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의학박사 학위논문

염증성 장질환 모델에서 soluble Siglec-9의 NF-κB 신호전달 차단을 통한 장염 호전 효과

Soluble Siglec-9 alleviates intestinal inflammation through inhibition of the NF-κB pathway

2021년 2월

서울대학교 대학원
의학과 내과학전공
강 은 애

A thesis of the Degree of Doctor of Philosophy

Soluble Siglec-9 alleviates intestinal inflammation through inhibition of the NF-κB pathway

February 2021

The Department of Internal Medicine,
Seoul National University
College of Medicine
Eun Ae Kang

Soluble Siglec-9 alleviates intestinal inflammation through inhibition of the NF-κB pathway

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Submitting a Ph.D. Dissertation of Internal Medicine

October 2020

Graduate School of Medicine

Seoul National University

Internal Medicine

Eun Ae Kang

Confirming the Ph.D. Dissertation written by

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지도교수 김 주 성

이 논문을 의학과 박사 학위논문으로 제출함

2020년 10월

서울대학교 대학원
의학과 내과학전공
강 은 애

강은애의 의학박사 학위논문을 인준함

2021년 1월

위 원 장 박 규 주 (인)
부 위 원 장 김 주 성 (인)
위 원 고 재 성 (인)
위 원 김 윤 준 (인)
위 원 김 유 선 (인)

Abstract

Soluble Siglec-9 alleviates intestinal inflammation through inhibition of the NF-κB pathway

Eun Ae Kang

Internal Medicine

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Background: Sialic acid-binding immunoglobulin-like lectins (Siglecs) are a superfamily of immunoreceptors recognizing sialic acid. Siglec-9 has been shown to mediate inhibitory immune responses. The aim of this study was to evaluate the effect of a soluble form of Siglec-9 (sSiglec-9) on inflamed intestinal epithelial cells (IECs), murine macrophages, and experimental murine colitis models.

Methods: COLO 205 human IECs and RAW 264.7 murine macrophages were pretreated with sSiglec-9 and then stimulated with TNF-α or lipopolysaccharides, respectively. The expression of proinflammatory cytokines such as IL-8 and TNF-α was measured using real-time RT-PCR and ELISA. To demonstrate the inhibitory effects of sSiglec-9 on the NF-κB pathway, IκB

α phosphorylation/degradation was determined using western blotting and the DNA binding activity of NF-κB was evaluated using an electrophoretic mobility shift assay. Further, mouse models with dextran sulfate sodium-induced acute colitis and piroxicam-induced IL-10-/- chronic colitis were generated. Intraperitoneal injections of sSiglec-9 were performed, and body weight, colon length, and histopathologic findings were examined.

Results: sSiglec-9 suppressed IL-8 and TNF-α gene expression in stimulated COLO 205 and RAW 264.7 cells. sSiglec-9 inhibited IκBα phosphorylation/degradation and the DNA binding activity of NF-κB. sSiglec-9 injections significantly ameliorated weight loss, colon shortening, and the severity of intestinal inflammation in acute and chronic colitis mouse models.

Conclusion: sSiglec-9 may inhibit NF-κB activation in IECs and macrophages and alleviate experimental colitis in mice, suggesting that sSiglec-9 is a potential therapeutic agent for the treatment of inflammatory bowel disease.

Key words: Siglec–9, NF–κB, experimental colitis, inflammatory bowel diseases

Student Number: 2017-34540

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Introduction

Inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis, are characterized by chronic inflammation of the gastrointestinal tract; their pathogenesis is attributed to many causes, including genetic, immunological, and environmental factors. Variable mechanisms involved in IBD development have been targeted by numerous new treatment candidates [1-4]. Immunologic dysregulation and disruption of intestinal homeostasis play an important role in the pathogenesis of IBD [5]. The nuclear factor kappa beta (NF-kB) pathway is one of the most important mechanisms of inflammation in IBD. Inhibitors of NF-kB signaling may function as potential therapeutics for IBD [6].

Immune cells express specific glycan molecules on their cell surface. Sialic acid-binding immunoglobulin-like lectins (Siglecs) are a family of transmembrane receptors found on immune cells, including neutrophils, monocytes, dendritic cells, and macrophages [7]. Sialic acid is a ligand for Siglecs, which regulate cell to cell interaction and innate and acquired immune responses [8]. A total of 14 Siglecs have been identified in humans and mice [7]. Siglecs can mediate either pro-inflammatory or anti-inflammatory responses based on the type of Siglec. In particular, Siglec-9 plays an inhibitory role in inflammation through immunoreceptor tyrosine-based inhibitory motifs (ITIMs) [9] and can promote IL-10 production in macrophages [10]. Normal colon epithelial cells are known to express ligands for Siglec-9, which is present on the surface of mucosal macrophages [11]. Soluble Siglec-9 (sSiglec-9), which is an extracellular domain of Siglec-9, has been shown to have an anti-inflammatory effect on rheumatoid arthritis models, altering the polarization of macrophages from M1 to M2 [12]. Herein, we investigated the anti-inflammatory effects of sSiglec-9

on a murine colitis model and intestinal epithelial cells (IECs), specifically evaluating its ability to inhibit the NF-κB signaling pathway.

Materials and methods

1. Materials and reagents

Recombinant human Siglec-9 Fc chimera protein and TNF-α were purchased from R&D Systems (Minneapolis, MN, USA). Dextran sulfate sodium (DSS) was purchased from MP Biochemicals (Irvine, CA, USA). Lipopolysaccharide (LPS; from *Escherichia coli* O127:B8), dimethyl sulfoxide (DMSO), and piroxicam were provided from Sigma-Aldrich (St. Louis. MO, USA). Anti-IκB α antibody, anti-β-actin antibody, and goat anti-mouse IgG were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-phosphorylated IκBα was supplied by Cell Signaling (Danvers, MA, USA).

2. Cell culture

The human IECs (COLO 205; Korean Cell Line Bank, 10222, Seoul, Korea) and murine macrophage cells (RAW 264.7; Korean Cell Line Bank, 40071, Seoul, Korea) were seeded in 6-well plates and cultured overnight in Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, MO, USA) with 10% fetal bovine serum, 2 mM glutamine, and antibiotics (100 units/mL penicillin and 100 μg/mL streptomycin) [13-16].

3. Real-time reverse transcription polymerase chain reaction

Real-time reverse transcription polymerase chain reaction (RT-PCR) was used to measure the expression of pro-inflammatory cytokines such as IL-8 and TNF-α from COLO 205 cells and RAW 264.7 cells pretreated with sSiglec-9 for 24 h. After pretreatment, vehicle (DMSO) and treatment (2 ng/mL or 10

ng/mL of sSiglec-9) groups were stimulated with either 10 ng/mL of TNF-α (for COLO 205 cells) or 10 μg/mL of LPS (for RAW 264.7 cells) for 4 h. The expression of β-actin and IL-8 was measured from COLO 205 cells; the expression of β-actin and TNF-α was determined in RAW 264.7 cells.

