RESEARCH

Lactobacillus acidophilus and propionate attenuate Sjögren's syndrome by modulating the STIM1-STING signaling pathway

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Abstract

Background Sjögren's syndrome (SS) is an autoimmune disease characterized by inflammation of the exocrine gland. An imbalance of gut microbiota has been linked to SS. However, the molecular mechanism is unclear. We investigated the effects of *Lactobacillus acidophilus* (*L. acidophilus*) and propionate on the development and progression of SS in mouse model.

Methods We compared the gut microbiomes of young and old mice. We administered *L. acidophilus* and propionate up to 24 weeks. The saliva flow rate and the histopathology of the salivary glands were investigated, and the effects of propionate on the STIM1-STING signaling pathway were evaluated in vitro.

Results Lactobacillaceae and *Lactobacillus* were decreased in aged mice. SS symptoms were ameliorated by *L. acidophilus*. The abundance of propionate-producing bacterial was increased by *L. acidophilus*. Propionate ameliorated the development and progression of SS by inhibiting the STIM1-STING signaling pathway.

Conclusions The findings suggest that *Lactobacillus acidophilus* and propionate have therapeutic potential for SS.

Keyword Sjögren's syndrome, *Lactobacillus acidophilus*, Propionate, Stromal interaction moleculae 1 (STIM1), Stimulation of interferon genes (STING)

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Background

Sjögren's syndrome (SS), first described in 1933 [1], is an autoimmune disease mediated by the infiltration of lymphocytes into the exocrine glands, including the salivary and lacrimal glands [2, 3]. Lymphocyte infiltration leads to destruction of glandular tissue and dysfunctional glandular secretion, followed by dry eyes and mouth [4, 5]. Although SS does not affect life expectancy, it reduces the quality of life. As with other autoimmune diseases, there is no cure for SS, and treatment focuses on relieving symptoms.

Recent studies have revealed dysbiosis of the microbiome in animal models of, and patients with, autoimmune diseases, including SS [6, 7]. Moon et al. reported that the phylum *Bacteroidetes* and the genus *Bifidobacterium* were increased, whereas the *Firmicutes:Bacteroidetes* ratio, an indicator of gut dysbiosis, was decreased, in patients with SS. Cano-Ortiz et al. reported lower diversity and richness of gut microbiota in patients with SS [8]. Supplementation with probiotics can enhance gut-associated immunity [9, 10]. *Lactobacillus acidophilus*, a common probiotic [11, 12], is a candidate immunomodulator [13, 14]. *L. acidophilus* attenuates intestinal inflammation by inhibiting endoplasmic reticulum (ER) stress [15] and modulating the Th17:Treg balance [16]. In addition, in one study, *L. acidophilus* supplementation had a therapeutic effect in lupus-prone mice by regulating the specific intracellular adhesion molecule-3 grabbing non-integrin homolog-related 3 (SIGNR3) pathway [17]. SIGNR3 is a critical mediator of the interaction between *L. acidophilus* and host immune cells [17–19].

Bacteria produce metabolites, including vitamins and short-chain fatty acids (SCFAs), from dietary substrates [20, 21]. SCFAs reportedly have a therapeutic effect in autoimmune diseases [22, 23]. Wen et al. showed that concentrations of SCFAs—including acetate, propionate, butyrate, isobutyrate, valerate, and isovalerate—were markedly decreased in mice with DSS-induced colitis, and butyrate regulated the Treg:Th17 balance [24]. We reported that butyrate ameliorated SS by inducing interleukin-10 (IL-10)-producing B cells [7] and attenuated rheumatoid inflammation in an animal model of rheumatoid arthritis [25]. Propionate is produced by the gut microbiota, including *Ruminococcaceae*, *Clostridiaceae*, and *Prevotellaceae* [26–28]. Tedelind et al. showed that propionate has anti-inflammatory effects on inflammatory bowel diseases [29]. Furthermore, Filippone et al. reported that propionate has anti-inflammatory and anti-oxidant effects [30].

Type I interferon (IFN) has antiviral activity and is induced by stimulation of interferon genes (STING) and the ER signaling adaptor and suppressed by stromal interaction molecule 1 (STIM1), an ER Ca^{2+} sensor [31, 32].

The type I IFN pathway is involved in the onset of autoimmune diseases, such as SS, systemic lupus erythematosus (SLE), systemic sclerosis, and RA [33, 34]. Type I IFN stimulates B cells to produce autoantibodies [35, 36], a hallmark of autoimmune diseases. It shows potential as a therapeutic target for autoimmune diseases [37, 38].

The roles of microbiota/SCFAs in type I IFN production is controversial. Villena et al. reported that *Lactobacillus rhamnosus* induces type I IFN production [39]. Also, Yang et al. reported that microbiota-derived butyrate suppresses type I IFN [40], whereas acetate lead to increase type I IFN production [41]. However, the effect of L. acidophilus and propionate in type I IFN production has not been studied. In this study, we evaluated the role of *L. acidophilus* and propionate in the development and progression of SS and their therapeutic potential.

Methods

Animals

We purchased female NOD/ShiLtJ mice from Jackson Laboratories (Bar Harbor, ME, USA). The mice were housed under specific-pathogen-free conditions at the Catholic Research Institute of Medical Science, The Catholic University of Korea, and were fed a diet sterilized by gamma ray (TD 2018S; Harlan Laboratories, Tampa, FL, USA) and autoclaved water.

Gut microbiome analysis

Mice with average pain data and histopathology results for each group were analyzed. Samples were analyzed in Chun-lab; results are available at http://www.ezbiocloud.net/apps. The cecal microbiome was analyzed to the family and genus levels.

Measurement of salivary secretion in NOD/ShiLtJ mice

Mice were anesthetized by inhalation of isoflurane (2%). Whole saliva was collected for 7 min from the oral cavity, starting 90 s after intraperitoneal injection of pilocarpine (100 µg/mouse; Sigma-Aldrich, St. Louis, MO, USA). Saliva flow rates were expressed as microliters of saliva secreted per gram body weight per min (µL/g/min).

Supplementation with *L. acidophilus* or propionate

L. acidophilus was purchased from CNS Pharm Korea (Jincheon, Korea). *L. acidophilus* was resuspended in saline a concentration of 125 mg/mL (2×10^{11} CFU/mL) and heat killing at 80 °C for 30 min. Twelve-week-old NOD mice were orally administered 50 mg/kg of *L. acidophilus* daily for 12 weeks in saline ($N=10$) or saline alone ($N=15$). Twelve-week-old NOD mice were intraperitoneally administered 200 mg/kg sodium propionate

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(Sigma) in saline ($N=4$) three times per week for 10 weeks or saline alone ($N=5$).

Histopathological analysis

Tissue was fixed in 10% formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (H&E). Inflammation of the salivary gland was scored as described previously [42]. The scoring criteria were as follows: score 0, no infiltrate; score 1–1.5, 1–2 foci per section; score 2–2.5, 3–5 foci per section; score 3, 6–10 foci per section; and score 4, > 10 foci per section.

Immunohistochemistry

Immunohistochemistry was performed with a Vectastain ABC Kit (Vector Laboratories, Burlingame, CA, USA). Tissue sections were incubated with anti-IL-6, -IL-17, -TNF-α, -STIM1, or -phospho-STING primary antibodies at 4 °C overnight followed by the appropriate biotinylated secondary antibody. Next sections were incubated with streptavidin–peroxidase complex for 1 h. DAB chromogen (Dako, Carpinteria, CA, USA) was added as the substrate. Stained cells were visualized by microscopy (Olympus, Center Valley, PA, USA). Antibodies are listed in Supplementary Table 1.

