Gallbladder-derived surfactant protein D regulates gut commensal bacteria for maintaining intestinal homeostasis

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Contributed by Tadatsugu Taniguchi, August 13, 2017 (sent for review July 21, 2017; reviewed by Ruslan Medzhitov and Satoshi Uematsu)

The commensal microbiota within the gastrointestinal tract is essential in maintaining homeostasis. Indeed, dysregulation in the repertoire of microbiota can result in the development of intestinal immune-inflammatory diseases. Further, this immune regulation by gut microbiota is important systemically, impacting health and disease of organ systems beyond the local environment of the gut. What has not been explored is how distant organs might in turn shape the microbiota via microbe-targeted molecules. Here, we provide evidence that surfactant protein D (SP-D) synthesized in the gallbladder and delivered into intestinal lumen binds selectively to species of gut commensal bacteria. SP-D-deficient mice manifest intestinal dysbiosis and show a susceptibility to dextran sulfate sodium-induced colitis. Further, fecal transfer from SP-D-deficient mice to wild-type, germ-free mice conveyed colitis susceptibility. Interestingly, colitis caused a notable increase in *Sftpd* gene expression in the gallbladder, but not in the lung, via the activity of glucocorticoids produced in the liver. These findings describe a unique mechanism of interorgan regulation of intestinal immune homeostasis by SP-D with potential clinical implications such as cholecystectomy.

surfactant protein D | gut microbiota | gallbladder | colitis | glucocorticoids

The composition of the gut commensal microbiota reflects the coevolution of host and microorganisms to achieve intestinal homeostasis that is symbiotic or mutually beneficial (1, 2). In this context, local interactions between commensal microbiota and intestinal cells are central to the regulation of the intestinal immune system (1, 2). Imbalances in the microbiota, termed dysbiosis, underlie the development and/or aggravation of immune-related diseases even in distant organs from the gastrointestinal tract (1, 2). Given the importance of this connection to human health, there has been much focus on the elucidation of the mechanisms by which the symbiosis is maintained.

One focus on this process has been the study of soluble, immunoregulatory molecules produced in the intestinal lumen by immune and epithelial cells that maintain intestinal immune homeostasis (1, 3). However, in this context, little attention has been paid to the role of immunoregulatory molecules produced by distant organs. During the course of our study on the role of the IRF3 transcription factor in intestinal homeostasis (4), we observed the presence of surfactant protein D (SP-D) in the intestinal lumen. SP-D belongs to the collectin family of soluble innate immune receptors and is a component of lung surfactant proteins that are continuously and specifically produced in the lungs by alveolar type II epithelial cells (5–7). Thus, SP-D protein is constitutively secreted into the alveolar fluid for the protection against aerally

transmitted pathogens (5–7). SP-D binds carbohydrates, lipids, and nucleic acids with a broad spectrum of specificity (5–7). To date, the physiological role of SP-D in other organs remains totally unknown.

In this study, we show that SP-D is produced by the gallbladder and plays a critical role in maintaining symbiotic status of the commensal bacteria. We show SP-D expression in the gallbladder epithelial cells and provide evidence that the gallbladder-produced SP-D, secreted into the bile and delivered to the intestinal lumen, is the major source of intestinal SP-D. Our results also indicate that SP-D selectively affects populations of commensal bacterial species through its direct binding to the bacteria, which interferes with their replication and causes a cascade of events that skew the repertoire of bacterial species in the intestine.

Indeed, mice deficient in the *Sftpd* gene (*Sftpd*^{−/−} mice) show intestinal dysbiosis and sensitization to dextran sulfate sodium (DSS)-induced colitis. The importance of the dysbiosis resulting

Significance

The imbalance in the intestinal microbiota repertoire, called dysbiosis, underlies the development of inflammatory diseases in the intestine and distant organ systems. Whether and how distant organs regulate the commensal gut bacteria and intestinal homeostasis via microbe-targeted molecules has remained poorly explored. In this study, we show that the gallbladder regulates intestinal immune homeostasis through the production of surfactant protein D (SP-D) that is delivered to the intestine via bile. The importance of the gallbladder-derived SP-D was underscored by an intestinal dysbiosis in SP-D-deficient mice and susceptibility of these mice to dextran sodium sulfate sodium-induced colitis. Our study thus revealed a unique interorgan regulation of intestinal homeostasis by SP-D with potential clinical implications such as cholecystectomy.