4. Western blot

A western blot assay was used to demonstrate the effects of sSiglec-9 on proteins of the NF-κB pathway in COLO 205 and RAW 264.7 cells. COLO 205 cells and RAW 264.7 cells were pretreated with sSiglec-9 for 4 h, followed by LPS stimulation for 30 min. The levels of β-actin, IκBα, and phosphorylated IκBα were determined using the appropriate antibodies. Experiments were carried out with a control (not stimulated with LPS), vehicle (with DMSO), low dose of sSiglec-9 (2 ng/mL), and high dose of sSiglec-9 (10 ng/mL).

5. Electrophoretic mobility shift assay

COLO 205 cells were pretreated with 2 ng/mL or 10 ng/mL of sSiglec-9 for 24 h, followed by stimulation with 10 ng/mL of TNF-α for 1 h. Nuclear extracts from COLO 205 cell were used for LightShift® chemiluminescent electrophoretic mobility shift assay (EMSA, Thermo Scientific Inc., Rockford, IL, USA). A biotin-3'-end-labeled DNA oligonucleotide probe corresponding to the sequence of NF-κB was incubated with the nuclear extracts. Anti NF-κB p50 antibody was used to detect NF-κB activity by DNA binding. A bounded DNA band for NF-κB was visualized by chemiluminescence after electrophoresis using a 5% polyacrylamide gel and a nylon membrane. The

specificity of the NF-κB probe was demonstrated using a mutant probe.

6. ELISA

To evaluate the secretion of pro-inflammatory cytokines, IL-8 in COLO 205 cells was measured using R&D Systems' Quantikine ELISA Kit.[13, 16] After culture of each cell line, pretreatment was performed with low dose (2ng/mL of sSiglec-9) and high dose (10ng/ml of sSiglec-9) for 24 h and then stimulated with TNF-α. Supernatants from cell culture were obtained and transferred to ELISA plates for analysis.

7. Mice

C57BL/6 specific pathogen free (SPF) wild type (WT) mice were used for the acute murine colitis model (Young-Bio, Seongnam, Korea). SPF IL-$10^{-/-}$ C57BL/6 mice were used for the chronic colitis model (Biomedical Center for Animal Resource Development of Seoul National University, Seoul, Korea) [13, 16, 17]. The mice were 6–7 weeks of age with body weights ranging from 20–22 g.

8. DSS-induced acute murine colitis

Six-week-old SPF C57BL/6 WT mice were fed with regular chow. DSS (4%) was administrated in drinking tap water from day 0 to day 7 continuously. Mice were randomly assigned to four groups: normal control (n = 3; no injection), vehicle with PBS administration (n = 9), 10 ng/g of sSiglec-9 (n = 9), and 50 ng/g of sSiglec-9 (n = 9). sSiglec-9 and PBS were administrated

via intraperitoneal injection every other day at day -2, day 0, day 2, day 4, and day 6. Mice were euthanized on day 8. Body weight was measured daily. Both the colon length and the histologic scores based on hematoxylin and eosin (H&E) staining were measured after sacrifice [19].

9. Chronic colitis of IL-10 $^{-/-}$ mice

Seven-week-old SPF IL-10-/- mice with a body weight of 20 ± 1 g were used for generating the chronic murine colitis model. Chronic colitis was induced with 200 ppm of piroxicam mixed with chow from day 0 to day 14 [20]. Mice were randomly assigned to three groups: a negative control group without intervention (n = 5), a vehicle group receiving intraperitoneal injections of PBS (n = 9), and a treatment group receiving intraperitoneal injections of 50 ng/g of sSiglec-9 (n = 9). Injections were administrated every other day from day 14 to day 28. Mice were euthanized on day 28. As with the DSS-induced acute colitis model, body weight, colon length, and histologic scores were evaluated.

10. Ethical considerations

This study with mice was approved by the Institutional Animal Care and Use Committees (IACUC) of Seoul National University, Seoul, Korea (IACUC No. SNU-170404-22). All procedures in this study were conducted in accordance with the Guide for the Care and Use of Laboratory Animals by National Institute of Health (NIH publication no. 80–23, revised in 1978).

11. Statistical analysis

All data were analyzed with SPSS for Windows version 22 (SPSS, Inc., Chicago, IL, USA) and Prism software (Graph Pad, La Jolla, CA, USA). Body weight and colon length of the mice were compared using repeated one-way ANOVA with Tukey's post-hoc test. Data are presented as the mean ± standard deviation (SD). P value < .050 was considered significant.

Results

sSiglec-9 inhibited pro-inflammatory cytokine gene expression in COLO 205 cells and RAW 264.7 cells

To investigate the effect of sSiglec-9 on the gene expression of pro-inflammatory cytokines, IL-8 from COLO 205 cells and TNF-α from RAW 264.7 cell were examined after pretreatment with sSiglec-9 and stimulation with TNF-α and LPS, respectively. IL-8 and TNF-α gene expression was reduced in the sSiglec-9-treated groups compared with that of the vehicle-treated group in a dose-dependent manner (Figure 1). Gene expression of β-actin was not different among groups.

sSiglec-9 suppressed IκBα phosphorylation and degradation in COLO 205 cells and RAW 264.7 cells

The NF-κB signaling pathway is activated by LPS stimulation through IκBα phosphorylation. Western blot was used to evaluate the inhibitory effects of sSiglec-9 on the NF-κB pathway in regard to IκBα phosphorylation and degradation. COLO 205 cells and RAW 264.7 cells were treated with sSiglec-9 (2 ng/mL or 10 ng/mL) and stimulated with LPS. The levels of phosphorylated IκBα were decreased in the sSiglec-9-treated groups compared with those in vehicle-treated cells. Levels of the free form of IκBα were increased in the sSiglec-9-treated groups compared with those in vehicle-treated cells (Figure 2).

