A C-Type Lectin MGL1/CD301a Plays an Anti-Inflammatory Role in Murine Experimental Colitis

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Inflammatory bowel disease is caused by abnormal inflammatory and immune responses to harmless substances, such as commensal bacteria, in the large bowel. Such responses appear to be suppressed under healthy conditions, although the mechanism of such suppression is currently unclear. The present study aimed to reveal whether the recognition of bacterial surface carbohydrates by the macrophage galactose-type C-type lectin-1, MGL1/CD301a, induces both the production and secretion of interleukin (IL)-10. Dextran sulfate sodium salt (DSS) was orally administered to mice that lacked MGL1/CD301a (*Mgl1*^{-/-} mice) and their wild-type littermates. *Mgl1*^{-/-} mice showed significantly more severe inflammation than wild-type mice after administration of DSS. MGL1-positive cells in the colonic lamina propria corresponded to macrophage-like cells with F4/80-high, CD11b-positive, and CD11c-intermediate expression. These cells in *Mgl1*^{-/-} mice produced a lower level of IL-10 mRNA compared with wild-type mice after the administration of DSS for 2 days. Recombinant MGL1 was found to bind both *Streptococcus sp.* and *Lactobacillus sp.* among commensal bacteria isolated from mesenteric lymph nodes of DSS-treated mice. Heat-killed *Streptococcus sp.* induced an increase in IL-10 secretion by MGL1-positive colonic lamina propria macrophages, but not the macrophage population from *Mgl1*^{-/-} mice. These results strongly suggest that MGL1/CD301a plays a protective role against colitis by effectively inducing IL-10 production by colonic lamina propria macrophages in response to invading commensal bacteria. (*Am J Pathol* 2009, 174:144–152; DOI: 10.2353/ajpath.2009.080235)

response to these antigens is delicately regulated. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are known to be caused by an abnormal mucosal immune response to ordinarily harmless antigens. However, the mechanisms of the pathogenesis of inflammatory bowel diseases remains unclear and effective methods to prevent or treat these diseases are not established.

Oral administration of dextran sulfate sodium salt (DSS) is widely used as a model for ulcerative colitis. In this model, the colonic epithelial barrier has been shown to be disrupted and abnormal infiltration of commensal bacteria was observed.¹ Germ-free mice were previously reported to have less severe inflammation in this model, indicating that interactions between the host and intestinal bacteria play an important role during the pathogenesis of colitis.² Not all bacteria were harmful, however, and administration of several species of *Lactobacilli* ameliorated experimental colitis,^{3,4} although the mechanism of the anti-inflammatory effect of these beneficial commensal bacteria is unknown. Interleukin (IL)-10 is one candidate because *IL-10*-deficient mice have been reported to develop colitis spontaneously, indicating that this cytokine acts as a suppressing regulatory factor for experimental colitis.⁵ Indeed, therapeutic administration of the *IL-10* gene has been shown to be effective to treat DSS-induced colitis,⁶ although which cells produce IL-10 and how IL-10 inhibits the pathogenesis of colitis remain unknown.

Macrophages and dendritic cells (DCs) are known to have distinct properties in the intestine compared with other organs, and serve as candidates for the identification of IL-10-producing cells. These cells have been shown to possess an immune suppressive function and

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The gastrointestinal tract is continuously exposed to exogenous and endogenous antigens, and the immune

were shown to monitor external antigens without any inflammatory responses. Furthermore, human intestinal macrophages were reported to uniquely induce inflammatory anergy, in which these cells showed unresponsiveness toward inflammatory stimuli but retained the ability to be phagocytic and bacteriocidal.⁷ Interestingly, DCs and macrophages in the intestinal tract have been reported to express a higher amount of IL-10 when responding to inflammatory stimuli than DCs from the spleen.⁸ Thus, the anti-inflammatory response should be considered a unique characteristic of intestinal macrophages and DCs.

We hypothesized that the anti-inflammatory response of intestinal macrophages and DCs was mediated by cell surface molecules recognizing bacterial components. Lectins, carbohydrate recognition proteins, therefore, serve as one of the candidates of these recognition molecules. In the present report, we tested the role of macrophage galactose-type C-type lectin (MGL/CD301), a type II transmembrane lectin that specifically recognizes terminal galactose (Gal) and *N*-acetylgalactosamine (GalNAc) residues as monosaccharides in a calcium-dependent manner.^{9–11} Although other C-type lectins on macrophages and DCs, such as macrophage mannose receptor and DC-specific intracellular adhesion molecules-3 grabbing nonintegrin (DC-SIGN), were previously proposed to play an important role in the elimination of invading pathogens through their function as endocytic receptors, signal transducers, or signal modulators,^{12–14} definite proofs of the role of these lectins in the intestinal protection *in vivo* with knockout mice has not yet been obtained.