Author contributions: H.S.-K., H.N., J.N., A.K., and T.T. designed research; H.S.-K., H.N., J.N., W.S., Y.N., M.Y.-K., K.I., S.K., and N.E. performed research; H.K. and M.H. contributed new reagents/analytic tools; H.S.-K., H.N., J.N., W.S., Y.N., M.Y.-K., K.I., S.K., N.E., H.Y., M.A., H.K., M.H., A.K., and T.T. analyzed data; and H.N., J.N., and T.T. wrote the paper.

Reviewers: R.M., Yale University School of Medicine; and S.U., Chiba University.

The authors declare no conflict of interest.

Data deposition: The sequence reported in this paper has been deposited in the GenBank database (accession no. BioProjectID PRJDB5950).

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1712837114/-DCSupplemental.

from the absence of SP-D is further underscored by fecal transfer experiments, wherein germ-free mice receiving feces from *Sftpd*^{-/-} mice are more susceptible to DSS-induced colitis compared with those that received the feces of *Sftpd*^{+/-} mice. Of note, there was a significant reduction of Foxp3 expression in regulatory T cells (Tregs) in the intestinal lamina propria of *Sftpd*^{-/-} mice, which may account for the increased susceptibility to colitis.

In addition, we also found a notable elevation of SP-D expression during the development of DSS-induced colitis in the gallbladder, but not in the lung. We provide evidence that colitis enhances synthesis of glucocorticoids in the liver, which is secreted into the bile to induce *Sftpd* mRNA expression in gallbladder epithelial cells. Taken together, our findings reveal the hitherto unknown role of SP-D in the interorgan regulation of intestinal commensal microbiota. We discuss the significance of these findings as well as the clinical implications such as cholecystectomy.

Results

Expression of SP-D in Gallbladder. When we examined relative *Sftpd* gene expression in various organs by quantitative RT-PCR (qRT-PCR), a notably high expression level of *Sftpd* mRNA was observed in the gallbladder compared with other digestive organs, including the stomach, intestines, appendix, liver, and pancreas (Fig. 1A). Further, SP-D protein expression was detected by immunohistochemical staining in the gallbladder epithelium, but not in lamina propria (Fig. 1B). As shown in Fig. 1C, SP-D protein was measured in the bile, suggesting that it reaches the intestinal lumen through its secretion into the bile. Consistent with this, bile duct ligation performed to inhibit bile flow into the intestine resulted in a marked reduction in SP-D protein levels in the fecal suspension (Fig. 1D), indicating that gallbladder is the main source of SP-D found in the intestinal lumen.

Alteration of Intestinal Bacterial Population by the Absence of SP-D. Since SP-D has been reported to play an important role in the elimination of pathogenic bacteria such as *Pseudomonas aeruginosa* and *Staphylococcus aureus* within the alveoli of the lungs (5, 7), we asked if expression of SP-D affects populations of commensal bacteria in the intestine. To address this question, we first performed a comparative microbiome analysis on feces from wild-type (WT) and *Sftpd*^{-/-} mice.

Interestingly, significant differences in the bacterial population and diversity were observed between WT and *Sftpd*^{-/-} mice (Fig. 1E and Fig. S1A and B). Since the sequence analysis of 16S ribosomal RNA (rRNA) revealed that most of the altered bacteria species have been poorly characterized, these data are presented using operational taxonomic units (OTUs) (Fig. 1F and Fig. S1C). In this analysis, we observed that species more closely related to *Clostridia* cluster IV and XIVa were markedly decreased while species more closely related to the *Lactobacillus salivarius* cluster were increased in *Sftpd*^{-/-} mice. Among the latter cluster, OTU00007 is *Lactobacillus murinus* as the sequence showed a similarity of 100% (8). On the other hand, commensal fungi, which have also been implicated to be bound by SP-D (9, 10), were not detected in our specific-pathogen-free (SPF) mice, an observation similarly reported in the SPF mice (11) (Fig. S2).