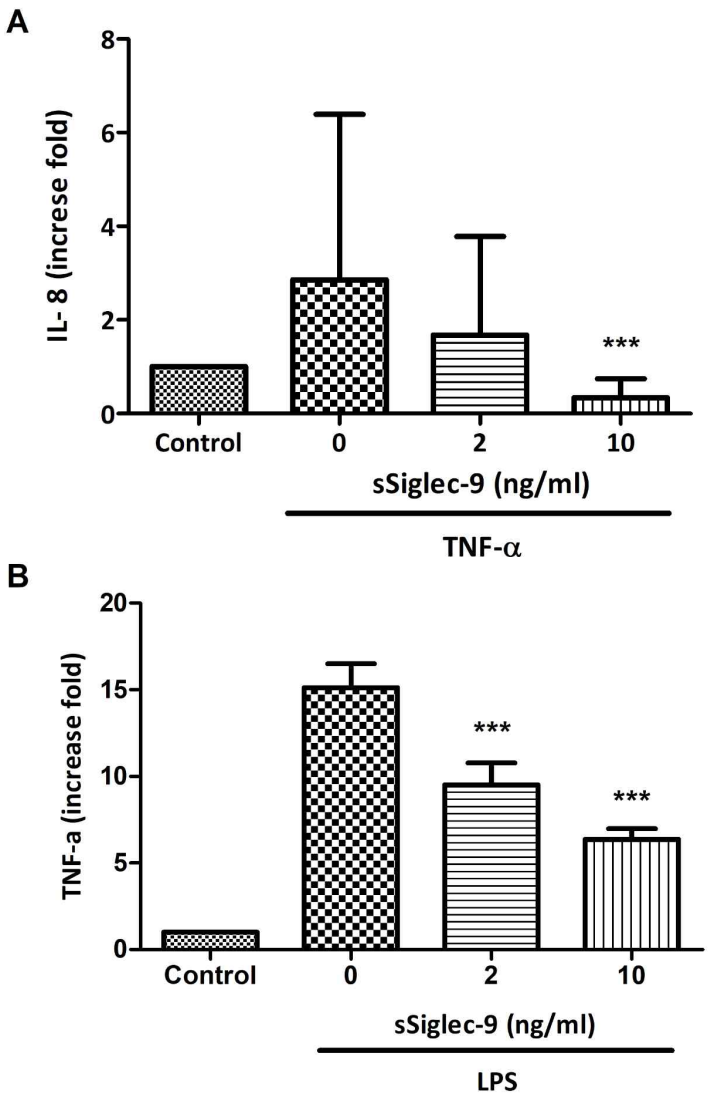


Figure 1. Soluble Siglec-9 (sSiglec-9) suppressed the expression of proinflammatory cytokines in COLO 205 and RAW 264.7 cells. (A) COLO 205 cells were pretreated with two doses of sSiglec-9 (2 ng/mL or 10 ng/mL) for 24 h, followed by stimulation with TNF-α (10 ng/mL) for 4 h. The expression of IL-8 in COLO 205 cells was measured using real time RT-PCR (***, $P < 0.0001$; compared with vehicle; one-way ANOVA with

Tukey's multiple comparison test). (B) RAW 264.7 cells were pretreated with two doses of sSiglec-9 (2 ng/mL or 10 ng/mL) for 24 h, and then stimulated with lipopolysaccharide (LPS, 10 μg/mL) for 4 h. The expression of TNF-α was measured using real time RT-PCR (***, P < 0.0001; compared with vehicle; one-way ANOVA with Tukey's multiple comparison test).

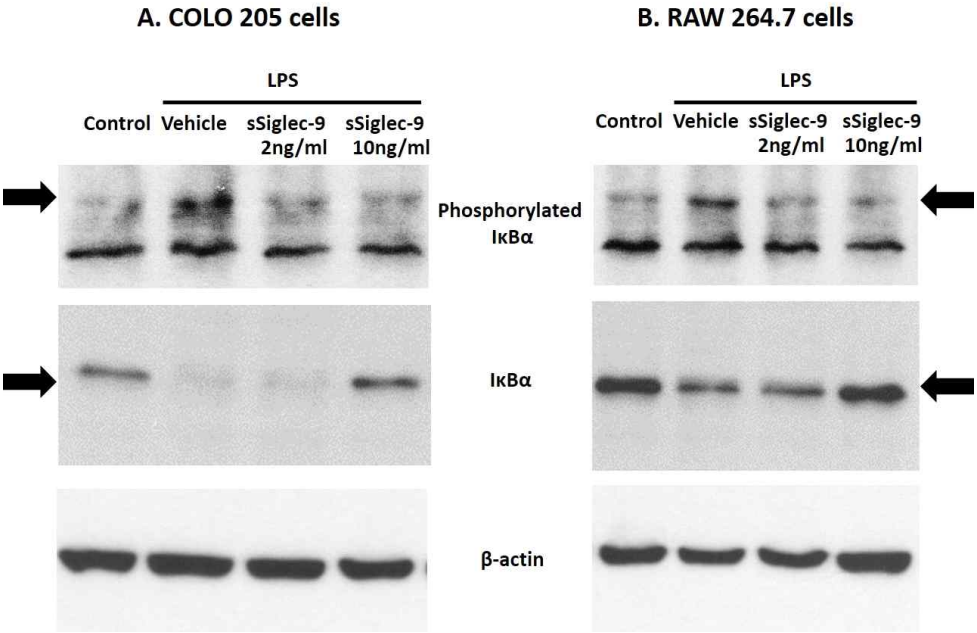


Figure 2. Soluble Siglec-9 (sSiglec-9) reduced IκBα phosphorylation and degradation in COLO 205 and RAW 264.7 cells. Western blot of IκBα and phosphorylated form of IκBα in COLO 205 cells (A) and RAW 264.7 cells (B). COLO 205 cells and RAW 264.7 cells were pretreated with two doses of sSiglec-9 (2 ng/mL or 10 ng/mL) for 4 h and followed by the stimulation with 10 μg/mL of lipopolysaccharide (LPS) for 30 min. sSiglec-9 attenuated I κBα phosphorylation and degradation in stimulated COLO 205 cells and RAW 264.7 cells.

sSiglec-9 inhibited DNA binding activity of NF-κB in COLO 205 cells

Nuclear extracts from COLO 205 cells pretreated with sSiglec-9 and stimulated by TNF-α were examined using an anti-NF-κB p50 antibody. The nuclear binding activity of NF-κB gradually decreased in a dose-dependent manner with addition of sSiglec-9 (Figure 3).

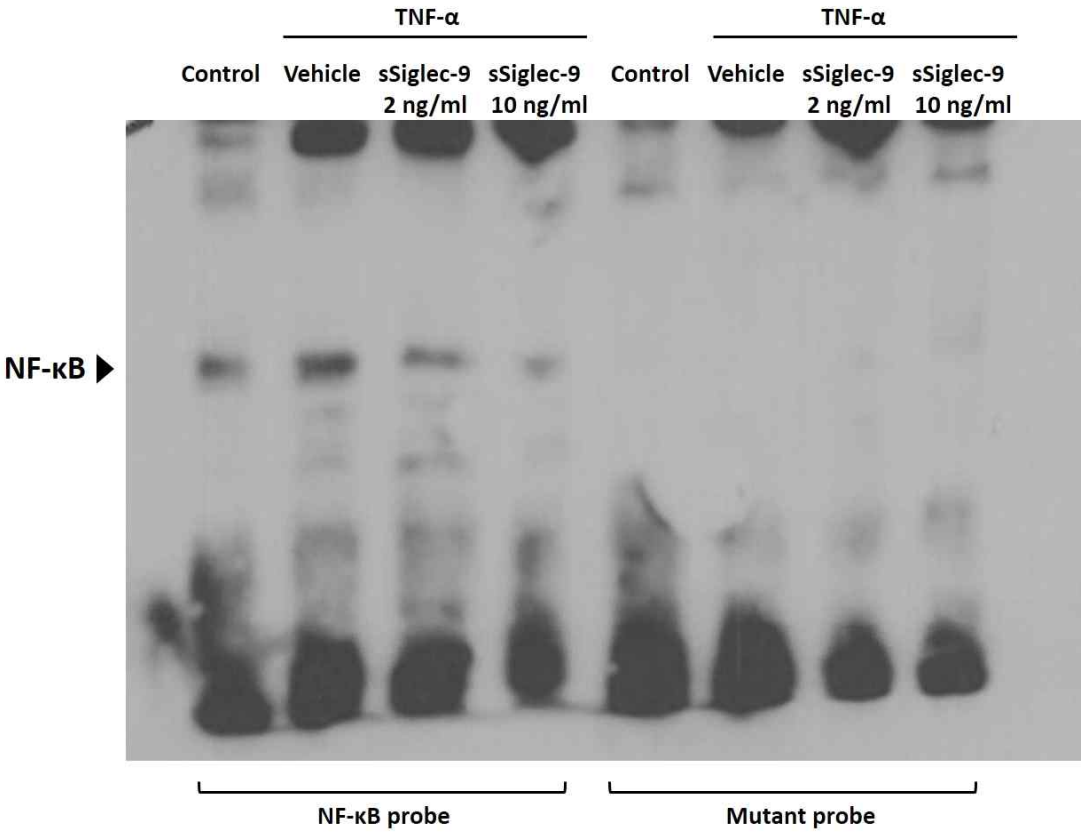


Figure 3. Soluble Siglec-9 (sSiglec-9) inhibited DNA binding activity of NF-κB in COLO 205 cells. COLO 205 cells were pretreated with two doses of sSiglec-9 (2 ng/mL or 10 ng/mL) for 24 h and stimulated with TNF-α (10