Mgl1-deficient mice were used in the present study. The *Mgl* family is known to have two homologous genes in mice, *Mgl1* and *Mgl2*, and these two lectins have distinct carbohydrate recognition specificities, although their distinct roles have not yet been defined.^{15,16} In our previous reports, we found that MGL1 and/or MGL2 were mainly expressed on macrophages and immature DCs, and that these cells were observed mainly in the connective tissue of various organs, especially in skin, large intestines, and lymph nodes.¹⁷ These lectins were found to be involved in the uptake of mucin-like GalNAc-conjugated polymers by murine bone marrow-derived and human monocyte-derived DCs,^{18,19} which was thought to be an important process of antigen processing. *Mgl1*-deficient mice did not show obvious defects in lymphoid and erythroid homeostasis.²⁰ In an *in vivo* study with mouse embryos, MGL1 was shown to function as an endocytic receptor for X-irradiation-induced apoptotic cells, whereas *Mgl1*-deficient mice showed retarded clearance of apoptotic cells in neural tubes.²¹ It was also suggested that MGL1 regulated trafficking of MGL1-expressing cells from skin to lymph nodes.^{22,23} Antigen-induced inflammatory tissue formation in skin was abrogated in *Mgl1*-deficient mice,²⁴ suggesting that MGL1 functioned under inflammatory conditions.

The present study strongly suggests that MGL1 expressed on intestinal lamina propria macrophages functions through its interaction with commensal bacteria by magnifying the IL-10 production by these cells. DSS-

induced experimental colitis caused by infiltration of bacteria was more severe in *Mgl1*-deficient mice than in wild-type mice, probably because of insufficient suppression of inflammation by the shortage of IL-10.

Materials and Methods

Mice

Mgl1-deficient mice and littermate wild-type mice (C57BL/6J strain) were maintained under specific pathogen-free conditions at the Graduate School of Pharmaceutical Sciences of the University of Tokyo. They were fed and housed according to the guidelines of the Bioscience Committee of the University of Tokyo.

Induction and Assessment of Colitis

Colitis was induced in 6- to 8-week-old female mice by feeding them with water containing 2.5% (w/v) DSS (molecular weight, 35,000 to 44,000; ICN Biomedicals, Irvine, CA) for 7 days as previously described.¹ Body weights were measured, and stools were collected daily. Stool blood was assessed by the use of guaiac reaction.²⁵ Histological score was assessed by the criteria described previously.^{26,27} Two sections of the colon were assessed for each mouse.

Immunohistochemical Staining

MGL1-positive cells were immunohistochemically detected in 10- μ m-thick cryostat sections of the large intestine, modified as previously described.¹⁷ Nonspecific bindings were blocked using phosphate-buffered saline (PBS) containing 2% normal goat serum and 3% bovine serum albumin (BSA). The sections were treated with the first antibodies at 4°C for 16 hours, and with alkaline phosphatase-conjugated goat anti-rat IgG (Invitrogen, Eugene, OR). Visualization was performed with Histomark RED (KPL, Gaithersburg, MD). For the staining of isolated cells, cells were attached on poly-L-lysine-coated glass slides on a Cytospin (Thermo Fisher Scientific, Waltham, MA). Cells were fixed with 4% paraformaldehyde for 5 minutes, and stained as described above, except for the use of Alexa-488 streptavidin (Invitrogen). Antibodies used in this study were anti-MGL1 monoclonal antibody (LOM-8.7), anti-CD11b (eBioscience, San Diego, CA), and anti-IL-10 monoclonal antibody (JES5-2A5).