Confocal microscopy

Salivary gland tissue was stained with anti-CD4 and -IL-17 antibodies for Th17 cells and anti-CD4, -CD25, and -FoxP3 antibodies for Treg cells at 4 °C overnight. Secondary antibodies conjugated with FITC, APC, and PE were incubated at room temperature for 2 h. Nuclei were stained with 4′,6-diamidino-2-phenylindole. Confocal images were obtained with an LSM 700 confocal microscope (Zeiss, Oberkochen, Germany) at 200× magnification. Antibodies are listed in Supplementary Table 1.

Cell culture

Human submandibular gland (HSG) cells were cultured with recombinant human TNF-α (2 ng/mL) and human IL-17 (20 ng/mL) in the absence or presence of *L. acidophilus* (10 or 100 µg/mL) for 48 h. Mouse splenocytes were cultured with anti-CD3 (0.5 µg/mL) in the absence or presence of *L. acidophilus* (10 or 100 µg/mL) or propionate (0.2 or 1 mM) for 48 h. Mouse non-T cells from spleen were cultured with LPS (100 ng/mL) in the absence or presence of *L. acidophilus* (10 or 100 µg/mL) for 48 h.

mRNA isolation, cDNA synthesis, and real-time quantitative PCR

mRNA was extracted with TRI Reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions. PCR amplification was performed with an Applied Biosystems StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) and SensiFAST SYBR Hi-ROX (Bioline USA, Taunton, MA, USA) according to the manufacturer's instructions. Expression was normalized to that of β-actin. Primer sequences are listed in Supplementary Table 2.

Intracellular staining and flow cytometry

Cells were isolated from the spleen and salivary glands and stimulated with 25 ng/mL phorbol myristate acetate and 250 ng/mL ionomycin (Sigma-Aldrich) in the presence of GolgiStop (BD Biosciences, San Jose, CA, USA) for 4 h. Cells were reacted with anti-CD4, -CD19, or -CD11c antibodies at 4 °C for 30 min; permeabilized with Cytofix/Cytoperm solution (BD Pharmingen, Franklin Lakes, NJ, USA); and stained intracellularly with anti-IFN-γ, -IL-4, -IL-17, or -IFN-α antibodies. Samples were analyzed with a FACS Calibur (BD Pharmingen) fluorescence-activated cell sorting (FACS) instrument, and data were analyzed with FlowJo (Tree Star, Ashland, OR, USA). Antibodies are listed in Supplementary Table 1.

Ethics approval and consent to participate

Animal procedures were performed in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Guidelines and Policies for Rodent Experiments of the Institutional Animal Care and Use Committee of the School of Medicine, The Catholic University of Korea (no. 2021-0277-02).

Statistical analysis

Data are means ± standard deviation (SD). Statistical analyses were performed in Prism version 5 for Windows (GraphPad Software, San Diego, CA, USA). Normally distributed continuous data were analyzed by parametric Student's *t* test. Differences in means among groups were subjected to one-way analysis of variance (ANOVA). $p < 0.05$ was taken to indicate statistical significance.

Results

Dysbiosis of the gut microbiome

NOD/ShiLtJ mice spontaneously develop inflammatory lesions in the salivary gland at 10 weeks old. Therefore, we examined the cecal content of young (4-week-old) and old (18-week-old) NOD/ShiLtJ mice. The *Bacteroides:Firmicutes* ratio was increased in old mice (Fig. 1A), and the gut microbiota differed markedly at the family level between young and old mice (Fig. 1B). The abundance of *Lactobacillaceae* was markedly decreased in old mice (Fig. 1B). The gut microbiota also differed at the genus level (Fig. 1C). The Chao1 and
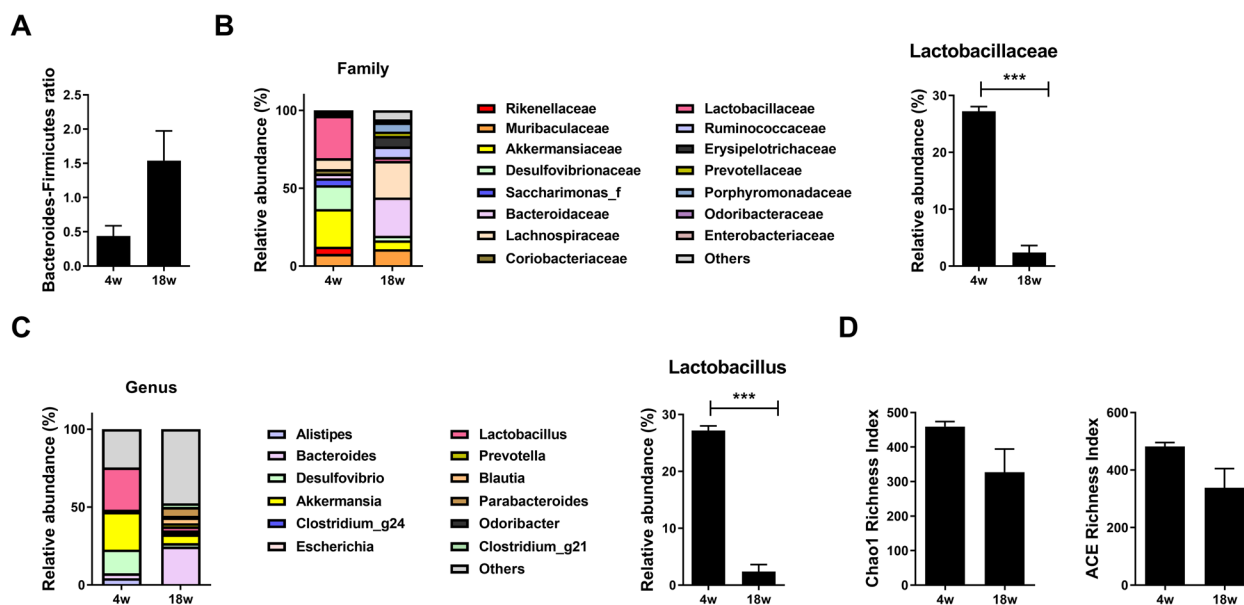
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Fig. 1 Changes in the microbiome with age. Cecal microbiome analyses of 4- and 18-week-old NOD mice. **A** *Bacteroides:Firmicutes* ratio in 4- and 18-week-old NOD mice. **B** Abundance (family level) of the gut microbiome (left) and *Lactobacillaceae* (right) in 4- and 18-week-old NOD mice. **C** Abundance (genus level) of the gut microbiome (left) and *Lactobacillus* (right) in 4- and 18-week-old NOD mice. **D** Chao1 and ACE indices. Data are means $\pm$ SD (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$)

ACE indices suggested lower species richness in the gut microbiomes of old mice compared to young mice (Fig. 1D). These data suggest that dysbiosis of the gut microbiome is related to SS.