ng/mL) for 1 h. Nuclear extracts from the stimulated COLO 205 cells were obtained and measured for DNA binding activity using NF-κB p50 antibody. sSiglec-9 ameliorated NF-κB signaling pathway in COLO 205 cells.

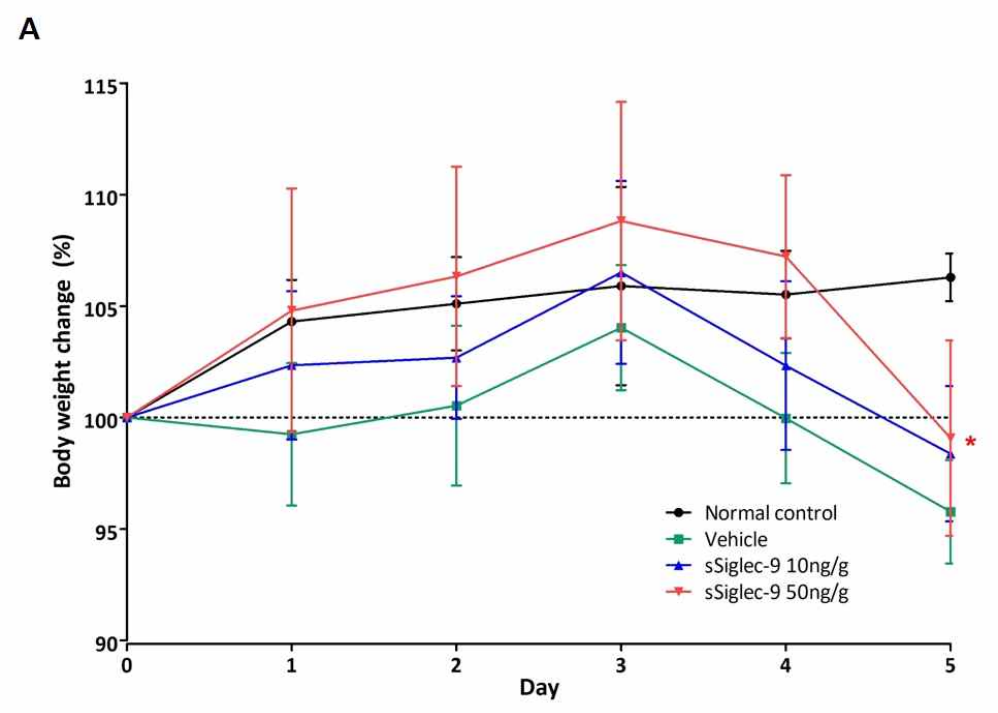
ELISA

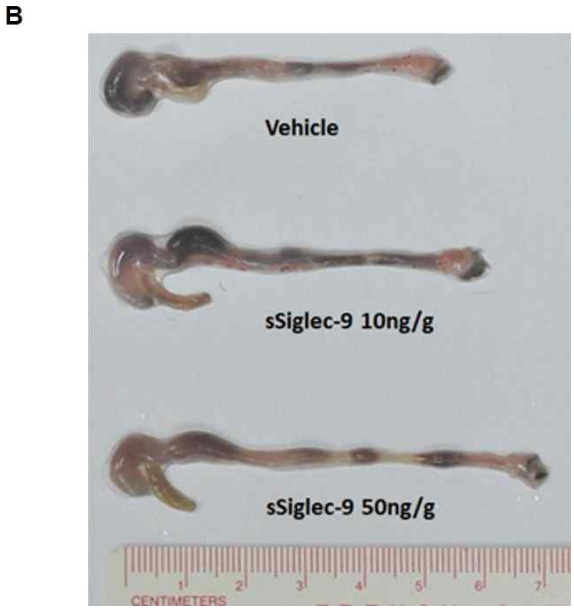
Secretion of pro-inflammatory cytokine, IL-8, was measured in COLO 205 cells using ELISA. IL-8 levels of control, vehicle, low dose of sSiglec-9 (2 ng/ml) and high dose of sSiglec-9 (10 ng/ml) were compared following the stimulation with TNF-α. IL-8 secretion was significantly reduced in the 10 ng/ml of Siglec-9-treated group than in the vehicle (data not shown).

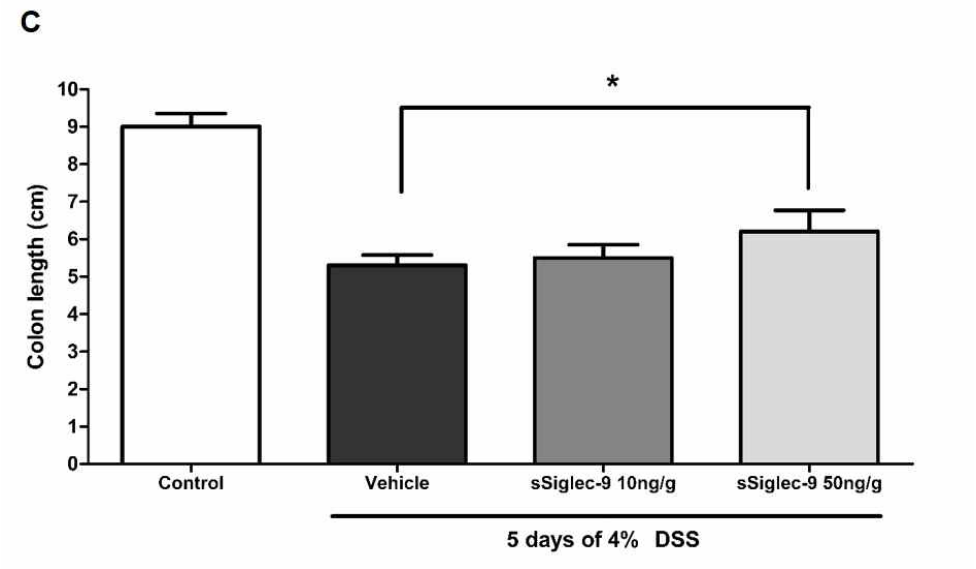
sSiglec–9 prevented DSS–induced acute colitis

