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The Membrane-Bound Mucin Muc1 Regulates T Helper 17-Cell Responses and Colitis in Mice

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Abstract

BACKGROUND & AIMS—T helper (Th) 17 cells produce the effector cytokine interleukin (IL)-17, along with IL-22, which stimulates colonic epithelial cells to produce a membrane-bound mucin, Muc1. Muc1 is a component of the colonic mucus, which functions as a lubricant and a physiologic barrier between luminal contents and mucosal surface. The gene *MUC1* has been associated with susceptibility to inflammatory bowel disease; we investigated the role of Muc1 in development of colitis in mice.

METHODS—*Muc1* and *RAG1* were disrupted in mice (*Muc/RAG* double knockout mice); Th1-mediated colitis was induced by intravenous injection of CD4⁺CD45RB^{high} T cells. We also studied Th2-mediated colitis using mice with disruptions in *Muc1* and *T-cell receptor α chain* (*Muc/TCR* double knockout mice).

RESULTS—Muc1 deficiency led to the development of more severe forms of Th1- and Th2-induced colitis than controls. Loss of Muc1 increased colonic permeability and the Th17-cell, but not Th2 or Th1 cell, response in the inflamed colon. Loss of Muc1 also promoted expansion of an innate lymphoid cell population (Lin[−] ckit[−] Thy1⁺ Sca1⁺) that produces IL-17. The expansion of Th17 adaptive immune cells and innate lymphoid cells required the commensal microbiota.

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Supplementary Materials

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at doi:10.1053/j.gastro.2011.

Conflicts of interest:

The authors disclose no conflicts.

CONCLUSIONS—Muc1, which is up-regulated by Th17 signaling, functions in a negative feedback pathway that prevents an excessive Th17 cell response in inflamed colons of mice. Disruption of this negative feedback pathway, perhaps by variants in Muc1, might contribute to inflammatory bowel disease in patients.

Keywords

Susceptibility; Risk Factor; Mouse Model; Immune Regulation

Inflammatory bowel disease (IBD) is a chronic intestinal inflammatory condition, which is classified into 2 major forms: Crohn's disease (CD) and ulcerative colitis (UC).^{1–3} Recent advances on genome-wide association studies (GWAS) have identified 71 susceptibility loci in CD⁴ and 49 susceptibility loci in UC.⁵ Some genes are specific to each disease, and others are common in both diseases, suggesting that CD and UC are mediated by both common and different mechanisms.^{1–3} The currently identified susceptibility genes highlight at least 3 major pathways in IBD, including epithelial defense against commensal microbiota, interleukin (IL)-23 receptor (IL-23R)/T helper cell (Th) 17 axis, and immune regulation.^{2,6,7}

An attractive cell population that links the major IBD pathways together may be Th17 T-cell subset. Th17 subset develops through a reciprocal differentiation. Individual T-cell precursor can differentiate into effector Th17 cells vs forkhead box P3 (Foxp3⁺) regulatory T cells,^{8–10} suggesting a relation of Th17 subset to the initial event of immune regulation pathway in IBD. Polymorphisms of IL-23 receptor are negatively associated with the development of IBD.¹¹ Indeed, pathogenic role of IL-23 has been well documented in several IBD models,¹² and beneficial effect of anti-IL-12p40 monoclonal antibodies (mAbs), which can neutralize both IL-12 (p35/p40) and IL-23 (p19/p40) activities, has been shown in active CD patients.¹³ The IL-23 is required for the maintenance of Th17 cells,^{8–10} highlighting the involvement of Th17 cells in the IL-23 pathway in IBD.^{1–3,7,12} In addition, Th17 cells are considered to closely participate in the pathway of epithelial defense through production of IL-22.⁸ Polymorphisms of IL-10R2, which is shared by IL-22R1 to make a heterodimer receptor for IL-22, are positively associated with IBD,¹⁴ and IL-22 gene is located within an UC susceptibility locus.⁵ Indeed, we and others previously demonstrated the protective role of IL-22 in some colitis models, including Th2-mediated colitis of T-cell receptor α knockout (TCR α KO) mice¹⁵ and Th1-mediated colitis of CD45RB model.¹⁶ IL-22 serves as a major activator of signal transducer and activator of transcription 3 (STAT3) in epithelial cells under intestinal inflammatory conditions^{15,17} and possesses the ability to stimulate the epithelial cells to produce a membrane-bound mucin, Muc1, which is heavily O-glycosylated protein, represents one of the major components in colonic mucus that functions as a lubricant and a physiologic barrier between luminal contents and mucosal surface.^{19,20} Most notably, a recent GWAS has identified Muc1 as a potential susceptibility gene of CD.⁴ However, little information on the role of Muc1 in chronic colitis and intestinal Th17 responses is currently available.