Isolation of Lamina Propria Mononuclear Cells (LPMCs)

Dissected large intestines were cut into small pieces and washed with calcium- and magnesium-free Hanks' balanced salt solution (CMF/HBSS). The epithelium was removed by two consecutive treatments with 5 mmol/L ethylenediaminetetraacetic acid (EDTA) in CMF/HBSS containing 10% fetal calf serum for 15 minutes at 37°C. Intestinal tissue pieces were digested with 1 mg/ml of

Table 1. Primers Used for Conventional and Real-Time PCR

Gene	Forward	Reverse
Conventional PCR		
<i>Mgl1</i>	5'-TCTCTGAAAGTGGATGTGGAGG-3'	5'-CACTACCCAGCTCAAACACAATCC-3'
<i>Mgl2</i>	5'-TCTCTGAAAGTGGATGTGGAGG-3'	5'-GCTATAAGTTGTGGGGAGTGGGC-3'
<i>Il-10</i>	5'-ATGCAGGTCTTTAAGGGTACTTGGGT-3'	5'-ATTTCGGAGAGAGGTACAAACGAGGTTT-3'
<i>β-Actin</i>	5'-CTTCTACAATGAGCTGCGTGTGG-3'	5'-TGATGACCTGGCCGTCAGGCA-3'
Real-time PCR		
<i>Il-10</i>	5'-AGGCGCTGTCATCGATTTCTC-3'	5'-TGGCCTTGTTAGACACCTTGGTC-3'
<i>β-Actin</i>	5'-TCCAGCCTTCCTTCTTGGGA-3'	5'-CAGCACTGTGTTGGCATAGAGG-3'

collagenase (Wako, Tokyo, Japan) and 100 U/ml of DNase I (Roche, Basel, Switzerland) in RPMI 1640 medium containing 10% fetal calf serum at 37°C for 120 minutes. Cells were washed with CMF/HBSS containing 5 mmol/L EDTA and 10% fetal calf serum. LPMCs were further purified by Percoll (GE Health Care, Uppsala, Sweden) density gradient centrifugation.

Flow Cytometry and Cell Sorting

Cells were incubated with fluorescein isothiocyanate-conjugated anti-CD11b, CD11c, MHC class II, phycoerythrin-conjugated F4/80 (eBioscience), and biotin-conjugated anti-MGL1 mAb LOM-8.7 for 30 minutes on ice. Rat IgG2a and rat IgG2b were used as isotype controls. Allophycocyanin-conjugated streptavidin (eBioscience) was used for the detection of biotin-conjugated antibodies. As a marker of viable cells, 7-amino-actinomycin D (eBioscience) was used. All antibodies and streptavidin were diluted by PBS containing 0.1% (w/v) BSA and 0.1% (w/v) sodium azide. Analysis was performed by FACS Aria (BD, Franklin Lakes, NJ) and analyzed with FlowJo software (Tree Star, Ashland, OR).

Incorporation of Latex Beads

Cells were incubated with fluorescent-labeled latex beads (0.1 μ m; Sigma, St. Louis, MO) at 37°C in the CO₂ incubator for 4 hours. Cells were washed with cold PBS and cyto-spun onto poly-L-lysine-coated glass slides. Cells were counterstained with TOTO-3 (Invitrogen), and observed on a confocal microscope, MRC1024 (Bio-Rad, Hercules, CA).

Esterase Staining

Cells were placed on poly-L-lysine-coated glass slides and dried at room temperature. Nonspecific esterase staining was performed by using 1-naphthylacetate in 2-methoxyethanol as a substrate. Cells were counterstained with methyl green and examined on a light microscope.

Conventional and Real-Time Polymerase Chain Reaction (PCR)

Total RNA was extracted from the sorted cells by using a RNeasy mini kit (Qiagen, Valencia, CA). Total RNA was reverse-transcribed into cDNA by Superscript II (Invitro-

gen). All procedures were performed according to the manufacturers' instructions. Quantitative real-time PCR was performed on an ABI Prism 7700 (Applied Biosystems, Foster City, CA) using Power SYBR Green master mix (Applied Biosystems). The primers used for the reaction are listed in Table 1.

Culture of Infiltrated Intestinal Bacteria

Infiltrated intestinal bacteria were cultured from mesenteric lymph nodes of DSS-treated mice obtained on day 7. All procedures were conducted under sterile conditions. Mesenteric lymph nodes were homogenized, plated on MacConkey agar and sheep's blood agar plates (BD Bioscience, San Jose, CA), and cultured at 37°C for 24 hours under aerobic or anaerobic conditions. Bacteria species were determined by Gram staining and selective media. For harvesting bacterial bodies, *Streptococcus* sp. and *Lactobacillus* sp. were plated on a sheep's blood agar plate and an MRS agar plate (BD Bioscience), respectively, for 48 hours at 37°C under anaerobic conditions. Bacterial bodies were washed with PBS twice, and heat-killed bacteria were prepared by incubating them at 100°C for 20 minutes. For *in vitro* stimulation of cells, heat-killed bacteria were added into the cell culture at a concentration of 10 μ g/ml, and incubated for 16 hours at 37°C.