SP-D Binding to Intestinal Bacteria and Its Effect on Bacterial Growth.

To gain further insight into the intestinal dysbiosis resulting from the absence of SP-D, we next examined SP-D binding to commensal bacteria in the feces from WT mice. For this, fecal bacteria from WT mice were stained with anti-SP-D antibody and subjected to flow cytometric analysis. As shown in Fig. 2A, commensal microbiota contained about 2% of SP-D binding bacteria and 16S rRNA sequencing further revealed that 61 OTUs were increased in SP-D-positive fraction with significant difference compared with SP-D-negative fraction (Fig. S3). Among them, significant SP-D binding was observed with *L. murinus* (OTU00007 and

OTU00840) and *Lactobacillus animalis* (OTU01291) (Fig. 2B and C), whose relative amounts were increased in *Sftpd*^{-/-} mice (Fig. S1), whereas such binding was not observed with OTUs closely related to *Clostridia* IV and XIVa (OTU00194 and OTU00272) (Fig. 2C), which showed a decrease in these mice (Fig. S1). Additionally, OTU00009 (*Lactobacillus johnsonii*) and OTU00069 (*Lactobacillus reuteri*), which were unchanged in mutant mice, did not show significant binding to SP-D (Fig. 2C).

When we next examined the effects of recombinant SP-D (rSP-D) on the growth of *L. murinus* in vitro, a dose-dependent inhibition was observed, suggesting that SP-D directly interferes with the growth of *L. murinus* in the intestine (Fig. 2D, Left). Growth inhibition was also observed against another *L. murinus* strain ATCC35020 (Fig. S4A). Of note, we found features of cell lysis of *L. murinus* cells incubated with rSP-D using analysis by scanning electron microscopy (Fig. S4B). In contrast, no growth inhibition was observed for *L. johnsonii*, which is not bound by SP-D (Fig. 2C and D, Right). In this regard, it is interesting that *L. murinus* interferes with the growth of the *Clostridia* species in vitro (Fig. S5).

Development of Intestinal Immune Cells in the Absence of SP-D. As changes in the composition of intestinal microbiota can affect the immune system (1, 2), we next asked whether immune cell populations in the intestinal lamina propria are altered by the absence of SP-D. No overt change was observed in the frequencies of immune cells in the intestinal lamina propria of *Sftpd*^{-/-} mice (Fig. S6). However, the majority of Tregs in *Sftpd*^{-/-} mice showed significantly lower expression levels of Foxp3 compared with Tregs from control mice (Fig. 3A). On the other hand, no abnormality was observed in Tregs in the spleen of the mutant mice (Fig. 3B). These data suggest that intestinal dysbiosis caused by the absence of SP-D selectively affects the development of intestinal Tregs, which are shown to be critical to the regulation of intestinal inflammatory responses (12).

Susceptibility of the SP-D-Deficient Mice to Experimental Colitis.

Given the intestinal dysbiosis that is associated with abnormal Treg development in *Sftpd*^{-/-} mice, we next examined the ability of these mice to control colonic inflammation. A model of DSS-induced colitis showed that *Sftpd*^{-/-} mice had a marked increase in the susceptibility to colitis compared with WT mice as evidenced by a rapid decrease in body weight (Fig. 3C) and shortened length of the colon (Fig. 3D). Consistent with this, disease activity index (DAI) was significantly higher in *Sftpd*^{-/-} mice (Fig. 3E).

To further examine the link between intestinal dysbiosis and aggravated colitis in *Sftpd*^{-/-} mice, we next performed a fecal microbiota transplant experiment in which feces from *Sftpd*^{+/-} or *Sftpd*^{-/-} mice were transplanted to germ-free WT mice and then challenged with DSS. As shown in Fig. 3F, a significant loss of the body weight was observed in the mice that received the feces from *Sftpd*^{-/-} mice compared with those that received feces from *Sftpd*^{+/-} mice. The different phenotype observation in the germ-free mice supports the notion that, in this experimental condition, the status of microbiota remains unchanged in the germ-free mice, which have the intact *Sftpd* gene. These results indicate that the dysbiotic microbiota present in *Sftpd*^{-/-} mice indeed accounted for the susceptibility to the colitis development, at least in part.