L. acidophilus attenuates SS and the infiltration of lymphocytes into the salivary gland

To determine the role of *Lactobacillus* in SS, we administered *L. acidophilus* to 12-week-old NOD/ShiLtJ mice. *L. acidophilus* improved the saliva flow rate and tissue inflammation (Fig. 2A, B). Furthermore, the infiltration of inflammatory cytokine (such as IL-6, IL-17, and TNF-α)-producing cells into the salivary gland was decreased by *L. acidophilus* (Fig. 2C), and infiltration of Th1, Th2, Th17, and B17 cells into salivary gland and spleen was also decreased by *L. acidophilus* (Fig. 2D, E), whereas Treg cells were increased by *L. acidophilus.* Our results show that *L. acidophilus* has immunomodulatory (anti-inflammatory) effects in SS.

L. acidophilus induces the SIGNR3 pathway

SIGNR3 regulates intestinal immunity [19]. Expression of SIGNR3 and the number of IL-10-producing cells in the salivary gland were increased by *L. acidophilus* (Fig. 3A, B), as were mRNA levels of SINGR3, PD-L1, IDO, and IL-10 (Fig. 3C, D). These data suggest that *L. acidophilus* ameliorates SS by enhancing immunomodulation of the SINGR3 pathway.

L. acidophilus attenuates SS by inhibiting the STIM1-STING pathway

STING induces type I IFN, a key mediator of autoimmune diseases, including SS. To investigate the effects of *L. acidophilus* on the STING pathway, we measured the expression of STIM1, a negative regulator of STING, and phospho-STING in salivary glands. STIM1 and phospho-STING expression were increased and decreased, respectively, by *L. acidophilus* (Fig. 3E, F). In addition, *L. acidophilus* decreased the number of type I IFN–producing cells

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Fig. 2 Therapeutic effects of *L. acidophilus* in SS. *L. acidophilus* was administered orally to NOD mice, which were monitored for 24 weeks. Salivary glands and the spleen were harvested. **A** Saliva flow rate at 12, 16, 20, and 24 weeks. **B** Salivary glands were stained with H&E. The histological score (left) and inflammation area (right) are shown. **C** Salivary gland tissues were stained for IL-6, IL-17, and TNF-α. Numbers of cells positive for IL-6 (left), IL-17 (center), and TNF-α (right) are shown. **D** Splenocytes (top) and salivary glands (bottom) analyzed by flow cytometry for percentages of Th1 (CD4+IFN-γ+), Th2 (CD4+IL-4+), Th17 (CD4+IL-17+), and B17 (CD19+IL-17+) cells. **E** Salivary glands were analyzed by confocal microscopy. Representative images of Th17 (CD4+IL-17+; top) and Treg (CD4+CD25+FOXP3.+; bottom) cells are shown. Bar graphs show the numbers of cells positive for Th17 and Treg. Data are means $\pm$ SD (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$)
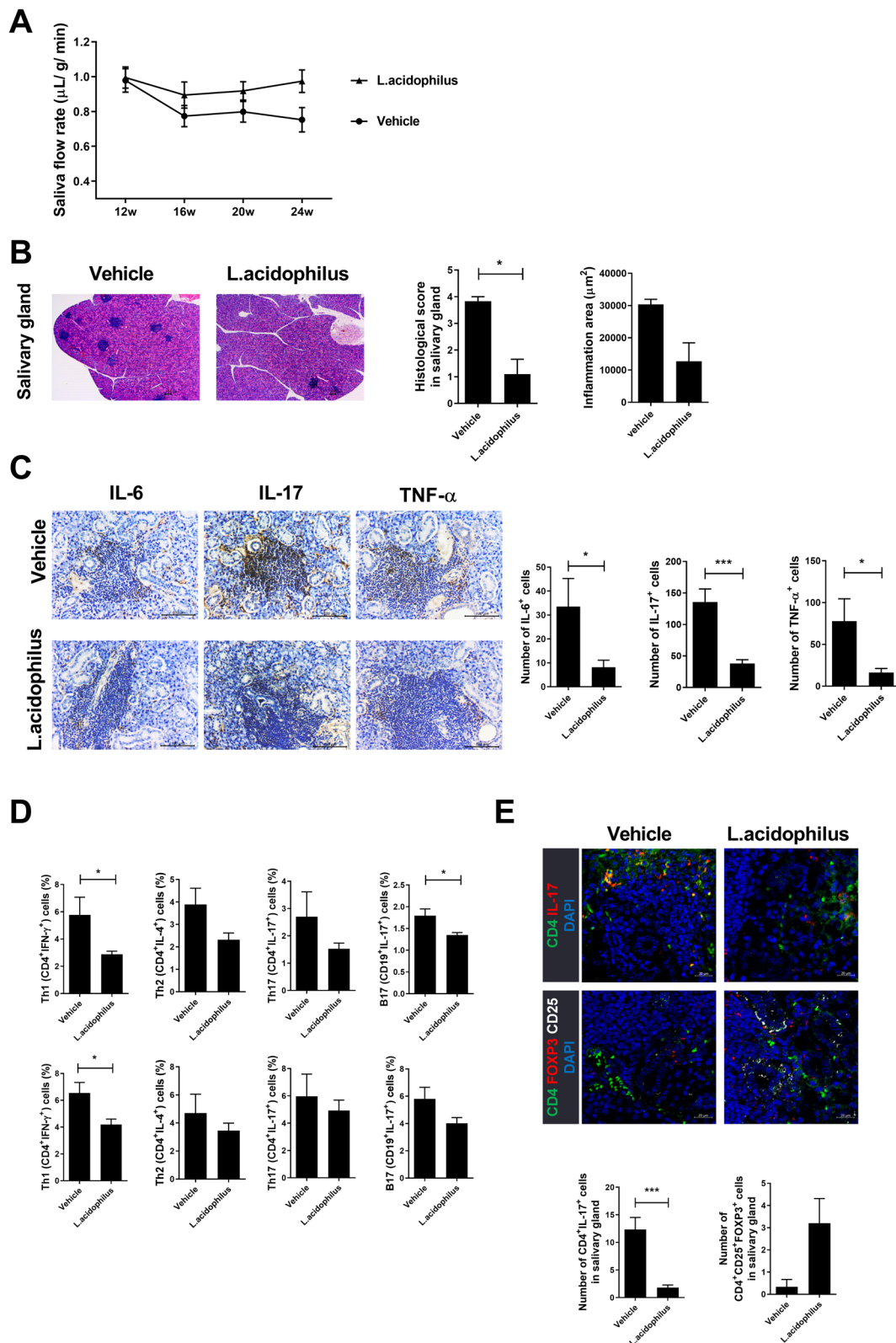
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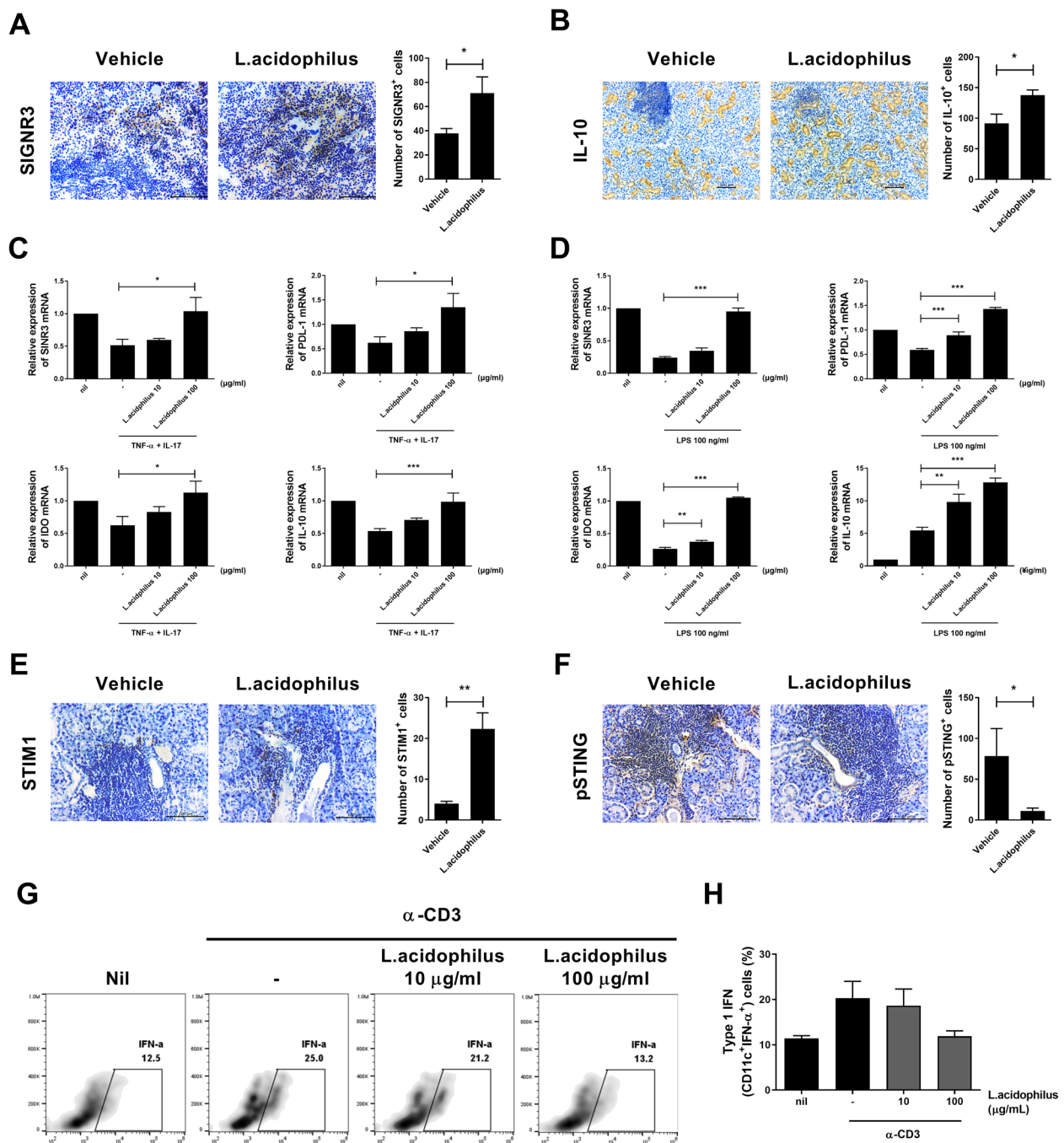Woo *et al. Cell Communication and Signaling*   (2023) 21:135