Binding Assays

Recombinant MGL1 (rMGL1) was prepared as previously described¹⁰ and immobilized onto 96-well plates (Greiner, Frickenhausen, Germany) for 16 hours at 4°C. Inhibition of binding was performed with 100 mmol/L of Gal or mannose or 5 mmol/L EDTA by pre-incubation of immobilized rMGL1 with these carbohydrates at room temperature. Heat-killed bacteria were suspended in Dulbecco's modified PBS (DPBS; containing 0.91 mmol/L CaCl₂ and 0.49 mmol/L MgCl₂), and incubated at room temperature for 1 hour. After mild washing with DPBS, bacteria were fixed with 0.25% glutaraldehyde (Wako) and stained with crystal violet. After washing with water, crystal violet was eluted with a mixture of water, ethanol, and methanol (5:4:1) and absorbance was measured at 550 nm. For the uptake assays, CHO cells stably expressing MGL1 were used.²³ Heat-killed bacteria were labeled with the PKH-26 red fluorescent cell linker kit (Sigma) according to the manufacturer's instructions.

Cells were incubated with labeled bacteria for 60 minutes at 37°C and analyzed by flow cytometry on a FACSaria.

Preparation of Bacterial Cell Walls

Bacterial bodies were harvested and ultrasonicated for 30 minutes on ice. Residual cell pellets were removed by centrifugation at $5000 \times g$ for 30 minutes at 4°C. Supernatants were collected and centrifuged at $18,000 \times g$ for 30 minutes at 4°C. Precipitates were dissolved in 4% sodium dodecyl sulfate and boiled for 40 minutes. Cell walls were collected by centrifugation at $18,000 \times g$ at 4°C for 30 minutes and washed three times with distilled water.

Enzyme-Linked Immunosorbent Assay

Cell walls were immobilized on a 96-well plate (Greiner) by loading cell wall solutions at a concentration of 10 $\mu\text{g}/\text{ml}$ in PBS at 4°C for 16 hours. After blocking with 2% BSA in DPBS, biotinylated rMGL1 (brMGL1) that was pre-incubated with and without 1 mmol/L of Gal or mannose at 4°C for 1 hour was incubated with immobilized cell walls for 2 hours. brMGL1 was detected with horseradish peroxidase-conjugated streptavidin (1:1000, Invitrogen), and 1 mmol/L 2,2'-amino-bis(3-ethylbenzthiazoline-6-sulfonic acid) ammonium solutions containing 0.34% H_2O_2 in 0.1 mol/L sodium citrate buffer (pH 4.3). The absorbance at 405 nm was measured.

Statistics

Data are presented as mean $\pm$ SD, where n represents the number of mice per study. Data were compared using either a Student's t -test or a Mann-Whitney U -test, and the differences were statistically significant when P values were <0.05 .

Results

Mgl1-Deficient Mice Showed More Severe Inflammation

To assess the difference in the sensitivity of $Mgl1^{-/-}$ and littermate wild-type mice to colitis formation, the mice were fed with water containing 2.5% DSS for 7 days and the body weights were monitored daily. A decrease in the body weight was observed earlier in $Mgl1^{-/-}$ mice than in wild-type mice, although the difference at each time point was not statistically significant between these two groups (Figure 1A). Stool blood level was assessed as an indicator of severity of the inflammation. An increase in blood in feces was observed with $Mgl1^{-/-}$ mice earlier than with $Mgl1^{+/+}$ mice, and on day 4, $Mgl1^{-/-}$ mice (2.29 ± 0.76 , $n = 7$) showed a significantly higher score of bleeding than $Mgl1^{+/+}$ mice (1.43 ± 0.53 , $n = 7$; $P < 0.05$) (Figure 1B). By histological assessment, marked cellular infiltrations and ulcer formations were observed in the large intestine of $Mgl1^{-/-}$ mice, whereas the epithelial structure remained intact in $Mgl1^{+/+}$ mice on day 7 (Figure 1C).

Figure 1. DSS-induced colitis in wild-type mice and $Mgl1$ -deficient mice. $Mgl1$ -deficient mice and littermate wild-type mice were fed with 2.5% DSS for 7 days. **A:** Body weights of $Mgl1^{+/+}$ (filled circle; $n = 5$) and $Mgl1^{-/-}$ (open circle; $n = 5$) mice. **B:** Blood contents in stools of $Mgl1^{+/+}$ (filled circle; $n = 7$) and $Mgl1^{-/-}$ (open circle; $n = 7$) mice. **C:** H&E-stained sections of colon from $Mgl1^{+/+}$ (left) and $Mgl1^{-/-}$ (right) mice. **D:** Histological scores of damage of colonic walls on day 7. Colon from $Mgl1^{+/+}$ (filled circle) and $Mgl1^{-/-}$ (open circle) were compared. * $P < 0.05$. Data represent mean $\pm$ SD. Scale bar = 200 μm .