Interorgan Regulation of *Sftpd* Gene Expression in Colitis Development.

In view of the importance of gallbladder-derived SP-D to control DSS-induced colitis, we then examined whether intestinal inflammation affected *Sftpd* gene expression in the gallbladder. Interestingly, we found that *Sftpd* mRNA and SP-D protein were markedly enhanced in the gallbladder but not lungs of WT mice made to develop DSS-induced colitis (Fig. 4A and Fig. S7A). Since the *Sftpd* gene is known to be induced by fibroblast growth factors (FGFs), glucocorticoids, and endotoxin in the lung epithelial cells (13–15), we tested whether these signaling pathways

† indicates bacteria species classified into *Clostridia* Cluster IX or XIVa.

Fig. 1. Gallbladder-specific expression of the *Sftpd* gene in digestive organs and dysbiosis in *Sftpd*^{-/-} mice. (A) Quantitative RT-PCR (qRT-PCR) analysis of *Sftpd* mRNA expression in lungs, stomach (St), small intestine (SI), large intestine (LI), appendix (App), pancreas (Pan), liver, and gallbladder (GB) from wild-type (WT) mice (*n* = 5). (B) Immunohistochemical analysis of SP-D protein in gallbladder from WT mice. (Scale bar, 100 μ m.) (C) Concentration of SP-D protein in the bile from WT (*n* = 5) or *Sftpd*-deficient (*Sftpd*^{-/-}) mice (*n* = 4). (D) Immunoblot analysis of SP-D protein in the feces from WT mice with sham operation or bile duct ligation (BDL) (Left). Fresh fecal suspensions from these mice were subjected to immunoblot analysis after immunoprecipitation (IP) with anti-SP-D antibody. Relative band intensity is shown (Right). (E) Principal coordinate analysis (PCoA) of unweighted UniFrac distance in the fecal microbiota from WT (blue, *n* = 8) and *Sftpd*^{-/-} mice (red, *n* = 9) (Left). Mean unweighted UniFrac distances within and between the groups is shown (Right). (F) Phylogenetic tree analysis of operational taxonomic units (OTUs) whose read counts are significantly increased (red) or decreased (green) in *Sftpd*^{-/-} mice compared with WT mice. Bacteria on the yellow background belong to *Firmicutes* and those on the green background belong to *Bacteroidetes*. **P* < 0.05, ****P* < 0.001. NS, not significant.

could induce *Sftpd* mRNA in a gallbladder-derived epithelial cell line, OCU-1. As shown in Fig. 4B and Fig. S7B, we found *Sftpd* mRNA is induced in a dose-dependent manner by synthetic glucocorticoids, but neither by FGFs nor LPS. Interestingly, this induction was not observed in a hepatocyte cell line Huh7 (Fig. S7C), suggesting the cell-type-specific *Sftpd* gene induction by glucocorticoids.

Consistent with this, DSS-induced colitis enhanced the concentrations of corticosterone, a major glucocorticoid in mouse, in the bile, while serum concentrations exhibited only a slight

increase (Fig. 4C, Left). This was also accompanied by a significant increase in the volume of bile (Fig. 4C, Right). Further, corticosterone levels were also increased in the liver under conditions of intestinal inflammation, but not within the gallbladder itself (Fig. 4D), suggesting the local production of corticosterone occurs specifically in the liver. Indeed, corticosterone levels were enhanced in the supernatants of liver tissues cultured from DSS-treated mice, which was suppressed by addition to the cultures of metyrapone, an inhibitor for corticosterone-producing enzyme, Cyp11b1 (16) (Fig. 4E). Thus, these findings suggest a feedback