In this paper, we demonstrate that Muc1 serves as a major determinant of colonic Th17 responses under inflammatory conditions, and absence of Muc1 leads to the exacerbation of chronic inflammations in both Th1-mediated and Th2-mediated colitis models. Therefore, it is possible that Muc1 expressions, which can be induced by Th17 response, in turn provide a negative feedback on this response.

Materials and Methods

Mice

TCR α KO and recombination activating gene (RAG)1 KO mice have been described previously.²¹ Muc1 KO mice²² were obtained from Dr S. J. Gendler. All mice were C57BL/6 background. Muc1-deficient TCR α double knockout (Muc/TCR DKO) and Muc1-deficient RAG1 double knockout (Muc/RAG DKO) mice were generated by crossing Muc1 KO mice with TCR α KO mice or RAG1 KO mice. All mice were maintained in the specific pathogen-free facility at Massachusetts General Hospital. All experiments were approved by Subcommittee on Research Animal Care of Massachusetts General Hospital.

Induction and Evaluation of Colitis

Muc1 KO, TCR α KO, and Muc/TCR DKO mice were killed at 6 months of age. For CD45RB model, T cells were first enriched from the wild-type (WT) spleen using T-cell enrichment column (R&D system, Minneapolis, MN). After staining with fluorescein isothiocyanate-anti-CD4 and phycoerythrin (PE)-anti-CD45RB mAbs, CD4⁺CD45RB^{high} T cells were then purified using flow cytometric sorting. The purity was over 99%. CD4⁺CD45RB^{high} T cells (5×10^5) were intravenously transferred into RAG KO vs Muc/RAG DKO mice, and the recipients were killed at 8 weeks after cell transfer. The disease score was assessed by a combination of gross and histologic scores as previously described.^{15,21,23} Myeloperoxidase assays were performed using Myeloperoxidase fluorometric detection kit (Enzo Life Science Inc, Farmingdale, NY). Immunohistochemical analysis using anti-Gr1 mAb was performed as previously described.²⁴

Colonic Cell Isolation

Please see detailed Supplementary Materials.

Colonic Permeability

Colonic permeability was determined in vivo by measuring the appearance in blood of rectally administered horseradish peroxidase (HRP) as previously described.²⁵ After anesthesia of mice, 50 μ L of phosphate-buffered saline containing 0.6 mg of HRP (Sigma-Aldrich, St. Louis, MO) was rectally administered with a feeding needle. Blood samples were then collected from the tail vein at 0, 45, and 120 min after HRP administration, and serum HRP concentrations were measured by enzyme-linked immunosorbent assay.

Depletion of Enteric Flora

Development of colitis was assessed by presence of diarrhea. Enteric flora was then depleted in mice with sign of colitis by treatment with a combination of ampicillin (1 g/L), vancomycin (500 mg/L), neomycin sulfate (500 mg/L), and metronidazole (1 g/L) in drinking water for 3 weeks.^{24,26}

Coculture Experiments

CD11c⁺ dendritic cells (DC) were purified from the inflamed colon of TCR α KO vs Muc/TCR DKO mice as previously described,²⁴ and they were pulsed overnight with 10 μ g/mL ovalbumin (OVA) 323–339 peptides in the presence of lipopolysaccharide. CD4⁺ T cells were purified from the spleen of DO11.10 transgenic RAG2 KO mice (Taconic Farm, Hudson, NY) that have only monoassociated CD4⁺ T cells specific to OVA 323–339. After wash, OVA-pulsed DCs were cocultured with the purified CD4⁺ T cells at 1:2 ratio for 3 days as previously described.²⁷ The T cells were then restimulated with phorbtor-12,13-dibutylate for 4 hours in the presence of Brefeldin A and subjected to intracellular fluorescence-activated cell sorter (FACS) analysis.

Flow Cytometric Analysis

Please see detailed Supplementary materials.

Real-time Polymerase Chain Reaction Analysis

Please see detailed Supplementary materials.