The severity of colitis was evaluated by histological scoring, and $Mgl1^{-/-}$ mice (3.13 ± 0.83 , $n = 8$) showed a significantly higher score than $Mgl1^{+/+}$ mice (2.2 ± 0.91 , $n = 10$; $P < 0.05$) (Figure 1D).

Properties of Lamina Propria MGL1-Positive Cells

To identify the cell populations that express MGL1 in the steady state, colonic LPMCs were prepared from untreated mice and analyzed by flow cytometry for the expression of cell surface markers using anti-MGL1 mAb LOM-8.7. Cells expressing MGL1 were shown to express CD11b, CD11c, a high level of F4/80, and MHC class II (Figure 2A). CD11b $^{+}$ and F4/80-high cells expressed MGL1, whereas CD11b $^{+}$ and F4/80-intermediate cells were negative for MGL1 (Figure 2B).

Based on these results, cells with high levels of MGL1 expression in wild-type mice were sorted with CD11b and F4/80, and the presence of $Mgl1$ and $Mgl2$ mRNA was determined by RT-PCR. The mRNA was detected in CD11b $^{+}$ F4/80-high cells but not in CD11b $^{+}$ F4/80-intermediate cells (Figure 2C). Nearly a 100% of the sorted cells that expressed a combination of high levels of CD11b and F4/80 were shown to incorporate fluorescein isothiocyanate-labeled latex beads *in vitro* (Figure 2D). These cells were also positively stained for a nonspecific esterase (Figure 2E). Therefore, the predominant populations of LPMCs expressing MGL1 were considered to be macrophages, although they expressed CD11c.

Figure 2. Characterization of lamina propria macrophages. **A:** Lamina propria macrophages isolated from the large intestine were analyzed by flow cytometry with the anti-MGL1 mAb LOM-8.7 and anti-CD11c, anti-CD11b, anti-MHC class II, or anti-F4/80. **B:** Expression of *MGL1* on CD11b-positive F4/80-high cells (R1) and CD11b-positive F4/80-intermediate cells (R2) was examined. **C:** Expression of *Mgl1*, *Mgl2*, and IL-10 in R1 and R2 cells was examined by RT-PCR. **D:** Incorporation of fluorescent-labeled latex beads (green) by CD11b-positive F4/80-high (R1) and CD11b-positive F4/80-intermediate (R2) cells was assessed under a confocal microscope. Nuclei were counterstained with TOTO-3 (blue). **E:** Nonspecific esterase staining was performed with R1 and R2 cells. Esterase-positive cells stained in dark red. Nucleus was counterstained with methyl green. **F:** Percentage of R1 fraction was examined in *Mgl1*^{+/+} (filled bar) and *Mgl1*^{-/-} (open bar) mice. Data represent mean $\pm$ SD. Original magnifications, $\times$ 100.

Because many reports previously suggested that IL-10 plays a key role in the modulation of DSS-induced experimental colitis in mice as well as Crohn's disease and ulcerative colitis in humans,²⁸ the expression of IL-10 mRNA by MGL1-positive macrophage-like cells was investigated. CD11b⁺ and F4/80-high cells, which consist of the MGL1-positive cells, were shown to contain significantly higher levels of IL-10 mRNA than F4/80-intermediate MGL1-negative cells (Figure 2C). These intestinal macrophage populations were likely to play an immune suppressive role through their IL-10 production, yet the number of these cells in lamina propria was not significantly different in *Mgl1*^{-/-} mice (Figure 2F).

Expression of MGL1 Was Gradually Reduced in the Lamina Propria as Inflammation Proceeded

To further characterize the cells expressing MGL1, untreated or DSS-treated large intestines were stained with the anti-MGL1 mAb LOM-8.7. In untreated tissue, cells expressing MGL1 were observed in the lamina propria and the submucosa (Figure 3A) where immune cells, putatively macrophages, DCs, and lymphocytes, were present. After induction of colitis, cells expressing MGL1 were observed only in the edematous submucosa and were almost absent in the lamina propria of the severe

ulcer region, where CD11b⁺ and MGL1-negative cells were observed (Figure 3A).