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Fig. 3 Regulation of SINGR3 expression by *L. acidophilus*. Salivary glands were harvested from the vehicle and *L. acidophilus* groups. **A** Representative images of SINGR3-positive cells in salivary glands and average numbers of SINGR3-positive cells. **B** Representative images of IL-10-positive cells in salivary glands and average numbers of IL-10-positive cells. **C** HSG cells were stimulated with TNF-α (2 ng/mL) and IL-17 (20 ng/mL) in the absence or presence of *L. acidophilus* (10 or 100 μg/mL) for 48 h and harvested for mRNA extraction and real-time PCR. Bar graphs show mRNA levels of SINGR3 (top and left), PD-L1 (top and right), IDO (bottom and left), and IL-10 (bottom and right). **D** Mouse non-T cells from the spleen were stimulated with LPS (100 ng/mL) in the absence or presence of *L. acidophilus* (10 or 100 μg/mL) for 48 h and harvested for mRNA extraction and real-time PCR. mRNA levels of SINGR3 (top and left), PD-L1 (top and right), IDO (bottom and left), and IL-10 (bottom and right) are shown. **E** Representative images of STIM1 in the salivary gland and average numbers of STIM1-positive cells. **F** Representative images of phosphor-STING in the salivary gland and average numbers of phosphor-STING-positive cells. **G** Splenocytes were stimulated with anti-CD3 (0.5 μg/mL) in the absence or presence of *L. acidophilus* (10 or 100 μg/mL) for 48 h and harvested for flow cytometry. FACS plots show percentages of type I IFN–producing dendritic cells. **H** Bar graphs show average percentages of type I IFN–positive cells. Data are means ± SD ($*p < 0.05$, $***p < 0.001$)

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(Fig. 3G, H). These data suggest that *L. acidophilus* ameliorates SS by inhibiting the production of type I IFN.

Effects of *L. acidophilus* on the gut microbiome

The *Bacteroides:Firmicutes* ratio and species richness and diversity were improved by *L. acidophilus* (Fig. 4A-C)). The abundance of propionate-producing bacteria, such as *Ruminococcaceae*, *Clostridiaceae*, and *Prevotellaceae*, was significantly increased by *L. acidophilus* (Fig. 4D).

These data suggest that *L. acidophilus* ameliorates SS by altering the gut microbiome.

Propionate attenuates SS and the infiltration of lymphocytes into the salivary gland