Results

Exacerbation of Th2-Mediated Colitis by Absence of Muc1

We previously demonstrated that IL-22, which contributed to the suppression of colitis in TCR α KO mice, enhanced the STAT3-dependent production of Muc1 in the colonic epithelial cells.¹⁵ However, the role of Muc1 in this Th2-mediated colitis sharing some features with human UC³ still remains unknown. Colonic expressions of IL-22 have been shown to increase with the development of colitis in TCR α KO mice.¹⁵ Similarly, expressions of Muc1 in colonic epithelial cells were upregulated in the inflamed colon of TCR α KO mice (Figure 1A). Therefore, we generated Muc1-deficient TCR α double knockout (Muc/TCR DKO) to know the role of Muc1. As shown in Figure 1B and C, Muc/TCR DKO mice spontaneously developed more severe form of colitis at 6 months of age as compared with TCR α KO mice. In contrast, colitis was not observed in Muc1 KO mice. Interestingly, absence of Muc1 altered the histologic characteristics of colitis with TCR α KO mice. As previously demonstrated,²⁴ Th2-mediated colitis of TCR α KO mice was characterized by elongation of epithelial crypts with inflammatory cell infiltration restricted to the colonic mucosa (Figure 1B, middle panel). In contrast, Muc/TCR DKO mice exhibited more extended inflammatory cell infiltration through the submucosa of colon (Figure 1B, right panel). In addition, more severe epithelial cell damages as indicated by loss of crypts and severe dysplastic changes were observed in the colon of Muc/TCR DKO mice (Figure 1B, right panel).

Consistent with the histology, there was significant increase in the absolute numbers of cells in the inflamed colon of Muc/TCR DKO mice as compared with TCR α KO mice (Figure 1D). Immunohistochemical analysis using anti-Gr1 mAbs suggested that the enhanced inflammatory cell infiltration in Muc/TCR DKO mice is associated with an increase in neutrophils (Figure 1E). Indeed, myeloperoxidase activity (a marker of neutrophil infiltration) was significantly up-regulated in the inflamed colon of Muc/TCR DKO mice as compared with TCR α KO mice (Figure 1F). Taken together, these findings suggest that Muc1 plays a protective role in the Th2-mediated chronic colitis.

Enhanced Colonic IL-17 Responses by Absence of Muc1

We found that absolute numbers of CD4⁺ T cells also increased in the inflamed colon of Muc/TCR DKO mice as compared with TCR α KO mice (Figure 2A). To see the cytokine profile of the increased CD4⁺ T cells, colonic lamina propria cells were isolated from the diseased colon of Muc/TCR DKO vs TCR α KO mice, stimulated in vitro, and subjected to FACS analysis. Enhanced Th2 response has been well documented in TCR α KO mice by many groups.^{28,29} Unexpectedly, absence of Muc1 altered the cytokine profile to Th17 dominant response in Muc/TCR DKO mice (Figure 2B). In addition, immunohistochemical analysis showed that CD4⁺ IL-17⁺ T cells diffusely exist throughout the inflamed colon of Muc/TCR DKO mice (Figure 2C). In contrast, there was no significant difference in Th2 responses as judged by IL-4 production in the inflamed colon of TCR α KO vs Muc/TCR DKO mice (Figure 2D). In addition, absence of Muc1 did not alter the colonic Th1 responses as judged by interferon (IFN)- γ productions (Figure 2D). To confirm further the unexpected result, real-time polymerase chain reaction analysis was next performed using freshly isolated and purified CD4⁺ T cells from the inflamed colon of TCR α KO vs Muc/

TCR DKO mice. Again, significant increases in the expression of IL-17A and IL-17F as well as RAR related orphan receptor gamma (ROR γ t) (the major factor of Th17 cell development) were observed in the colonic CD4⁺ T cells from Muc/TCR DKO mice as compared with TCR α KO mice (Figure 2E). In contrast, there was no significant difference in the expression of IL-4, GATA binding protein 3 (GATA3) (a transcription factor of Th2), IFN- γ , or T-bet (a transcription factor of Th1) (Figure 2E). These findings suggest the ability of Muc1 to suppress Th17, but not Th2 or Th1, response in Th2-mediated intestinal inflammation.