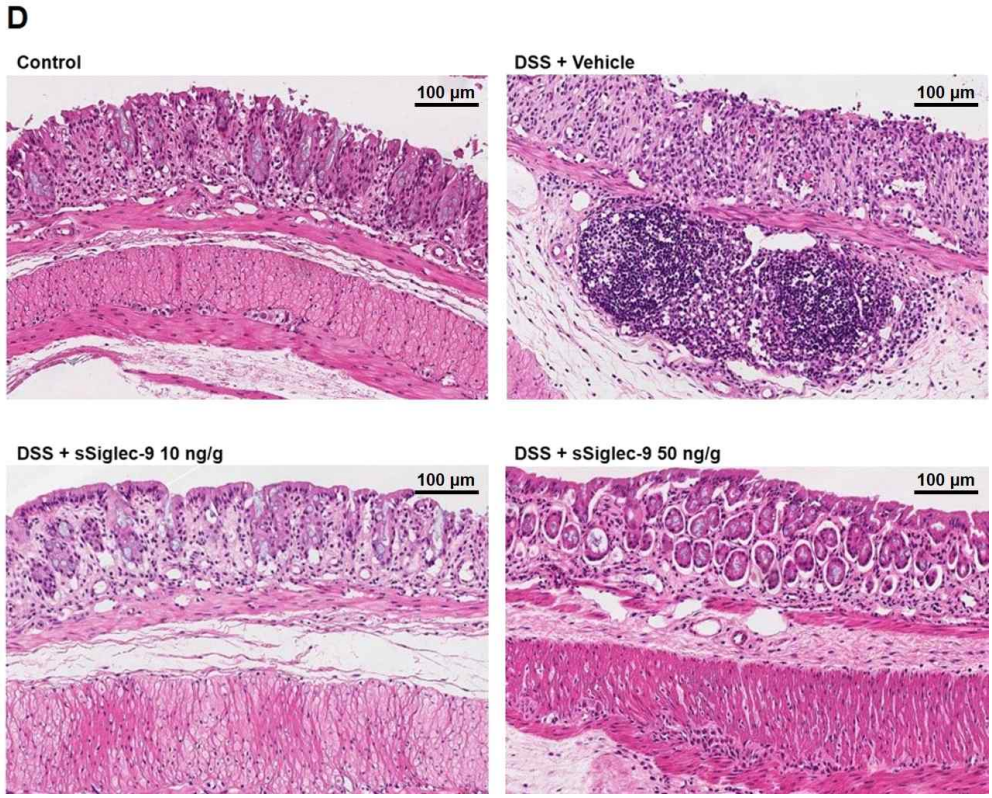
DSS-induced colitis in WT mice manifests as acute colitis, which is limited to inflammation of the mucosa, resulting in weight loss and colon shortening. The preventive effects of two different doses of sSiglec-9 (10 ng/g or 50 ng/g) on the acute colitis were investigated by measuring daily body weight, colon length, and the histologic scores of H&E stained slides. Intraperitoneal administration of sSiglec-9 significantly reduced weight loss compared with the vehicle (Figure 4A). However, the difference in weight change between the low and high doses of sSiglec-9-treated groups was not significant. Shortening of colon length was significantly more prominent in vehicle group compared with the sSiglec-9 groups and the negative control (Figure 4B, 4C). Based on H&E stained slides of colon tissues, sSiglec-9 attenuated mucosal denudation and inflammation compared with the vehicle (Figure 4D). The histologic scores of colon specimen were compared and showed significant

differences (Figure 4E)






C
Colon length (cm)
10
9
8
7
6
5
4
3
2
1
0
*
Control
Vehicle
sSiglec-9 10ng/g
sSiglec-9 50ng/g
5 days of 4% DSS


D
Control
100 μm
DSS + Vehicle
100 μm
DSS + sSiglec-9 10 ng/g
100 μm
DSS + sSiglec-9 50 ng/g
100 μm