Colonic Lamina Propria Macrophages of *Mgl1*^{-/-} Mice Expressed a Smaller Quantity of IL-10 mRNA than Those in Wild-Type Mice at the Early Phase of DSS-Induced Colitis

IL-10 mRNA expression levels were measured in colonic lamina propria macrophages from wild-type mice and the equivalent cells in *Mgl1*^{-/-} mice. mRNA obtained from lamina propria macrophages was examined for the relative quantity of *IL-10* by real-time PCR. The level of IL-10 in lamina propria macrophages was not significantly different on day 0. However, IL-10 from wild-type mice was 2.1-fold higher than that from *Mgl1*^{-/-} mice on day 2 ($n = 3$) (Figure 3B). The results indicate that the cell population with MGL1 on the surface was capable of producing IL-10 and that the level was significantly lower when MGL1 was absent.

MGL1 Interacts with Colonic Commensal Bacteria Isolated from Mice

It is known that interaction between host cells and commensal bacteria plays a crucial role in the pathogenesis

Figure 3. Cells expressing MGL1 in healthy and inflamed colon. **A:** Distribution of cells expressing MGL1 was investigated by immunohistochemical staining with the specimen from DSS-untreated (day 0) and treated mice (day 2 and day 7). MGL1-positive or CD11b-positive cells were detected with mAb LOM-8.7 or anti-CD11b, respectively, and stained in red. Nuclei were counterstained with hematoxylin. **B:** Total RNA was isolated from CD11b-positive F4/80-high cells of DSS-treated mice on day 0 (left) and day 2 (right), and IL-10 mRNA was quantified by real-time PCR. IL-10 mRNA levels in the cells from *Mgl1*^{-/-} (open bar) were significantly lower than *Mgl1*^{+/+} (filled bar). **P* < 0.05. Data represent mean $\pm$ SD.

of DSS-induced colitis. We tested whether MGL1 recognized infiltrating bacteria during the experimental colitis. Because bacterial penetration through the intestinal wall from the lumen seemed to be important,²⁶ commensal bacteria were isolated from mesenteric lymph nodes of DSS-treated mice on day 7. The bacteria obtained were identified as *Escherichia coli*, *Enterococcus* sp., *Streptococcus* sp., and *Lactobacillus* sp. according to morphological examination and growth characteristics in selection media. No bacteria were isolated from lymph nodes of healthy untreated wild-type mice. Binding of these bacteria to immobilized MGL1 is shown in Figure 4A with background binding to immobilized BSA. Of all bacteria isolated from the mice, heat-killed *Streptococcus* sp. and heat-killed *E. coli* bound to immobilized MGL1. The binding was significantly reduced by the addition of 100 mmol/L Gal but not mannose (Figure 4A). The binding was also abrogated by the addition of 5 mmol/L EDTA, indicating that the interaction between the bacteria and MGL1 was calcium-dependent (Figure 4B). To evaluate the interaction of bacteria with MGL1 on cell surfaces,

Figure 4. MGL1 binding to intestinal commensal bacteria. **A:** Commensal bacteria were isolated from mesenteric lymph nodes of DSS-treated mice on day 7. Heat-killed bacterial bodies were applied on microtiter plates immobilized with recombinant MGL1 or BSA. Bound bacteria were detected with crystal violet and measured by absorbance at 550 nm. Hatched bar, plates immobilized with BSA; open bar, plates immobilized with MGL1; filled bar, plates immobilized with MGL1 plus 100 mmol/L Gal; gray bar, plates immobilized with MGL1 plus 100 mmol/L mannose. **B:** Inhibition of binding of bacteria to immobilized MGL1 by incubations with EDTA solutions. Open bar, without EDTA; filled bar, with EDTA. **C:** Engulfment of fluorescent-labeled bacteria examined by flow cytometry. CHO cells transfected with mock (gray) or MGL1 (black line) were tested. **D:** Binding of MGL1 to bacterial cell walls measured by enzyme-linked immunosorbent assay. Cell walls from *Streptococcus* sp. and *Lactobacillus* sp. were immobilized, and reacted with biotinylated recombinant MGL1. **P* < 0.05, ***P* < 0.005, *n* = 3. Data represent mean $\pm$ SD.

uptake of fluorescent-labeled bacteria by CHO cells transfected with *Mgl1* was examined. These cells engulfed *Streptococcus* sp., but not *E. coli* or *Enterococcus* sp. (Figure 4C), suggesting that *Streptococcus* sp. was one of the candidates of bacteria that interact with MGL1 during the pathogenesis of experimental colitis.