Expansion of IL-17-Producing Innate Lymphoid Cells in Absence of Muc1

Interestingly, we found that enhanced production of IL-17 can be observed not only in CD4⁺ cell population but also in CD4⁻ cell population in the inflamed colon of Muc/TCR DKO mice (Figure 3A). TCR $\gamma\delta$ T cells have recently been shown to produce IL-17,³⁰ and a marked increase in TCR $\gamma\delta$ T cells is a characteristic feature with the colitis of TCR α KO mice.²⁴ These previous findings prompted us to develop a hypothesis that TCR $\gamma\delta$ T cells may be another major source of IL-17 in the inflamed colon of Muc/TCR DKO mice. However, IL-17 production was observed in TCR $\gamma\delta$ ⁻ cell population but not in TCR $\gamma\delta$ ⁺ cell population (Figure 3B). In addition, immunohistochemical analysis found that some IL-17⁺ CD4⁻ cells tend to focally exist in the inflamed colon of Muc/TCR DKO mice (Supplementary Figure 1).

More recently, a previously unappreciated innate lymphoid cell population, which is characterized by lineage marker (Lin)⁻ cKit⁻ Sca1⁺ Thy1⁺ phenotype, was identified to produce IL-17 in the colon.³¹ Interestingly, our subsequent studies found a significant increase in the Lin⁻ cKit⁻ Sca1⁺ Thy1⁺ innate lymphoid cells within the inflamed colon of Muc/TCR DKO mice as compared with TCR α KO mice (Figure 3C and D). As previously demonstrated,³⁰ they were indeed able to produce IL-17 (Figure 3E). These findings suggest that Muc1 also contributes for suppressing the expansion of Lin⁻ cKit⁻ Sca1⁺ Thy1⁺ innate lymphoid cells.

Requirement of Commensal Microbiota for the Enhanced Th17 Response by Absence of Muc1

Muc1 serves as one of the major components in the mucus that provides the first barrier against microorganisms.²⁰ Alternatively, hypoglycosylated Muc1 per se has previously been proposed to stimulate T cells by serving as an antigen.³² Therefore, to test whether the enhanced Th17 responses by absence of Muc1 is mediated by innate (commensal microbiota) or adaptive (antigenic activity of Muc1) immune mechanism, we depleted enteric bacteria in Muc/TCR DKO mice by administration of a combination of antibiotics as previously described.^{23,26} As the result, we found that the depletion of enteric bacteria significantly reduced the number of IL-17-producing CD4⁺ T cells in the colon (Figure 4A). In addition, significant decrease in the absolute number and proportion of Lin⁻ cKit⁻ Sca1⁺ Thy1⁺ innate lymphoid cells were also observed in the colon of Muc/TCR DKO mice after antibiotics treatment (Figure 4B). The suppressed development of IL-17-producing cell populations by antibiotics treatment was associated with the significant reduction of inflammatory cell infiltration in the inflamed colon (Figure 4C). These findings suggest that commensal microbiota is responsible for the enhanced development of Th17 T cells and innate lymphoid cells in absence of Muc1.

Increased Colonic Permeability by Absence of Muc1

Because enteric bacteria were required for the enhanced Th17 response in the absence of Muc1, we next performed bacterial invasion and colonic permeability assays to see whether absence of Muc1 alters the epithelial barrier system. Interestingly, colonic epithelial

permeability, as evidenced by detection of elevated serum levels of the tracer HRP following rectal administration, was significantly increased in Muc/TCR DKO mice as compared with TCR α KO mice (Figure 4D). In contrast, there was no significant difference in the bacterial translocation into the spleen and colon of these mouse groups (data not shown). These findings suggest that absence of Muc1 enhances the translocation of some molecules such as bacterial products from intestinal lumen to colonic lamina propria.

No Effect of Muc1 on the Function of Antigen-Presenting Cells

The Th responses are determined by interaction of T cells with antigen-presenting cells such as DCs. To see whether the enhanced Th17 response in Muc/TCR DKO mice results from altered DC function, an in vitro coculture system was employed. Briefly, OVA-specific CD4⁺ T cells from DO11.10 transgenic RAG2 KO mice were cocultured with OVA-loaded colonic CD11c⁺ DCs from TCR α KO vs Muc/TCR DKO mice. Interestingly, both DCs induced the considerable production of IFN- γ but not IL-17 or IL-4 by OVA-specific CD4⁺ T cells (Figure 4E). These findings suggest that fundamental function of DCs is not altered by absence of Muc1. Therefore, it is possible that the enhanced Th17 response by Muc1 deficiency is mediated by some specific antigens that may be enriched in the colonic lamina propria because of the increased colonic permeability or by altered colonic environment that may further condition DCs in vivo to induce Th17 responses.