To investigate the role of propionate in SS, we administered propionate to 12-week-old NOD/ShiLtJ mice. Propionate improved the saliva flow rate and tissue inflammation (Fig. 5A, B). Furthermore, it decreased the
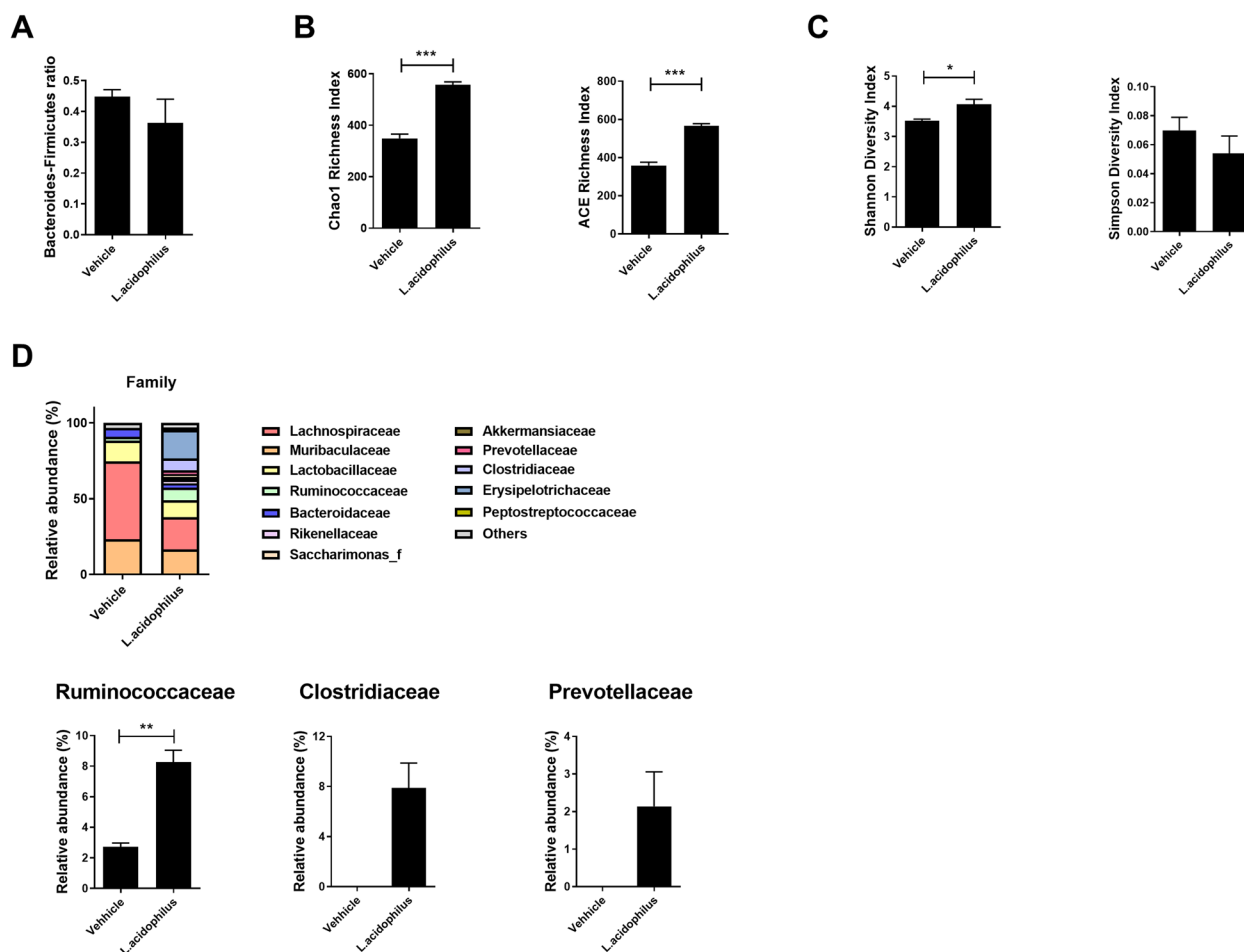
Fig. 4 *L. acidophilus* affects the gut microbiota. Cecal microbiome analyses of the vehicle and *L. acidophilus* groups. **A** *Bacteroides:Firmicutes* ratio. **B** Chao1 and ACE indices. **C** Shannon and Simpson diversity indices. **D** Composition of gut microbiota at the family level (top). Bar graphs at the bottom show the abundance of *Ruminococcaceae* (left), *Clostridiaceae* (center), and *Prevotellaceae* (right). Data are means $\pm$ SD (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$)

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Fig. 5 Therapeutic effects of propionate in SS. Propionate (200 mg/kg) was administered intraperitoneally to 12-week-old NOD mice, which were monitored for 24 weeks. Salivary glands and the spleen were harvested. **A** Saliva flow rate at 12, 16, 19, and 23 weeks. **B** Salivary glands were stained with H&E. Bar graphs show the histological score (left) and inflammation area (right). **C** Salivary gland tissue stained for IL-6, IL-17, and TNF-α and numbers of cells positive for IL-6 (left), IL-17 (center), and TNF-α (right). **D** Salivary glands were visualized by confocal microscopy. Representative images of Th17 (CD4^{+}IL-17^{+}; top) and Treg (CD4^{+}CD25^{+}FOXP3.[+]; bottom) cells are shown. Bar graphs show numbers of Th17- and Treg-positive cells. Data are means $\pm$ SD (*$p < 0.05$, **$p < 0.005$, ***$p < 0.001$)
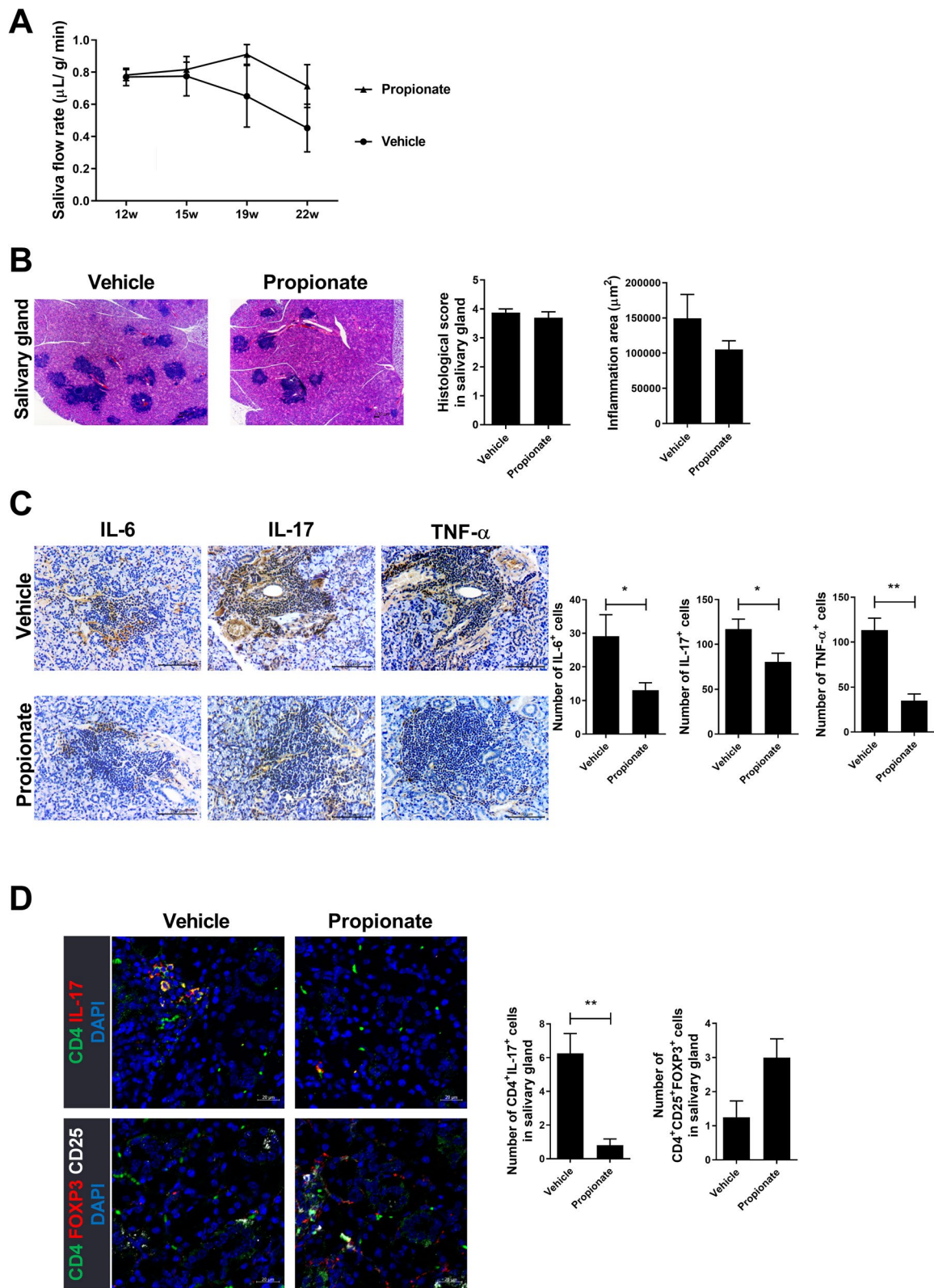
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infiltration of inflammatory cytokine (IL-6, IL-17, and TNF-α)-producing cells into the salivary gland (Fig. 5C). The Th17:Treg balance (decreased Th17 cells and increased Tregs) in the spleen was improved by propionate (Fig. 5D). These results suggest that propionate has therapeutic potential based on its anti-inflammatory activity.