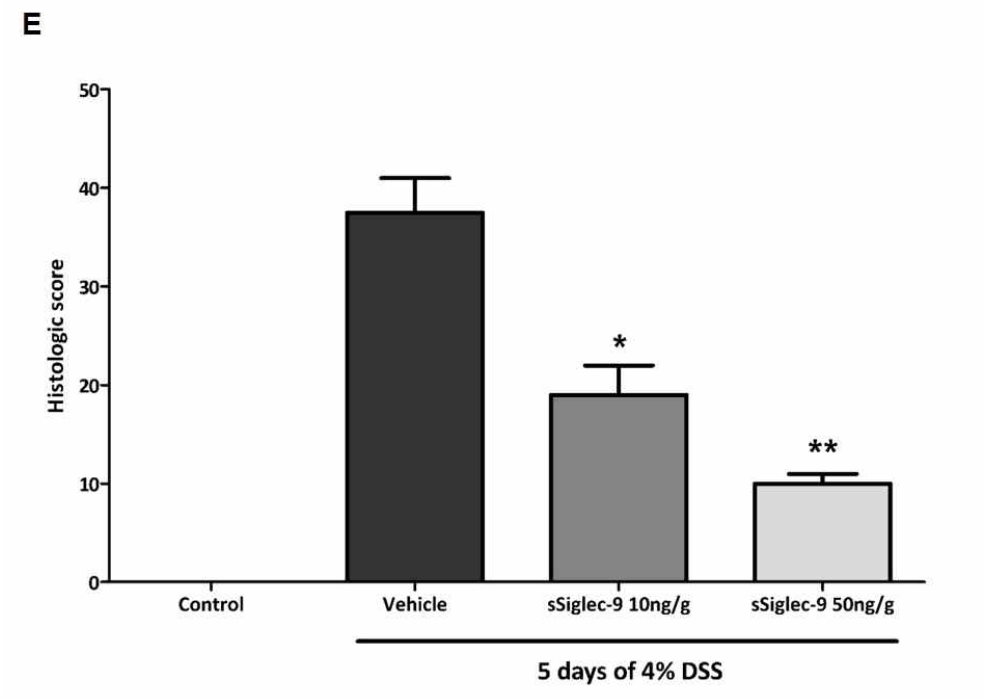


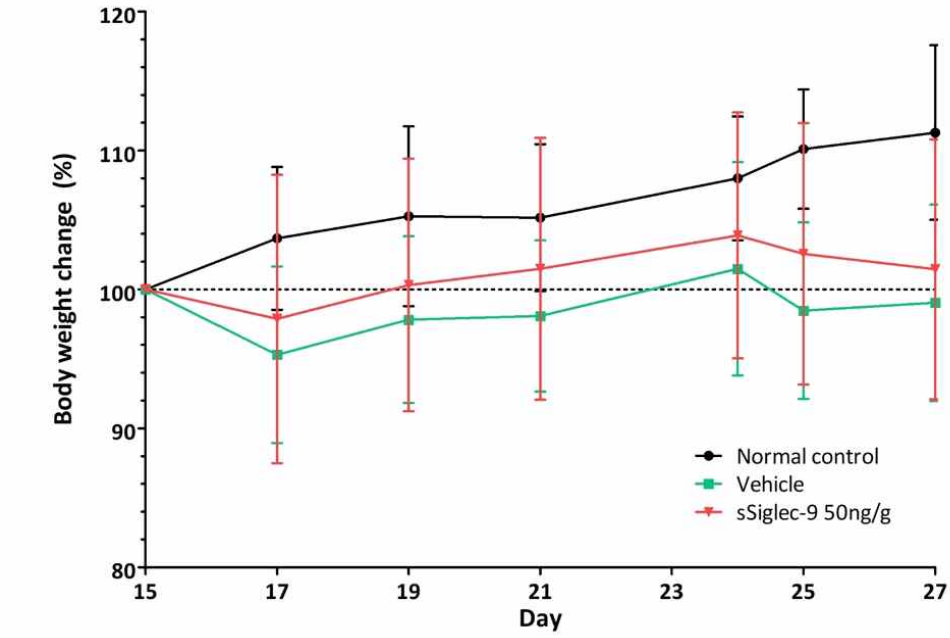
Figure 4. Soluble Siglec-9 (sSiglec-9) showed a preventive effect on dextran sulfate sodium (DSS)-induced acute colitis model. DSS (4%) was administrated in drinking tap water from day 0 to day 7. Two days before DSS administration, vehicle (PBS), 10 ng/g, or 50 ng/g of sSiglec-9 was injected into the abdomen. Injections were then repeated every other day. (A) Daily body weight was measured in each group: normal control (black line, n = 3), vehicle (PBS; green line, n = 9), 10 ng/g of sSiglec-9 (blue line, n = 9), and 50 ng/g of sSiglec-9-treated group (red line, n = 9). sSiglec-9 significantly reduced weight loss compared with the vehicle-treated group (*, P = 0.0028; Repeated measures ANOVA with Tukey's multiple comparison test). (B) Changes in colon length after the euthanasia of mice with DSS-induced colitis. sSiglec-9 prevented colon shortening compared with the vehicle group. (C) Colon length between vehicle and 50 ng/g of

sSiglec-9-treated groups showed significant difference (*, $P < 0.001$; one-way ANOVA with Tukey's multiple comparison test). (D) sSiglec-9 alleviated mucosal inflammation compared with vehicle. Representative histological sections of colon tissue with hematoxylin and eosin staining (magnification, 100 ×; scale bar represented 100 μm) were shown in normal control, vehicle, 10 ng/g of sSiglec-9, and 50 ng/g of sSiglec-9. DSS induced the colonic epithelial architectural destruction with crypts loss and disruption of epithelial integrity and intense infiltration of inflammatory cells. Pretreatment with sSiglec-9 ameliorated this morphological damage. (E) The histologic score of acute colitis in the preventive model. Pretreatment of sSiglec-9 improved the histologic score of acute colitis compared with the vehicle. The bars indicate the mean ± standard deviation (*, $P < 0.010$; **, $P < 0.001$; one-way ANOVA with Tukey's multiple comparison test).

sSiglec-9 alleviated chronic colitis in IL-10$^{-/-}$ mice

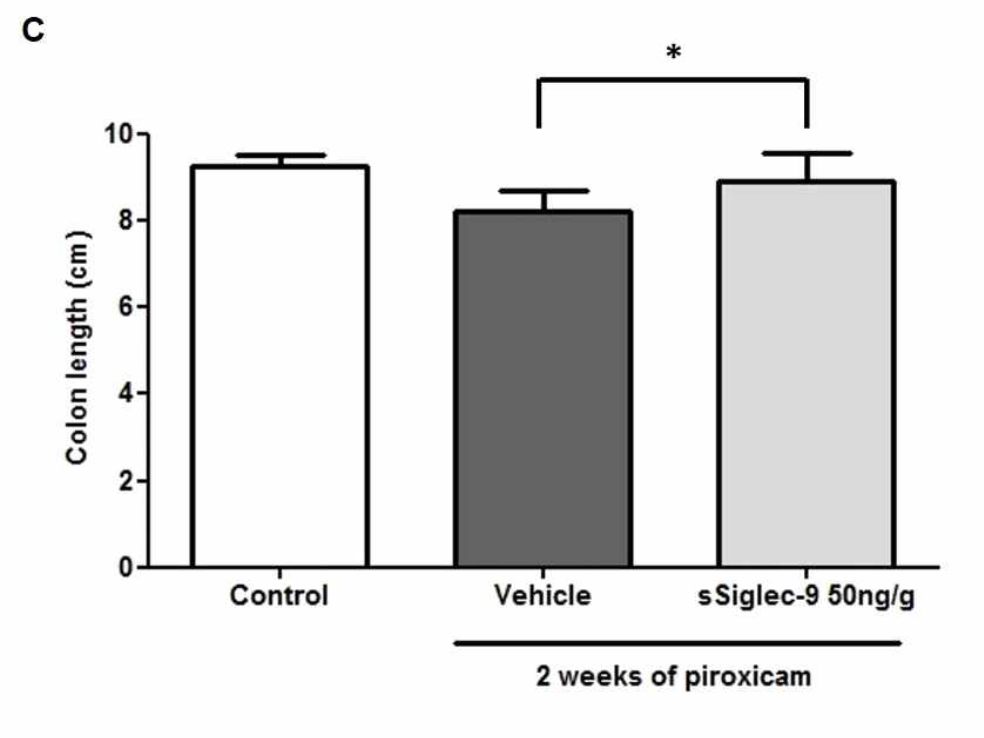
IL-10$^{-/-}$ mice developed colitis spontaneously due to loss of anti-inflammatory function. Chronic colitis in IL-10$^{-/-}$ mice was promoted by administration of piroxicam for 14 days. After chronic colitis was induced, 50 ng/g of sSiglec-9 or PBS was injected into the abdominal cavity of the mice every other day for 14 days. Body weight change was not significantly different between the sSiglec-9 and the vehicle groups (Figure 5A). However, colon length was significantly shorter in the mice of the vehicle group than in those treated with sSiglec-9 (Figure 5B, 5C). Histologic score and inflammation of colon tissue were significantly improved in the sSiglec-9-treated group compared with the vehicle group based on H&E staining (Figure 5D, 5E).

A


120
110
100
90
80
Body weight change (%)
15
17
19
21
23
25
27
Day
Normal control
Vehicle
sSiglec-9 50ng/g

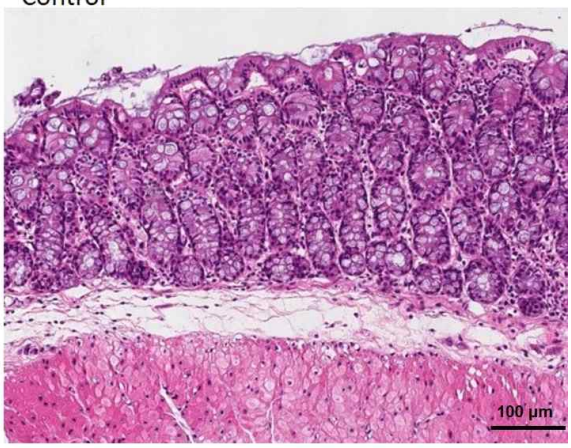
B


N/C
Vehicle
sSiglec-9 50ng/g
CENTIMETERS
FRENCH CATHETER SCA

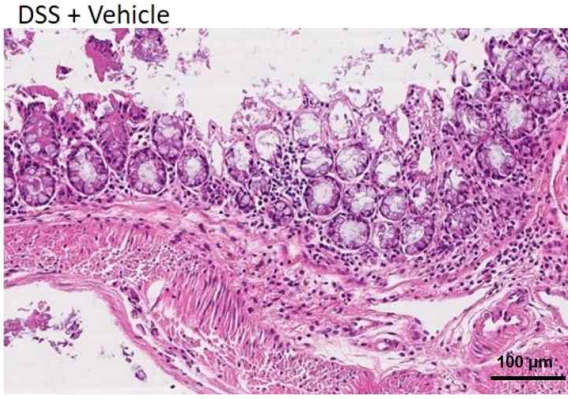

C
*
10
8
6
4
2
0
Colon length (cm)
Control
Vehicle
sSiglec-9 50ng/g
2 weeks of piroxicam