Contribution of Muc1 for Suppression of a Th1-Mediated Colitis

Muc1 has recently been identified as a potential CD susceptibility gene,⁴ and a protective role of IL-22, which enhances the production of Muc1, has also been demonstrated in a Th1-mediated colitis model (CD45RB model)¹⁶ that shares many gene signatures with human CD.³³ We confirmed that Muc1 expression was up-regulated in the colonic epithelial cells of CD45RB model as compared with RAG1 KO mice (Figure 5A). To dissect the role of Muc1 in this Th1-mediated colitis, we next generated Muc1-deficient RAG1 double knockout (Muc/RAG DKO) mice by crossing Muc1 KO mice with RAG1 KO mice. FACS purified CD4⁺ CD45RB^{high} T cells from the spleen of WT mice were then transferred into the Muc/RAG DKO vs RAG KO mice. As previously demonstrated,¹² the recipient RAG1 KO mice developed colitis with different severities at 8 weeks after cell transfer. Alternatively, all Muc/RAG DKO mice with transfer of WT CD4⁺ CD45RB^{high} T cells uniformly developed very severe colitis (Figure 5B and D). The exacerbation of colitis in the recipient Muc/RAG DKO mice was supported by the high incidence of granuloma development (Figure 5C). Seven out of 8 recipient Muc/RAG DKO mice developed granulomas, whereas granulomas were unrecognized in recipient RAG1 KO mice. These findings suggest that Muc1 may contribute to the improvement of a Th1-mediated colitis developing in CD45RB model.

Ability of Muc1 to Suppress Th17, but not Th1, Response in Th1-Mediated Colitis

We next examined the cytokine profile of colonic CD4⁺ T cells from RAG1 KO vs Muc/RAG DKO mice after transfer of WT CD4⁺ CD45RB^{high} T cells to see whether absence of Muc1 alters the Th1 responses in the CD45RB model.⁹ As the result, we found that Th17 response was significantly enhanced in the inflamed colon of Muc/RAG DKO mice as compared with RAG1 KO mice (Figure 6A and B). In contrast, there was no significant difference in Th1 responses (IFN- γ production) among these 2 mouse groups (Figure 6A and B). These data were further confirmed by real-time polymerase chain reaction analysis using freshly isolated and purified CD4⁺ T cells from the inflamed colon. Enhanced expressions of IL-17A, IL-17F, and ROR γ t were observed in the colonic CD4⁺ T cells from recipient Muc/RAG DKO mice as compared with recipient RAG1 KO mice (Figure 6C). In contrast, there was no significant difference in the expression of IFN- γ or T-bet (Figure 6C).

As seen in Th2-mediated colitis model (Figure 3), absence of Muc1 again led to the significant increase in the absolute number of IL-17-producing Lin⁻ cKit⁻ Sca1⁺ Thy1⁺ innate lymphoid cells in the inflamed colon of the CD45RB model (Figure 6D and E). Taken together with the data from Th2-mediated colitis model, these findings suggest that Muc1 is involved in the suppression of IL-17-producing cells in both Th1-mediated colitis and Th2-mediated colitis models.

Discussion

We herein demonstrate that a membrane-bound mucin, Muc1, serves as a major determinant of Th17, but not Th1 or Th2, response in the inflamed colon of both Th1-mediated and Th2-mediated colitis models. In addition, absence of Muc1 enhanced the expansion of recently identified Lin⁻ cKit⁻ Sca1⁺ Thy1⁺ innate lymphoid cells that produce IL-17 and boosts Th17 responses.^{8,31} Th17 T cells produce not only IL-17 but also IL-22 that has previously been demonstrated by us and others to stimulate the STAT3-dependent production of Muc1 in colonic epithelial cells.^{15,18} Therefore, we herein propose that Muc1 expressions, which are enhanced by Th17 response, in turn contribute to the termination of this response. Thus, a breach in this negative feedback pathway may lead to the consequent exacerbation of IBD. Indeed, IL-22, IL-10R2 (a component of IL-22 receptor), STAT3, and Muc1, all of which are involved in the Th17-associated epithelial defense pathway, have been previously and recently identified as potential IBD susceptibility genes.^{2,4,5,14}