Fig. 3. Critical role of SP-D in the control of intestinal homeostasis. (A) Flow cytometric analysis of Foxp3 protein expression in CD4⁺Foxp3⁺ cells from colonic lamina propria of *Sftpd*^{+/-} (*n* = 7) or *Sftpd*^{-/-} mice (*n* = 6). Representative histogram (Left) and pooled data of relative mean fluorescence intensity (MFI) of Foxp3 (Right) are shown. Relative MFI was calculated by dividing the MFI of each mouse by the average of MFI of *Sftpd*^{+/-} mice. (B) Relative MFI of Foxp3 in CD4⁺Foxp3⁺ cells from spleen of *Sftpd*^{+/-} (*n* = 4) and *Sftpd*^{-/-} mice (*n* = 3). (C–E) Colitis was induced by oral administration of 3–3.5% of DSS. Body weight (WT, *n* = 5; *Sftpd*^{-/-}, *n* = 6) (C), pictures of colons on day 0 and 8 and reduction rate of colon length (*n* = 4 per group) (D) and disease activity indices (DAIs) (WT, *n* = 5; *Sftpd*^{-/-}, *n* = 4) (E) are shown. (F) WT germ-free mice were inoculated with the fecal suspension from *Sftpd*^{+/-} (*n* = 5) or *Sftpd*^{-/-} (*n* = 5) mice. Colitis was induced by administration of 2.5% of DSS in drinking water for 6 d. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

On the other hand, we did not see a strong correlation between SP-D binding to bacteria species and reduced prevalence of the bacteria in vivo (Figs. S1 and S3). As such, a complex cascade of events, involving interbacterial interactions and/or host–bacterial interactions, may bring about intestinal dysbiosis in the absence of SP-D. Although unlikely, it is not excluded that the absence of SP-D may also affect bile composition, thereby contributing to the intestinal dysbiosis.

The composition of commensal bacteria in the gut is known to affect development of specific immune cell subsets. This was the case for our study in which we observed alterations of lamina propria Tregs in *Sftpd*^{-/-} mice. Indeed, Tregs were not significantly reduced in absolute number, but showed lower expression of Foxp3 (Fig. 3A and Fig. S6). Since it has been shown that an attenuated Foxp3 expression in Tregs results in a loss of immunosuppressive activity of these cells (21, 22), exacerbation of DSS-induced colitis found in *Sftpd*^{-/-} mice may be attributed, at least in part, to the abnormality of intestinal Tregs in these mice. It is interesting in this context that functional Tregs are induced by *Clostridia cluster IV* and *XIVa* species in the intestine (23) and that some of these species are reduced in *Sftpd*^{-/-} mice (Fig. 1F and Fig. S1). The question for how this abnormal Treg development, within the lamina propria, might contribute to the increased susceptibility to the DSS-induced colitis in *Sftpd*^{-/-} mice will be an important issue to address in the future.

It is interesting that *Sfipd* gene expression is further enhanced by DSS-induced colitis in the gallbladder (Fig. 4A), suggesting a

feedback regulation between the intestines, liver, and gallbladder organs in response to intestinal immune inflammation. SP-D is involved in this feedback mechanism, as its expression is enhanced by glucocorticoids such as corticosterone produced by the liver and delivered to gallbladder in response to colitis (Fig. 4 B–D). In fact, an elevation of biliary corticosterone was observed showing there is a cross-talk between these organs (Fig. 4C). To our knowledge, glucocorticoid synthesis by the liver was previously unknown. Endotoxin and inflammatory cytokines, such as LPS, TNF- α , and IL-1 β , are known to activate local corticosterone production in skin and lung (24). We found LPS administration in the mouse indeed caused an enhancement of liver corticosterone accompanied by *Sftpd* mRNA expression in the gallbladder (Fig. S8). Therefore, the enterohepatic circulation-mediated delivery of LPS and/or other inflammatory molecules produced during the development of colitis may account for the enhancement of liver corticosterone production.

At present, the physiological significance of the feedback regulation of SP-D during colitis development remains unclear. In this context, it is interesting to note that in WT mice, the number of SP-D binding bacteria is significantly increased during DSS-induced colitis (Fig. S9A). Interestingly, however, the population of *L. murinus* was significantly decreased (Fig. S9B), which is consistent with the growth inhibition of *L. murinus* by SP-D (Fig. 2D). Thus, although further work will be required, a view emerges in which corticosterone-mediated induction of SP-D in the gallbladder may be important for the proper balancing of intestinal bacteria for maintaining homeostasis.