D

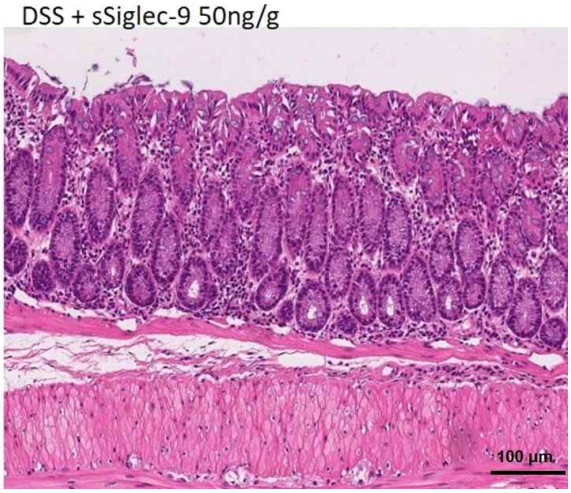
Control



DSS + Vehicle



DSS + sSiglec-9 50ng/g



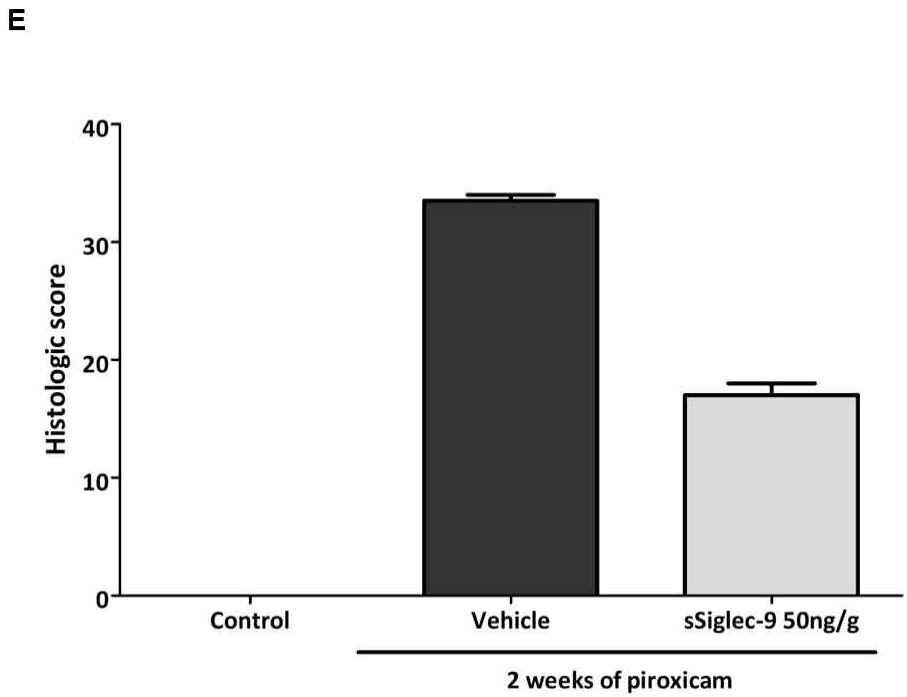


Figure 5. Soluble Siglec-9 (sSiglec-9) had a therapeutic effect on IL-10$^{-/-}$ chronic colitis model. Piroxicam was administered for 2 weeks to induce chronic colitis in IL-10$^{-/-}$ mice. Normal control-, vehicle-, and 50 ng/g of sSiglec-9-treated mice were compared for 2 weeks. Treatments were administered through the intraperitoneal injection every other day. (A) Daily body weight was measured in each group: normal control (black line, n = 5), vehicle (green line, n = 9), and 50 ng/g sSiglec-9 (red line, n = 9). Treatment with sSiglec-9 resulted in less weight loss than vehicle alone, but was not statistically significant. (B) Colon shortening was attenuated in the sSiglec-9-treated group. (C) The colon length between vehicle-and 50 ng/g of sSiglec-9-treated groups was significantly different (*, $P < 0.050$; one-way ANOVA with Tukey's multiple comparison test). (D) Representative histologic sections of colon tissue from each group indicated that sSiglec-9 improved

histopathologic damage of colon tissues compared with vehicle (hematoxylin and eosin stain; magnification, 100 ×; scale bar represented 100 μm). Colon tissue of chronic colitis showed destruction of the epithelial architecture, deep ulceration, and intense infiltration of inflammatory cells in submucosa. sSiglec-9 promoted recovery of chronic colitis in IL-$10^{-/-}$ mice. (E) The histologic score of colitis tissue showed that sSiglec-9 attenuated colitis in IL-$10^{-/-}$ mice with statistical significance (*, $P < 0.001$; one-way ANOVA with Tukey's multiple comparison test).

Discussion

IBD is an immune-mediated chronic inflammatory disease with varied pathophysiology. IECs and macrophages play an important role in regulating mucosal immunity, mucosal integrity, and innate immune responses [21, 22]. New therapeutic options are being developed that target various pathophysiological stages, but drugs are still needed that treat IBD at a fundamental immunological level.

We investigated the anti-inflammatory effects of sSiglec-9 on experimental murine colitis models, IECs, and murine macrophages to determine whether sSiglec-9 affects the NF-κB pathway to alleviate colon inflammation. We induced inflammation in COLO 205 and RAW 264.7 cells by treatment with TNF-α and LPS, respectively. Our data showed that the expression of pro-inflammatory cytokines such as IL-8 and TNF-α decreased in COLO 205 and RAW 264.7 cells when treated with sSiglec-9. To demonstrate the relationship between sSiglec-9 and the NF-κB pathway, IκBα phosphorylation was measured using western blot. LPS-induced IκBα phosphorylation was suppressed by pretreatment of sSiglec-9 in a dose-dependent manner. The DNA binding activity of NF-κB was evaluated using DNA oligonucleotide probes in COLO 205 and RAW 264.7 cells treated with sSiglec-9 at different doses. Results indicated reduced band thickness in the sSiglec-9-treated cells compared with untreated LPS-stimulated cells. Moreover, sSiglec-9 had protective effects in the DSS-induced colitis model and therapeutic effects in the IL-10-/- chronic colitis model. This is the first study to evaluate the anti-inflammatory effects of sSiglec-9 on colon inflammation.