Because *Lactobacillus* sp. showed autoaggregation and could not be tested with the binding assays or the uptake assays, binding of MGL1 to these cell wall fractions was measured by enzyme-linked immunosorbent assay. The cell walls of *Streptococcus* sp. also bound MGL1, and this binding was blocked by 1 mmol/L Gal, suggesting that the cell wall fractions contained the MGL1-binding determinants. The cell wall of *Lactobacillus* sp. was also reactive with MGL1 in a carbohydrate-dependent manner (Figure 4D).

Figure 5. Expression of IL-10 in colonic lamina propria macrophages. **A:** Isolated colonic lamina propria macrophages from the large intestine were incubated with or without heat-killed *Streptococcus* sp. for 16 hours. mRNA of cells from *Mgl1*^{+/+} and *Mgl1*^{-/-} mice were isolated and IL-10 mRNA was measured by real-time PCR. Relative expression levels were normalized to the expression of β -actin, and fold increase of the cytokine expression was normalized to untreated cells. Open bar, medium (med); filled bar, heat-killed *Streptococcus* sp. (St.). **B:** Isolated colonic lamina propria macrophages were co-cultured with or without heat-killed *Streptococcus*, and the cells were stained with anti-IL-10 monoclonal antibody (green). Nuclei were counterstained with DAPI (blue). Scale bar = 10 μ m.

Production of IL-10 by Colonic Lamina Propria Macrophages Expressing MGL1

Colonic lamina propria macrophages in *Mgl1*^{-/-} mice were shown to express a smaller amount of IL-10 mRNA than those in wild-type mice on day 2, as shown in Figure 3B. Therefore, we hypothesized that MGL1 modulates the response of lamina propria macrophages to MGL1-reactive commensal bacteria. Intestinal CD11b⁺ and F4/80-high cells were prepared from LPMCs isolated from untreated wild-type or *Mgl1*^{-/-} mice and considered as colonic lamina propria macrophages. These cells were cultured in the presence or absence of heat-killed *Streptococcus* sp. for 16 hours. IL-10 mRNA was compared by real-time PCR. Cells from wild-type mice co-incubated with heat-killed *Streptococcus* sp. showed higher levels of IL-10 expression than control cells (2.6-fold) (Figure 5A). Cells from *Mgl1*^{-/-} mice showed only a slight increase in IL-10 mRNA (1.3-fold) (Figure 5A). To confirm that this mRNA up-regulation lead to an increase in IL-10 protein, colonic lamina propria macrophages were co-cultured with or without 10 μ g/ml of heat-killed *Streptococcus* sp., and stained with anti-IL-10 monoclonal antibodies. The levels of IL-10 were significantly elevated when lamina propria macrophages were cultured with *Streptococcus* sp. (Figure 5B). Such elevation was not observed with the equivalent cells from *Mgl1*^{-/-} mice.

Discussion

The hallmark of inflammatory bowel diseases, including Crohn's disease and ulcerative colitis, is abnormal inflammation of the gastrointestinal tract, but the pathogenesis has not been fully elucidated. In the present study, *Mgl1*-deficient mice exhibited a more severe inflammation than wild-type mice in an experimental model for ulcerative colitis. The mechanistic basis for this difference was determined to be a change in cytokine production in response to intestinal commensal bacteria. The predominant portion of cells expressing MGL1 in colonic lamina propria were found to be macrophages, and these cells were shown to produce IL-10 in response to the bacteria. IL-10 was previously considered a crucial cytokine for the maintenance of intestinal homeostasis because *IL-10*-deficient mice spontaneously developed colitis.⁵ The present study clearly shows that lamina propria macro-

phages lacking *Mgl1* produced less IL-10 than these cells expressing MGL1.