Fig. 4. Feedback regulation of *Sftpd* gene expression by colitis. (A) *Sftpd* mRNA expression in the gallbladder (Left) and concentration of SP-D protein in the bile (Right) from WT mice ($n = 4$) treated with 3.5% of DSS for 4 or 8 d. (B) *Sftpd* mRNA expression in gallbladder epithelial cell line OCUG-1 treated with dexamethasone (Dex; 1 μ M), FGF7 (100 μ g/mL), FGF10 (100 μ g/mL), or EGF (100 μ g/mL) for indicated time periods. (C) Corticosterone concentration in the serum or bile from WT mice ($n = 5$) treated with 3.5% DSS for 7 d and water for an additional 1 d (Left). Volume of bile is shown at Right. (D) Concentration of corticosterone in the liver (Left) or gallbladder (Right) from WT mice ($n = 5$) treated with 3.5% DSS for 9 d (Left). (E) Concentration of corticosterone in the tissue culture supernatant of liver from mice ($n = 5$) treated with 3.5% DSS for 8 d (Right). MET; 200 μ g/mL of metyrapone, PF; 100 μ M of PF-915275. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. NS, not significant.

Considering the critical role of SP-D in the protection of the lung against pathogens, it is possible that SP-D also plays an important role in the protection against pathogen in the gallbladder. As infection is the main cause of biliary tract inflammation such as cholecystitis and cholangitis (25), this issue may have clinical importance to be addressed in further studies. In addition, our findings may raise a possible concern for the treatment of gallbladder-related diseases such as gallstone and gallbladder cancer. Usually, gallbladder removal or cholecystectomy is a common treatment of these diseases. Although the side effect of cholecystectomy is thought to be modest, significant change of intestinal microbiota is reported in patients treated with this procedure (26). Therefore, our study may have clinical implication on the pathogenesis of intestinal diseases, possibly with therapeutic potential of SP-D in intestinal diseases.

Materials and Methods

Mice. FVB/Njcl (FVB) were purchased from Clea Japan, Inc. SP-D-deficient (*Sftpd*^{−/−}) mice, described previously (27), were kindly gifted by Dr. Jeffery A. Whitsett, Cincinnati Children's Hospital Medical Center, Cincinnati, and maintained on a FVB genetic background in SPF conditions. Germ-free C57BL/6N mice were purchased from Sankyo Labo Service Corporation, Inc. or Clea Japan, Inc. All animal experiments were done in accordance with guidelines of the University of Tokyo and Osaka University.

Immunoprecipitation and Immunoblot Analysis for SP-D. The fresh feces from the mice were suspended in the lysis buffer (1% Nonidet P-40, 10 mM Tris-HCl pH 7.8, 150 mM NaCl, 1 mM EDTA) and subjected to immunoprecipitation with anti-SP-D antibody (H-120; Santa Cruz) and Dynabeads Protein G (Invitrogen) according to the manufacturer's protocol. Immunoblot analysis for SP-D was performed by SDS/PAGE with SP-D antibody (ab17781; Abcam).

Bacteria. *L. murinus* (ATCC35020) was obtained from American Type Culture Collection. *Clostridium* 17 strain mix was described before (23). Endogenous *L. murinus* and *L. johnsonii* were isolated from feces from SPF mice and identified by sequencing of 16S ribosomal RNA genome.

Analysis of SP-D Binding Bacteria. Fecal pellets were suspended in 1 mM CaCl₂/PBS, weighed, and filtered by a 40-μm cell strainer. Flowthrough was washed in

1 mM CaCl₂/PBS with 8,000 × g and left on ice for 20 min. After washing in 1% BSA/1 mM CaCl₂/PBS twice, it was resuspended and incubated for blocking in 2% BSA/1 mM CaCl₂/PBS for 20 min on ice. Bacterial concentration was adjusted by resuspending bacterial pellet from 10 mg feces in 100 μL of 2% BSA/1 mM CaCl₂/PBS. The 100 μL of bacterial suspension was incubated with 2 μg of anti-SP-D antibody (sd13980; Santa Cruz) or rabbit IgG for 15 min on ice. After washing with 8,000 × g at 4 °C twice, bacterial suspension was resuspended in 100 μL of 2% BSA/1 mM CaCl₂/PBS containing 1 μg of APC-conjugated anti-rabbit IgG (sc8346; Santa Cruz) for 15 min on ice. After being washed, bacterial suspension was subjected for sorting of SP-D-positive or -negative bacteria by FACS Aria cell sorter (BD).