Propionate attenuates SS by inhibiting the STIM1-STING pathway

STIM1 and phospho-STING expression were increased and decreased, respectively, by propionate (Fig. 6A, B). In addition, propionate decreased the number of type I IFN–producing cells (Fig. 6C, D). These data suggest that propionate ameliorates SS by inhibiting the production of type I IFN.

Discussion

SS is characterized by immune cell infiltration and tissue destruction, leading to dry eyes and mouth. It is most commonly diagnosed in adults older than 40 years

old and is more frequent in women. There is no cure for SS; treatment focuses on relieving symptoms [4]. In this study, we propose a microorganism (probiotic) and metabolite (SCFA) for the treatment of SS.

The gastrointestinal system and oral cavity are rich in microorganisms [43]. Probiotics reportedly have an immunomodulatory effect in inflammatory diseases [9, 13]. Because SS involves inflammation of the salivary and lacrimal glands, probiotics may have therapeutic potential. We reported that *L. acidophilus* improves intestinal inflammation in a model of DSS-induced IBD [16]. *L. acidophilus* decreases levels of inflammatory cytokines in the intestine and regulates the Th17:Treg balance. In addition, it has a therapeutic effect in lupus-prone mice by regulating the SIGNR3 pathway [17]. Manirarora et al. showed that lactobacilli have therapeutic potential for lupus by enhancing immunoregulation [44]. In this study, *L. acidophilus* ameliorated the development and progression of SS by decreasing the infiltration of inflammatory cytokine–producing cells into the salivary gland
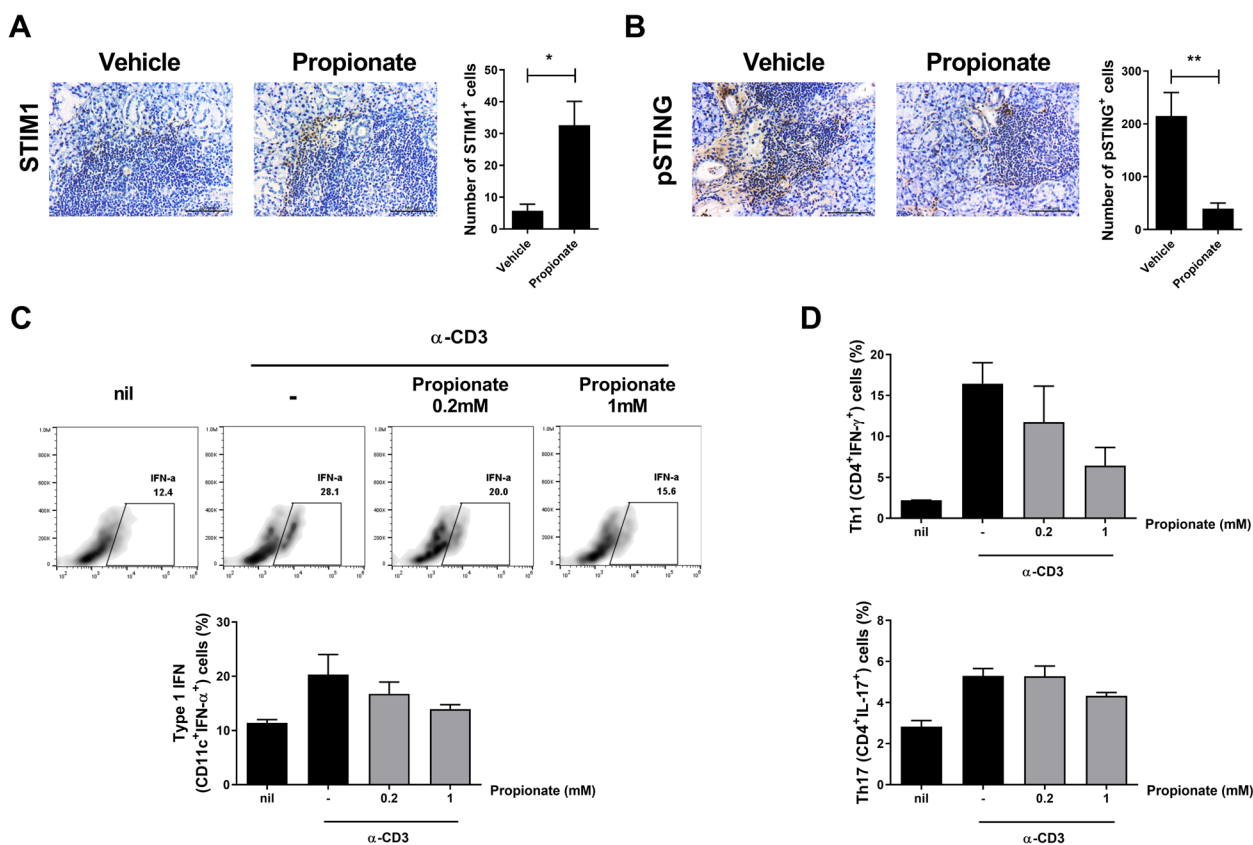

Fig. 6 Propionate regulates expression of STIM1 and STING. Salivary gland tissue was stained for STIM1 and phospho-STING. **A** Representative images of STIM1 in the salivary gland and average numbers of STIM1-positive cells. **B** Representative images of phosph0-STING in the salivary gland and average numbers of phosphor-STING-positive cells. **C** Splenocytes were stimulated with anti-CD3 (0.5 μg/mL) in the absence or presence of propionate (0.2 or 1 mM) for 48 h and harvested for flow cytometry. FACS plots show percentages of type I IFN–positive cells. Bar graphs show average percentages of type I IFN–producing dendritic cells. **D** Percentages of Th1 (CD4+IFN-γ+) and Th17 (CD4+IL-17.+) cells. Data are means ± SD (**p* < 0.05, ****p* < 0.001)

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and improving the Th17:Treg balance. SIGNR3 regulates intestinal immunity [19]. *L. acidophilus* increases expression of SIGNR3. Our data suggest that *L. acidophilus* ameliorates SS by modulating SIGNR3 signaling.

Probiotics alter the microbial composition of the gut [45, 46]. Alcon-Giner et al. reported that induction of *Bifidobacterium* changes the composition of the gut microbiota [47]. Shi et al. showed that *Lactobacillus* supplementation restores the gut microbiota [48]. In this study, we found the therapeutic potential of administration of *L. acidophilus* through restored the gut microbiota in the SS animal model. Administration of *L. acidophilus* led to change *Bacteroides:Firmicutes* ratio as well as species richness and diversity. The species richness and diversity mean the number of species which reflect the gut microbiome health. Our data indicated that *L. acidophilus* ameliorate SS through altering gut environment. Furthermore, *L. acidophilus* increased the abundance of *Ruminococcaceae, Clostridiaceae*, and *Prevotellaceae* which are the propionate-producing bacterial taxa. Tedelind et al. reported that propionate has a therapeutic effect in inflammatory bowel disease [29]. Mizuno et al. showed that propionate ameliorates the severity of EAE in an animal model [49]. In this study, supplementation with propionate attenuated the development and progression of SS by decreasing the infiltration of inflammatory cytokine–producing cells into the salivary gland. Therefore, propionate ameliorates SS by regulating the Th17:Treg balance and thus has therapeutic potential for SS. Also, propionate is known to regulate insulin production. NOD mice used in this study often accompany diabetes with high blood glucose level. Increasing propionate by administration of *L. acidophilus* may be possible mechanism in regulating SS pathogenesity.