IL-10 produced by a variety of cells, such as macrophages, DCs, and T cells, plays an important role in the pathogenesis of colitis. In macrophage-depleted mice, DSS-induced colitis was more severe, and IL-10 mRNA from the whole colon of these mice was decreased compared with sham-treated mice, indicating that colonic macrophages secrete IL-10 during colonic inflammation.²⁹ However, the most important target of IL-10 remains unclear. IL-10 is likely to influence the functions of a wide range of immune cell populations by suppressing pro-inflammatory responses.²⁹ For example, IL-10 is known to suppress production of IL-12, tumor necrosis factor- α , and reactive oxygen species by macrophages and DCs. It also inhibits Th1 and Th2 responses, which aggravate pathogenic inflammation. Colonic epithelial cells are not likely to act as the target of IL-10. IL-10 has been shown to inhibit MHC class II expression on epithelial cell lines, but it does not affect the chemokine secretion responsible for the recruitment of neutrophils and monocytes to injured sites.^{30,31}

By immunohistochemical analysis, MGL1-expressing cells were observed in the lamina propria and in the submucosa, where many other types of immune cells were also observed. After the induction of colitis, cells expressing MGL1 were absent from this area. Although it is possible that cells expressing MGL1 migrated to other regions, it is likely that MGL1 expression was down-modulated on CD11b⁺ cells, considering that CD11b⁺ cells with similar morphology were present in this ulcerated region. Furthermore, when isolated lamina propria macrophages were exposed to heat-killed *Streptococcus* sp., these cells significantly reduced the cell surface expression of MGL1, as indicated by the binding of monoclonal antibody LOM-8.7 (data not shown). Thus, the disappearance of MGL1 was likely to be attributable to its down-regulation, although the possibility that MGL1-positive macrophages migrated to other regions could not be eliminated. Similarly, expression of MGL on bone marrow-derived DCs was previously shown to be abrogated after maturation by lipopolysaccharide,¹⁹ and absence of MGL1 expression in the late phase of colitis might be a consequence of activation with several stimuli. The immunological significance of this down-modulation of MGL1 remains unclear.

To date, many reports have shown that C-type lectins interact with microorganisms and are involved in endocytosis, signal transduction, and opsonization.¹² Pathogenic microorganisms have been shown to use these lectins for their infection,³² as observed with human MGL acting as an entry site for filovirus.³³ MGL1 should be considered unique among the C-type lectins expressed on macrophages and DCs because of its distinct carbohydrate specificity. Mannose or glucose residues on the surface of pathogens have been found to be reactive with lectins, such as macrophage mannose receptor, DC-SIGN, and dectin-1, whereas MGL1 binds specifically to terminal Gal and GalNAc residues as a monosaccharide. Previously, a soluble lectin expressed in the intestine, intelectin, was shown to bind to *Nocardia rubra* and the binding was inhibited by the addition of Gal,³⁴ although the biological consequence of the binding of bacteria to this lectin is unknown. The present report is the first to demonstrate the role of a Gal-type C-type lectin in the recognition of commensal bacteria.

The presence of the Gal/GalNAc residue in the cell wall polysaccharide of streptococci has been reported.³⁵ Co-aggregation of *Streptococcus viridans*, a member of the oral flora, was inhibited by the addition of oligosaccharides containing Gal and/or GalNAc,³⁶ strongly suggesting that Gal/GalNAc residues were exposed on the surfaces of *Streptococci*. Another study showed that Gal and GalNAc residues were present in an exo-polysaccharide or a capsular polysaccharide produced by *Lactobacillus*.³⁷ Bacteria have been known to produce many types of glycoproteins. The carbohydrate structures are different depending on the species and environments,³⁸ although it is still unclear which glycoprotein is reactive to MGL1.

Possible mechanisms of regulation of cytokine expression by MGL1 may be mediated by the YXXL motif in the cytoplasmic tail. MGL1, MGL2, Dectin-1, and DC-SIGN have been previously shown to contain similar motifs in the cytoplasmic domain, and IL-10 expression has been reported to be induced by the signals from the YXXL motif of Dectin-1.^{39,40} However, possible signal transduction through MGL1/2 was not previously reported. Alternatively, MGL1 might be involved with the modulation of signals through pathogen recognition receptors. Constitutive signals from commensal bacteria through TLRs and MyD88 were reported to be necessary to suppress colitis.^{26,41} Notably, a mutation in *Nod-2* was shown to be a major genetic factor in determining the susceptibility to Crohn's disease.^{42,43} DC-SIGN was also reported to modulate signals from bacterial components and to induce IL-10 expression, although the mechanism was YXXL motif-independent.⁴⁴ Collectively, signaling through these endogenous lectins may be essential for the maintenance of intestinal homeostasis.

In the present study, we provide new insight into the role of a C-type lectin, MGL1, in the pathogenesis of colitis. MGL1 is expressed on lamina propria macrophages of colon and is responsible for the interaction of these cells with commensal bacteria. The received signals from bacterial carbohydrates enhance IL-10 produc-

tion in these cells, resulting in the suppression of intestinal inflammation.

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