Our data reveal that the enhanced Th17 response in absence of Muc1 is abrogated by depletion of enteric bacteria, supporting the concept that development of intestinal Th17 response can be induced by commensal microbiota.^{8,34,35} Some bacteria-derived adenosine 5'-triphosphate stimulates the development of intestinal Th17 T cells,³⁴ and a segmented filamentous bacterium leads to the appearance of intestinal Th17 cells.³⁵ Interestingly, absence of Muc1 increased the colonic epithelial permeability and specifically enhanced Th17, but not Th1 or Th2, responses under intestinal inflammatory conditions. Therefore, it is possible that Muc1 participates in the defense against selected bacterial products favoring Th17 responses through the charge density of glycosylated Muc1. In addition, the data from TCR α KO mice may raise an attractive possibility regarding the bacteria-derived antigen for inducing intestinal Th17 development. CD4⁺ T cells in TCR α KO mice express functional TCR β chains without TCR α chains.³⁶ The physiologic relevance of this unique T-cell population is highlighted by recent studies, including a single cell analysis showing that approximately 20% of peripheral TCR β ⁺ T cells in WT mice express nonfunctional (out-frame) TCR α genes³⁷ and a genomic study identifying TCR β ⁺ T cells without functional expressions of TCR α chains in humans.³⁸ The unique T cells can recognize superantigens or superantigen-like molecules rather than conventional antigens.³⁹ Therefore, it is possible that enteric bacteria-derived superantigens possess the ability to induce the development of colonic Th17 responses particularly under inflammatory conditions. Indeed, a bacteria-derived, CD-specific superantigen termed *I2* has been identified.⁴⁰

We herein demonstrate that Muc1 contributes for the improvement of both Th1-mediated (CD45RB model) and Th2-mediated (TCR α KO mice) chronic colitis. The major function of Muc1 is the defense against enteric microorganisms as indicated by the fact that absence of Muc1 increases the susceptibility of mice to intestinal infection.⁴¹ Therefore, it is highly likely that Muc1-dependent suppression of both Th1-mediated and Th2-mediated colitis may be initially mediated by the reduction of host/microbial interaction. Indeed, commensal microbiota is required for the induction and progression of the majority of chronic colitis models, including the 2 models used.^{1,3,6,36} In contrast, commensal microbiota plays opposite role in acute intestinal damage as indicated by the exacerbation of dextran sulfate sodium-induced acute intestinal injury in the absence of enteric bacteria.²⁶ Consistent with

this, Muc1 KO mice were recently proposed to be resistant to the dextran sulfate sodium-induced acute intestinal injury.⁴² Taken together, these findings suggest the active involvement of Muc1 for controlling host/microbial interactions in any type of intestinal inflammation.

Muc1, which is heavily O-glycosylated protein, forms a static external barrier at the epithelial cell surface.^{19,20} Previous studies demonstrated that overexpressions of “hypoglycosylated” Muc1 exacerbated another Th1-mediated chronic colitis developing in IL-10 KO mice.^{32,43} Therefore, it is possible that complete glycosylation is required for the protective function of Muc1, and the hypoglycosylated Muc1 makes a breach in the static barrier, resulting in the exacerbation of colitis. Indeed, impaired glycosylation in the mucus has been demonstrated to exacerbate colitis.^{44,45} In addition, we recently confirmed that IL-22 stimulates colonic epithelial cells to express not only Muc1 but also core 3 β 1,3-N-acetylglucosaminyl transferase responsible for the O-glycosylation in colonic mucus (our unpublished data). Taken together, these findings suggest that the protective function of Muc1 in chronic colitis may be determined not only by the expression levels but also by the glycosylation state.

In summary, we herein demonstrate the protective function of a potential CD susceptibility gene product Muc1 in both Th1-mediated and Th2-mediated colitis models. In addition, we propose that Muc1, which functions as a distal event in Th17-induced epithelial defense pathway, provides a negative feedback to prevent excessive intestinal Th17 responses under inflammatory conditions. Therefore, it is possible that IBD susceptibility genes negatively and positively interact with each others to maintain appropriate balance of innate and adaptive immunity for preventing the induction and/or progression of IBD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used in this paper

CD	Crohn's disease
DC	dendritic cells
DKO	double knockout
FACS	fluorescence-activated cell sorter
GWAS	genome-wide association studies
HRP	horseradish peroxidase
IBD	inflammatory bowel disease
IL	interleukin

IFN	interferon
KO	knockout
mAbs	monoclonal antibodies
Muc1	mucin 1
Muc/TCR DKO	Muc1-deficient TCR α double knockout
Muc/RAG	Muc1-deficient RAG1 double knockout
OVA	ovalbumin
RAG	recombination activating gene
TCR	T-cell receptor
Th	T helper
UC	ulcerative colitis
WT	wild type

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Figure 1.