DSS-Induced Colitis. DSS-induced colitis was performed as described before with modification (4). Age-matched animals were administered 3.5% DSS (MP Biomedicals) in drinking water for 7–9 d. For body weight change studies, the percentage of body weight was determined by the following equation: 100 × (body weight at the indicated day)/(body weight before DSS treatment). In fecal transfer experiment, 0.01 g of feces from *Sftpd*^{+/−} or *Sftpd*^{−/−} mice was orally inoculated into germ-free mice (C57BL/6N; Sankyo Lab). After 4 d, mice were treated with 2.5% DSS for 6 d and water for additional 2 d. DAI of DSS colitis was assessed by a previously described method (28).

Measurement of Tissue Corticosterone. To examine tissue-contained corticosterone, tissues were homogenized in PBS (liver; 150 mg/mL, gallbladder; 1 tissue in 0.5 mL) by Shakeman3 BMS-SMN03 (Biomedical Science) and supernatants were subjected to ELISA with corticosterone ELISA kit (Enzo Life Sciences). For tissue culture, liver was sectioned into small pieces (around 3 mm square) and cultured as 100 mg tissue/mL with 199 medium (Sigma) including 5% charcoal stripped FBS (Gibco) and 1% antibiotic-antimycotic (Gibco). After a 15-h incubation at 37 °C, culture supernatants were subjected to ELISA.

All other information is available in [Supporting Information](#).

ACKNOWLEDGMENTS. We thank K. Honda, K. Atarashi, T. Tanoue, H. Takamatsu, and H. Kikutani for helpful discussion and M. Sugahara, M. Taniguchi, and N. Fujii for technical assistance. This work was supported in part by Grant-In-Aid for Scientific Research (S) 15638461 from the Ministry of Education, Culture, Sports, Science; Japan Agency for Medical Research and Development 15656877; the Nakajima Foundation; and Uehara Memorial Foundation. H.S.-K., Y.N., and N.E. are research fellows of the Japan Society for the Promotion of Science. The Department of Molecular Immunology is supported by BONAC Corporation and Kyowa Hakko Kirin Co.