Type I IFN is produced by STING activation and is implicated in the pathogenesis of rheumatic diseases, including SLE, SS, and RA [50]. Type I IFNs have immunostimulatory properties, including the activation of B cells to produce autoantibodies [51]. Suppression of local type I IFN by the gut microbiota has been reported [40]. However, the role of the gut microbiota or metabolites in type I IFN expression is unclear. *L. acidophilus* and propionate increase expression of STIM1, a negative regulator of STING, and decrease expression of STING, an inducer of type I IFN. In addition, *L. acidophilus* and propionate decrease type I IFN expression in splenocytes. Our data suggest that *L. acidophilus* and propionate ameliorate SS by inhibiting the STIM1–STING–type I IFN axis. As we said, however, the role of microbiota and its metabolite in type I IFN production is still controversial. The effect of microbiota and metabolite varies by disease type, disease severity. It still requires further study to understand mechanism of regulating type I IFN production through microbiota and metabolite.

Conclusions

Our results suggest that *L. acidophilus* and propionate have therapeutic potential for SS. Dysbiosis of the gut microbiota promotes SS by increasing the infiltration of lymphocytes into salivary glands and activating the STIM1–STING–type I IFN axis. Our findings demonstrate the therapeutic potential of *L. acidophilus* and propionate for SS.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12964-023-01141-0.

Additional file 1: Supplementary Table 1. List of antibodies usAQed in this study. **Supplementary Table 2.** List of primers used in this study.

Availability of data and materials
The authors confirm that the data supporting the findings of this study are available within the article. Gut microbiome analysis was deposited in the BioProject and Sequence Read Archive (SRA; https://www.ncbi.nlm.nih.gov/sra) under the accession numbers PRJNA863566.

Declarations

Ethics approval and consent to participate
Animal procedures were performed in accordance with the Laboratory Animals Welfare Act, the Guide for the Care and Use of Laboratory Animals, and the Guidelines and Policies for Rodent Experiments of the Institutional Animal Care and Use Committee of the School of Medicine, The Catholic University of Korea (no. 2021-0277-02).

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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References

1. Murube J. Henrik Sjogren, 1899–1986. Ocul Surf. 2010;8(1):2–7.
2. Nguyen CQ, Yin H, Lee BH, Carcamo WC, Chiorini JA, Peck AB. Pathogenic effect of interleukin-17A in induction of Sjogren's syndrome-like disease using adenovirus-mediated gene transfer. Arthritis Res Ther. 2010;12(6):R220.
3. Kakan SS, Edman MC, Yao A, Okamoto CT, Nguyen A, Hjelm BE, et al. Tear miRNAs identified in a murine model of Sjogren's Syndrome as potential diagnostic biomarkers and indicators of disease mechanism. Front Immunol. 2022;13: 833254.
4. Brito-Zeron P, Baldini C, Bootsma H, Bowman SJ, Jonsson R, Mariette X, et al. Sjogren syndrome. Nat Rev Dis Primers. 2016;2:16047.
5. Soret P, Le Dantec C, Desvaux E, Foulquier N, Chassagnol B, Hubert S, et al. A new molecular classification to drive precision treatment strategies in primary Sjogren's syndrome. Nat Commun. 2021;12(1):3523.
6. Zdziarski P, Pasciak M, Gamian A. Microbiome analysis and pharmacovigilance after inhaled glucocorticoid: oral dysbiosis with the isolation of three Rothia species and subsequent Sjogren's Syndrome. Front Pharmacol. 2022;13: 636180.
7. Kim DS, Woo JS, Min HK, Choi JW, Moon JH, Park MJ, et al. Short-chain fatty acid butyrate induces IL-10-producing B cells by regulating circadian-clock-related genes to ameliorate Sjogren's syndrome. J Autoimmun. 2021;119: 102611.
8. Cano-Ortiz A, Laborda-Illanes A, Plaza-Andrades I, Membrillo Del Pozo A, Villarrubia Cuadrado A, Rodriguez Calvo de Mora M, et al. Connection between the gut microbiome, systemic inflammation, gut permeability and FOXP3 expression in patients with primary Sjogren's Syndrome. Int J Mol Sci. 2020;21(22):8733.
9. Liu Y, Alookaran JJ, Rhoads JM. Probiotics in autoimmune and inflammatory disorders. Nutrients. 2018;10(10):1537.
10. Maldonado Galdeano C, Cazorla SI, LemmeDumit JM, Velez E, Perdigon G. Beneficial effects of probiotic consumption on the immune system. Ann Nutr Metab. 2019;74(2):115–24.
11. Olsson LM, Boulund F, Nilsson S, Khan MT, Gummesson A, Fagerberg L, et al. Dynamics of the normal gut microbiota: a longitudinal one-year population study in Sweden. Cell Host Microbe. 2022;30(5):726–39.
12. Beak JA, Park MJ, Kim SY, Jhun J, Woo JS, Choi JW, et al. FK506 and Lactobacillus acidophilus ameliorate acute graft-versus-host disease by modulating the T helper 17/regulatory T-cell balance. J Transl Med. 2022;20(1):104.
13. Cristofori F, Dargenio VN, Dargenio C, Miniello VL, Barone M, Francavilla R. Anti-inflammatory and immunomodulatory effects of probiotics in gut inflammation: a door to the body. Front Immunol. 2021;12: 578386.
14. Morshedi M, Hashemi R, Moazzen S, Sahebkar A, Hosseinifard ES. Immunomodulatory and anti-inflammatory effects of probiotics in multiple sclerosis: a systematic review. J Neuroinflamm. 2019;16(1):231.
15. Kim DH, Kim S, Lee JH, Kim JH, Che X, Ma HW, et al. Lactobacillus acidophilus suppresses intestinal inflammation by inhibiting endoplasmic reticulum stress. J Gastroenterol Hepatol. 2019;34(1):178–85.
16. Park JS, Choi JW, Jhun J, Kwon JY, Lee BI, Yang CW, et al. Lactobacillus acidophilus improves intestinal inflammation in an acute colitis mouse model by regulation of Th17 and Treg cell balance and fibrosis development. J Med Food. 2018;21(3):215–24.
17. Kim DS, Park Y, Choi JW, Park SH, Cho ML, Kwok SK. Lactobacillus acidophilus supplementation exerts a synergistic effect on tacrolimus efficacy by modulating Th17/Treg balance in lupus-prone mice via the SIGNR3 pathway. Front Immunol. 2021;12: 696074.
18. Lugo-Villarino G, Neyrolles O. SIGNing a symbiotic treaty with gut microbiota. EMBO J. 2015;34(7):829–31.
19. Lightfoot YL, Selle K, Yang T, Goh YJ, Sahay B, Zadeh M, et al. SIGNR3-dependent immune regulation by Lactobacillus acidophilus surface layer protein A in colitis. EMBO J. 2015;34(7):881–95.
20. Silva YP, Bernardi A, Frozza RL. The role of short-chain fatty acids from gut microbiota in gut-brain communication. Front Endocrinol (Lausanne). 2020;11:25.
21. Parada Venegas D, De la Fuente MK, Landskron G, Gonzalez MJ, Quera R, Dijkstra G, et al. Short chain fatty acids (SCFAs)-mediated gut epithelial