Siglecs are a superfamily of immunoglobulins that recognize sialic acid and modulate immune function. Siglecs are important in regulation of infection,

inflammation, and carcinogenesis [23]. Siglecs have N-terminal Ig-like V-type domains which recognize sialic acid and varying numbers of Ig-like C2-type domains [24]. Among Siglecs, CD33-related Siglecs have similarity in their intracellular and extracellular domains. Siglec-9 is one of the CD33-related immunoregulatory receptors expressed on human monocytes, macrophages, neutrophils, a fraction of NK cells, B cells, and a minor subset of CD8+ T cells [25]. Siglecs with ITIM or ITIM-like domains deliver inhibitory signals through Src homology 2 tyrosine phosphatases (SHP)-1 and SHP-2 activations [26-28]. Siglec-9 inhibits TNF-α production and enhances IL-10 production when stimulated by LPS in macrophages Siglec-9 is known to be expressed in the human aorta, airway, and colon epithelium [11, 29, 30]. Siglec-9 present on neutrophils, which interact with sialic acid in the airways, can ameliorate active inflammation. According to a previous study, ongoing inflammation of human airway cells results in the upregulation of ligands for Siglec-9 through the NF-κB pathway [30]. Increased levels of inhibitory Siglec-9 can alleviate active inflammation to maintain homeostasis.

Based on previous studies, Siglec-9 has an important role in innate immunity [31, 32]. Siglec-9 mediates intercellular interaction with adhesive functions. Sialylated glycans, which could be recognized by Siglecs, are expressed on the surface of several pathogens including Neisseria meningitides [33]. Furthermore, endogenous sialylated glycans could influence the binding activities of Siglecs. Siglecs can recognize exogenous and endogenous sialic acids and adjust immunological signals. sSiglec–9 changed the polarization of macrophages from M1 to M2 macrophages for anti–inflammatory effects in a rheumatoid arthritis model and spinal cord injury model [12, 34].

In the present study, sSiglec-9 suppressed inflammation of IECs and macrophages by inhibiting the NF-κB pathway. NF-κB is a major pathway of

pro-inflammatory signaling in IBD [35]. Degradation of IκBα by phosphorylation is crucial for NF-κB activation. sSiglec-9 blocked IκBα phosphorylation and degradation and disturbed the DNA binding activity of NF-κB. By inhibiting IκBα phosphorylation, sSiglec-9 mitigated colonic inflammation in the experimental colitis models. To investigate whether sSiglec-9 improves colonic inflammation in vivo, we generated two different types of experimental colitis models: a DSS-induced acute colitis model and a piroxicam-induced IL-10-/- chronic colitis model [16-18]. DSS can induce acute distal colitis through the activation of the innate immune system by increasing macrophage-derived pro-inflammatory cytokines. Acute colitis induced by DSS is independent of the activation of B cells and T cells [36]. This model represents ulcerative colitis. IL-10-/- mice can develop spontaneous colitis gradually over several months due to a lack of anti-inflammatory cytokine, IL-10. This chronic colitis can be promoted by administration of piroxicam, resulting in colon inflammation after approximately 2 weeks. The Th1-mediated adaptive immune response is activated in the chronic colitis model of IL-10-/- [20]. IL-10-/- chronic murine colitis model represents Crohn's disease. Using these in vivo models, we demonstrated the therapeutic effect of sSiglec-9 on murine colitis.

The beneficial effects of sSiglec-9 on experimental colitis have been demonstrated in this study, but the specific mechanisms of action that inhibit NF-κB are not fully understood. Siglecs recognize various sialic acid-containing carbohydrates and might have different effects on inflammation and tumors [37]. However, a previous study showed that Siglec-9 can have an anti-tumorigenic effect by modulating the innate immune response to cancer [38]. In order to apply sSiglec-9 to the treatment for IBD, it is necessary to address safety issues, especially those related to the risk of cancer. Further studies are warranted to evaluate a variety of

anti-inflammatory and antitumor effects that may be induced by Siglec-9.

In conclusion, sSiglec-9 blocked the NF-κB signaling pathway and alleviated colon mucosal inflammation in IECs, murine macrophages, and acute and chronic experimental colitis models. sSiglec-9 is a candidate for a new therapeutic for IBD.

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국문 초록

배경: 시알산 (Sialic acid)에 결합하는 면역글로불린 유사 렉틴 (Siglec)은 면역세포의 표면에 존재하는 수용체이다. Siglec의 일종인 Siglec-9은 억제 면역 반응을 매개하며 항염증 효과를 가지고 있다. 하지만 Siglec-9의 수용성 형태인 soluble Siglec-9 (sSiglec-9)이 장내 염증반응에 대해 항염증 효과를 나타내는지는 잘 알려져 있지 않다. 본 연구에서는 sSiglec-9이 염증을 유발한 장상피세포, 대식세포에 미치는 영향과 마우스 대장염 모델에 미치는 영향을 평가하고 nuclear factor-kappa B (NF-κB)신호전달체계에 미치는 영향을 알아보고자 하였다.

방법: 인체 장상피세포주인 COLO 205, 쥐의 대식세포주인 RAW 264.7 에 sSiglec-9을 전처치 후 염증을 유발하여 real time RT-PCR, 효소면역측정법을 통해 IL-8, TNF-α 와 같은 염증성 사이토카인의 분비를 측정하였다. sSiglec-9이 NF-κB 신호전달체계에 미치는 영향을 알아보기 위하여 wetern blot과 electrophorectic mobility shift assay 방법을 통해 IκBα 인산화와 분해 정도를 측정하였다. 동물실험으로 Dextran sulfate sodium (DSS)를 경구투여하여 급성 장염을 유도한 마우스 장염 모델과 IL-$10^{-/-}$ 마우스 모델에 piroxicam을 경구투여하여 만성 장염을 유도한 모델에 sSiglec-9 의 효과를 시험하였다. 복강내 주사로 sSiglec-9을 투여하여 대조군과 비교하여 체중변화, 장길이, 병리학적 소견을 평가하였다.

결과: sSiglec-9은 염증을 유발한 COLO 205세포주와 RAW 264.7세포주에서 IL-8, TNF-α의 유전자 발현을 감소시켰다. sSiglec-9은 IκBα 인산화와 분해를 억제하여 NF-κB 활성을 저해하였다. 동물실험에서 sSiglec-9을 복강 내 투여하였을 때 체중감소, 장 길이 단축, 병리학적 염증 소견이 모두

호전되는 양상을 보였다. 이러한 결과는 급성 장염의 예방모델 (DSS-induced acute colitis model)과 만성 장염의 치료모델 (IL-$10^{-/-}$ chronic colitis model)에서 모두 확인되었다.

결론: sSiglec-9은 장상피세포와 대식세포에서 NF-κB 신호전달체계를 억제하며 대장염을 유발한 마우스에서 장염 억제 효과를 보였다. 향후 sSiglec-9이 염증성 장질환의 치료에 활용될 수 있는 새로운 후보 물질로 개발될 수 있을 것으로 기대된다.

주요어: Siglec-9, NF-κB, 대장염 모델, 염증성 장질환

학번: 2017-34540