulation of *Sftpd* in WT mice treated with DSS (**Fig. 3, A-B**). There were no difference in histologic damage or colon length between WT and KO (**Fig. 3, C-D**).

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Fig. 1: **A)** Correlation of immunoscore and inflammatory activity in surgical specimens from IBD patients. **B)** Immunostaining for SP-D in a patient with UC. **C)** Immunostaining for SP-D in a patient with CD

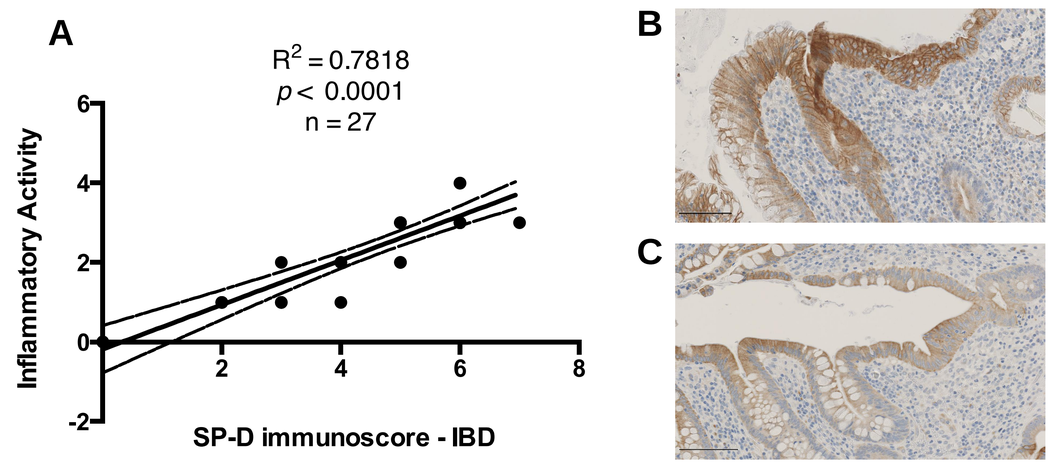


Fig. 2: A) Weight loss. **B)** DAI score. All data are presented with SEM.