22. Bell KJ, Saad S, Tillett BJ, McGuire HM, Bordbar S, Yap YA, et al. Metabolite-based dietary supplementation in human type 1 diabetes is associated with microbiota and immune modulation. Microbiome. 2022;10(1):9.
23. Tilly G, Cadoux M, Garcia A, Morille J, Wiertlewski S, Pecqueur C, et al. Teriflunomide treatment of multiple sclerosis selectively modulates CD8 memory T Cells. Front Immunol. 2021;12: 730342.
24. Wen S, He L, Zhong Z, Zhao R, Weng S, Mi H, et al. Stigmasterol restores the balance of Treg/Th17 cells by activating the Butyrate-PPARgamma axis in colitis. Front Immunol. 2021;12: 741934.
25. Kim DS, Kwon JE, Lee SH, Kim EK, Ryu JG, Jung KA, et al. Attenuation of rheumatoid inflammation by sodium butyrate through reciprocal targeting of HDAC2 in osteoclasts and HDAC8 in T cells. Front Immunol. 2018;9:1525.
26. Shimizu J, Kubota T, Takada E, Takai K, Fujiwara N, Arimitsu N, et al. Propionate-producing bacteria in the intestine may associate with skewed responses of IL10-producing regulatory T cells in patients with relapsing polychondritis. PLoS ONE. 2018;13(9): e0203657.
27. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature. 2013;500(7461):232–6.
28. Poeker SA, Geirnaert A, Berchtold L, Greppi A, Krych L, Steinert RE, et al. Understanding the prebiotic potential of different dietary fibers using an in vitro continuous adult fermentation model (PolyFermS). Sci Rep. 2018;8(1):4318.
29. Tedelind S, Westberg F, Kjerrulf M, Vidal A. Anti-inflammatory properties of the short-chain fatty acids acetate and propionate: a study with relevance to inflammatory bowel disease. World J Gastroenterol. 2007;13(20):2826–32.
30. Filippone A, Lanza M, Campolo M, Casili G, Paterniti I, Cuzzocrea S, et al. The anti-inflammatory and antioxidant effects of sodium propionate. Int J Mol Sci. 2020;21(8):3026.
31. Srikanth S, Woo JS, Wu B, El-Sherbiny YM, Leung J, Chupradit K, et al. The Ca(2+) sensor STIM1 regulates the type I interferon response by retaining the signaling adaptor STING at the endoplasmic reticulum. Nat Immunol. 2019;20(2):152–62.
32. Vassilieva EV, Li S, Korniychuk H, Taylor DM, Wang S, Prausnitz MR, et al. cGAMP/Saponin adjuvant combination improves protective response to influenza vaccination by microneedle patch in an aged mouse model. Front Immunol. 2020;11: 583251.
33. Muskardin TLW, Niewold TB. Type I interferon in rheumatic diseases. Nat Rev Rheumatol. 2018;14(4):214–28.
34. Conigliaro P, Perricone C, Benson RA, Garside P, Brewer JM, Perricone R, et al. The type I IFN system in rheumatoid arthritis. Autoimmunity. 2010;43(3):220–5.
35. Ali S, Mann-Nuttel R, Schulze A, Richter L, Alferink J, Scheu S. Sources of type I interferons in infectious immunity: plasmacytoid dendritic cells not always in the driver's seat. Front Immunol. 2019;10:778.
36. Hadjadj J, Yatim N, Barnabei L, Corneau A, Boussier J, Smith N, et al. Impaired type I interferon activity and inflammatory responses in severe COVID-19 patients. Science. 2020;369(6504):718–24.
37. Psarras A, Emery P, Vital EM. Type I interferon-mediated autoimmune diseases: pathogenesis, diagnosis and targeted therapy. Rheumatology (Oxford). 2017;56(10):1662–75.
38. Decout A, Katz JD, Venkatraman S, Ablasser A. The cGAS-STING pathway as a therapeutic target in inflammatory diseases. Nat Rev Immunol. 2021;21(9):548–69.
39. Villena J, Chiba E, Vizoso-Pinto MG, Tomosada Y, Takahashi T, Ishizuka T, et al. Immunobiotic Lactobacillus rhamnosus strains differentially modulate antiviral immune response in porcine intestinal epithelial and antigen presenting cells. BMC Microbiol. 2014;14:126.
40. Yang K, Hou Y, Zhang Y, Liang H, Sharma A, Zheng W, et al. Suppression of local type I interferon by gut microbiota-derived butyrate impairs antitumor effects of ionizing radiation. J Exp Med. 2021;218(3): e20201915.
41. Antunes KH, Fachi JL, de Paula R, da Silva EF, Pral LP, Dos Santos AA, et al. Microbiota-derived acetate protects against respiratory syncytial virus infection through a GPR43-type 1 interferon response. Nat Commun. 2019;10(1):3273.
42. Hwang SH, Woo JS, Moon J, Yang S, Park JS, Lee J, et al. IL-17 and CCR9(+)alpha4beta7(-) Th17 cells promote salivary gland inflammation,

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dysfunction, and cell death in Sjogren's Syndrome. Front Immunol. 2021;12: 721453.

43. Kodukula K, Faller DV, Harpp DN, Kanara I, Pernokas J, Pernokas M, et al. Gut microbiota and salivary diagnostics: the mouth is salivating to tell us something. Biores Open Access. 2017;6(1):123–32.

44. Manirarora JN, Kosiewicz MM, Alard P. Feeding lactobacilli impacts lupus progression in (NZBxNZW)F1 lupus-prone mice by enhancing immunoregulation. Autoimmunity. 2020;53(6):323–32.

45. Bloemendaal M, Szopinska-Tokov J, Belzer C, Boverhoff D, Papalini S, Michels F, et al. Probiotics-induced changes in gut microbial composition and its effects on cognitive performance after stress: exploratory analyses. Transl Psychiatry. 2021;11(1):300.

46. Hemarajata P, Versalovic J. Effects of probiotics on gut microbiota: mechanisms of intestinal immunomodulation and neuromodulation. Therap Adv Gastroenterol. 2013;6(1):39–51.

47. Alcon-Giner C, Dalby MJ, Caim S, Ketskemety J, Shaw A, Sim K, et al. Microbiota supplementation with bifidobacterium and lactobacillus modifies the preterm infant gut microbiota and metabolome: an observational study. Cell Rep Med. 2020;1(5): 100077.

48. Shi Y, Zhai Q, Li D, Mao B, Liu X, Zhao J, et al. Restoration of cefixime-induced gut microbiota changes by Lactobacillus cocktails and fructooligosaccharides in a mouse model. Microbiol Res. 2017;200:14–24.

49. Mizuno M, Noto D, Kaga N, Chiba A, Miyake S. The dual role of short fatty acid chains in the pathogenesis of autoimmune disease models. PLoS ONE. 2017;12(2): e0173032.

50. Di Domizio J, Cao W. Fueling autoimmunity: type I interferon in autoimmune diseases. Expert Rev Clin Immunol. 2013;9(3):201–10.

51. Xiao ZX, Miller JS, Zheng SG. An updated advance of autoantibodies in autoimmune diseases. Autoimmun Rev. 2021;20(2): 102743.

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