- Mowat AM, Agace WW (2014) Regional specialization within the intestinal immune system. *Nat Rev Immunol* 14:667–685.
- Goldschmid RS, Trinchieri G (2012) The price of immunity. *Nat Immunol* 13:932–938.
- Hancock RE, Haney EF, Gill EE (2016) The immunology of host defence peptides: Beyond antimicrobial activity. *Nat Rev Immunol* 16:321–334.
- Negishi H, et al. (2012) Essential contribution of IRF3 to intestinal homeostasis and microbiota-mediated Tslp gene induction. *Proc Natl Acad Sci USA* 109:21016–21021.
- Wright JR (2005) Immunoregulatory functions of surfactant proteins. *Nat Rev Immunol* 5:58–68.
- Kishore U, et al. (2006) Surfactant proteins SP-A and SP-D: Structure, function and receptors. *Mol Immunol* 43:1293–1315.
- Crouch EC (2000) Surfactant protein-D and pulmonary host defense. *Respir Res* 1:93–108.
- Sun Z, et al. (2015) Expanding the biotechnology potential of lactobacilli through comparative genomics of 213 strains and associated genera. *Nat Commun* 6:8322.
- Iliev ID, et al. (2012) Interactions between commensal fungi and the C-type lectin receptor dectin-1 influence colitis. *Science* 336:1314–1317.
- Dollive S, et al. (2013) Fungi of the murine gut: Episodic variation and proliferation during antibiotic treatment. *PLoS One* 8:e71806.
- Tang C, et al. (2015) Inhibition of dectin-1 signaling ameliorates colitis by inducing Lactobacillus-mediated regulatory T cell expansion in the intestine. *Cell Host Microbe* 18:183–197.
- Boehm F, et al. (2012) Deletion of Foxp3+ regulatory T cells in genetically targeted mice supports development of intestinal inflammation. *BMC Gastroenterol* 12:97.
- Mason RJ, et al. (2002) Maintenance of surfactant protein A and D secretion by rat alveolar type II cells in vitro. *Am J Physiol Lung Cell Mol Physiol* 282:L249–L258.
- Rucka Z, et al. (2013) Differential effects of insulin and dexamethasone on pulmonary surfactant-associated genes and proteins in A549 and H461 cells and lung tissue. *Int J Mol Med* 32:211–218.
- Bachurski CJ, Ross GF, Ikegami M, Kramer BW, Jobe AH (2001) Intra-amniotic endotoxin increases pulmonary surfactant proteins and induces SP-B processing in fetal sheep. *Am J Physiol Lung Cell Mol Physiol* 280:L279–L285.
- Gold EM, Diraimondo VC, Forsham PH (1960) Quantitation of pituitary corticotropin reserve in man by use of an adrenocortical 11-beta hydroxylase inhibitor (SU-4885). *Metabolism* 9:3–20.
- Turpin W, Humblot C, Thomas M, Guyot JP (2010) Lactobacilli as multifaceted probiotics with poorly disclosed molecular mechanisms. *Int J Food Microbiol* 143: 87–102.
- Alok A, et al. (2017) Probiotics: A new era of biotherapy. *Adv Biomed Res* 6:31.
- Sarao LK, Arora M (2017) Probiotics, prebiotics, and microencapsulation: A review. *Crit Rev Food Sci Nutr* 57:344–371.
- Delucchi L, Fraga M, Perelmutter K, Cella CD, Zunino P (2014) Effect of native Lactobacillus murinus LBP2 administration on total fecal IgA in healthy dogs. *Can J Vet Res* 78:153–155.
- Rudensky AY (2011) Regulatory T cells and Foxp3. *Immunol Rev* 241:260–268.
- Wan YY, Flavell RA (2007) Regulatory T-cell functions are subverted and converted owing to attenuated Foxp3 expression. *Nature* 445:766–770.
- Atarashi K, et al. (2013) Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. *Nature* 500:232–236.
- Talabér G, Jondal M, Okret S (2013) Extra-adrenal glucocorticoid synthesis: Immune regulation and aspects on local organ homeostasis. *Mol Cell Endocrinol* 380:89–98.
- Bornscheuer T, Schmiedel S (2014) Calculated antibiosis of acute cholangitis and cholecystitis. *Viszeralmedizin* 30:297–302.
- Sauter GH, et al. (2002) Bowel habits and bile acid malabsorption in the months after cholecystectomy. *Am J Gastroenterol* 97:1732–1735.
- Korfhagen TR, et al. (1998) Surfactant protein-D regulates surfactant phospholipid homeostasis in vivo. *J Biol Chem* 273:28438–28443.
- Kim JJ, Shajib MS, Manocha MM, Khan VI (2012) Investigating intestinal inflammation in DSS-induced model of IBD. *J Vis Exp* 3678.
- Chiba S, et al. (2014) Recognition of tumor cells by dectin-1 orchestrates innate immune cells for anti-tumor responses. *Elife* 3:e04177.
- Martorell P, Querol A, Fernández-Espinar MT (2005) Rapid identification and enumeration of Saccharomyces cerevisiae cells in wine by real-time PCR. *Appl Environ Microbiol* 71:6823–6830.
- Ueno M, et al. (2010) Assessment and improvement of methods for microbial DNA preparation from fecal samples. *Handbook of Molecular Microbial Ecology II: Metagenomics in Different Habitats*, ed de Bruijn FJ (John Wiley & Sons, Inc., Hoboken, NJ), pp 191–198.
- Nishio J, et al. (2015) Requirement of full TCR repertoire for regulatory T cells to maintain intestinal homeostasis. *Proc Natl Acad Sci USA* 112:12770–12775.