Protective role of Muc1 in Th2-mediated colitis: (A) Real-time polymerase chain reaction analysis shows the expression levels of Muc1 in the colonic epithelial cells from WT mice (*open bars*) and TCR α KO mice without (*shaded bars*) or with (*solid bars*) colitis. $*P < .05$. (B) Histology of colon (original magnification, $\times 4$) from Muc1 KO (*left panel*), TCR α KO (*middle panel*), and Muc/TCR DKO (*right panel*) at 6 months of age is shown. Colitis in TCR α KO mice is characterized by elongation of epithelial crypt, whereas epithelial cell damage with more exacerbated inflammatory cell infiltration is observed in the colon of Muc/TCR DKO mice. (C) The disease scores of Muc1 KO, TCR α KO, and Muc/TCR DKO mice are summarized. Each *dot* represents an individual mouse. $**P < .0005$. (D) Absolute

numbers of infiltrated cells into the colon of Muc1 KO (n = 8), TCR α KO (n = 8), and Muc/TCR DKO (n = 8) mice are shown. * P < .05, ** P < .005. (E) Immunohistochemical analysis of colon from TCR α KO (*left panel*) vs Muc/TCR DKO (*right panel*) mice using anti-Gr1 monoclonal antibodies is shown. (F) Myeloperoxidase assay suggests the increased neutrophil activity in the inflamed colon of Muc/TCR DKO mice as compared with TCR α KO mice.

Figure 2.

Enhanced Th1, but not Th2, response in Th2-mediated colitis by absence of Muc1. (A) Absolute numbers of CD4⁺ T cells in the inflamed colon of TCR α KO (n = 6) vs Muc/TCR DKO (n = 6) mice are shown. ***P* < .001. (B) CD4⁺ T cells from the inflamed colon of TCR α KO (left panel) vs Muc/TCR (right panel) mice were stimulated with phorb-12, 13-dibutylate and subjected to fluorescence-activated cell sorter analysis. Representative figures on the expressions of IL-17 vs IL-4 in the colonic CD4⁺ T cells are shown. (C) Immunofluorescent staining shows the expressions of IL-17F (green, left panel) vs CD4 (red, middle panel) in the inflamed colon of Muc/TCR DKO mice. The merge image is shown in right panel. The result is representative of 9 Muc/TCR DKO mice. (D) Absolute

numbers of colonic CD4⁺ T cells expressing IL-17 (*Th17, left panel*), IL-4 (*Th2, middle panel*), or IFN- γ (*Th1, right panel*) are summarized. * $P < .01$. (E) Real-time polymerase chain reaction analysis shows the expression levels of IL-17A, IL-17F, ROR γ t, IL-4, GATA3, IFN- γ , and T-bet in the colonic CD4⁺ T cells from TCR α KO (*open bars*) vs Muc/TCR DKO (*solid bars*) mice. The data represent the average ($n = 5$) of the values of 2^{-CT} of target molecules/ 2^{-CT} of β -actin. * $P < .05$, ** $P < .0005$.

Figure 3.

Increased IL-17-producing innate cell population in the absence of Muc1. (A) Colonic cells from inflamed colon of Muc/TCR DKO mice were stimulated with phorbtor-12,13-dibutylate and subjected to cytokine fluorescence-activated cell sorter analysis. IL-17 productions are observed in both CD4⁺ and CD4⁻ cell populations. (B) Expressions of IL-17 vs TCRγδ in total cells from the inflamed colon of Muc/TCR DKO mice after stimulation with phorbtor-12,13-dibutylate are shown IL-17 productions are recognized in TCRγδ⁻ but not TCRγδ⁺ cell population. (C) Representative figures of Sca1⁺ Thy1⁺ cells in the gated lin⁻ cKit⁻ cell population from the inflamed colon of TCRαKO (*left panel*) vs Muc/TCR (*right panel*) mice are shown. (D) Absolute numbers of Lin⁻ cKit⁻ Sca1⁺ Thy1⁺ cells in the inflamed colon of TCRαKO (n = 3) and Muc/TCR (n = 4) are summarized. ***P* < .0005 (E) Expression of IL-17 in the colonic Lin⁻ cKit⁻ Sca1⁺ Thy1⁺ cells of Muc/TCR DKO mouse is shown. This observation was representative in 8 separate experiments.