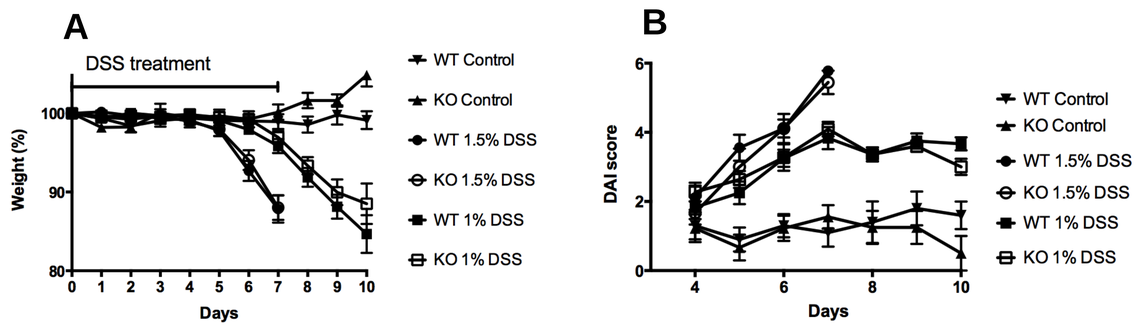
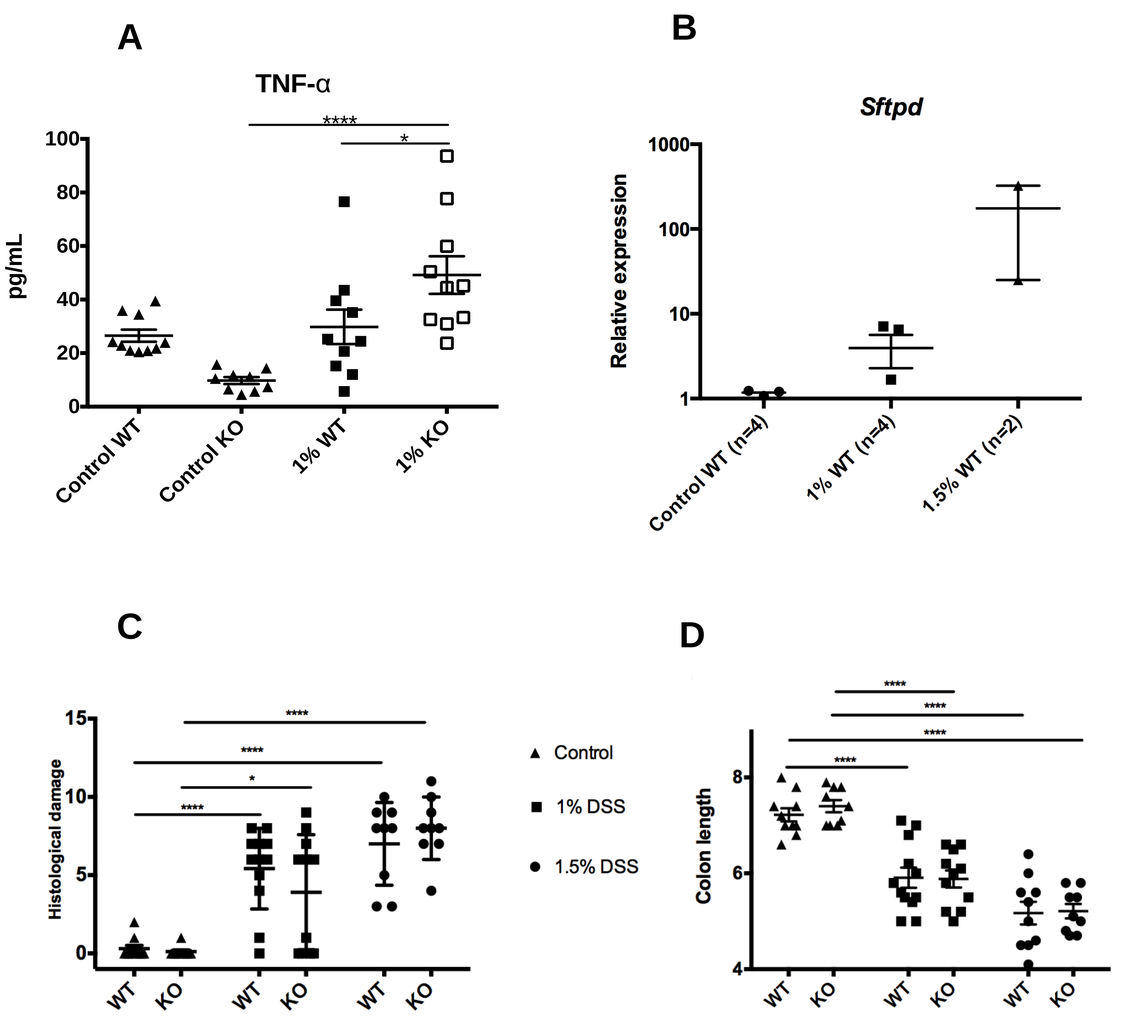


Fig. 3: A) ELISA analysis showed a significant difference between colonic levels of TNF-α in WT compared to KO in the restitution phase. **B)** Quantative real-time PCR of murine expression of SP-D in the colon. **C)** Histological damage score. **D)** Length of colon. All data are presented with SEM.



CONCLUSION

- Limited anti-inflammatory effects of SP-D in DSS-induced inflammation in mice.
- Positive correlation between inflammatory activity and immunoscore for SP-D observed in surgical specimens from IBD patients.
- SP-D is upregulated in clinical disease.

REFERENCES
1. Madsen et al. J Immunol. 2000;164(11):5866–70
2. Z. Lin et al., Ann. Hum. Genet. 75, 559–568 (2011).
3. M. Tanaka et al., Inflamm. Bowel Dis. 15, 918–925 (2009).

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