Figure 4.

Requirement of commensal microbiota for the enhanced Th17 response by absence of Muc1. Muc/TCR DKO mice with colitis were treated with a combination of antibiotics for 3 weeks. (A) Expressions of IL-17 in colonic CD4⁺ T cells from Muc/TCR DKO mice without (*left panel*) vs with (*middle panel*) antibiotics treatment are shown. Absolute numbers of colonic Th17 cells in Muc/TCR DKO mice without (n = 5) vs with (n = 5) antibiotics treatment are summarized in right panel. **P* < .05. (B) Representative figures of Sca1⁺Thy1⁺ cells in the gated Lin[−] cKit[−] cell population from the colon of Muc/TCR DKO mice without (*left panel*) or with (*middle panel*) antibiotics treatment are shown. The absolute numbers of Lin[−] cKit[−] Sca1⁺Thy1⁺ cells are summarized in right panel. ***P* < .0005. (C) Absolute numbers of inflammatory cell infiltrates into the colon of Muc/TCR DKO mice without vs with antibiotics treatment are summarized. **P* < .01. (D) After rectal administration of horseradish peroxidase, serum horseradish peroxidase concentrations in TCRαKO (*open bars*) vs Muc/TCR DKO (*solid bars*) mice were measured at 0, 45, and 120 minutes. ***P* < .005. (E) OVA-loaded colonic DCs from TCRαKO mice and Muc/TCR

DKO mice induce Th1, but not Th17 or Th2, responses of OVA-specific CD4⁺ T cells. The data are representative in 2 individual mice.

Figure 5.

Protective role of Muc1 in Th1-mediated colitis. (A) Real-time polymerase chain reaction analysis shows the expression levels of Muc1 in the colonic epithelial cells from WT mice (open bar) and RAG1 KO mice without (shaded bar) or with (solid bar) transfer of CD4⁺CD45RB^{high} T cells. * $P < .005$. (B) Colonic histology (original magnification, $\times 4$) of recipient RAG KO (top panel) vs Muc/RAG DKO (bottom panel) at 8 weeks after adoptive transfer of CD4⁺CD45RB^{high} T cells from WT mice is shown. More severe colitis with granulomas (arrow) is recognized in the recipient Muc/RAG DKO mice. (C) High magnification (original magnification, $\times 40$) of representative granuloma seen in Muc/RAG DKO mice with transfer of WT CD4⁺CD45RB^{high} T cells is shown. (D) Disease scores of

recipient RAG KO vs Muc/RAG DKO at 8 weeks after adaptive transfer of WT CD4⁺CD45RB^{high} T cells are summarized. Each *dot* represents an individual mouse. * $P < .001$.

Figure 6.

Enhanced Th17, but not Th1, response in Th1-mediated colitis by absence of Muc1. (A) Expressions of IFN- γ vs IL-17 in colonic CD4⁺ T cells from RAG KO (*left panel*) vs Muc/RAG DKO (*right panel*) at 8 weeks after transfer of WT CD4⁺CD45RB^{high} T cells are shown. (B) Absolute numbers of colonic CD4⁺ T cells producing IL-17 (Th17) or IFN- γ (Th1) in RAG KO (n = 5) vs Muc/RAG DKO (n = 5) after transfer of WT CD4⁺CD45RB^{high} T cells are summarized. **P* < .05. (C) Real-time polymerase chain reaction analysis shows the expression levels of IL-17A, IL-17F, ROR γ t, IFN- γ , and T-bet in the colonic CD4⁺ T cells from RAG KO (*open bars*, n = 5) vs Muc/RAG DKO (*solid bars*, n = 5) after transfer of WT CD4⁺CD45RB^{high} T cells. The data represent the average of the values of 2^{-CT} of target molecules/2^{-CT} of β -actin. **P* < .05, ***P* < .005. (D) Representative figures of Sca1⁺ Thy1⁺ cells in the gated lin⁻ cKit⁻ cell population from the colon of RAG

KO (*left panel*) vs Muc/RAG DKO (*right panel*) at 8 weeks after transfer of WT CD4⁺CD45RB^{high} T cells are shown. (E) The absolute numbers of Lin⁻ cKit⁻ Sca1⁺ Thy1⁺ cells are summarized. ** $P < .005$.